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Etude de la composition chimique et du potentiel biologique de *Pistacia lentiscus*, plante médicinale traditionnelle d'Algérie

Chabha Sehaki

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Chabha Sehaki. Etude de la composition chimique et du potentiel biologique de *Pistacia lentiscus*, plante médicinale traditionnelle d'Algérie. Autre. Université de Picardie Jules Verne; Université Mouloud Mammeri (Tizi-Ouzou, Algérie), 2022. Français. NNT : 2022AMIE0058 . tel-04566259

HAL Id: tel-04566259

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Thèse de Doctorat en Cotutelle

Mention: Chimie
Spécialité: Chimie thérapeutique

Présentée à *l'Ecole Doctorale en Sciences Technologie et Santé (ED 585)* de
l'Université de Picardie Jules Verne

et

à *l'Ecole doctorale de chimie* de
L'Université Mouloud Mammeri de Tizi-Ouzou
par

Chabha SEHAKI

pour obtenir le grade de

Docteur de l'Université de Picardie Jules Verne

Et

Docteur de l'Université Mouloud Mammeri de Tizi-Ouzou

*Etude de la composition chimique et du potentiel biologique
de Pistacia lentiscus, plante médicinale traditionnelle
d'Algérie*

Soutenance le 15 décembre 2022, après avis des rapporteurs, devant le jury d'examen:

M. F. Mesnard, Professeur, Univ. de Picardie Jules Verne
M^{me}. D. Laurain-Matter, Professeur, Univ. de Lorraine
M. A. Aziz, Professeur, Univ. de Reims
M. K. Houali, Professeur, Univ. de Tizi-Ouzou
M^{me}. D. Mezioug, Professeur, Univ. USTHB d'Alger
M. E. Gontier, Professeur, Univ. de Picardie Jules Verne
M^{me}. F. Fernane, Professeur, Univ. de Tizi-Ouzou
M. N. Jullian, Professeur, Univ. de Picardie Jules Verne

Président
Rapporteur
Rapporteur
Examineur
Examineur
Directeur de thèse
Co-directeur de thèse
Co-encadrant

Avant-propos

Cette Thèse en cotutelle est issue de travaux conjoints du laboratoire de ressources naturelles à l'Université de Mouloud Mammeri de Tizi-Ouzou, en Algérie, et de celui de Biologie des Plantes et Innovation BIOPI UMRT BioEcoAgro INRAE1158, à l'Université de Picardie Jules Verne, en France.

Le premier de ces deux laboratoires travaille depuis de nombreuses années dans la caractérisation et la valorisation des plantes endémiques de la région de Tizi-Ouzou. Le second laboratoire est spécialisé dans l'étude des molécules issues de plantes médicinales notamment les huiles essentielles et les polyphénols. Ce laboratoire travaille également sur la compréhension des modifications de la paroi cellulaire et du métabolisme au cours du développement végétal. Il met en avant des modèles de développement ciblés (graine, hypocotyle...), en conditions contrôle/stress, sur des espèces modèles (*Arabidopsis*) ou des espèces d'intérêt agro-économique (*Lin*).

Ce projet de Thèse porte sur l'étude de la composition chimique et du potentiel biologique de la plante *Pistacia lentiscus*. Ce travail de thèse est devisé en deux grands volets : une étude des huiles essentielles et une étude des polyphénols.

Dans un tout premier temps, l'échantillonnage de la plante *Pistacia lentiscus* a été réalisé à Tizi-Ouzou, en Algérie.

L'étude sur les huiles essentielles est initiée par l'extraction des huiles essentielles des feuilles, au laboratoire de ressources naturelles. Ensuite la caractérisation de ces huiles par chromatographie en phase gazeuse couplée à la spectrométrie de masse et l'évaluation de leur potentiel antioxydant ont été effectuées au laboratoire BIOPI (pôle Sciences, UPJV-Amiens).

Pour le second volet de la Thèse portant sur l'étude des 'polyphénols', la préparation des extraits phénoliques et l'évaluation de leur potentiel antidiabétique ont été effectuées au laboratoire BIOPI. L'analyse phytochimique de ces extraits phénoliques a été réalisée à la plateforme analytique de l'UPJV. Le traitement des données a été réalisé à la faculté de pharmacie au laboratoire BIOPI

Ce mémoire est construit autour de ces deux volets d'activité de recherche et pour chaque thème, une synthèse bibliographique est présentée afin de mettre en place l'étude expérimentale et l'analyse prospective des résultats. Ce sont donc quatre chapitres qui vont être présentés autour de chacun des articles constituant le bilan scientifique de cette Thèse.

Nous terminerons par une conclusion d'ensemble suivie d'une analyse en perspective.

REMERCIEMENTS

Je tiens à remercier en tout premier lieu mes directeurs de thèse, M. Eric GONTIER, Mme Farida FERNANE et Mme Nathalie PAWLICKI-JULLIAN de m'avoir soutenue et encouragée tout au long de cette thèse. J'ai pris un grand plaisir à travailler avec vous.

Ensuite, j'aimerais exprimer un grand merci aux membres du jury qui m'ont fait l'honneur de bien vouloir évaluer mon travail.

J'adresse également mes remerciements à Roland Molinié, David Mathiron, Elodie Choque, Jean-Xavier Fontaine, Rebecca Dauwe, Serge Pilard, Francois Mesnard et tous les membres des laboratoires BIOPI de la faculté des sciences, de la faculté de pharmacie et de la plateforme analytique, avec qui, j'ai réalisé ce travail de Thèse.

Je continuerai à remercier infiniment tout le personnel du laboratoire BIOPI, les permanents et les nombreux stagiaires, pour leur accueil toujours aussi chaleureux.

Merci à l'équipe technique pour le soutien qu'elle m'a apporté, et en particulier à Romain Roulard pour la GC-MS et Solène BASSARD pour sa bonne humeur, ses conseils et ses réponses à toutes mes questions pendant ces trois années. Merci à Véronique Dulin pour votre travail et votre sympathie.

Je veux aussi remercier le personnel du Laboratoire de Ressources Naturelles en particulier Mme Ayati Fadila et Mme Saadoun Noria pour ses précieux conseils concernant l'échantillonnage de la plante surtout au début de ma thèse. Aussi Mme Benhouhou Amina professeur à l'INSA d'El Harrach pour l'identification botanique de la plante étudiée dans le cadre de ce projet de thèse.

Je remercie les membres du comité de suivi individuel de ma thèse; Monsieur Alain Hehn, Madame Sevser Sahpaz et Madame Catherine Sarazin, pour la bonne évaluation de mon travail de thèse pendant ces trois années et pour leurs conseils précieux qui m'ont permis de faire mieux que le travail initialement prévu.

J'exprime également ma gratitude à Campus France et aux personnels administratifs des deux établissements UPJV et UMMTO pour la bonne orientation, le bon accueil et pour l'aboutissement du financement et de la mobilité pendant ma thèse.

Merci à vous tous !

Enfin, à mes parents, mon Père, ma mère, mes sœurs,

un très très grand merci à vous pour votre amour et votre soutien !!!

Un très grand merci à mon père SEHAKI Ahmed, pour sa longue contribution à l'échantillonnage de la plante

Résumé

Cette thèse vise à caractériser et à évaluer la composition chimique de *Pistacia lentiscus* provenant de la région algérienne de Tizi-Ouzou. Le travail est construit à partir de prélèvements réalisés en montagne et en plaine littorale au cours des quatre saisons de l'année 2019. En parallèle, ce travail de recherche met en évidence des propriétés biologiques potentiellement valorisables; en particulier des activités antioxydantes et une activité antidiabétique *in vitro* (effet inhibiteur de l' α -glucosidase).

Une étude bibliographique exhaustive a été effectuée sur la phytochimie, les propriétés pharmacologiques et l'usage traditionnel de la plante *Pistacia lentiscus*. Les données extraites ont visé à répondre à la problématique de cette Thèse.

Les travaux expérimentaux sont basés sur deux principaux volets :

- Le premier volet porte sur l'étude de la variabilité de la composition et du potentiel antioxydant de l'huile essentielle sous l'effet de l'interaction entre l'altitude et la saison de récolte de lentisque. L'extraction des huiles essentielles a été faite par hydrodistillation Clevenger. La caractérisation de leur composition chimique a été effectuée par GC-MS. L'activité antioxydante de ces huiles a été évaluée avec les trois tests ABTS, DPPH, et FRAP. Les résultats montrent que la saison et l'altitude de récolte ont un effet significatif sur la variation chémotypique et le potentiel antioxydant des huiles essentielles de lentisque.

- Le second volet a été consacré au criblage phytochimique des composés inhibiteurs de l' α -glucosidase dans différentes parties de *Pistacia lentiscus* en utilisant une étude chimiométrique qui relie l'activité biologique et les données UHPLC-ESI-HRMS. En outre, ce travail s'intéresse également à l'évaluation de la variation de l'intensité de l'inhibition de l'activité de l' α -glucosidase en fonction de l'habitat de la plante (montagne et littoral) et de l'organe (feuilles, écorces de tige, fruits). Les résultats de cette partie révèlent que l'écorce des tiges récoltées au littoral présente la plus forte activité inhibitrice sur l' α glucosidase. Cette activité est comparable à celle du médicament de référence « l'acarbose ». L'analyse UHPLC-ESI-HRMS a permis d'identifier 6 métabolites inhibiteur de l' α -glucosidase dont les dérivés d'epigallocatechine.

Cette thèse constitue une phase initiale qui doit contribuer à la valorisation du lentisque. En s'appuyant sur les différents facteurs ayant un impact sur la variabilité i) de la composition chimique et ii) du rendement de ces métabolites étudiés, il devient possible d'optimiser leur production. Cela permettra de développer des applications scientifiques-technologiques industrielles basées sur les activités biologiques des différents extraits isolés à partir des ressources de *Pistacia lentiscus*.

Mots-clés: *Pistacia lentiscus*, littoral, montagne, huile essentielle, GC-MC, chémotypes, activité antioxydante, polyphénols, UHPLC-ESI-HRMS, α -glucosidase.

Abstract

This thesis aims to characterize and evaluate the variation of the chemical composition of *Pistacia lentiscus* from Tizi-Ouzou region (Algeria) from samples collected in the mountains and coastal plain during the four seasons of 2019 year. In parallel, it aims to highlight the potentially valuable biological properties such as antioxidant activities and *in-vitro* anti-diabetic activity (the inhibitory effect of α -glucosidase).

An extensive bibliographic study was carried out on the phytochemistry, the pharmacological properties and the traditional use of the plant *Pistacia lentiscus*. The extracted knowledge led to the development of the experimental part aiming to answer the problematic of this thesis.

The experimental work carried out in this thesis is based on two main parts:

- The first part concerns the study of the variability of the composition and the antioxidant potential of the essential oil under the effect of the interaction between altitude and the harvesting season of lentisk. The extraction of essential oils was done by Clevenger hydrodistillation. The characterization of their chemical composition was performed by GC-MS. The antioxidant activity of these oils was evaluated by ABTS, DPPH, and FRAP tests. The results of this work showed that season and altitude of harvest have a significant effect on the chemotypic variation and on the antioxidant potential of the essential oils of lentisk.

- The second part was devoted to the phytochemical screening of α -glucosidase inhibitory compounds in different parts of *Pistacia lentiscus* using a chemometric study that links biological activity and UHPLC-ESI HRMS data. In addition, this work is also interested in evaluating the variation in the intensity of inhibition of α -glucosidase activity as a function of plant habitat (mountain and coastal) and organ (leaves, stem bark, fruit). The results of this section reveal that stem bark harvested from the coastline has the strongest inhibitory activity on α -glucosidase. This activity is comparable to that of the reference drug "acarbose". The UHPLC-ESI-HRMS analysis identified 6 α -glucosidase inhibitory metabolites including epigallocatechin derivatives.

This thesis constitutes an initial phase that could contribute to the valorization of lentisk. Based on the different factors that have an impact on the variability of the chemical composition and yield of these studied metabolites, it would be possible to optimize their production. This would allow the development of industrial scientific & technological applications based on the biological activities of plant extracts isolated from the resources of *Pistacia lentiscus*.

Key words: *Pistacia lentiscus*, essential oil, chemotype, antioxidant activity, polyphenols, GC-MC, UHPLC-ESI-HRMS, littoral, mountain

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Liste des abréviations

ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic) acid

AFB 1: Aflatoxin B1

ANOVA: Analysis of Variance

DPPH: 2,2-diphenyl 1-picrylhydrazyl

EO: Essential Oil

FAME: Fatty Acid Methyl Ester

FRAP: Ferric reducing power

HPLC: High-performance liquid chromatography

HPLC-DAD-MS: High-performance liquid chromatography (HPLC)–diode array detection (DAD)–mass spectrometry (MS) analysis

HPLC-ESI-QTOF-MS: High-performance liquid chromatography with diode array coupled to electrospray ionization mass spectrometry

HPLC-DAD: High-performance liquid chromatography with diode array detection analysis

UHPLC-ESI-HRMS: Ultra high-performance liquid chromatography (UHPLC), coupled to a hybrid mass spectrometer, equipped with an electrospray ionization source (ESI)

HPLC UV/Vis PDA: High-performance liquid chromatography (HPLC) coupled with UV/Vis PDA detector

ICP-MS: Inductivity Coupled Plasma-Mass Spectrometry

GC-MS: Gas Chromatography-Mass spectrometry

LDL: Low-Density Lipoprotein

LOX: *Lipoxygénase*

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

MUFA: Mono Unsaturated Fatty Acid

ND: Not determined

PCA: Principal component analysis

P.l.: *Pistacia lentiscus*

pNPG: para-Nitro Phenyl- α -D-Glucopyranoside

P.v.: *Pistacia vera*

PUFA: Poly unsaturated fatty acids

RT: Retention time

SFA: Saturated Fatty Acid

Introduction générale

Les métabolites secondaires (aussi plus récemment appelés métabolites spécialisés) sont des molécules organiques complexes synthétisées et accumulées en petites quantités par les plantes (Belhachat et *al.*, 2019). Ils présentent plusieurs familles moléculaires dont les trois principales sont: les polyphénols, les terpènes et les alcaloïdes (Belhachat et *al.*, 2019).

Les terpènes sont des hydrocarbures formés par assemblage de deux ou plusieurs unités isopréniques. Ce sont des polymères de l'isoprène, de formule brute générale: $(C_5H_8)_n$. Selon le nombre d'unités associées, on distingue les monoterpènes en (C_{10}) , les sesquiterpènes en (C_{15}) , les diterpènes (C_{20}) , les triterpènes (C_{30}) , les tétraterpènes (C_{40}) et les polyterpènes (Belhachat et *al.*, 2019). L'unité de base de la biosynthèse des terpènes est l'*isopentényl-diphosphate* IPP et son isomère le *diméthylallyl-diphosphate* DMAPP. Deux voies de biosynthèse conduisent à ces unités de base à 5 atomes de carbone (Singh et *al.*, 1990). La première est la voie du mévalonate. La seconde voie, est la voie du méthylérythritol phosphate (MEP) ou encore nommée voie non mévalonique.

Parmi tous les extraits végétaux possibles, l'un des plus riches en terpènes est sans aucun doute l'huile essentielle. Les huiles essentielles contiennent principalement des monoterpènes, des sesquiterpènes et peu de diterpènes (Belhachat et *al.*, 2019). Les huiles essentielles (HEs) sont des extraits volatils et odorants, généralement de composition complexe (Belhachat et *al.*, 2019). Divers organes sont utilisés pour la production de l'huile essentielle: fleurs, feuilles, écorce, bois, rhizomes, fruits, grain (Kaloustian et *al.*, 2013). Les composants des huiles essentielles sont synthétisés et accumulés dans des cellules spécifiques. Ces cellules sécrétrices sont rarement à l'état isolé. Elles sont le plus souvent regroupées dans des poches, dans des canaux sécréteurs ou dans des poils sécréteurs. Ces cellules sont le plus souvent situées à la périphérie des organes extérieurs de la plante (Belhachat et *al.*, 2019) et jouent un rôle important dans les relations écologiques de la plante et de son environnement.

Les polyphénols appartiennent au groupe des phénylpropanoïdes. Ils constituent le groupe le plus important et le plus largement distribué dans le règne végétal avec plus de 8000 structures phénoliques connues (Lugasi et *al.*, 2003). La désignation « polyphénols », ou encore « composés phénoliques », est fréquemment utilisée pour nommer l'ensemble des composés phénoliques. Ils désignent à la fois les mono-, les di- et les polyphénols dont les molécules comportent respectivement une, deux ou plusieurs fonctions phénoliques. Ces molécules peuvent posséder ou non d'autres fonctions (alcool: OH, acide carboxylique:

COOH...). Les polyphénols sont présents dans toutes les parties des végétaux supérieurs: racine, tiges, feuilles, fleurs, fruits (Boizot et al, 2006). Les composés phénoliques sont classés selon le nombre d'atomes de carbone dans le squelette de base, pouvant aller du simple phénol en C6 à des formes très polymérisées avec différents degrés d'oxydation, d'hydroxylation, de méthylation.... Ces molécules peuvent être également diversement substituées (glucides, lipides, protéines et autres métabolites secondaires pouvant être ou non des composés phénoliques). En s'appuyant sur la structure carbonée de base, plusieurs classes de composés phénoliques peuvent être distinguées; acides phénoliques, lignanes, lignines, stilbènes, flavonoïdes, isoflavonoïdes...

A l'échelle de la cellule, les composés phénoliques se localisent principalement dans deux compartiments: les vacuoles et la paroi. La plupart des molécules phénoliques sont formées à partir de deux acides aminés aromatiques, la tyrosine et la phénylalanine. Ces acides aminés sont formés à partir de la voie de l'acide shikimique (Macheix et al., 2005). La biosynthèse des polyphénols se fait selon deux voies principales: la voie de l'acide shikimique (Haslam et al., 1994; Dewick et al., 1995), et la voie de l'acétate (Martin et al., 2002).

1. L'espèce *Pistacia lentiscus*

L'Algérie abrite une importante richesse en plantes aromatiques et médicinales. De nombreuses plantes sont utilisées dans les remèdes traditionnels. Parmi celles-ci, se trouve *Pistacia lentiscus* L., aussi appelée: pistachier lentisque, lentisque, et arbre à mastic (relativement à sa résine utilisée pour la préparation d'une gomme à odeur prononcée). Cette plante appartient à la famille des "Anacardiaceae" (Belhachat et al., 2019). En Algérie, le genre *Pistacia* est représenté par quatre espèces, *Pistacia lentiscus*, *Pistacia terebinthus*, *Pistacia vera* et *Pistacia atlantica* (Quezel et Santa, 1962 cité par Belachat et al., 2019).

Pistacia lentiscus L. est un arbuste des maquis et des garrigues de toute la région Méditerranéenne (Figure 1). Il occupe toutes les altitudes entre 0 et 1200 m (Doga et al., 2003). Cette plante pousse sur différents types de sols tels que des sols sablo-argileux-limoneux, argilo-limoneux, sableux et argileux (Doga et al., 2003).



Figure1: individu arbustif algérien de *Pistacia lentiscus* (Marzougui et al., 2015)

Pistacia lentiscus L. est connu pour ses usages traditionnels depuis l'antiquité (Zitoini et al., 2016); il occupe une place appréciable dans la médecine traditionnelle et pharmaceutique de plusieurs régions méditerranéennes avec différentes utilisations que nous allons détailler spécifiquement dans la suite de ce manuscrit au travers d'un article de revue (article 1 de la Thèse).

2. Etude chimique et pharmacologique de l'espèce *Pistacia lentiscus*

Des travaux de recherche ont été menés sur la phytochimie des différents produits de la plante *Pistacia lentiscus*. Ces produits regroupent principalement la résine, l'huile végétale, l'huile essentielle et les polyphénols. Diverses compositions ont été observées. Cette plante présente également un potentiel pharmacologique riche et variable. Des informations détaillées sur l'aspect chimique et pharmacologique de cette espèce seront étudiés en profondeur dans les articles 1 et 3 qui constituent une partie de la suite de ce mémoire.

3. Organisation de la thèse

L'objectif général de cette thèse porte sur l'étude phytochimique approfondie de *Pistacia lentiscus*, plante médicinale traditionnelle d'Algérie. Ainsi, nous nous intéressons à la composition des huiles essentielles et à l'analyse des polyphénols du lentisque. Nous initierons une première évaluation du potentiel biologique de ces deux catégories de produits naturels issues de la plante.

Notre étude vise à apporter des éléments de réponse à la problématique de développement d'une filière de valorisation du lentisque avec un volet de recherche amont centré sur cette question: « Quel est l'effet des facteurs environnementaux (site d'échantillonnage et saison de récolte) sur la composition chimique et le potentiel biologique des métabolites du lentisque de Tizi-Ouzou? ».

Cette thèse est rédigée sous un format hybride entre le format classique et la thèse d'articles. Les travaux de synthèse et de recherche réalisés sont ainsi présentés et répartis en quatre articles consécutifs (présentés en quatre chapitres):

a. Objectif du premier article

Compte-tenu de l'étendue des recherches publiées concernant la plante *Pistacia lentiscus*, les usages traditionnels, la phytochimie et la pharmacologie de ses différents produits, le premier article de cette thèse présente la première revue bibliographique rédigée dans le but d'accéder à une vue d'ensemble actualisée des caractéristiques botaniques de cette plante, ainsi que des aspects chimiques et pharmacologiques de l'huile essentielle de lentisque. Une partie importante de cette revue est consacrée à l'étude de la variabilité interspécifique de l'huile essentielle de lentisque. Cette partie constitue en outre, une source d'informations riche et utile pour l'élaboration du travail expérimental de recherche réalisé ici (article 2).

b. Objectif du deuxième article

Cet article de recherche concerne les travaux effectués durant la première phase de la thèse. Une partie de ces travaux est initiée en Algérie notamment l'échantillonnage de la plante en montagne et au littoral de Tizi-Ouzou, sur une année (2019) et pendant les 4 saisons. Ensuite, l'extraction des huiles essentielles a été effectuée après chaque récolte. Le travail de cette partie a été poursuivi en France pour la caractérisation de la composition chimique des huiles essentielles par chromatographie en phase gazeuse couplée à la spectrométrie de masse. Ensuite l'évaluation de l'activité antioxydante des huiles essentielles a été réalisée. Finalement, le profilage en acides gras des lipides acylés (acyl-lipids) des feuilles de lentisque a été abordé.

c. Objectif du troisième article

L'article 3 est une seconde revue bibliographique. Elle porte sur les polyphénols chez le lentisque. L'objectif de ce travail de synthèse est de présenter un aperçu actualisé des profils phénoliques des différents types d'extraits de *Pistacia lentiscus* collectés dans les pays

méditerranéens. Cette revue se concentre sur la phytochimie, la pharmacologie, la variabilité de la composition qualitative et quantitative et les différents facteurs qui peuvent induire-expliquer cette variabilité dans la région méditerranéenne. L'article 3 sert à compléter l'article 1 et à construire une base de données pour développer l'approche expérimentale décrite dans l'article 4.

d. Objectif du quatrième article

Ce dernier article de recherche porte sur l'étude et la caractérisation du profil phytochimique de *Pistacia lentiscus* en lien avec son potentiel hypoglycémiant décrit en médecine traditionnelle. Les travaux réalisés dans le cadre de cet article présentent la deuxième phase de cette thèse. L'ensemble des travaux de cette phase sont effectués en France. La première partie de ce travail consiste à évaluer l'effet inhibiteur de l' α -glucosidase (activité antidiabétique *in vitro*) des feuilles, tiges (écorces) et fruits de *Pistacia lentiscus* récoltés en 2019 en montagnes et en zone littorale de Tizi-Ouzou en Algérie. Ensuite, le profilage phytochimique des extraits a été réalisé par UHPLC-ESI-HRMS. Dans cette étude, nous avons adopté une approche de métabolomique pour caractériser les inhibiteurs potentiels de l' α -glucosidase dans le lentisque.

La dernière partie de ce manuscrit sera consacrée à une discussion générale et à la réflexion autour des perspectives scientifiques et des avancées concrètes vers une possible valorisation industrielle basée sur des ressources végétales de la région de Tizi-Ouzou.

Chapitre I:

Revue bibliographique sur les utilisations traditionnelles, la phytochimie, la pharmacologie des extraits de *Pistacia lentiscus*

Résumé de l'étude

Introduction

Pistacia lentiscus est une espèce végétale appartenant à la famille des Anacardiacees. Le genre *Pistacia* ainsi que l'espèce *Pistacia lentiscus* sont largement distribués dans les régions méditerranéennes. De nombreuses caractéristiques biologiques et phytochimiques ont été décrites pour la seule espèce *Pistacia lentiscus* (*P.l.*). À ce jour, 37 000 publications incluant "*Pistacia lentiscus*" sont signalées dans la base de données Google Scholar. Des études ethnobotaniques et ethnopharmacologiques ont précisé les différentes utilisations traditionnelles de *P.l.* dans la vaste zone périméditerranéenne. Les produits de *P.l.* étaient principalement utilisés pour soigner les ulcères et les troubles respiratoires, pour traiter les allergies pulmonaires, la bronchite, la toux, les vomissements, la diarrhée. Des données détaillées ont été élaborées sur la composition chimique et les activités pharmacologiques de l'huile essentielle. Différentes compositions ont été observées. Des applications pharmacologiques intéressantes ont été mises en évidence ; en particulier des activités antimicrobiennes. Sur la base d'un tel intérêt scientifique pour cette espèce végétale, notre travail de synthèse vise à illustrer une vue d'ensemble actualisée sur les caractéristiques botaniques, les utilisations traditionnelles, ainsi que sur la phytochimie et la pharmacologie des produits de *Pistacia lentiscus*.

Huile essentielle de lentisque (HE)

L'huile essentielle de *Pistacia lentiscus* est obtenue par hydrodistillation à partir des parties aériennes telles que les feuilles, les rameaux, les fleurs et les baies (Catalani et *al.*, 2017). Elle est également extraite de la gomme de mastic (résine) (Negro et *al.*, 2015). Elle a une couleur jaune, une odeur aromatique et un goût piquant (Negro et *al.*, 2015).

Selon la littérature, il existe des rendements variables en huile essentielle de lentisque. On peut observer que pour 100g de matériel végétal frais, le rendement moyen en huile essentielle peut varier de 0,05 à 0,4% selon l'origine de la plante, la nature des parties de lentisque, la période de récolte, le taux d'humidité et la méthode d'extraction. Des travaux scientifiques ont identifié plusieurs compositions chimiques de l'huile essentielle de lentisque. La variabilité de cette composition est le résultat d'interactions entre le génotype, et des facteurs biotiques et abiotiques liés à l'environnement de la plante.

Variabilité de la composition de l'huile essentielle de *Pistacia lentiscus*

Les huiles essentielles de *Pistacia lentiscus* (*P.l.*) présentent des différences chimiques intra spécifiques dans leur composition. Ces différences sont définies comme «chémotypes» (Agha *et al.*, 2013). Ce terme fait référence à la «race chimique» ou aux principaux composés chimiques. Différents constituants ont été détectés dans les HEs de *P.l.* (voir le Tableau 2 dans l'article ci-après). Cette variabilité de la composition chimique est évidemment influencée par de nombreux facteurs tels que: origine géographique, partie de la plante utilisée, période de récolte et cycle végétatif, facteurs environnementaux, sexe de la plante, qualité du sol, méthode d'extraction. Afin d'obtenir une meilleure vue de la composition des différentes HEs de *P.l.*, en nous appuyant sur les données quantitatives de la littérature, nous avons réalisé une analyse en composantes principales (ACP, voir figure 2 dans la revue ci-après). Cette analyse statistique a été réalisée sur les HEs de feuilles qui étaient l'organe le plus étudié (*i.e.* pour lequel il existe le plus de données disponibles; données extraites du Tableau 2). L'ACP (score plot et loading plot) a été réalisée sur 50 compositions (HEs) de *P.l.* d'origine méditerranéenne (voir le score plot). Ces compositions proviennent de la littérature couvrant la période de 1999 à 2021 (Tableau 2).

Notre Analyse en Composantes Principales permet d'établir un lien entre l'origine géographique des HEs et la nature des chémotypes qui les composent. Les résultats de l'ACP montrent 4 clusters différents correspondant à 4 chémotypes distincts ; (i) terpinen-4-ol, α -phellandène et terpinéol pour les huiles essentielles provenant principalement d'Algérie, (ii) cubébol, α -amorphène, 8-cadinène caractérisant les huiles d'origine tunisienne, (iii) myrcène, α -pinène, limonène, terpinène-4-ol et α -phellandène pour les huiles provenant du Maroc, (iiii) β -caryophyllène, β -pinène, 3-carène, β -phellandène et germacrène D pour les HEs d'Espagne et quelques huiles d'Italie.

Selon notre analyse ACP obtenue sur les données de la littérature, nous pouvons mettre en évidence 4 chémotypes différents qui distinguent les HEs de 7 pays méditerranéens. Cette variabilité peut être due à l'effet de l'origine géographique; mais aussi, à d'autres paramètres car certaines origines sont présentes dans différents clusters, et, une variabilité relativement importante se produit au sein de chaque cluster. Cette analyse met en évidence que (et partiellement « comment ») la période de récolte de la plante, la qualité du sol, le sexe et la nature génétique de la plante jouent un grand rôle dans la variabilité du profil des HEs.

Activités pharmacologiques

Des investigations scientifiques ont été menées sur le potentiel pharmacologique de l'huile essentielle de *Pistacia lentiscus*. Ces extraits de lentisque ont des applications pharmacologiques intéressantes; en particulier des activités antimicrobiennes, antioxydantes, anti-inflammatoires et anticancéreuses.

Parmi toutes les activités biologiques recensées, l'activité antimicrobienne est assurément l'activité la plus marquée des HEs de lentisque. Le potentiel antimicrobien a été testé contre différentes souches bactériennes et fongiques. L'activité antimicrobienne des HEs de *P.l.* (Tableau 2) a montré que les souches à Gram-négatif étaient plus résistantes que les souches à Gram-positif. L'inhibition bactérienne est importante sur *Proteus mirabilis* (Bouyahya et al., 2019), *Salmonella sp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus sp.*, *Enterococcus faecalis*, *Mycobacterium aurum*, *Mycobacterium smegmatis* (Hafsé et al., 2013). *Bacillus subtilis* était la souche la plus sensible aux deux HEs, tandis que *Pseudomonas aeruginosa* était la plus résistante (Haloui et al., 2015).

L'huile essentielle de lentisque a également un effet antifongique remarquable contre *Aspergillus flavus*, *Rhizoctonia solani*, *Penicillium commune*, *Fusarium oxysporum*, *Aspergillus flavus* (Barra et al., 2007), *Candida albicans* et *Candida glabrata* (Milia et al., 2021).

Discussion

Aucun article de synthèse n'avait jusque-là discuté de la variabilité de la composition des huiles essentielles décrites dans les investigations scientifiques récentes. De plus, nous n'avons trouvé aucun rapport sur les investigations détaillées menées sur l'huile comestible et la variation de sa composition ainsi que sur la résine. En outre, aucune revue récente n'a été développée sur les enquêtes ethnopharmacologiques les plus récentes et sur les utilisations traditionnelles du lentisque et de ses produits. Par conséquent, la présente revue (ci-après) vise à présenter les utilisations traditionnelles, la phytochimie, la pharmacologie des extraits de *Pistacia lentiscus* en particulier la résine, l'huile végétale et l'huile essentielle.

A review on Mediterranean *Pistacia lentiscus* L.: traditional uses, essential oils chemical composition variability and pharmacological effects

Chabha SEHAKI^{1,2}, Farida FERNANE² and Eric GONTIER^{1*}

¹ BIOPI-UPJV Laboratory UMRT BioEcoAgro INRAE1 158, SFR Condorcet FR CNRS 3417, UFR of Sciences, University of Picardie Jules Verne, 33 Rue Saint Leu, 80000 Amiens, France

² Laboratory of Natural Resources, University Mouloud Mammeri of Tizi-Ouzou, Tizi-Ouzou 15000, Algeria

* Correspondence: eric.gontier@u-picardie.fr; Tel.: +33-6-59-21-81-08

Context: *Pistacia lentiscus* is a plant species belonging to the Anacardiaceae family. It is spontaneously growing all around the Mediterranean area. Since antiquity, *P. lentiscus* is considered and traditionally used as a medicinal plant. Different parts and products of/from the plants have been described for health purposes. Mastic, fruit oils, dried leaves (for infusion-decoction), and essential oils from leaves were used and have been studied in more than 37 000 publications since the mid of twentieth century. Within that large literature dedicated to *P. lentiscus*, it becomes difficult to get a clear view about what is common and what is subjected to very large variabilities.

Objectives: Here, we want to try to develop a specific point of view about the possibilities to valorize the naturally growing *Pistacia lentiscus*. we first decided to establish a dedicated overview of the actual knowledge on *Pistacia lentiscus*, its medicinal uses and the variability between the different biological Mediterranean resources. This review aims to support a future effective valorization of this abundant natural resource for scientifically driven health applications. This strategy is needed for an economical sustainable development based on *P. lentiscus*.

Methods: Then, this article aims to review and update what is known about *Pistacia lentiscus*. The collected data illustrate and summarize the most important literature on the plant, and mostly on essential oil obtained from this plant. The main items here presented are focused on botanical data the species *P. lentiscus*. Selected references are also based on lentisk traditional uses, phytochemistry, pharmacology, essential oils chemical compositions, and, on the different factors that may induce variability in the Mediterranean area. These sources were collected via Elsevier, Google Scholar, MDPI, Taylor and Francis, and Springer databases. The data are organized in order to (i) illustrate traditional uses described through ethnopharmacological studies, (ii) show the chemical diversity of lentisk essential oils and, (iii) link and correlate pharmacological effects to specific compounds. A further dedicated statistical analysis has been realized in order to correlate chemical compositions and bioactivities.

Results: *Pistacia lentiscus* L. is an evergreen shrub with year-round foliage. As a dioecious plant, *P. lentiscus* populations are represented by 1 to 5 meters heights. Ethnobotanical and ethnopharmacological studies specified the different traditional uses of *P. lentiscus* in the wide Mediterranean area. Lentisk products were mainly used to heal ulcers and respiratory disorder, to treat pulmonary allergies, bronchitis, coughing, vomiting, diarrhea. The chemical composition and pharmacological activities of *Pistacia lentiscus* essential oils have been published as detailed data. Largely variable compositions were observed. Some interesting pharmacological applications were proved; in particular antimicrobial activities. The statistical analysis from literature data could distinguish 4 different chemotypes characterizing *P. lentiscus* essential oils from 7 different Mediterranean countries.

Conclusion-perspectives: This review emphasizes the main traits of essential oils from *Pistacia lentiscus* all around the Mediterranean area. It clarifies the traditional medicine allegations and tends to associate chemotypes with bioactivities. It is a first step toward choice, harvest, and then, optimization of molecules extraction and isolation in quantities large enough for further evaluations of pharmacological activities and mechanisms of action. This review thus highlights the way for the development of a micro-industrial sector based on the valorization of the metabolites issuing from this plant.

Keywords: *Pistacia lentiscus*; traditional uses; ethnopharmacological studies; Mediterranean area; essential oils; chemotypes; pharmacological activities

1. Introduction

Pistacia lentiscus L. is a plant species largely distributed in Mediterranean area. It has been largely used for medicinal application concerning a diversity of health treatments [1]. This plant is spontaneously growing in the north of African continent but also all around the Mediterranean area [2]. Depending on the region, similar uses have spread with the knowledge associated with human civilization exchanges [3]. This is true for *P. lentiscus* but also for other *Pistacia* species belonging to the Anacardiaceae family [3]. The same uses could be described and, if the efficiency of the treatment were obviously true, then, they were empirically based on the phytochemical composition of the different plant parts [3]. As far as Anacardiaceae family is composed of 81 genera (800 species) [4], and the *Pistacia* genus represents 11 species localized in very different natural areas [5]. Then, many biological and phytochemical traits have been described for the only *Pistacia lentiscus* species. To date, 104 000 publications including “*Pistacia*” are reported in the Google Scholar database. From those, almost 37 000 of these publications and scientific reports deal with the *Pistacia lentiscus* species.

Based on such a scientific interest of this plant species, it is then necessary to clarify what is known, what is common, and what is varying between the different *P. lentiscus* occurrences. This is an absolute need to be able to develop -science based, and thus- rational applications for medicine, pharmacy or other industrial uses based on biological activities of plant extract isolated from the *P. lentiscus* resources.

Different folk medicine uses have been described: management of dental, liver, gastrointestinal and respiratory disorders [9]. But, only a few reviews have summarized the studies conducted on *Pistacia lentiscus* [10,11,12,13,14]. These reviews focused on a brief general description of the phytochemical composition and pharmacological activities of products from *P. lentiscus* and other *Pistacia* species. As such, the venereal use of *Pistacia* plants was also noted [12] in association with the chemical composition of leaf and fruit extracts and their anti-inflammatory and antioxidant activities [14]. Nevertheless, no review article has discussed the variability in essential oil composition described in several result articles. Furthermore, no report about recent ethnopharmacological investigations on traditional uses of lentisk.

Therefore, the present review aims to present an updated overview of the botanical characteristics, the taxonomic classification, the traditional uses of *Pistacia lentiscus* and its essential oil phytochemistry and pharmacology. One part of this review focuses on the phytochemical variability and yield of essential oil under the effect of environmental parameters. A statistical analysis has been set up to show the chemical diversity and to visualize its different groups of chemotypes as described within the Mediterranean area. This review article also aims to make a link between the pharmacological effects attributed to lentisk essential oils and their chemical compositions.

2. Methodology research

The writing of this review was based on the consultation of articles published on the plant *Pistacia lentiscus*. The selection of articles was made according to the objectives conveyed in this review. The selected articles are about the botanical belonging of lentisk, traditional uses and essential oils. All the illustrated data were obtained from journals, books and some theses. These sources were collected via Elsevier, Google Scholar, MDPI, Taylor and Francis, and Springer databases. The search conducted in this review article was carried out by searching the following keywords or combinations: *Pistacia lentiscus*, Anacardiaceae, genus *Pistacia*, botanical study, essential oil, chemical composition, environmental factors, intra-specific variability, pharmacological activity, traditional use, yield, Mediterranean regions.

3. The *Pistacia lentiscus* species

3.1. Botanical description and Taxonomy

Pistacia lentiscus (Anacardiaceae family) is also called “lentisk” or “mastic tree”. It is a dioecious shrub with male or female individuals. They are thermophilic trees of 1 to 5 meters height [15]. *P. lentiscus* grows in the wild in both scrub and scrubland in the Mediterranean basin [16]. It is characterized by dark green and evergreen leaves. Its flowers appear at spring in the form of small bunches (March to May). The female flowers are yellowish green; while the male ones are dark red. Whatever the sex, the bark is reddish on the young branches and grey on the old ones. On female trees, the fruit is rounded and first red before becoming black on ripening, at autumn [15]. An aromatic resin flows from the

trunk and the main branches of *P lentiscus*. The species is so called “mastic tree” in reference to this resin [17]. A plate of pictures presenting the different parts of *P. lentiscus* is presented below in Figure 1, 2, 3.

Table 1: Lentisk taxonomy according to Linne [18].

• <i>Kingdom</i>	<i>Plants</i>
• <i>Branch</i>	<i>Spermaphytes</i>
• <i>Under a branch</i>	<i>Angiosperms</i>
• <i>Class</i>	<i>Dicotyledons</i>
• <i>Order</i>	<i>Sapindales</i>
• <i>Family</i>	<i>Anacardiaceae</i>
• <i>Genus</i>	<i>Pistacia</i>
• <i>Species</i>	<i>Pistacia lentiscus</i> L.



Figure 1: Top part of *Pistacia lentiscus* trees. Pictures of two female specimens from Tizi-Ouzou region (Tigzirt, GPS: 36°29'58.3 "N, 4°04'43.4 "E) at late winter (February 2019).



Figure 2: Typical male flowers (left image) and female flowers (right image) of lentisk. Origin: Tizi-Ouzou (Ait Irane, GPS 36°53'43.0"N, 4°11'00.4"E); Date: April 201



a



b

Figure 3: Red fruits on female *Pistacia lentiscus* trees at two maturation levels: the fruits become black at maturity in autumn. Origin:Tizi-ouzou (3.a: Tigzirt, GPS: 36°29'58.3 "N, 4°04'43.4 "E/3.b: Ait Irane, GPS: 36°53'43.0 "N, 4°11'00.4 "E); Date: October 2019

3.2. Traditional uses

Pistacia lentiscus has been known for its medicinal properties since antiquity [19]. In recent times, ethnobotanical studies have been conducted (see below) on *Pistacia lentiscus* with herbalists, pharmacists and populations living in contact with this plant. These studies have allowed specifying the different traditional uses of *Pistacia lentiscus* by the populations.

In terms of applications, *P. lentiscus* was mainly used against diseases of the digestive system [14,20,21]. It was used for healing ulcers [22,23] and respiratory system [20,21,24,25,26]. This plant has also been indicated for the treatment of circulatory system disorders such as hypertension or heart disease [20,24] and also for the treatment of type 2 diabetes [27]. Pulmonary allergies, bronchitis, coughing, vomiting, diarrhea have also been treated by products from *P. lentiscus* [28] with also skin problems, acne, skin allergy and burns [21,24]. The ripe fruits of the mastic tree are very effective in the treatment of stomach diseases and respiratory infections [29]. Gum Mastic was used against gastralgia [30,31,32].

In order to administer (use) the active ingredients, different *P. lentiscus* products and methods have been described: decoction, infusion or direct use of crude matter or powder. Leaves or leafy stem in decoction or maceration was done in milk, in water, or other vegetable oil for health uses. Decoctions of leaves are used in mouthwash to treat toothache [33]. *P. lentiscus* fruit oil has also been used in its direct form for massage or ingestion [23]. Chewing the resin was used to freshen the breath [23]. The use of resin has also been reported for lip care against chapping [33].

Other non-medicinal uses of lentisk derivatives have also been described. Young branches of *P. lentiscus* are commonly used for the mechanical and (empiric) biochemical cleaning of water tanks [34]. The stems and leaves of lentisk are used as aromatic herb and for their potentially preservative properties of hardened olives [35].

4. Essential oil of *Pistacia lentiscus*

Essential oils (EOs) are a showcasing family of plant products composed of secondary metabolites and mainly synthesized in the secreting cells of aromatic plants [36]. These are liquid complex mixtures of natural molecules highly volatile, each with its characteristic odor [37]. Hydrodistillation remains the most used extraction process for essential oil; especially for pharmaceutical and cosmetic purposes. Depending on the plant species, EOs can be

obtained from leaves, seeds, buds, twig flowers, bark, wood, roots, stems or fruit as well as from gums from tree trunks [38]. They are mainly composed of terpenes and terpenoids [39]. *Pistacia lentiscus* L. is one of the plant species used for the production of EO. The *P. lentiscus* EO is obtained by hydro-distillation from the aerial parts such as leaves, twigs, flowers and berries [36]. It is also extracted from mastic gum [40]. It has a yellow color, aromatic odor, and a pungent taste [40]. *P. lentiscus* EO was used in the perfumery, pharmaceutical and food industries [41] and as flavorings in beverages [42].

4.1. The Yield of *Pistacia lentiscus* essential oil

Depending on harvest/post-harvest and extraction conditions, variable yields of essential oil can be obtained from the literature. As such, in Morocco, the authors determined different values of the yield [43,44,45]. The hydrodistillation of *P. lentiscus* leaves led to a yield of 0.2% and 0.3% [44,45]. Twigs, yielded to 0.15% and 0.5% [44,45]. From December to June for three sites in Morocco, the *Pistacia lentiscus* EO yields varied between 0.14 and 0.21% [46]. The highest EO contents were obtained during the flowering period for all three sites [46]. In Morocco, an EO yield of 0.14% in winter (February) was obtained by Amahmadi et al. [45].

In Algeria, the average yield of EO extracted from leaves and twigs of *P. lentiscus* was 0.39% and 0.25% from lentisks growing in the province of Mostaganem and Boumerdes respectively [47].

In Tunisia, the yield of EO obtained from fresh leaves at the end of fruiting was 0.14% (v/w) [48].

In Italy, EO of all aerial parts harvested between April and October yields averaged 0.17% [49]. For EOs extracted from leaves and berries by supercritical CO₂ extraction coupled with a fractional separation technique (SFE), the yield was 0.45% in leaves and only 0.2% in berries in Sardinia [50].

In France, yields of essential oil from fresh material (leaves and twigs) varied from 0.05 to 0.1% in Corsica [51].

From these results, we can observe that for 44g of fresh plant material, the average yield of essential oil can vary from 0.05 to 0.4% depending on the origin of the plant individual, the nature of the plant parts, the period of harvest, the humidity level (fresh or dry)

and the extraction method. Comparing these EOs yields of *P. lentiscus* with those of *Pistacia vera*, fresh unripe fruits were richer in EO (0.5%, w/w) in *P. vera*, when its leaves led to only 0.1%, w/w yields [52].

4.2. Chemical composition and pharmacological activities

The following Table 2 presents the main compositions of *Pistacia lentiscus* EOs as a function of plant geographical origin, part of the plant used, method of extraction, major compounds detected. Pharmacological activities are also indicated. These data are organized in order to show (i) the chemical diversity of the *P. lentiscus* EOs, and, (ii) to try to link the observed pharmacological effects to the chemical composition. The interpretation of this Table is done via the two sub-headings that will follow.

Table 2: Chemical composition of essential oils from *Pistacia lentiscus* originating from different Mediterranean and non-Mediterranean areas (pharmaceutical activities indicated when available).

Provenance of the plant	Part of the plant used and method of extraction if other than hydrodistillation	Main compound	Pharmacological activities	References
Algeria	Leaves (two algerian populations)	Terpinene-4-ol (17-34%), α -terpineol (10-11%), germacrene D (8-15%)		[53]
	Aerial part			
Algeria	Alger	Longifolene (12%)		
(three regions in Algeria)	Tizi-ouzou	Longifolene (16%)		[54]
	Oran	α -Pinene (19%)		
Algeria	Leaves	Limonene (25-43%), myrcene (23-33%), α -pinene (20-34%),		[55]
Algeria	Leaves	β -Caryophyllene (12%), δ -cadinene (10%), cubebol (10%), β -bisabolene (7%), α -pinene (6%), caryophyllene oxide (6%)		[56]

Algeria	Leaves	Terpinene-4-ol (41%), β -caryophyllene (12%), β -myrcene (10%), α -pinene (9%), limonene (9%), p-cymene (8%), α -terpineol (7%)	Good antioxidant activity with DPPH essay	[57]
Algeria	Leaves	α -Pinene (15 %), limonene (14 %), β -myrcene (9%)	Strong antimicrobial activity of leaves essential oil against <i>Helicobacter pylori</i>	[58]
Algeria	Aerial parts	β -Linalool (52%), β -pinene (6%), terpene-4-ol (1%)		[59]
Egypt	Leaves	δ -3-Carene (65%)		[60]
Egypt	Leaves	α -Pinene (38%), α -phellandrene (10%), D-limonene (11%), γ -muurolene (7%)		[61]
	Leaves			
	Extraction by supercritic CO2	Caryophyllene (31%), germacrene D (12%), cadinene (6%)		[50]
Italy	Extraction by hydrodistillation	α -Pinene (18%), caryophyllene (13%), α -phellandrene (12%)		

	Leaves	α -Pinene (16-25%), germacrene D (9-14%), limonene (6-12%), terpinene-4-ol (7-12%)	
Italy	Branches	α -Pinene (34-46%), limonene (8-13%), myrcene (6-11%)	[62]
	Fruits	Myrcene (68-71 %), α -pinene (7-11%), limonene (9-19%)	
	All aerial parts (leaves, juvenile branches and flowers) harvested between April and October	α -Pinene (14-22%), terpinen-4-ol (14-28%), β -myrcene (1-19%), p-cymene (1-16%)	1. Antifungal activity against <i>Aspergillus flavus</i> 2. Inhibition of mycelial growth of <i>A. Flavus</i> . 3. Fairly good antioxidant activity eliminated by DPPH test
Italy	Leaves and twigs	α -Pinene (18 %), sabinene (13%), myrcene (9%)	[49]
		α -Pinene, thujene, camphene, sabinene, myrcene, α -phellandrene, α -terpinene, para-cymene	
Italy	Leaves		[64]
		α -Pinene (9-24%), terpineol (7-14%), β -phellandrene (4-11%), terpineol (4-8%), β -pinene (1-8%), β -myrcene (07-9%)	
Italy	Leaves		[65]

Italy	Aerial part (leaves, twigs and berries)	Myrcene (25%), α -pinene (18%), 4-terpineol (7%)	Cytotoxic action against the cancerous cell lineage "follicular thyroid carcinoma" [36] The viability of the cells was controlled by the WST-8 test
Italy	Branches	Terpinene-4-ol (25%), α -phellandrene (11%), β -phellandrene (10%), γ -terpinene (10%)	High fungal activity against <i>Cryptococcus neoformans</i> [66]
Italy	Leaves	α -Pinene (16%), terpinen-4-ol (16%), sabinene (7%), α -phellandrene (7%), γ -terpinene (6%)	1. Antibacterial activity against: <i>Streptococcus gordonii</i> <i>Actinomyces naeslundii</i> [14] <i>Fusobacterium nucleatum</i> <i>Porphyromonas gingivalis</i> <i>P. gingivalis Clinical</i> <i>Tannerella forsythia</i> <i>T. forsythia</i>

			2. Antifungal activity against: <i>Candida albicans</i> <i>Candida albicans</i> <i>Candida glabrata</i> <i>Candida glabrata</i>	
Italy	Leaves	α -Pinene (16%), terpinen-4-ol (16%), sabinene (7%), α -phellandrene (7%), γ -terpinene (6%)	<i>Candida glabrata</i> <i>Candida glabrata</i>	[14]
			3. Anti-inflammatory activity: inhibitory capacity toward COX-2 and LOX inflammatory enzymes	
Greece	Leaves	Myrcene (20%), germacrene D (13%), E-caryophyllene (8%), α -cadinol (7%), cadinene (7%)		[67]
Greece	Leaves	α -Pinene (9-24%), limonene (9-17%), germacrene D (3-13%), terpinen-4-ol (6-10%)		[17]
Greece	Leaves	δ -Cadinene (17%), α -amorphene (10%), δ -germacrene (9%), trans-caryophyllene (6%), α -cubebene (5%)		[68]
Morocco	Aerial part (03 harvesting sites Oulmes, Chaouen, Mehdia)	α -Pinene (16-38%), terpinene-4-ol (14-19%), β -myrcene (10-11%), limonene (6-9%), bornyle acetate (6-10%), caryophyllene oxide (6-10%), limonene (6-8%)		[46]

Morocco	Leaves	Myrcene (39%), limonene (10%), germacrene (4%), pinene (2%), muurolene (2%), humulene (2%),	[47]
			Antibacterial activity against: <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumonia</i> <i>salmonella typhi</i> <i>Staphylococcus aureus</i> , <i>Staphylococcus intermedius</i> , <i>Enterococcus faecalis</i> , <i>Bacillus sphericus</i> <i>Enterobacter aerogens</i>
Morocco	Leaves	α -Pinene (24%), β -pinene (12%), limonene (7%), terpinen-4-ol (6%), terpineol (4%), caryophyllene (3%), verbenol (3%), linalool (2%), camphene (2%), myrcene (2%)	[69]
			Important antibacterial effect against: <i>Salmonella sp.</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Bacillus sp.</i> , <i>Enterococcus faecalis</i> , <i>Mycobacterium aurum</i> , <i>Mycobacterium smegmatis</i>
Morocco	Leaves/stems	Limonene (9/15%), D-germacrene (9/2%), terpinene-4-ol (6/9%), b-gurjunene (6/2%), α -pinene (3/1%)	[43]
Morocco	Leaves	Limonene, α -pinene, terpineol and β -caryophyllene	[70]

Morocco	Leaves	Tricyclene (7%), terpinen-4-ol (7%), sabinene (6 %), p-cymene (5%), caryophyllene (6%), caryophylleneoxide (6%)	<i>Antibacterial activity against :</i>
	Twigs	α -Pinene (19%), tricyclene (8%), caryophyllene (6%), 3-carene (5%), germacrene D (5%)	<i>Bacillus subtilis,</i> <i>Staphylococcus aureus</i> [44] <i>Escherichia coli</i>
Morocco	Branches and leaves	Germacrene D (8-22%), α -pinene (10-19%), sabinene (9-19%)	Antibacterial activity:
			- weak against <i>Streptococcus agalactiae,</i> <i>Pseudomonas areuginosa</i> [71] - high against <i>Staphylococcus aureus,</i> <i>Acinetobacter baumannii,</i> <i>Klebsiella pneumoniae,</i> <i>Escherichia coli</i>
Morocco	Leaves	Myrcene (33%), α -pinene (19%)	1. Interesting antioxidant effects (especially fruit oil) revealed by DPPH, FRAP, and ABTS trials
	fruits	α -Pinene (20%), Limonene (18%)	2. Antileishmanial activity of the two oils [72] against: <i>Leishmania infantum,</i> <i>Leishmania major,</i> and <i>Leishmania tropica</i> using the MTT test.

			3. High antibacterial activity of these two oils on <i>Proteus mirabilis</i> .
Morocco	Leaves	Myrcene (33%), α -pinene (19%)	
	fruits	α -Pinene (20%), limonene (18%)	4. Remarkable cytotoxic effects on RD and L20B adenocarcinoma cell lines of fruit oil by MTT assay.
	Leaves	α -Pinene (79%), β -myrcene (3%)	
Spain	Unripe fruits	α -Pinene (22%), β -myrcene (54%)	[72]
	Ripe fruit	β -Myrcene (72%), α -pinene (11%)	
Spain	Leaves and galls	Caryophyllene (13%), cadinene (8%), germacrene D (6%)	[73]
Spain	Leaves	α -Pinene (19%), germacrene D (11%), β -caryophyllene (11%), sabinene (6%), β -pinene (6%), 3-carene (7%), limonene (8%), terpinen-4-ol (5%)	[74]
	Calcareous soil		

Spain	Leaves Siliceous soil	Germacrene D (17%), β -caryophyllene (12%), Myrcene (11%), terpinen-4-ol (6%), epi- α -muurolol (9%)	[74]
Spain	Leaves	α -Pinene (2-39%), germacrene-D (3-20%), myrcene (0.6-59%), β -phellandrene (0-42%), terpinen-4-ol (0.2-19%)	[75]
Tunisia	Leaves	α -Pinene (17%), terpinene-4-ol (12%), γ -terpinene (9%)	Antibacterial activity against: <i>Staphylococcus aureus</i> <i>Enterococcus faecalis</i> <i>Salmonella enteritidis</i> <i>Salmonella typhimurium</i> <i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i> [76]
Tunisia	Leaves	Germacrene D (11,9%), α -pinene (9,9%), limonene (8,5%), caryophyllene (8,2%), δ -cadinène (8,5%), terpinene-4-ol (5,1%)	Moderate antioxidant activity determined [2] with: free radical scavenging activity (DPPH), carotene bleaching test, ferric reducing power (FRAP)

	Males		
	Leaves	α -Limonene (7-18%), α -pinene (4-10%), le germacrene-D (8-13%), α -terpinen-4-ol (3-9%), β -caryophyllene (7-8%), δ -cadinene (7-9%)	
	Stems	α -Limonene (4.2-12%), α -pinene (3-15%), germacrene-D (6-14%), α -terpinen-4-ol (4-14%), δ -cadinene (2-8%)	
	Flowers	β -Caryophyllene (12%), germacrene-D (5%), α -terpinen-4-ol (7%), δ -cadinene (5%)	
Tunisia			[15]
	Femals		
	Leaves	Germacrene-D (10-20%), α -limonene (5-29 %), α -pinene (5-11%), β -myrcene (5-9 %),	
	Stems	α -Limonene (9-36%), α -pinene (7-13%)	
	Flowers	α -Limonene (28%), germacrene-D (23%)	
	Fruits unripe	β -Myrcene (87%), germacrene-D (23%), α -pinene (3 %)	
	Fruits ripe	β -Myrcene (75%), α -Pinene (12%)	
Tunisia	Fruits	β -Myrcene (11%), 3-pentadecylphenol (8%)	[77]

Turkey	Branches	Sabinene (23%), α -pinene (19%), germacrene D (14%), limonene (6%), β -phellandrene (6%), terpinene-4-ol (5%)	[78]
	Leaves	Terpinene-4-ol (29%), β -caryophyllene (29%), p-cymene (7%)	

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

COX: cyclooxygenase

DPPH: 2,2-diphenyl 1-picrylhydrazyl

FRAP: Ferric reducing antioxidant power

LOX: lipoxygenase

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

4.2.1. Variability of the composition of lentisk essential oils

Essential oils of *Pistacia lentiscus* present intraspecific chemical differences in their compositions, depending on both environmental and living conditions within the same plant species. These differences are defined as chemotype [79]; this term refers to the “chemical race” or the major chemical compounds. Different constituents were detected in EOs of *P. lentiscus* (Table 2). This variability in chemical composition is obviously influenced by many factors as described above.

4.2.1.1. Geographical origin:

According to Table 2, α -pinene is the first major compound for most of the essential oils extracted from the leaves in Algeria, Morocco, Tunisia, Spain, Italy and Greece. In Italy and Morocco EOs are richer in myrcene [43,45]; β -caryophyllene in Algeria [56], in tricyclene in Morocco [44], in limonene and germacrene D in Tunisia [2,15]. In addition, even EOs from different geographical sites of the same country can show variable chemotypes [46,53,54,74].

In order to obtain a better view of the composition of the different EOs from *P. lentiscus*, we realized a principal component analysis (PCA) using the package FactoMineR under R (version 3.6.1). This statistical analysis was done on EOs from leaves which were the most studied organ (*i.e.* more data available; & data extracted from Table 2). The PCA (score plot and loading plot) was performed on 50 compositions of EOs from *P. lentiscus* of Mediterranean origin (see the score plot). These compositions come from the literature covering from 1999 to 2021 (Table 2).

The two first axes of the PCA discriminate only 30% of the total variability. Nevertheless, the score plot can separate the essential oil profiles into 4 different clusters presented in various colors on figure 4a. Each cluster gathers EOs with a close chemical composition (*i.e.* with similar chemotypes). The loading plot (figure 4b) presents the main compounds constituting the four clusters of chemotypes observed during these studies. The combined analysis of the score plot and loading plot allow the establishment of a correlation between the geographical origin of the EOs and the nature of the chemotypes that compose them.

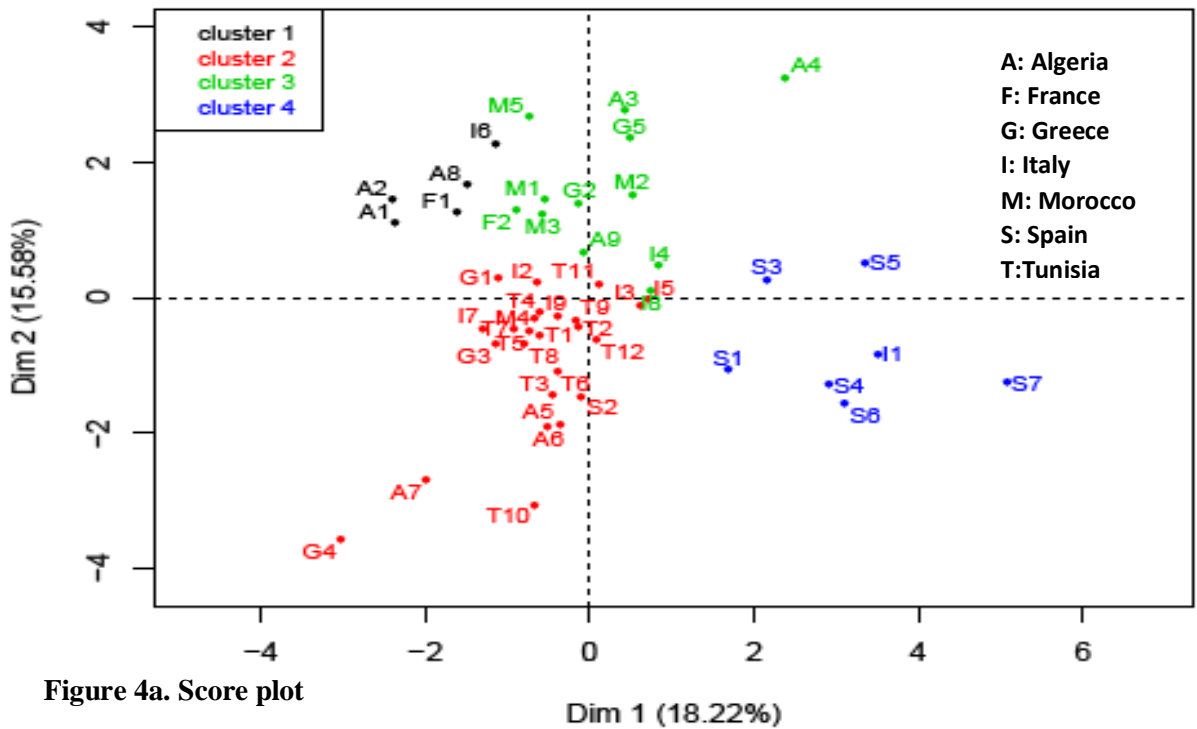


Figure 4a. Score plot

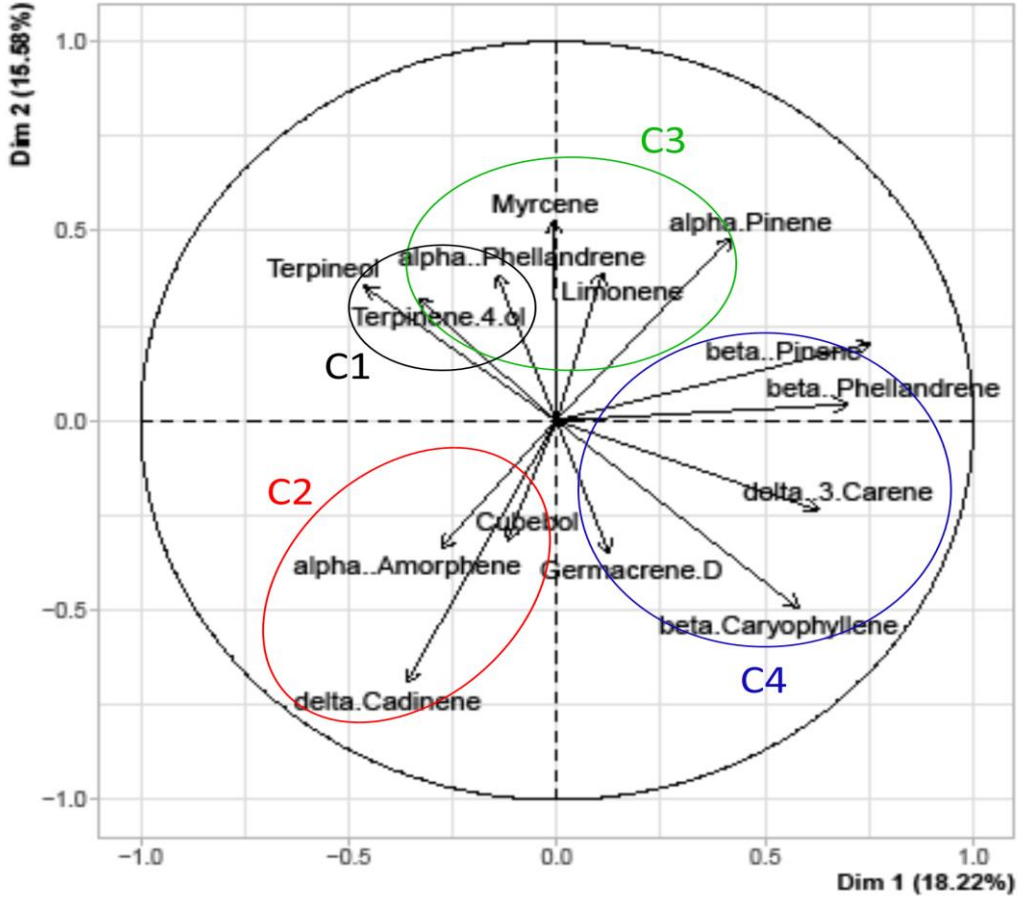


Figure 4b. Loading plot

Figure 4a, 4b: PCA analysis performed on main compounds from the essential oils of *Pistacia lentiscus* leaf samples from several Mediterranean countries (score plot and loading plot)

According to these two graphs, the four clusters present 4 different chemotypes:

-The first cluster (black) includes essential oils from Algeria, France and Italy. These oils are mainly rich in terpinen-4-ol, α -phellandrene and terpineol.

-The second cluster (red) presents essential oils from Algeria, Italy, and also Tunisia, Greece, Morocco. The majority of EOs in this cluster are of tunisian origin. This may (at least slightly) impact the results of the PCA. The oils of cluster 2 are composed of cubebol, α -amorphene, 8-cadinene as major components.

-The third cluster (green) consists principally of oils from Morocco. There are also some oils from Algeria, France, Italy and Greece. The compositions of these EOs are mainly characterized by the presence of myrcene, α -pinene, limonene, terpinen-4-ol, and α -phellandrene.

-The fourth cluster (blue), integrates EOs from Spain and some oils from Italy. These oils are characterized by their richness in β -caryophyllene, β -pinene, delta-3-carene, β -phellandrene and germacrene D.

Furthermore, within each cluster, the distribution of EOs scores (*i.e.* position on the fig 4a PCA score plot) is spread, thus indicating relatively important differences of molecules contents within each chemotype.

According to our PCA study obtained on data from literature, we can highlight 4 different chemotypes that distinguish the EOs from 7 Mediterranean countries. This variability can be due to the effect of geographical origin; but also, to other parameters because some origins are present in different clusters, and, relatively large variability occurs within each cluster.

The harvesting period of the plant, the soil quality, the gender and the genetic nature of the plant most probably play a key role in the variability of EOs profile. Nevertheless, our actual PCA was done on selected (*i.e.* representative & useful) literature data carried out on the leaves. Consecutively, our PCA excludes the analysis of the part of the plant as a factor influencing the EOs composition.

Detailed variations in the chemical composition of lentisk are presented in Table 2. In the following parts of that review, we are going to illustrate sources of variability other than geographic origin.

4.2.1.2. Part of the plant used:

The chemical composition of the *P. lentiscus* EO highly depends of the organ it has been extracted from (Table 2). Based on the analysis of 40 publications, it can be assumed as follows:

Leaves: most EOs extracted from leaves are richer in α -pinene followed by other compounds that differ according to geographical origin: β -myrcene [46,73], α -phellandrene [50], limonene [62], γ -terpinene [76], sabinene [63], β -pinene [69], and other compounds (see Table 2). The PCA carried out above (Figures 4a, 4b) has clearly shown the different chemotypes that characterize the *P. lentiscus* EO of leaves in the Mediterranean area.

Stems: For stems (also mentioned twigs), Haloui et al. [44] also detected α -pinene as the main compound; it was followed by tricyclene and caryophyllene in EOs extracted from stems in Morocco. α -Limonene, α -pinene, germacrene-D were identified in the EOs of stems in Tunisia [15]. In the EOs of lentisk twigs, sabinene, α -pinene, germacrene D are the main compounds identified by Kivack et al. [78] in Turkey, While, Marengo et al. [66] detected terpinene-4-ol followed by α -phellandrene and β -phellandrene in their Italian EOs.

Fruits: the *P. lentiscus* EOs from fruit are richer in α -pinene followed by β -myrcene in the reports of [15,62,73]. Oppositely, Bouyahia et al. [72] reported limonene as the major compound.

Flowers: germacrene-D, β -caryophyllene, α -terpinen-4-ol and α -Limonene were detected in essential oils of lentisk's trees [15].

Aerial part: The EOs obtained from the whole aerial parts (leaves, stems and, flowers & fruits) of lentisk tree presented variable compositions. Longifolene was the major compound detected in lentisk oils from Algeria [54]. Other EOs are rich in α -pinene followed by β -myrcene in Tunisia and Italy [46,49] and myrcene followed by pinene in Italy [36].

Yosr et al. [15] studied the chemical composition of essential oils from different parts of *Pistacia lentiscus* (leaves, stems, fruits and flowers). This study confirms the variation of the chemical composition of the extracted oils depending on the organ.

4.2.1.3. Harvesting period and vegetative cycle:

The period of the vegetative cycle also influences the proportion of each chemical compound in *P. lentiscus* EO. The contents of the main compounds (α -pinene (14-22%), β -myrcene (1-19%), p-cymene (1-16%)) of lentisk harvested in Italy from April to October varied according to the month of harvest [108]. The contents of α -limonene (7-18 %), pinene (4-10%) and D-germacrene (8-13%) changed according to the developmental stage of the lentisk harvested at 4 different vegetative stages during the year in Tunisia [15]. In addition, differences in content of the major compounds were found in samples from different origins

and seasonal variability. This was observed in the study of [15,41,49].

4.2.1.4. Sex:

The sex of the plant also plays an important role in the chemical composition of EOs. Germacrene-D, β -caryophyllene, α -terpinen-4-ol were detected in EOs from male lentisk's trees. In opposite, α -limonene, α -pinene, germacrene-D were the main compounds detected in female ones [15].

4.2.1.5. Environmental factors:

Many environmental factors are likely to influence its development: daylight, temperature, humidity, day-night duration, sunshine, wind, nature of the soil. These factors can significantly influence the quantity and quality of the EO produced by causing important biochemical and metabolic changes within the plant [2]. Aissi et al. [2] showed the effect of environment on the chemical composition of the *P. lentiscus* EOs on several populations of lentisk exposed to variable climatic factors.

4.2.1.6. Soil quality:

The nature of the soil can influence the secondary metabolites profile of plants. Llorens et al. [74] showed that the chemical compositions of lentisk's EO are rich in α -pinene when the plant grew on calcareous soil. EOs from siliceous soil areas have high levels of myrcene followed by caryophyllene and terpinen-4-ol.

4.2.1.7. Method of extraction:

Extraction is an important step in the processing of plant material. It can lead to significant changes in quantity and quality of the EO. The different methods of extraction expose the raw material to relatively aggressive conditions in terms of temperature, acidity and heat, resulting in potential isomerization, racemization, oxidation, rearrangement, hydrolysis reactions. Congiu et al. [50] showed the effect of the method of extraction on the quality of essential oil extracted from lentisk leaves. EO extracted by hydrodistillation was rich in α -pinene (18%), α -phellandrene (12%) and caryophyllene (13%). For EOs extracted by supercritical CO₂ method, the major compounds detected were caryophyllene (31%), germacrene D (12%) and cadinene (6%).

4.2.1.8. Literature and possible prediction of EO's profile and levels:

From this comparative study, we can synthesize that the composition of lentisk

essential oil is first of all the result of the expression of the genetic heritage of this species. But it also depends on external factors to which the plant is subjected throughout its development. Among these factors, some are linked to the environment: climate, soil, humidity, altitude, temperature. Others are linked to human intervention: harvest period, part of the plant used, drying, and extraction practices. It appears that most of the EOs from *Pistacia lentiscus* are characterized by the presence of monoterpenes as the main component. However, several qualitative and quantitative differences are recorded according to the factors mentioned above. These factors influence the biosynthetic pathways of the plant and therefore the nature of the major components and their levels. In other words, they do influence the chemotype of the essential oil of *Pistacia lentiscus*.

These observations also show that it is possible to study and know the variations in the chemical composition of the essential oil through the seasons and thus to define the best time for harvesting by the best balance between yield and chemical composition.

It is important to know the factors influencing the composition of the essential oil. This helps to control those possible (harvest period, harvest altitude, extraction method, sex of the plant). This makes it possible to anticipate (i.e. predict partially) the composition of the EO obtained. This interests industrial aromatherapists who are looking for good yields in the extraction of specific molecules or a stable chemical composition.

5. Pharmacological activities

Authors have reported that the essential oil of *Pistacia lentiscus* has interesting pharmacological applications, in particular antimicrobial activities (Table 2). The scientific results also revealed the antioxidant, anti-inflammatory and anticancer activities of the essential oil of lentisk.

Antimicrobial activities: antibacterial and antifungal

The *P. lentiscus* EOs were tested against different bacterial and fungal strains (see Table 2). EOs from the leaves and fruits showed the strongest antibacterial inhibition on *Proteus mirabilis* with diameter inhibition values of 35mm and 33mm respectively [72]. The crude EOs of both leaves and twigs were active against strains *Salmonella sp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus sp.*, *Enterococcus faecalis*, *Mycobacterium aurum*, *Mycobacterium smegmatis*. Thus, *M. aurum* and *M. smegmatis* were more sensitive to twigs essential oil [43]. The difference in intensity of antibacterial activities of these two EOs could be attributed to a difference in chemical composition according the plant part used

(leaves / stems) [43]. *S. aureus* was found to be sensitive to EOs rich in camphen and carophyllene [76]. Douissa et al. suggested that camphene and carophyllene may act synergistically or additively against *S. aureus* bacteria [77]. *P. lentiscus* EOs (from leaves and twigs) have remarkable antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* [44]. *Bacillus subtilis* was the most sensitive strain to both EOs, while *Pseudomonas aeruginosa* was the most resistant [44]. Furthermore, the antimicrobial activity of *P. lentiscus* EOs against the tested bacteria in previous studies (Table 2) showed that Gram-negative strains were more resistant compared to Gram-positive strains.

Whether a relatively large set of data, the Antibacterial activities of *P. lentiscus* EOs remains difficult to correlate with one or few specific compounds [44]. EO with high terpene contents (tricyclene, 3-carene, trans- β -ocimene, and D-germacrene) are known to have relatively low antibacterial activity [80]. The sensitivity of the different bacteria tested with lentisk EOs could be associated with the high concentration of hydrocarbon terpenoids and in particular of α -pinene. These compounds are known for their antimicrobial activity [76]. In addition, many reports have shown that α -terpineol, has an inhibitory effect against *E. coli* [44].

The essential oil of lentisk has also been tested for its antifungal activity against *Aspergillus flavus*, *Rhizoctoniasolani*, *Penicillium commune*, *Fusarium oxysporum*. This EO is effective against *Aspergillus flavus* [49]. In addition, terpinenol and R-terpineol, two of the main components of *P. lentiscus* EO, totally inhibited the mycelial growth of *A. flavus* [49]. Previous study showed the antifungal activity of lentisk EO *Candida albicans* and *Candida glabrata* [14].

Antioxidant, anti-inflammatory and anti-cancer activities *in vitro*

The essential oil from *Pistacia lentiscus* fruits showed more interesting antioxidant effects than *P. lentiscus* EOs from leaves in Morocco. Its antioxidant capacity values measured as IC₅₀ were 29/38/73 μ g/mL as revealed by DPPH/ FRAP/ ABTS assay respectively [72]. Good antioxidant capacity of the leaves EO was reported for different origins and harvesting times [49]. Leaf EO from Italy exhibits high seasonal variability of DPPH radical inhibition as equivalent antioxidant capacity of Trolox (TEAC) [49]. It is difficult to correlate the antioxidant potential to a single compound. Up to now, it is rare also to observe strong correlations between individual metabolites and antioxidant activities [81,82].

Essential oils from lentisk leaves obtained in Italy were used topically in a rat model using

carrageenan-induced paw edema and cotton ball-induced granuloma. These EOs significantly inhibited granuloma development and serum TNF- (tumor necrosis factor -) and IL-6 (interleukin-6) levels in response to irritants [64].

The EOs obtained from the leaves of plants growing in Sardinia were tested against COX (cyclooxygenase) [14]. The results showed a higher inhibitory activity towards COX-2 (cyclooxygenase 2) compared to that produced towards COX-1 (cyclooxygenase 1). The IC₅₀ inhibition values were 10-4 g/mL and 6-2 g/mL of *P. lentiscus* EO for COX-1 and COX-2, respectively. Moreover, the inhibition of COX-2 by EO was similar to that recorded with ibuprofen used as a positive control [14].

Terpenes are able to inhibit several inflammatory molecules, for example IL-1, IL-6, TNF and COX- 2 [83,84,85], thus disrupting the amplification of inflammatory mechanisms. The anti-inflammatory effect of EO can be related to mixture of terpenoids composing the oil; with particular attention to pinene [64] and terpinene-4-ol, limonene, myrcene, caryophyllene, phellandrene and sabinene, which were highly represented hydrodistilled oil [14].

In vitro studies showed that the essential oil of lentisk is able to fight aggressive tumors, such as neuroblastoma [49], human fibroblasts [14] and some other important tumor cell lines [36,86].

Catalani et al. [36] showed selective cytotoxic action of the EO against different tumor cells. FTC-133 (follicular thyroid carcinoma) was the most sensitive cancer cell line, while AG-09429 healthy fibroblasts were the most resistant cells to *Pistacia lentiscus* administration. On the basis of the chemical composition, the monoterpenes myrcene and α -pinene were the most abundant compounds of this oil, suggesting that the antiproliferative effects of *Pistacia lentiscus* in this study might be possibly mediated by these two compounds [36]. In accordance, previous evidence indicated that myrcene and α -pinene might exert significant cytotoxic effects on different cancer cell lines [87,88]. Bouyahia et al. [72] reported that EOs from fruits of *Pistacia lentiscus* fruits showed remarkable cytotoxic effects on RD and L20B adenocarcinoma cancerous cell lines at IC₅₀ values of 26.4 μ g/mL and 33.02 μ g/mL, respectively.

Other activities

Eos from *Pistacia lentiscus* leaves showed an important antileishmanial (antiparasitic) effect against *Leishmania major* (IC₅₀=17 μ g/mL) than EOs from fruits. Oppositely, these lasts showed more activity against *Leishmania infantum* (IC₅₀= 8 μ g/mL) than leaves EOs. These results may be attributed to the major bioactive compounds present in essential oils

tested (α -pinene, myrcene and limonene) [72]. Essential oil from 14 populations in Tunisia was found to be effective inhibitory against acetylcholinesterase. Anti-acetylcholinesterase activity varied according to chemical composition (*i.e.* richness in monoterpenes) of the investigated EOs studied [2].

6. Conclusion and perspectives

The main objective of this review is to provide an overview of the large amount of published informations about *P. lentiscus* extracts. These informations cover botany, traditional uses, phytochemistry, pharmacology and essential oil compositional variability with the different factors that may induce-explain this variability in the Mediterranean area.

As such, this species from the Anacardiaceae family has been traditionally considered as a medicinal plant since antiquity. The most recent ethnobotanical studies showed that the organs (leaves, stems, flowers, fruits, roots), the resin, or even the whole plant have been used for medicinal properties. These therapeutic uses have been described in all Mediterranean regions where the plant naturally grows (Algeria, Tunisia, Morocco, Libya, Italy, Spain and Greece).

Pistacia lentiscus was mainly used against diseases of the digestive system for healing ulcers and respiratory system, to treat pulmonary allergies, bronchitis, coughing, vomiting, diarrhea; Also, to treat skin problems, acne, skin allergy and burns. Lentisk was also indicated for the treatment of hypertension or heart disease and also for the treatment of diabetes.

Comparing the different reports, a very high variability of terpenes profiles could be evidenced in the EOs from leaves of *Pistacia lentiscus* depending on Mediterranean area, but also to sub climate, to season and to (the dioic) plant sex. This variability is a limitation for a large and systemic valorization of *P. lentiscus* EOs. But it is also a very useful base of selection for extracting and valorizing specific bioactive products, if anticipated. This review aimed to illustrate that point, as far as possible. It then appeared clear that, future research should focus on optimizing the harvest, the extraction and the isolation of the compounds in sufficient quantities in order to study more in depth the pharmacological activities and their mechanisms of action. This could then allow the development of micro industrial sectors aiming at an eventual valorization of this plant. One of our very recent publication is still proposing data in that way [41].

Furthermore, this review shows that a need for experimental research on skin, muscle, ear, nose and throat applications, still exists, in order to validate traditional uses and move beyond the limitations due to uncertified traditional lentisk forms.

In addition, future research should focus on identifying the really active components and their potentially toxic counter parts in *Pistacia lentiscus* extracts. From that, studies in a preclinical setting could be really initiated; and could then be safely and economically translated into realistic equivalent doses for human health needs.

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Discussion-perspectives de l'étude

L'objectif de cette revue (cf « chapitre-article » précédent) était de clarifier ce qui est connu, ce qui est commun, et ce qui varie entre les différentes occurrences de *P. lentiscus*. Une bonne partie de cette revue se concentrait sur la variabilité phytochimique et le rendement en huile essentielle sous l'effet de paramètres environnementaux. Cette variabilité affecte particulièrement la composition de l'huile essentielle. Sur la base des données quantitatives issues de la littérature, nous avons entrepris une analyse statistique (ACP). Elle permet de montrer la diversité chimique et visualiser les différents groupes de chémotypes décrits jusque-là pour la région méditerranéenne.

A partir de notre étude comparative, nous pouvons synthétiser puis affirmer que la composition des HEs de *P.l.* est tout d'abord le résultat de l'expression du patrimoine génétique de cette espèce. Mais elle dépend également de facteurs externes auxquels la plante est soumise tout au long de son développement. Parmi ces facteurs, certains sont liés à l'environnement: climat, sol, humidité, altitude, température. D'autres sont directement liés à l'intervention humaine: période de récolte, partie de la plante utilisée, séchage, pratiques d'extraction. Il apparaît que la plupart des HEs de *Pistacia lentiscus* sont caractérisées par la présence de monoterpènes comme composants principaux. Cependant, plusieurs différences qualitatives et quantitatives sont enregistrées en fonction des facteurs mentionnés ci-dessus. Ces facteurs influencent les voies de biosynthèse de la plante. En d'autres termes, ils influencent le chémotype de l'huile essentielle de *Pistacia lentiscus*. Il serait intéressant d'examiner s'il existe de nouveaux chémotypes induits sous l'effet de l'interaction entre ces sources de variation. Il y aurait donc un intérêt à se focaliser sur les changements chimiques de l'huile essentielle de *Pistacia lentiscus* et en particulier, dans le cas de cette Thèse, de s'intéresser aux *P.l.* locaux-régionaux. C'est l'un des objectifs de la suite de notre travail de recherche (article 2). Le travail de synthèse réalisé (article 1) était une étape préliminaire qui nous a servi à développer une nouvelle recherche sur la variation de la composition en huile essentielle de lentisque sous l'effet de l'interaction entre la saison et l'altitude de récolte de lentisque. C'est l'intérêt du travail publié et développé dans le chapitre II.

Chapitre II:

Profilage de la composition en huiles essentielles et en acides gras des feuilles de *Pistacia lentiscus* de Tizi-Ouzou et étude du potentiel antioxydant

Résumé de l'étude

Introduction

Les huiles essentielles (HEs) sont des mélanges liquides complexes de molécules naturelles très volatiles; chacune ayant une odeur caractéristique (Adorjan et *al.*, 2010). L'hydrodistillation reste le procédé d'extraction le plus utilisé pour obtenir les huiles essentielles; notamment à des fins pharmaceutiques et cosmétiques (Ahmed et *al.*, 2021). Selon l'espèce végétale, les HEs peuvent être obtenues à partir de feuilles, de graines, de bourgeons, de fleurs de rameaux, d'écorces, de bois, de racines, de tiges, de fruits, voire de gommages de troncs d'arbres (Burt et *al.*, 2004). Elles sont principalement composées de terpènes et de terpénoïdes (Bakkali et *al.*, 2008).

Les terpènes et les terpénoïdes sont des produits naturels possédant un squelette carboné basé sur des unités isoprènes. La biosynthèse des terpénoïdes se fait à partir de l'acide mévalonique (Belhachat et *al.*, 2019). Leur importance touche un large éventail de fonctions dans la nature, notamment les antioxydants et les pigments responsables de la vision chez les animaux. Les terpénoïdes revêtent également une importance considérable sur le plan commercial et peuvent trouver des applications dans les arômes, les parfums, l'agrochimie et les produits pharmaceutiques (Belhachat et *al.*, 2019).

Pistacia lentiscus L. (*P.l.*) est l'une des espèces végétales utilisées pour la production de l'huile essentielle. L'HE de *P.l.* est obtenue par hydrodistillation à partir des parties aériennes telles que les feuilles, les rameaux, les fleurs et les baies (Catalani et *al.*, 2017). Elle est également extraite de la gomme de mastic (Negro et *al.*, 2015). Elle a une couleur jaune, une odeur aromatique et un goût piquant (Negro et *al.*, 2015). L'HE de *P.l.* a été utilisée en parfumerie, dans l'industrie pharmaceutique et alimentaire (Sehaki et *al.*, 2022) et comme arôme dans les boissons (Fernandez et *al.*, 2000).

Différentes compositions chimiques d'HEs provenant de plusieurs origines méditerranéennes (Algérie, Maroc, Tunisie, Italie, Espagne, Egypte, Grèce ou France) ont été décrites dans la littérature scientifique (revue précédente ; article1). Cette variabilité dépend de facteurs intrinsèques (génétique, mutation, variation somaclonale, voire épigénétique) et extrinsèques (environnement biotique et abiotique) discutés précédemment dans l'article1 de ce mémoire.

Dans le présent travail (article 2), nous avons visé à explorer la composition des HEs des feuilles de *P. lentiscus* de la grande région de Tizi-Ouzou, en Algérie. Nous avons ainsi étudié l'interaction de l'effet « altitude » et celui de la « saison de récolte » sur la variabilité du contenu en terpénoïdes de *P. lentiscus*. Au-delà de la caractérisation phytochimique fine, nous avons aussi étudié l'impact de la variabilité chimique sur les activités antioxydantes des HEs. En outre, le profilage des acyles gras des lipides foliaires a été réalisé en vue d'utiliser le taux d'acides gras (poly)insaturés comme marqueur de l'exposition au froid des plantes avant récolte. Une corrélation a ainsi pu être établie entre terpénoïdes et froideur saisonnière.

Résultats et discussion

Échantillonnage des plantes, extraction et analyse GC-MS des HEs

L'espèce végétale, *Pistacia lentiscus*, a été identifiée à l'ENSA (Ecole Nationale Supérieure d'Agronomie d'Algérie). Les échantillons de feuilles de *Pistacia lentiscus* ont été prélevés sur deux sites de la région algérienne de Tizi-Ouzou;

- 1) Ait-Irane, en montagne (altitude 876 m): son climat méditerranéen est caractérisé par un été sec et des températures relativement basses pendant l'hiver (Tableau S1; voir « Matériaux supplémentaires »).
- 2) Tigzirt, au littoral (altitude 13 m): ses températures estivales et hivernales sont moins extrêmes et une plus grande humidité est présente toute l'année (Tableau S2; voir « Matériaux supplémentaires »).

La Composition et les propriétés physico-chimiques des sols des deux stations:

Station 1 (Ait Irane) : Sol de texture limono-sableuse pauvre en matière organique

Station 2 (Tigzirt) : Sol de texture argilo-limoneuse très riche en matière organique

Les prélèvements sur ces deux sites ont été effectués sur les mêmes plantes pendant quatre saisons, en 2019, et à quatre stades végétatifs différents: hivernage végétatif (mi-février), pleine floraison (mi-avril), fructification précoce (mi-août) et fructification tardive (mi-octobre).

Les huiles essentielles (HEs) ont été extraites par hydrodistillation à l'aide d'un appareil de type Clevenger. Selon la période et l'altitude de la récolte, huit groupes d'huiles essentielles

ont été obtenus: montagne-hiver, montagne-printemps, montagne-été, montagne-automne, littoral-hiver, littoral-printemps, littoral-été et littoral-automne. Chaque groupe possède 3 HEs ce qui donne un total de 24 HEs. L'analyse chimique des HEs a été réalisée par spectrométrie de masse après chromatographie en phase gazeuse (GC-MS). Les conditions opératoires de cette analyse sont présentées dans l'article 2.

L'identification des composants de l'huile a été effectuée en comparant leurs indices de rétention (IR) avec les IR de la littérature (Hafsé et *al.*, 2013; Haloui et *al.*, 2015; Aissi et *al.*, 2016; Yosr et *al.*, 2018) et, avec ceux des composés authentiques disponibles dans le laboratoire des auteurs. De plus, l'identification a été confirmée en comparant les spectres de masse de chaque molécule avec (i) ceux disponibles dans la bibliothèque NIST 05 du système de données GC/MS et avec (ii) les spectres de masse publiés. 72 chromatogrammes ont ainsi été générés à partir de l'analyse GC-MS des HEs de lentisque. Quelques chromatogrammes sont présentés dans les données supplémentaires avec le détail de l'identification chimique réalisée (Figures S2,S3,S4,S5,S6,S7,S8,S9,S10 et Tableau S3)

Les résultats de l'analyse GC-MS des HEs sont illustrés dans le Tableau 1 de l'article 2. Quarante-sept pics chromatographiques (47 composés) ont pu être annotés (Tableau 1). Ces composés représentent 88% à 97% de la composition totale des huiles essentielles analysées. Ces huiles étaient caractérisées par leur richesse en hydrocarbures sesquiterpéniques. Les principaux composés détectés étaient les suivants: α -pinène (2-13%), β -caryophyllène (8-25%), β -myrcène (0,1-19%), acétate de bornyle (0,8-7%), δ -cadinène (3-8%), bisabolol (1-9%), β -pinène (0,9-7%), oxyde de caryophyllène (4-9%) et α -cadinol (3-11%).

Variations quantitatives des principaux composés et des classes terpéniques

L'abondance relative de chaque composé dans les différentes huiles essentielles (HE) a été déterminée sur la base des surfaces des pics chromatographiques mesurées en courant ionique total (TIC). Le Tableau 1 présente les teneurs moyennes (et les écarts types) pour chaque HE en fonction de leur origine: saison et site géographique. Pour les principaux pics chromatographiques (composés), des différences majeures peuvent être observées au sein des HEs (Tableau 1).

Une analyse de variance à deux facteurs (ANOVA) a été réalisée sur toutes les valeurs pour évaluer les effets de l'altitude du site (facteur 1), de la saison (facteur 2) et de l'interaction possible entre les deux facteurs sur chaque molécule. L'interaction entre l'altitude

et la saison (Tableau 1, colonne: Interaction Altitude * Saison) a eu un effet hautement significatif ($p < 0,001$) pour la plupart des molécules retrouvées dans les différentes huiles essentielles. Les différences significatives ($p < 0,05$) entre les niveaux moyens de chaque composé sont signalées dans chaque ligne du Tableau 1 par des lettres différentes. Ces données confirment que des différences et des similitudes sont apparues dans les profils chimiques de nos HEs.

Les différentes classes de terpènes ont également montré des variations significatives sur les quatre classes distinctes suivantes: hydrocarbures monoterpènes (MHs); hydrocarbures sesquiterpènes (SHs); monoterpènes oxygénés (OMs); sesquiterpènes oxygénés (OS).

Analyse multi variée de tous les composants détectés dans les HEs

Afin de mieux montrer la différenciation des compositions chimiques entre les différentes HEs, une analyse en composantes principales (ACP) a été réalisée sur les 47 composés majeurs annotés (Figure 1 de l'article 2). Les compositions des HEs analysées ont pu être subdivisées en cinq sous-chémotypes. Les HEs du site côtier correspondaient à quatre périodes de récolte: hiver, printemps, été et automne. Elles étaient caractérisées par des quantités élevées de **β -caryophyllène (C26)**. Les HEs du site de montagne étaient variables selon les saisons. En hiver, les HEs contenaient des quantités élevées de **δ -cadinène (C36)**, **de bisabolol (C46) et d'acétate de bornyle (C20)**. Les échantillons collectés au printemps et en été étaient caractérisés par des quantités élevées **d' α -pinène (C2) et de β -pinène (C5)**. Les échantillons prélevés en automne présentaient une forte proportion de **β -myrcène (C6)** suivie de **l' α -pinène (C2)**.

Activité antioxydante

Le changement d'altitude et la période de récolte affectent la nature « chimio-typique » des HEs de lentisque d'après l'analyse chémométrique réalisée. On s'est donc intéressé à l'étude de l'effet de cette variation remarquable de la composition de l'huile essentielle sur son potentiel antioxydant.

L'activité antioxydante des HEs a été évaluée à l'aide de trois techniques: le test de piégeage du radical DPPH, le test du pouvoir antioxydant ferrique réducteur (FRAP) et le test de réduction du cation radical ABTS. Toutes les mesures ont été déterminées et exprimées en capacité antioxydante équivalente de Trolox (TEAC). Le Trolox est l'équivalent hydrophile de la vitamine E et il a été utilisé comme standard pour les trois tests. La vitamine C (acide

ascorbique) a été testée comme témoin pour les trois tests ; les résultats sont exprimés en mg d'équivalent Trolox/g de vitamine C. Les résultats de l'évaluation de l'activité antioxydante sont présentés dans le Tableau 2 de l'article 2.

Selon l'origine de l'huile essentielle (c'est-à-dire la saison de récolte et l'altitude), l'analyse ANOVA a montré que l'interaction entre l'altitude et la saison avait un effet significatif ($p < 0,001$) sur les variations de l'activité antioxydante. Le test FRAP a toujours conduit à des valeurs plus élevées que les tests ABTS et DPPH. L'effet antioxydant (AOx) le plus élevé a été mesuré pour les échantillons de montagne parmi les trois tests. La capacité de réduction ferrique (FRAP) de ces HE variait de 9,6 (au printemps) à 22,3 mgTE/gEO (en été) pour le site de montagne et de 6,4 (en hiver) à 15,7 mgTE/gEO (en été) pour les échantillons du littoral.

Une exploration des corrélations potentielles entre les activités et les niveaux de chaque composant moléculaire dans les HE a été entreprise. Les coefficients de corrélation entre les valeurs FRAP, ABTS et DPPH et les composés majeurs des HEs ont ainsi été calculés. Les composés contribuant le plus à la variation des activités antioxydantes parmi les principaux composés des HEs sont:

- (1) le bisabolol, le δ -cadinène et le β -pinène. Pour le test FRAP;
- (2) le β -caryophyllène, l'acétate de bornyle, l'oxyde de caryophyllène, l' α -cadinol et les monoterpènes oxygénés pour le test FRAP, monoterpènes oxygénés pour le test DPPH;
- (3) oxyde de caryophyllène et α -cadinol pour le test ABTS.

Préparation des extraits d'acyl d'acides gras des feuilles de lentisque et analyse GC-MS

La biosynthèse des acyl gras (*i.e.* acides gras) chez les plantes est attribuée à l'expression de gènes (voie de biosynthèse des acides gras, enzymes de modification des AG (désaturases, élongases...), biosynthèse des phospholipides et triglycérides) qui peut être modulée sous l'effet des conditions climatique et plus particulièrement du changement de la température (Diamantoglou et *al.*, 1979; Martz et *al.*, 2003). La désaturation des acides gras augmente avec l'exposition progressive au froid des plantes. Pour cette raison, nous avons recherché une éventuelle corrélation entre la composition terpénique des HEs et le profil en acyles gras (sous forme d'esters méthyliques des acides gras obtenus par transestérification des acyl-lipides des feuilles de lentisque). Cela permet d'examiner si la variabilité des

chémotypes des HEs de lentisque est associée à la réponse physiologique des plantes aux températures basses (plus la plante s'est adaptée au froid, plus elle présente un taux d'acides gras polyinsaturés élevé dans ses lipides saponifiables).

Le profil en acyles gras des lipides saponifiables des feuilles a donc été analysé par GC-MS après extraction des lipides totaux et transestérification en esters méthyliques d'acides gras (FAME, en anglais Fatty Acid Methyl Esters). Les résultats sont présentés dans le Tableau 4 de l'article 2. La variation altitudinale et saisonnière du profil des acyles gras est présentée dans la figure 2 (art.2). Les principaux acides gras détectés dans les feuilles étaient l'acide linoléique, l'acide palmitique, l'acide linoléique et l'acide oléique. Les niveaux d'acides gras insaturés (c'est-à-dire C18:1, C18:2 et C18:3) variaient significativement avec la saison et l'altitude. Plus les conditions climatiques sont froides, plus le rapport relatif des acides gras poly-insaturés est élevé, et inversement (plus il y a de PUFA, plus les plantes ont été exposées récemment à des températures basses). Nous utilisons donc le taux de PUFA comme un marqueur biologique d'exposition au froid des plantes.

Les corrélations entre les principaux acyles gras dans les feuilles et les principaux terpénoïdes des HEs (Tableau 5) ont montré que le β -caryophyllène et le bisabolol étaient associés de manière opposée aux acides linoléique (C18:3) et oléique (C18:1). Lorsque les conditions sont plus froides, les niveaux de C18:3 et de bisabolol augmentent. En cas de températures moyennes, les niveaux de C18:1 et de β -caryophyllène étaient plus élevés. Aucun lien direct n'a été établi entre la voie de biosynthèse des acides gras et des terpénoïdes; mais cette corrélation a clairement démontré que la variation des températures entre le site de montagne et le site littoral expliquait la plupart des variations dans les compositions des HEs. Enfin, en fonction de la température, deux principaux chémotypes des HEs ont pu être distingués : un "chémotype d'hiver froid", caractérisé par des niveaux relativement élevés de bisabolol et un "chémotype tempéré" caractérisé par des niveaux élevés de β -caryophyllène.





Conclusion

Cet article a montré une grande variabilité dans les compositions en terpénoïdes (c'est-à-dire en huiles essentielles) et en acides gras des feuilles de *Pistacia lentiscus* collectées dans deux sites naturels contrastés de la région algérienne de Tizi-Ouzou. L'activité antioxydante des différentes HEs variait en fonction de la période de récolte et de l'altitude. La variation des activités antioxydantes peut être partiellement corrélée à la composition des huiles essentielles. La corrélation établie entre les principaux acyles gras dans les feuilles et les principaux terpénoïdes des HEs a clairement démontré que la différence des températures entre le site de montagne et celui du littoral expliquait la plupart des variations dans les compositions des HEs. La variabilité des chémotypes des HEs peut être associée à cette réponse physiologique des plantes aux températures.

Ce travail de recherche a développé des nouveaux résultats sur la composition de l'huile essentielle de lentisque. L'interaction de la saison et l'altitude de récolte a un effet significatif sur la variation chémotypique de l'huile essentielle de lentisque. A partir de ces résultats, une planification de la récolte des feuilles peut être menée pour imaginer de produire des HEs avec des chémotypes spécifiques. C'est la première étape dans la poursuite de la valorisation durable et rationnelle des ressources naturelles de la région de Tizi-Ouzou. C'est aussi l'occasion d'étudier plus en profondeur l'impact des changements climatiques sur la biosynthèse des terpénoïdes qui en résultent en utilisant des approches intégrant l'utilisation de réseaux de corrélation et l'analyse fluxomique.

Article

Profiling of Essential Oils from the Leaves of *Pistacia lentiscus* Collected in the Algerian Region of Tizi-Ouzou: Evidence of Chemical Variations Associated with Climatic Contrasts between Littoral and Mountain Samples

Chabha Sehaki ^{1,2}, Nathalie Jullian ¹, Elodie Choque ¹ , Rebecca Dauwe ¹, Jean Xavier Fontaine ¹ , Roland Molinie ¹ , Fadila Ayati ², Farida Fernane ² and Eric Gontier ^{1,*} 

¹ BIOPI-UPJV Laboratory UMRT BioEcoAgro INRAE1158, SFR Condorcet FR CNRS 3417, UFR de Sciences, Université de Picardie Jules Verne, 33 Rue Saint Leu, 80000 Amiens, France; chabha.sehaki@etud.u-picardie.fr (C.S.); nathalie.jullian@u-picardie.fr (N.J.); elodie.choque@u-picardie.fr (E.C.); rebecca.dauwe@u-picardie.fr (R.D.); jean-xavier.fontaine@u-picardie.fr (J.X.F.); roland.molinie@u-picardie.fr (R.M.)

² Laboratory of Natural Resources, University Mouloud Mammeri of Tizi-Ouzou, Tizi Ouzou 15000, Algeria; fadila.ayati@ummto.dz (F.A.); farida.fernane@ummto.dz (F.F.)

* Correspondence: eric.gontier@u-picardie.fr; Tel.: +33-6-59-21-81-08



Citation: Sehaki, C.; Jullian, N.; Choque, E.; Dauwe, R.; Fontaine, J.X.; Molinie, R.; Ayati, F.; Fernane, F.; Gontier, E. Profiling of Essential Oils from the Leaves of *Pistacia lentiscus* Collected in the Algerian Region of Tizi-Ouzou: Evidence of Chemical Variations Associated with Climatic Contrasts between Littoral and Mountain Samples. *Molecules* **2022**, *27*, 4148. <https://doi.org/10.3390/molecules27134148>

Academic Editor: Vincenzo De Feo

Received: 19 April 2022

Accepted: 23 June 2022

Published: 28 June 2022

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Abstract: Leaves of *Pistacia lentiscus* were collected from two Algerian sites in the mountains and the littoral of the Tizi-Ouzou region. The harvest was conducted in four consecutive seasons on the same selected set of trees. Essential oils (EOs) were extracted by hydrodistillation; then, they were analyzed by gas chromatography coupled mass spectrometry (GC-MS). Forty-seven constituents could be detected and quantified, including α -pinene (2–13%), β -caryophyllene (8–25%), β -myrcene (0.3–19%), bornyl acetate (0.8–7%), δ -cadinene (3–8%), bisabolol (1–9%), β -pinene (0.9–7%), caryophyllene oxide (4–9%), and α -cadinol (3–11%). Antioxidant (AOx) activities of the EOs were assessed by ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) assays. Significant differences in EO composition and AOx activities appeared dependent on the season and the site. Variations of AOx activities were significant for the FRAP and ABTS tests but not for DPPH. Characterization of the leaf fatty acyl (FA) profiles was performed by GC-MS. Variability appeared according to season and altitude. Polyunsaturated fatty acids levels were high (27–55%) at the coldest date and place. The levels of linolenic acyl in the leaves were significantly correlated with bisabolol levels in the EOs (Spearman's correlation coefficient: 0.818). Such results will be useful for the sustainable local valorization of wild *P. lentiscus*. These data also open new routes for further studies on terpenoid biosynthesis using correlation networks and fluxomic approaches.

Keywords: *Pistacia lentiscus*; essential oil; antioxidant activity; fatty acid

1. Introduction

Belonging to the Anacardiaceae family, *Pistacia lentiscus* (*P. lentiscus*) is one of at least 11 plant species of the *Pistacia* genus. Commonly called “lentisk” or “mastic tree” (in reference to its resin or so-called mastic), *P. lentiscus* is an evergreen shrub, largely distributed in Mediterranean countries [1]. It is a branched perennial thermophile shrub, measuring one to five meters in height. Its leaves are pinnate and evergreen. It is a dioecious species with distinct male and female plants harboring unisexual flowers. These flowers are approximately three millimeters wide and appear in spring (from March to May). They are aromatic, either male or female, and occur in small clusters. The female flowers are yellowish green. The male flowers are dark red. On female trees, the fruit is a rounded mono-sperm drupe of four to six millimeters in diameter. The fruit is first green, then

red, and becomes blackish when ripe in autumn [2]. A resin, also called mastic, is the best-known product of this plant. It is an aromatic and resinous substance that seeps from the trunk and the main branches [3]. The traditional use of different parts of the plant for therapeutic applications makes lentisk considered as a medicinal plant [4]. People in rural areas use the infusion of its leaves to treat gastric ulcers [5], respiratory disorders [6], and diabetes for its hypoglycemic effect [7]. They also use decoctions of lentisk aerial parts to treat tooth ache and throat infections [8]. The results of an ethnopharmacological study on lentisk, carried out in Algeria, have led to various therapeutic indications of this plant in the country [9]. The main components and pharmacological properties of the species were reviewed by Nahida (i.e., antimicrobial, hepatoprotective, antioxidant, and anticancer activities) [10] and Bozorgi (i.e., antimicrobial activity) [11]. The *Pistacia* species show variations in morphological, anatomical, chemical, and molecular traits across geographic origins and sexes [12].

Essential oils (EOs) have been produced from several parts of the *Pistacia* species [13]. Extracted from leaves, resin, mature or immature fruits, galls, buds, twigs, or flowers, lentisk EOs are generally a limpid liquid with a yellow color and an intense and piercing aromatic odor [13].

Different EOs from *Pistacia lentiscus* leaves have been produced by hydrodistillation [14]. Their chemical composition greatly depends on their origins (i.e., Algeria, Morocco, Tunisia, Italy, Spain, Egypt, Greece, or France). In Algeria, *P. lentiscus* EOs are mainly characterized by terpinen-4-ol (17%) and limonene (34%) [15,16]. Myrcene (39.2% [17]; 25.3% [18]) is the main compound reported in EOs from Morocco. In Tunisian and Italian EOs, α -pinene represented 9–18% of the total components [19,20]. Other studies reported the presence of monoterpene hydrocarbons [21–23] or sesquiterpene hydrocarbons [20,24] as the predominant fraction in lentisk EOs. The change in the chemical composition of the oils has been attributed (i) to the wide range of habitats in which lentisk grows [25]; (ii) to environmental factors; (iii) to seasons [26] and phenological stages [27]; (iv) to gender [2,28].

Lentisk EOs and derivatives have been used in perfumery [29] (e.g., Perfume “Accord Boisé” Minalys™ from the Olfactis Cie), in the food industry as flavoring in drinks [30,31] (e.g., liqueur Callicounis™ and Skinos™), and chewing gum [29] (e.g., Mastic Gum Elma™). *P. lentiscus* EOs have interesting biological activities. Indeed, antimicrobial effects against *Helicobacter pylori*, *Staphylococcus aureus*, *Sarcina lutea*, *Escherichia coli*, *Torulopsis glabrata*, and *Listeria monocytogenes* have been reported [21,29]. The use of *P. lentiscus* EOs as a natural fumigant against Lepidoptera, such as *Ectomyelois ceratoniae* and *Ephesia kuehniella*, had significant insecticidal effects [32]. The oil shows different levels of antioxidant properties [33].

These previous studies described various potential applications of *P. lentiscus* essential oils. Nevertheless, each use was based on the fine composition of essential oils and large variability occurred. It is also known that biological activity depends on the chemical composition of the EOs [34,35]. Most of the time, the main components are important, but sometimes synergic effects due to the different, eventually minor, compounds can lead to surprising enhanced (or decreased) biological activities.

Thus, in order to evaluate the potential of the natural *P. lentiscus* resources from the wide Algerian region of Tizi-Ouzou, it is of primary necessity to describe the profile of the essential oils from the leaves of wild plants. It is also needed to determine the variations of such profiles, and, if possible, to determine the main factors affecting such a profile.

This work aimed to explore the composition of wild *P. lentiscus* EOs extracted from leaves. Sample collection was conducted from two contrasting sites in the Tizi-Ouzou region (i.e., mountain and littoral); on the same selected tree from the bush, sampling was conducted four times a year to cover the four seasons (and yearly phenological stages of this perennial plant). We thus studied the variability of the terpenoid content in *P. lentiscus* and investigated the impact of this variability on its antioxidant activities as a function of both the harvesting period and altitude. In addition, the profiling of the fatty acyls from leaf lipids was performed. It allowed for proving that the main variations in the

EOs' composition were associated with the adaptation of the plants during the colder season in the Tizi-Ouzou Mountains.

2. Results and Discussion

2.1. Qualitative and Semi-Quantitative Composition of the Essential Oils

The different essential oils (EOs) were analyzed by GC-MS. Based on the previous literature and databases, 47 chromatographic peaks could be annotated (Table 1). These compounds corresponded to chromatographic peaks representing from 88% to 97% of the total area of all peaks. These oils were characterized by their richness in sesquiterpene hydrocarbons. This volatile fraction was the most abundant (29–46%) in almost all the different EOs (Table 1). Monoterpene hydrocarbons were present (5–42%) in all the samples. Oxygenated sesquiterpenes and oxygenated monoterpenes were in 13–37% and 3–9%, respectively. Within all samples, the major compounds detected were: α -pinene (2–13%), β -caryophyllene (8–25%), β -myrcene (0.1–19%), bornyl acetate (0.8–7%), δ -cadinene (3–8%), bisabolol (1–9%), β -pinene (0.9–7%), caryophyllene oxide (4–9%), and α -cadinol (3–11%).

Recent data from different Mediterranean countries have noted such a wide spectrum of chemical variability involving both the main compounds and the total amounts of the terpene classes [36,37]. For terpene classes, monoterpene hydrocarbons usually represented the main fraction: 75% in Egypt [37], 68% in Greece [3], 59% in Tunisia [24], and 56% in Italy [36]. However, in Tunisia, lentisk EOs were equally rich in monoterpene hydrocarbons (41%) and sesquiterpene hydrocarbons (40%) [19]. Lo-Presti et al. [20] and Llorens et al. [31] showed the prominence of sesquiterpenes in EOs from Italy (47%) and Spain (39%). Beyond terpene class variations, terpene levels varied as well. Thus, depending on the soil quality, different EO chemotypes have been described. In Spain, Llorens et al. [31], detected the presence of germacrene (10–17%), β -caryophyllene (11–12%), myrcene (1–11%), and terpinen-4-ol (5–6%) from calcicolous and siliceous soils. In Italy, Congiu et al. [38] detected β -caryophyllene (31%), germacrene (12%), and δ -cadinene (6%) as the main terpenes, while α -pinene and terpinen-4-ol were the chemotypes described in the (also Italian) EOs analyzed by Negro et al. [29]. EOs from Morocco varied in chemical composition; some were rich in myrcene (33–39%) [17,18,39], while others were mainly characterized by their 24% α -pinene level [40]. A number of other chemotypes rich in car-3-ene were also reported in Morocco [41]. The lentisk EOs in Tunisia were characterized by their high level of α -pinene (17%), terpinen-4-ol (12%), and δ -terpinene (9%) [24]. In addition, in Tunisia, germacrene D (11%), α -pinene (9%), limonene (8%), β -caryophyllene (8%), and δ -cadinene (8%) were found to be major compounds by Aissi et al. [19]. The (leaf) EOs from Egypt were described as rich in α -phellandrene [37]. It is thus clear that EOs from the leaves of *Pistacia lentiscus* presented variable compositions depending on the countries where the plants grew. Monoterpenes (i.e., α -pinene, terpineol, myrcene, limonene, and terpinen-4-ol) and sesquiterpenes (i.e., β -caryophyllene, δ -cadinene, and germacrene D) were the most common compounds. However, their levels greatly varied depending on the origin of the samples. The main factor contributing to this chemo-variability was attributed to the environmental conditions in the habitats of the *Pistacia lentiscus* species. Few or no data exist concerning genetic traits associated with the EO profiles. If *P. lentiscus* is an allogamous (dioic) species, we can assume that chemotypes were mostly (and naturally) selected essentially based on pedoclimatic traits. In our study, according to the harvest time (season) and place (altitude), various main chemotypes were detected. Except for the precise percentages, some of our main chemotypes correspond to other profiles previously described: caryophyllene [31,38], δ -cadinene [19], myrcene [18,39], and α -pinene [22]. Variations in EO profiles can be of interest. Nevertheless, it is important to determine the origins of variations before expecting a standardized production and a further economical use of such natural products, hence our interest in this littoral and mountain study.

Table 1. Compounds detected in the essential oils of *Pistacia lentiscus* leaves collected at two altitude sites and in four consecutive seasons. The levels of each compound are expressed as the chromatographic relative peak surface measured as the total ion current (area % TIC). The extraction yield for each EO was similar: 0.125 ± 0.005 g/100 g of dry matter.

Means % Content \pm Standard Deviation												
C	Compounds	IR	Mountain				Littoral				Altitude * Season Interaction	
			Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn		
C1	MH	tricyclene	923	0.2 \pm 0.1 ^{ab}	0.9 \pm 0.1 ^b	0.8 \pm 0.1 ^{ab}	0.5 \pm 0.1 ^a	0.1 \pm 0.1 ^c	0.3 \pm 0.1 ^d	0.2 \pm 0.1 ^d	0.2 \pm 0.1 ^{ab}	***
C2	MH	α -pinene	935	2.6 \pm 0.2 ^c	13.1 \pm 0.1 ^b	12.6 \pm 0.3 ^b	9.4 \pm 0.9 ^a	2.1 \pm 0.4 ^c	5.4 \pm 0.4 ^d	4.9 \pm 0.5 ^d	8.3 \pm 0.3 ^a	***
C3	MH	camphene	952	0.9 \pm 0.1 ^{cd}	3.1 \pm 0.1 ^b	3.2 \pm 0.0 ^d	1.6 \pm 0.0 ^a	0.6 \pm 0.1 ^e	0.8 \pm 0.1 ^f	1.1 \pm 0.1 ^f	1.0 \pm 0.0 ^{bc}	***
C4	MH	sabinene	975	0.5 \pm 0.0 ^e	1.4 \pm 0.0 ^c	1.2 \pm 0.1 ^c	3.0 \pm 0.4 ^a	0.3 \pm 0.1 ^f	1.1 \pm 0.1 ^d	1.0 \pm 0.1 ^{cd}	2.0 \pm 0.1 ^b	*
C5	MH	β -pinene	981	0.9 \pm 0.0 ^b	4.1 \pm 0.1 ^a	7.1 \pm 0.0 ^b	3.6 \pm 0.4 ^a	1.2 \pm 0.2 ^b	1.2 \pm 0.1 ^b	3.7 \pm 0.3 ^c	3.9 \pm 0.1 ^a	***
C6	MH	β -myrcene	993	0.1 \pm 0.0 ^c	0.5 \pm 0.0 ^c	0.3 \pm 0.0 ^c	19.0 \pm 0.9 ^b	0.5 \pm 0.1 ^d	5.0 \pm 0.1 ^b	4.8 \pm 0.9 ^{ab}	6.1 \pm 0.4 ^a	***
C7	MH	α -phellandrene	1011	0.0 \pm 0.0 ^{cd}	0.4 \pm 0.0 ^{bc}	0.0 \pm 0.0 ^{bc}	0.4 \pm 0.0 ^b	0.1 \pm 0.1 ^e	0.1 \pm 0.0 ^{de}	0.1 \pm 0.0 ^a	0.2 \pm 0.0 ^a	***
C8	Oth	o-cymene	1032	0.2 \pm 0.1 ^c	0.7 \pm 0.1 ^{ab}	0.9 \pm 0.1 ^{bc}	0.9 \pm 0.1 ^{ab}	0.3 \pm 0.1 ^d	0.3 \pm 0.1 ^{cd}	0.5 \pm 0.1 ^d	0.6 \pm 0.1 ^a	***
C9	Oth	p-cymene	1033	0.1 \pm 0.1 ^{ac}	0.2 \pm 0.1 ^a	0.4 \pm 0.1 ^{ab}	0.4 \pm 0.1 ^a	0.1 \pm 0.1 ^{bc}	0.1 \pm 0.1 ^a	0.2 \pm 0.1 ^c	0.3 \pm 0.1 ^a	
C10	MH	limonene	1036	0.5 \pm 0.1 ^f	3.8 \pm 0.1 ^f	2.7 \pm 0.0 ^b	4.2 \pm 0.1 ^b	1.4 \pm 0.2 ^e	4.7 \pm 0.1 ^d	1.1 \pm 0.2 ^c	4.9 \pm 0.3 ^a	***
C11	MH	γ -terpinene	1066	0.2 \pm 0.0 ^d	0.5 \pm 0.0 ^c	0.3 \pm 0.0 ^c	0.9 \pm 0.0 ^a	0.1 \pm 0.0 ^e	0.3 \pm 0.0 ^d	0.3 \pm 0.0 ^{bc}	0.4 \pm 0.0 ^b	***
C12	MH	α -terpinolene	1094	0.0 \pm 0.0 ^c	0.2 \pm 0.0 ^d	0.0 \pm 0.0 ^{bc}	0.2 \pm 0.0 ^b	0.1 \pm 0.0 ^d	0.2 \pm 0.0 ^d	0.1 \pm 0.0 ^a	0.1 \pm 0.0 ^a	***
C13	Oth	2-nonanone	1099	0.1 \pm 0.0 ^{cd}	0.3 \pm 0.0 ^b	0.1 \pm 0.0 ^{bc}	0.6 \pm 0.1 ^a	0.1 \pm 0.0 ^d	0.3 \pm 0.0 ^b	0.4 \pm 0.1 ^a	0.5 \pm 0.1 ^a	***
C14	Oth	nonanol	1109	0.2 \pm 0.1 ^{ac}	0.4 \pm 0.1 ^d	0.3 \pm 0.1 ^{bcd}	0.1 \pm 0.1 ^a	0.1 \pm 0.1 ^{ab}	0.5 \pm 0.1 ^{cd}	0.4 \pm 0.1 ^{ad}	0.3 \pm 0.1 ^{ac}	
C15	Oth	nonanal	1114	0.0 \pm 0.0 ^a	0.2 \pm 0.0 ^b	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.2 \pm 0.0 ^b	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	
C16	OM	menta-2-en-1-ol <E-p>	1137	0.0 \pm 0.0 ^{ac}	0.1 \pm 0.0 ^{ac}	0.0 \pm 0.0 ^{bc}	0.0 \pm 0.0 ^{ab}	0.0 \pm 0.0 ^c	0.0 \pm 0.0 ^c	0.0 \pm 0.0 ^c	0.0 \pm 0.0 ^a	
C17	OM	borneol	1187	0.1 \pm 0.0 ^{ab}	0.2 \pm 0.0 ^{ab}	0.1 \pm 0.0 ^{ab}	0.1 \pm 0.0 ^a	0.0 \pm 0.0 ^{ab}	0.1 \pm 0.0 ^c	0.1 \pm 0.0 ^{ab}	0.1 \pm 0.0 ^b	***
C18	OM	terpinen-4-ol	1207	1.3 \pm 0.1 ^{ce}	1.4 \pm 0.0 ^b	1.4 \pm 0.2 ^{de}	1.7 \pm 0.1 ^a	0.5 \pm 0.0 ^e	0.9 \pm 0.0 ^{cd}	1.5 \pm 0.2 ^{cd}	1.5 \pm 0.0 ^c	***
C19	OM	α -terpineol	1209	0.4 \pm 0.1 ^d	0.4 \pm 0.1 ^{cd}	0.9 \pm 0.1 ^c	0.5 \pm 0.1 ^a	0.3 \pm 0.1 ^c	0.5 \pm 0.1 ^b	0.5 \pm 0.1 ^e	0.6 \pm 0.1 ^{ab}	***
C20	OM	bornyl acetate	1297	6.2 \pm 0.2 ^a	7.0 \pm 0.1 ^b	4.9 \pm 0.3 ^b	1.5 \pm 0.1 ^c	2.8 \pm 0.1 ^b	1.9 \pm 0.1 ^f	1.9 \pm 0.0 ^d	0.8 \pm 0.0 ^e	***
C21	Oth	2-undecanone	1301	1.6 \pm 0.0 ^d	2.0 \pm 0.0 ^{bc}	0.9 \pm 0.1 ^{bc}	2.1 \pm 0.1 ^b	1.6 \pm 0.3 ^{cd}	2.0 \pm 0.1 ^c	1.9 \pm 0.2 ^a	2.5 \pm 0.2 ^b	***
C22	OM	α -terpinyl acetate	1358	0.2 \pm 0.0 ^d	0.3 \pm 0.0 ^f	0.0 \pm 0.0 ^c	0.0 \pm 0.0 ^e	1.0 \pm 0.1 ^a	1.5 \pm 0.1 ^c	0.3 \pm 0.0 ^a	0.5 \pm 0.1 ^b	***
C23	SH	α -copaene	1386	0.9 \pm 0.0 ^b	0.8 \pm 0.0 ^c	1.2 \pm 0.0 ^c	0.7 \pm 0.0 ^a	0.3 \pm 0.0 ^d	0.6 \pm 0.0 ^e	0.5 \pm 0.0 ^g	0.4 \pm 0.1 ^f	***
C24	SH	β -bourbonene	1395	0.0 \pm 0.0 ^{ab}	0.1 \pm 0.0 ^{de}	0.0 \pm 0.0 ^{ac}	0.0 \pm 0.0 ^e	0.1 \pm 0.0 ^{bcd}	0.1 \pm 0.0 ^{ce}	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	***
C25	SH	β -elemene	1398	1.1 \pm 0.0 ^d	1.2 \pm 0.0 ^{cd}	1.5 \pm 0.0 ^f	1.0 \pm 0.0 ^e	1.4 \pm 0.1 ^a	1.2 \pm 0.0 ^c	1.5 \pm 0.0 ^{ef}	1.3 \pm 0.0 ^b	***
C26	SH	β -caryophyllene	1435	8.8 \pm 0.1 ^e	10.7 \pm 0.2 ^d	10.8 \pm 0.5 ^f	13.2 \pm 0.9 ^f	24.9 \pm 1.0 ^c	17.2 \pm 0.6 ^b	25.4 \pm 0.8 ^b	21.7 \pm 0.1 ^a	***
C27	SH	β -cubebene	1444	0.8 \pm 0.1 ^b	0.8 \pm 0.0 ^{ab}	0.4 \pm 0.1 ^{ab}	0.8 \pm 0.0 ^{ab}	0.9 \pm 0.1 ^{ab}	0.9 \pm 0.1 ^{ab}	0.8 \pm 0.1 ^a	1.6 \pm 0.9 ^{ab}	

Table 1. Cont.

Means % Content ± Standard Deviation												Altitude * Season Interaction
C	Compounds	IR	Mountain				Littoral					
			Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn		
C28	Oth	isoamyl benzoate	1455	0.8 ± 0.1 <i>ab</i>	0.7 ± 0.1 <i>ab</i>	1.0 ± 0.1 <i>a</i>	0.6 ± 0.1 <i>b</i>	0.8 ± 0.1 <i>ab</i>	0.6 ± 0.1 <i>ab</i>	0.5 ± 0.1 <i>c</i>	0.6 ± 0.1 <i>b</i>	***
C29	SH	α-caryophyllene	1472	2.1 ± 0.0 <i>f</i>	2.0 ± 0.0 <i>e</i>	2.3 ± 0.1 <i>h</i>	1.7 ± 0.1 <i>g</i>	3.3 ± 0.0 <i>a</i>	2.5 ± 0.1 <i>b</i>	3.5 ± 0.0 <i>d</i>	2.8 ± 0.0 <i>c</i>	***
C30	SH	allo-aromadendrene	1477	0.8 ± 0.0 <i>a</i>	0.7 ± 0.0 <i>b</i>	0.7 ± 0.0 <i>b</i>	0.5 ± 0.0 <i>b</i>	0.6 ± 0.0 <i>a</i>	0.7 ± 0.0 <i>c</i>	0.6 ± 0.0 <i>c</i>	0.4 ± 0.0 <i>d</i>	***
C31	SH	γ-muurolene	1489	2.6 ± 0.0 <i>a</i>	1.9 ± 0.0 <i>d</i>	2.8 ± 0.1 <i>c</i>	1.2 ± 0.1 <i>c</i>	1.4 ± 0.1 <i>b</i>	1.7 ± 0.1 <i>e</i>	1.4 ± 0.0 <i>g</i>	1.0 ± 0.0 <i>f</i>	***
C32	SH	germacrene D	1498	2.5 ± 0.6 <i>bc</i>	2.8 ± 0.1 <i>d</i>	1.2 ± 0.3 <i>d</i>	4.0 ± 0.1 <i>d</i>	4.2 ± 0.3 <i>cd</i>	4.2 ± 0.3 <i>b</i>	4.2 ± 0.5 <i>a</i>	3.2 ± 0.2 <i>b</i>	***
C33	SH	α-muurolene	1514	1.9 ± 0.2 <i>b</i>	1.7 ± 0.0 <i>bc</i>	2.5 ± 0.0 <i>cd</i>	1.2 ± 0.0 <i>d</i>	1.8 ± 0.1 <i>a</i>	1.6 ± 0.0 <i>cd</i>	1.7 ± 0.1 <i>e</i>	1.4 ± 0.0 <i>d</i>	***
C34	SH	β-bisabolene	1520	1.1 ± 0.0 <i>a</i>	0.8 ± 0.0 <i>a</i>	0.4 ± 0.0 <i>a</i>	0.8 ± 0.4 <i>a</i>	0.4 ± 0.4 <i>a</i>	0.8 ± 0.2 <i>a</i>	0.6 ± 0.0 <i>a</i>	0.6 ± 0.0 <i>a</i>	*
C35	SH	γ-cadinene	1530	0.6 ± 0.1 <i>b</i>	0.8 ± 0.1 <i>b</i>	0.9 ± 0.0 <i>b</i>	0.4 ± 0.0 <i>c</i>	1.1 ± 0.0 <i>a</i>	0.8 ± 0.0 <i>b</i>	0.7 ± 0.1 <i>c</i>	0.6 ± 0.0 <i>b</i>	***
C36	SH	δ-cadinene	1534	8.5 ± 0.0 <i>a</i>	4.7 ± 0.1 <i>b</i>	3.6 ± 0.4 <i>b</i>	3.0 ± 0.3 <i>b</i>	4.8 ± 0.2 <i>a</i>	4.9 ± 0.1 <i>b</i>	4.6 ± 0.3 <i>a</i>	3.3 ± 0.1 <i>c</i>	***
C37	SH	calamenene	1540	2.3 ± 0.0 <i>a</i>	1.6 ± 0.2 <i>c</i>	1.5 ± 0.0 <i>b</i>	0.7 ± 0.0 <i>b</i>	0.9 ± 0.0 <i>a</i>	1.0 ± 0.0 <i>d</i>	0.9 ± 0.0 <i>d</i>	0.6 ± 0.0 <i>e</i>	***
C38	OS	elemol	1565	0.8 ± 0.0 <i>c</i>	0.2 ± 0.0 <i>f</i>	0.5 ± 0.0 <i>a</i>	0.5 ± 0.0 <i>d</i>	0.4 ± 0.0 <i>e</i>	0.6 ± 0.0 <i>b</i>	0.2 ± 0.0 <i>e</i>	0.3 ± 0.0 <i>g</i>	***
C39	OS	caryophyllene oxide	1573	8.7 ± 0.2 <i>c</i>	5.7 ± 1.2 <i>c</i>	7.9 ± 0.7 <i>bc</i>	4.5 ± 0.4 <i>d</i>	9.9 ± 0.5 <i>a</i>	7.8 ± 0.1 <i>ab</i>	7.5 ± 0.6 <i>c</i>	7.8 ± 1.0 <i>cd</i>	**
C40	OS	humulene epoxide II	1633	0.9 ± 0.1 <i>bc</i>	0.6 ± 0.1 <i>df</i>	0.7 ± 0.1 <i>b</i>	0.2 ± 0.1 <i>ef</i>	0.9 ± 0.1 <i>a</i>	0.8 ± 0.1 <i>bd</i>	0.5 ± 0.1 <i>cde</i>	0.5 ± 0.1 <i>f</i>	***
C41	OS	cubenol	1648	3.0 ± 0.1 <i>a</i>	1.2 ± 0.0 <i>d</i>	2.0 ± 0.1 <i>cd</i>	0.7 ± 0.1 <i>b</i>	1.0 ± 0.1 <i>a</i>	1.4 ± 0.0 <i>bc</i>	1.2 ± 0.1 <i>e</i>	0.8 ± 0.0 <i>f</i>	***
C42	OS	epi-Cadinol	1663	2.7 ± 0.0 <i>c</i>	1.8 ± 0.0 <i>cd</i>	2.8 ± 0.1 <i>de</i>	1.3 ± 0.1 <i>e</i>	2.8 ± 0.1 <i>a</i>	2.5 ± 0.1 <i>b</i>	2.7 ± 0.1 <i>e</i>	2.3 ± 0.0 <i>de</i>	***
C43	OS	δ-cadinol	1668	2.3 ± 0.1 <i>b</i>	1.1 ± 0.1 <i>cd</i>	1.3 ± 0.2 <i>cd</i>	0.6 ± 0.0 <i>d</i>	1.5 ± 0.1 <i>a</i>	1.4 ± 0.1 <i>bc</i>	1.2 ± 0.1 <i>cd</i>	0.9 ± 0.2 <i>e</i>	***
C44	OS	α-cadinol	1679	8.0 ± 0.1 <i>cd</i>	5.0 ± 0.9 <i>bc</i>	8.0 ± 0.2 <i>bc</i>	3.7 ± 0.3 <i>d</i>	11.9 ± 0.4 <i>a</i>	7.9 ± 0.1 <i>ab</i>	7.6 ± 0.8 <i>bc</i>	9.5 ± 3.2 <i>bc</i>	**
C45	OS	aromadendrene oxide	1684	1.0 ± 0.1 <i>a</i>	0.6 ± 0.0 <i>a</i>	1.1 ± 0.4 <i>a</i>	0.3 ± 0.0 <i>a</i>	0.5 ± 0.0 <i>a</i>	0.5 ± 0.0 <i>a</i>	0.4 ± 0.0 <i>b</i>	0.3 ± 0.0 <i>b</i>	**
C46	OS	bisabolol	1704	9.7 ± 0.3 <i>ab</i>	2.6 ± 0.0 <i>b</i>	1.2 ± 0.0 <i>a</i>	1.4 ± 0.1 <i>ab</i>	1.3 ± 0.1 <i>ab</i>	1.5 ± 0.0 <i>c</i>	1.1 ± 0.1 <i>ab</i>	1.2 ± 0.0 <i>d</i>	***
C47	Oth	benzyl benzoate	1797	0.4 ± 0.0 <i>ab</i>	0.2 ± 0.0 <i>c</i>	0.5 ± 0.0 <i>b</i>	0.3 ± 0.1 <i>c</i>	0.8 ± 0.2 <i>ab</i>	0.9 ± 0.0 <i>a</i>	0.4 ± 0.0 <i>b</i>	0.4 ± 0.0 <i>ab</i>	***
		Total (sum of C₁₋₄₇)		88.0 ± 0.6	91.1 ± 1.4	94.8 ± 0.6	94.0 ± 0.3	90.9 ± 0.8	90.9 ± 0.4	94.9 ± 0.3	96.6 ± 3.0	
		Terpene Classes										
MH		monoterpene hydrocarbons		5.8 ± 0.3 <i>c</i>	28.0 ± 0.1 <i>b</i>	28.2 ± 0.3 <i>b</i>	42.8 ± 2.6 <i>a</i>	6.4 ± 1.1 <i>d</i>	19.1 ± 1.0 <i>c</i>	17.4 ± 1.5 <i>c</i>	27.1 ± 1.2 <i>a</i>	***
SH		sesquiterpene hydrocarbons		33.8 ± 0.5 <i>c</i>	30.5 ± 0.6 <i>c</i>	29.8 ± 1.3 <i>d</i>	29.1 ± 1.4 <i>d</i>	45.9 ± 0.9 <i>a</i>	37.9 ± 0.9 <i>a</i>	46.5 ± 1.7 <i>a</i>	38.3 ± 0.4 <i>b</i>	***
OM		oxygenated monoterpenes		8 ± 0.3 <i>a</i>	9.3 ± 0.1 <i>a</i>	7.3 ± 0.5 <i>a</i>	3.8 ± 0.2 <i>a</i>	4.6 ± 0.4 <i>a</i>	4.9 ± 0.2 <i>a</i>	4.3 ± 0.3 <i>a</i>	3.4 ± 0.1 <i>a</i>	
OS		oxygenated Sesquiterpenes		37.0 ± 0.5 <i>bc</i>	18.8 ± 1.1 <i>c</i>	25.4 ± 0.6 <i>bc</i>	13.2 ± 1.0 <i>d</i>	30.3 ± 0.9 <i>a</i>	24.3 ± 0.4 <i>b</i>	22.4 ± 0.9 <i>c</i>	23.6 ± 4.4 <i>e</i>	***
Oth		Others		3.3 ± 0.1 <i>d</i>	4.5 ± 0.2 <i>cd</i>	4.1 ± 0.2 <i>bc</i>	5.1 ± 0.0 <i>ab</i>	3.7 ± 0.5 <i>d</i>	4.8 ± 0.1 <i>cd</i>	4.3 ± 0.1 <i>bc</i>	5.1 ± 0.1 <i>a</i>	

The means followed by different letters (in italic) within the same row were significantly different ($p < 0.05$). The main compounds name and levels are written in bold. Significance effect of the interaction between the altitude and season as determined by ANOVA, p -values: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

2.2. Quantitative Variations of the Major Compounds and Terpenic Classes

The relative abundance of each compound in the different essential oils (EOs) was determined based on chromatographic peak areas measured in the total ion current (TIC). As previously described [2], each relative abundance was then expressed as a percentage of the peak total area.

Table 1 presents the mean contents (and standard deviations) of each EO based on their origin: season and geographical site. Variations among sample origins clearly occurred. For the main chromatographic peaks, major differences can be observed (Table 1). As such, the highest percentages of α -pinene (12.6%) and β -pinene (7.1%) were, respectively, found in spring and summer at the mountain site. The highest amount of β -myrcene (19%) was detected in the mountains in autumn. In all EOs, the levels of β -caryophyllene exceeded 8% at both sites and harvest periods from 8.8% (winter, mountain) to 25.4% (summer, coast). δ -Cadinene (8.5%) and bisabolol (9.7%) harbored their highest levels in the EOs collected in winter in the mountains. In addition to these compounds, other molecules (also formatted in bold in Table 1) presented, at least once, a level superior to 5%, e.g., bornyl acetate (0.8–7.0%), caryophyllene oxide (4.5–9.9%), and α -cadinol (3.7–11.9%).

A two-factor analysis of a variance ANOVA test was performed with all the values to evaluate the effects of site altitude (Factor 1), season (Factor 2), and the possible interaction between both factors and each molecule. The significance of the effects are presented in Table 1 (column: Altitude * Season Interaction) with the following symbols: *** p -value < 0.001; ** p -value < 0.01; * p -value < 0.05. Most of the time, altitude and/or season had a significant ($p < 0.05$) or a highly significant ($p < 0.01$ to $p < 0.001$) effect on the variation of the EO component levels. The interaction between altitude and season (Table 1, column: Altitude * Season Interaction) had a highly significant effect ($p < 0.001$) for most of the molecules found in the different essential oils.

A Tukey test (post hoc) was applied for all the data. Significant differences ($p < 0.05$) between the mean levels of each compound are reported in each row of Table 1 as different letters. Homogeneous groups of the EO contents appeared among the eight altitudinal and seasonal conditions. These data confirm that differences and similarities occurred in the chemical profiles of our EOs. However, these data were not clear enough to determine the main rules on the implied variations in the individual compounds. The same analysis performed on the different terpene classes also showed variations over the four distinct classes of compounds (i.e., Table 1: monoterpene hydrocarbons (MHs); sesquiterpene hydrocarbons (SHs); oxygenated monoterpenes (OMs); oxygenated sesquiterpenes (OS)). MHs were higher in the mountains (28–42.8%) than in the littoral (6.4–27.1%), except for during winter (5.8%). For both sites, the highest rate of MHs was observed in autumn. MH levels in the mountains in autumn (42.8%) were one-and-a-half times the level of MHs from littoral samples in autumn (27.1%). For each site, the differences between levels of MHs were significant ($p < 0.05$) according to the season, except between spring and summer for both sites. Contrary to MHs, sesquiterpenes hydrocarbons (SHs) were higher ($p < 0.05$) in the littoral area (37.9–46.5%) than in the mountain (29.1–33.8%). SH levels were higher in the winter (mountain) and in the summer (littoral). Whatever the site or altitude, OS levels were higher in winter (mountain, 37.0%; littoral, 30.3%). OMs were present in smaller quantities than those of MH-SH-OS for all samples. Their variations were not significant according to the season and the altitude. For the four compound classes, the interaction between altitude and season (Table 1, Altitude * Season Interactions) had a significant ($p < 0.05$) or very significant ($p < 0.001$ for MH and SH) influence on the average levels. According to these results, we can conclude that the two parameters studied (i.e., altitude and harvesting season) contributed significantly to the variation in the chemical composition of the essential oils obtained from leaves of *Pistacia lentiscus* collected in the Algerian region of Tizi-Ouzou. Nevertheless, within all the variability detected for the composition of the different EOs, it was necessary (i) to determine the main traits of each oil and (ii) to compare them with other samples previously described in the literature. As such, the major EO constituents that were detected in lentisk leaves harvested

at the vegetative overwintering stage (February in Turkey) were previously described as terpinen-4-ol (29%; [42]) and β -myrcene (39%; [17]). For leaves collected at the flowering stage, in different places (i.e., countries), various EO chemotypes have also been described. The major compounds described were: α -pinene (25%), terpinen-4-ol (21%), δ -cadinene (11%), and β -caryophyllene (19%) by Negro et al. [29]; germacrene D (22%), α -pinene (17%), and sabinene (15%) by El Idrissi et al. [43]; tricyclene (7%), terpinen-4-ol (7%), sabinene (6%), and caryophyllene (6%) by Haloui et al. [23]. At a late fruiting stage (i.e., ~October), Mecherara et al. [16] detected the predominance of limonene (43%), α -pinene (34%), and myrcene (33.1 %) in EOs from lentisk leaves collected in Algeria. However, Ismail et al., (2012) in Tunisia reported the presence of α -pinene (20%), limonene (15%), β -pinene (9%), and germacrene D (8%) [22]. At early fruiting (i.e., ~June), the main EO compounds from lentisk leaves growing in the west Algerian region of Oran were terpinen-4-ol (41%), α -terpineol (7%), α -pinene (9%), limonene (9%), β -myrcene (10%), and p-cymene (8%) [44]. When comparing the composition of our EOs with those previously described in the literature and according to the harvest period, some similarities and differences can be raised. We note that some compounds, such as α -pinene, were similar at the flowering and early fruiting stages as was the case of Negro et al. [29], El Idrissi et al. [43], Haloui et al. [23], and Hamiani et al. [44]; similarities also occurred with β -myrcene in the late fruiting stage as described by Mecherara et al. [16], and β -pinene in the early fruiting stage was a main compound as shown by Ismail et al. [22].

However, our results largely differ from those of Zaouali et al. [2], who studied variations in the EOs' chemical composition according to the plant sex for different harvest periods in Tunisia. In female trees, α -limonene was mostly detected in leaves (26–29%) sampled at the early fruiting (August) and late fruiting (October) stages. Germacrene-D was dominant in leaves (20%) at the flowering and early fruiting stages, and δ -cadinene (15.6%) reached its highest content in leaves in the flowering (March) stage. In male trees, the highest amounts of germacrene-D (13%) were recorded in leaves at the flowering stage. Oppositely, for the variations in our results according to the (limited) literature regarding lentisk EOs, the influence of ecological factors may support an important part of the explanation. In other Mediterranean evergreen plant species, the influence of ecological factors on the chemical composition of EOs extracted from leaves has been clearly pointed out in *Juniperus oxycedrus* [45], *Rosmarinus officinalis* [46], *Myrtus communis* [47], and *Pinus halepensis* [48]. However, for *P. lentiscus*, no detailed information has until now been available to describe, and maybe explain, the precise relationship between environmental factors and the variations in EO composition. To our knowledge, only one unique study described the influence of the altitudinal gradient on the chemo-variation of *P. lentiscus* EO composition [26]. The samples were collected in Algeria, but with only one harvest period (September 2008). Data from our multisite/multiseason results are in accordance with Ait Said et al. [26]. The relationship between altitude and the chemotypes were commonly characterized by caryophyllene and by significant levels of monoterpene and sesquiterpene hydrocarbons.

2.3. Multivariate Analysis of All the Components Detected in the EOs

In order to better show the 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 1A) 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 1B) shows that the most discriminating compounds were the “major” compounds, which appeared at least once at a level greater than 5%.

Observations from the littoral samples, regardless of the season, were characterized by high quantities of β -caryophyllene (Group 1). The highest proportion of β -caryophyllene was found in the summer. The winter samples from the littoral group were characterized, in addition to β -caryophyllene, by significant contents α -cadinol and caryophyllene oxide (Group 2). EOs from the mountain site contained high amounts of δ -cadinene, bisabolol, and bornyl acetate in winter (Group 3). Samples collected in autumn had a high proportion of β -myrcene followed by α -pinene (Group 4), mainly from the mountain samples. The samples harvested in spring and summer at the mountain were characterized by high amounts of α -pinene and β -pinene (Group 5).

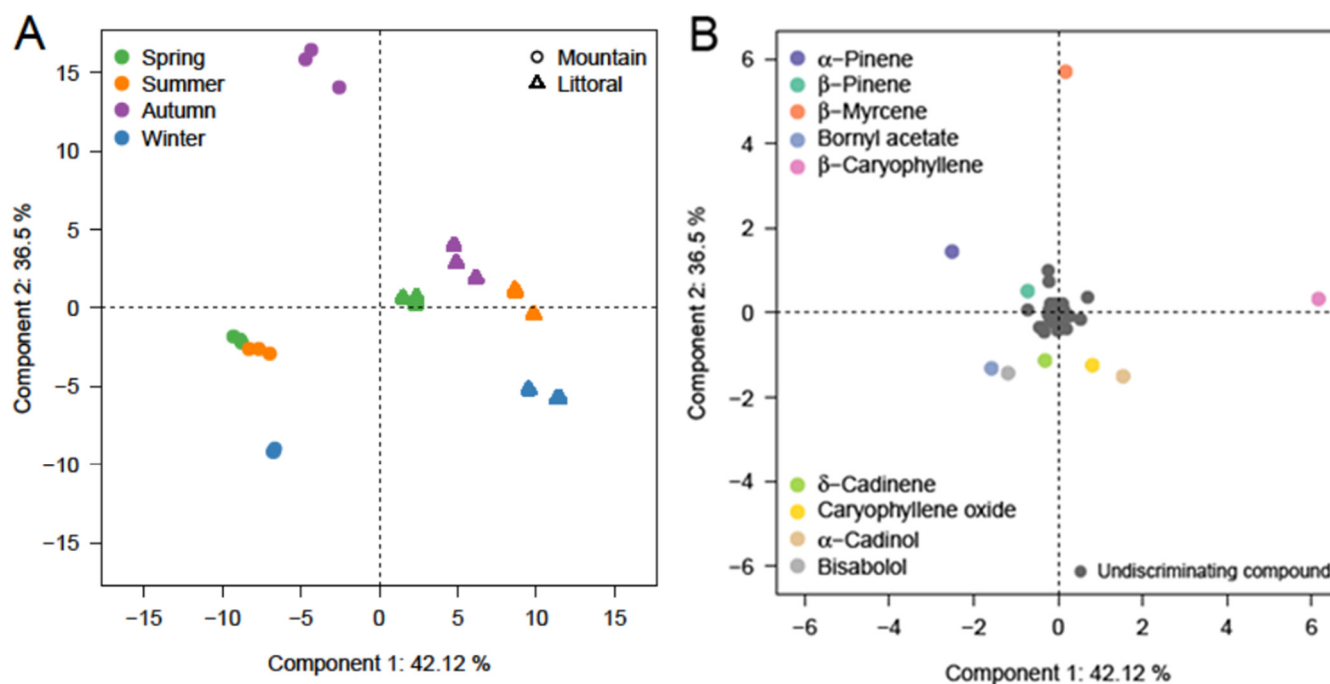


Figure 1. PCA analysis performed on all the compounds from the essential oils of *Pistacia lentiscus* leaf samples collected at the two altitude sites (Δ for littoral versus \circ for mountain) and in 4 consecutive seasons: (A) score plot of PC1 versus PC2 scores; (B) loading plot of PC1- and PC2-contributing EO compounds (the most discriminating compounds are represented in color).

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 [49]. 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 [49,50].

Abreu and Mazzafera [51] reported that lower temperatures at high altitudes can induce an overproduction of phytochemicals (*Hypericum brasiliense* Choisy). Previous studies have suggested that increasing altitude and subsequent changes in solar radiation and temperature in plant habitats can strongly correlate with the content of secondary metabolites, especially phenolics, due to the fact of their protective function against oxidative damage [52]. 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.

2.4. Antioxidant Activity

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 2.

Table 2. Evaluation of the antioxidant activities of *Pistacia lentiscus* essential oils using FRAP, ABTS, and DPPH tests.

		Antioxidant Assays		
Essential Oil Samples		FRAP (mg TE/g EO)	ABTS (mg TE/g EO)	DPPH (mgTE/g EO)
Mountain	Winter	11.3 ± 0.1 <i>c</i>	0.28 ± 0.01 <i>b</i>	0.12 ± 0.0 <i>a</i>
	Spring	9.6 ± 0.3 <i>a</i>	0.32 ± 0.0 <i>c</i>	0.44 ± 0.01 <i>a</i>
	Summer	22.3 ± 0.2 <i>c</i>	0.09 ± 0.01 <i>e</i>	0.08 ± 0.01 <i>b</i>
	Autumn	14.3 ± 0.1 <i>a</i>	0.24 ± 0.01 <i>a</i>	0.11 ± 0.0 <i>a</i>
Littoral	Winter	6.4 ± 0.9 <i>c</i>	0.09 ± 0.0 <i>c</i>	0.06 ± 0.0 <i>c</i>
	Spring	6.6 ± 1.1 <i>b</i>	0.23 ± 0.01 <i>e</i>	0.06 ± 0.0 <i>d</i>
	Summer	15.7 ± 0.5 <i>d</i>	0.30 ± 0.01 <i>a</i>	0.08 ± 0.0 <i>b</i>
	Autumn	14.8 ± 0.4 <i>b</i>	0.13 ± 0.01 <i>d</i>	0.06 ± 0.0 <i>c</i>
Altitude * Season Interaction		***	***	***
Vitamin C (mg TE/g vit C)		1080 ± 144	894 ± 88	1340 ± 192

Means followed by different letters (in italic) within the same row were significantly different ($p < 0.05$). Significance effect of the interaction between the altitude and season as determined by ANOVA, p -values: *** $p < 0.001$.

According to the origin of the essential oil (i.e., harvesting season and altitude), ANOVA analysis showed that the altitude and season interaction had a significant effect ($p < 0.001$) on variations in antioxidant activity. Significant variations ($p < 0.05$) in the different antioxidant activities can be observed using the post hoc test (Table 2). The FRAP test always led to higher values than the ABTS and the DPPH tests. As compared with the littoral samples, the highest antioxidant (AOx) effect was measured for the mountain samples among the three tests. The ferric reducing capacity (FRAP) of these EOs varied from 9.6 (spring) to 22.3 mgTE/gEO (summer) for the mountain site and from 6.4 (winter) to 15.7 mgTE/gEO (summer) for the littoral samples. Four conditions could be statistically separated (annotation as “a, b, c, and d” for the post hoc test in in Table 2, column: FRAP). Some of the highest FRAP values were recorded for samples collected during the summer period at both sites. However, they were higher at the mountain site (22.3 mgTE/gEO versus 15.7 for littoral). The antioxidant capacity determined by the two other tests, DPPH and ABTS, was quite comparable (but lower than FRAP). The extreme ABTS values were obtained from the mountain site. They were between 0.09 (summer) and 0.32 mgTE/gEO (spring). The post hoc test separated five significantly different homogeneous groups (annotated as “a, b, c, d, and e” in Table 2, column: ABTS). For the DPPH values, levels between 0.06 (winter, littoral) and 0.44 mgTE/gEO (spring, mountain) were found. Nevertheless, in the case of DPPH activity, the post hoc test distinguished significant differences (i.e., “a, b, c, and d”) between all the sample origins (altitude and season).

The antioxidant values of vitamin C (used as a positive control for validating the quantification method) showed that 1 g of Trolox reacted as ~1 g of vitamin C (i.e., 1.08, 0.89, and 1.34 gTE/g vitamin C for the FRAP, ABTS, and DPPH, respectively; Table 2). This confirmed that the measurements of antioxidant activity were correct.

According to the best knowledge of the authors, no report on this plant is available; therefore, comparisons were made with other species from the same genus. Studies on the antioxidant activity of essential oils extracted from the *Pistacia* species are not as numerous.

Barra et al. [53] showed a low antiradical capacity of essential oils obtained from *P. lentiscus* leaves collected from different origins and harvesting periods. In addition, Bouyahya et al., showed fairly good antioxidant effects (ABTS and DPPH tests) by the EOs from whole aerial parts of *P. lentiscus* harvested in Morocco [39]. In addition, EOs from *P. atlantica* [13] presented a low capacity for free radical neutralization and an interesting reducing power. On the other hand, Bampouli et al. [54] showed that *P. lentiscus* var. chia EOs did not exhibit antioxidant activity with the DPPH test.

An exploration of the potential correlations between AOx activities and the levels of each component in the EOs was undertaken. Table 3 shows the correlation coefficients between the FRAP, ABTS, and DPPH values and the components previously identified as the main compounds in EOs, as far as the sum of the oxygenated monoterpenes and oxygenated sesquiterpenes. According to this correlation analysis (Table 3), several observations can be noted. The compounds contributing most to the variation in antioxidant activities among the major compounds of EOs were: (1) bisabolol, δ -cadinene, and β -pinene for the FRAP test; (2) β -caryophyllene, bornyl acetate, caryophyllene oxide, α -cadinol, and oxygenated monoterpenes for the DPPH test; (3) caryophyllene oxide and α -cadinol for the ABTS test.

Table 3. Highest correlation coefficients between components of EOs and antioxidant activities.

Major Compounds	FRAP	ABTS	DPPH
α -Pinene	0.37	0.09	0.39
β -Pinene	0.62 **	−0.09	0.17
β -Myrcene	0.07	0.02	−0.33
Bornyl acetate	−0.18	0.27	0.56 **
β -Caryophyllene	−0.07	−0.18	−0.67 ***
δ -Cadinene	−0.54 **	0.33	0.15
Caryophyllene oxide	−0.24	−0.45 *	−0.46 *
α -Cadinol	−0.11	−0.54 **	−0.56 **
Bisabolol	−0.63 ***	0.36	0.43
Oxygenated monoterpenes	−0.21	0.30	0.55 **
Oxygenated sesquiterpenes	−0.17	−0.40	−0.27

Significant correlations: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

The change in antioxidant activity can be explained by the variation in the chemical composition of the EOs. However, it is nevertheless difficult to attribute these differences to a single component [53]. In general, strong correlations between individual metabolites and the antioxidant activity of plant extracts are rarely observed, even if multivariate models can show the predictive power of the metabolome for antioxidant activity and can indicate which groups of metabolites contribute most to this activity [55,56]. Sometimes, in addition to the major compounds, minor compounds can also play a significant role in the AOx activity of an EO. In fact, the reducing activity of EOs is obviously related to the oxygenated compounds and the terpenes with conjugated double bonds in these EOs [57].

2.5. Profiling of the Fatty Acids from the Leaf Acyl Lipids

Because we suspected a main link between climatic environment (i.e., sites and seasons) and EO composition, we decided to evaluate the fatty acyl (FA) profile of the leaves, with a more dedicated view toward the level of FA unsaturation, which is commonly associated with a physiological adaptation of plant tissues to lower temperatures. This was decided in order to correlate symptoms of low temperature exposure (fatty acid desaturases overexpression) to specific EO profiles, even if we do not suspect a direct metabolic link associating the terpene and fatty acid synthesis (i.e., no presumable fluxomic correlation). It was chosen to extract total lipids from leaves and to trans-esterify all the esterified acyls (from acyl lipids) using a TMAH reactant [58]. The resulting fatty acid methyl esters (FAMES) were analyzed by GC-MS. The FA composition of the different leaves is presented in Table 4.

Table 4. FAME analysis of the acyl lipids extracted from lentisk leaves. The levels of each compound are expressed as chromatographic relative peak surface measured as total ion current (area % TIC). The extraction yield for each lipid fraction was similar: 1.95 ± 0.05 g/100 g of dry matter.

Fatty Acid Methyl Ester (ME)	Corresponding acyl	Mountain (Site M)				Littoral (Site L)				Altitude * Season Interaction
		Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	
Myristic acid ME	C14:0	6.6 ± 1.7 <i>ac</i>	4.0 ± 0.2 <i>bc</i>	1.8 ± 0.1 <i>ac</i>	3.4 ± 0.1 <i>ab</i>	2.5 ± 0.1 <i>bc</i>	3.2 ± 0.1 <i>c</i>	2.7 ± 0.3 <i>a</i>	2.7 ± 0.5 <i>d</i>	***
Hexadecenoic acid ME	C16:1	1.2 ± 0.3 <i>c</i>	0.9 ± 0.1 <i>b</i>	1.2 ± 0.1 <i>bc</i>	1.1 ± 0.1 <i>a</i>	0.5 ± 0.1 <i>b</i>	1.1 ± 0.1 <i>ab</i>	1.3 ± 0.1 <i>bc</i>	1.5 ± 0.2 <i>bc</i>	***
Palmitic acid ME	C16:0	24.8 ± 2.3 <i>c</i>	28.3 ± 0.3 <i>c</i>	27.4 ± 0.5 <i>c</i>	30.3 ± 0.9 <i>a</i>	26.3 ± 1.1 <i>bc</i>	31.5 ± 0.4 <i>ac</i>	31.4 ± 1.0 <i>ab</i>	31.7 ± 2.0 <i>a</i>	
Margaric acid ME	C17:0	0.7 ± 0.1 <i>c</i>	0.8 ± 0.1 <i>b</i>	0.8 ± 0.1 <i>bc</i>	0.9 ± 0.1 <i>a</i>	0.6 ± 0.1 <i>b</i>	0.9 ± 0.1 <i>ab</i>	0.9 ± 0.1 <i>ab</i>	1.1 ± 0.1 <i>ab</i>	**
Linoleic acid ME	C18:2	20.1 ± 1.7 <i>ab</i>	20.0 ± 0.2 <i>c</i>	28.2 ± 8.7 <i>a</i>	13.9 ± 0.6 <i>c</i>	26.2 ± 0.9 <i>ab</i>	22.7 ± 0.5 <i>bc</i>	9.7 ± 0.2 <i>c</i>	14.1 ± 1.2 <i>bc</i>	***
Oleic acid ME	C18:1	2.8 ± 0.2 <i>c</i>	5.7 ± 0.4 <i>bc</i>	15.3 ± 8.3 <i>d</i>	10.8 ± 0.3 <i>c</i>	14.4 ± 0.3 <i>bc</i>	10.4 ± 0.6 <i>ab</i>	30.2 ± 1.2 <i>c</i>	15.3 ± 1.5 <i>a</i>	**
Linolenic acid ME	C18:3	35.5 ± 1.8 <i>d</i>	34.0 ± 1.2 <i>bc</i>	18.5 ± 0.2 <i>a</i>	31.8 ± 1.1 <i>c</i>	23.4 ± 0.2 <i>e</i>	21.7 ± 0.6 <i>e</i>	17.6 ± 0.7 <i>ab</i>	27.2 ± 2.8 <i>e</i>	***
Stearic acid ME	C18:0	1.9 ± 0.2 <i>bc</i>	2.3 ± 0.1 <i>ab</i>	2.6 ± 0.1 <i>c</i>	2.6 ± 0.1 <i>a</i>	2.1 ± 0.1 <i>bc</i>	2.2 ± 0.2 <i>ab</i>	2.8 ± 0.1 <i>bc</i>	2.6 ± 0.3 <i>a</i>	
Docosanoic acid ME	C22:0	0.9 ± 0.1 <i>ac</i>	1.2 ± 0.1 <i>c</i>	1.1 ± 0.1 <i>bc</i>	1.2 ± 0.1 <i>a</i>	0.9 ± 0.1 <i>bc</i>	1.3 ± 0.1 <i>c</i>	1.2 ± 0.2 <i>ac</i>	1.2 ± 0.2 <i>ab</i>	
C14; C16; C17; C18; C22	Total identified	94.3 ± 3.0	96.7 ± 0.7	96.8 ± 0.7	95.9 ± 0.6	96.9 ± 0.3	94.8 ± 0.9	97.8 ± 0.2	97.3 ± 0.3	
	Mean (Σ SFA)	34.8 ± 1.0 <i>d</i>	36.6 ± 0.4 <i>d</i>	33.6 ± 0.7 <i>d</i>	38.3 ± 1.1 <i>a</i>	32.4 ± 1.2 <i>cd</i>	39.0 ± 0.7 <i>bd</i>	39.0 ± 1.5 <i>ab</i>	39.6 ± 2.9 <i>abc</i>	**
	Mean (Σ MUFA)	3.9 ± 0.5 <i>c</i>	6.6 ± 0.4 <i>ac</i>	16.5 ± 8.3 <i>d</i>	11.9 ± 0.4 <i>bc</i>	14.9 ± 0.2 <i>ac</i>	11.5 ± 0.5 <i>ab</i>	31.5 ± 1.2 <i>c</i>	16.8 ± 1.7 <i>a</i>	*
	Mean (Σ PUFA)	55.5 ± 2.7 <i>b</i>	54.0 ± 1.1 <i>bc</i>	46.7 ± 8.6 <i>a</i>	45.7 ± 0.5 <i>bd</i>	49.6 ± 0.9 <i>bd</i>	44.3 ± 1.1 <i>cd</i>	27.3 ± 0.5 <i>bd</i>	41.2 ± 1.8 <i>d</i>	**

Means followed by different letters (in italic) within the same row were significantly different ($p < 0.05$). Significance effect of the interaction between altitude and season as determined by ANOVA, p -values: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Ten FAMES were identified (Table 4). They represented 94 to 97% (peak area % in TIC) of the extracts. The leaves were all characterized by important levels of polyunsaturated fatty acids (PUFAs): 27–55% linoleic and linolenic FA. The highest PUFA levels were detected in winter in the mountains (55%: linoleic 20% and linolenic 35%). Saturated fatty acids (SFAs), mainly palmitic and stearic FAs, reached 34 to 39%. The monounsaturated oleic acid varied between 3 and 31%. The relative amount of the FA classes were in the order PUFAs > SFAs > MUFAs. The main fatty acids detected in the leaf lipids were linolenic acid (17–35%), palmitic acid (24–31%), linoleic acid (9–28%), and oleic acid (2–30%).

In comparison with the few previous studies, our investigation is the first description of the variation in FA contents in leaves of Algerian lentisk according to the period and altitude at harvest. Our quantitative results are mostly comparable to those of Diamantoglou et al. [59], obtained in Greece, and those of Akdemir et al. [60], in Turkey. Our lentisk leaves from the Algerian Tizi-Ouzou region presented FA proportions comparable to those of the Greece and Turkish ones for C14:0 (myristic), C16:0 (palmitic), C16:1 (palmitoleic), C18:2 (linoleic), and C18:3 (linolenic). On the other hand, behenic (C22:0) was not detected in the samples from Greece and Turkey. Furthermore, the results of Harrat et al. [61] (*P. lentiscus* from Algeria) reported the presence of other minor fatty acids in the leaves such as C12:0, C13:0, C14:1, C15:0, and C20:0. We did not significantly observe the presence of these minor FAs in our samples. In our quantitative approach, the interaction between altitude and harvesting season was tested (Table 4, column: Altitude * Season Interaction). It had a significant effect on the variation of the levels of the main FAs. This was not true for C16:0, C18:0, and C22:0. This interaction was also significant on the variation of saturated fatty acid (SFA) and polyunsaturated fatty acid contents ($p < 0.01$). It was less significant for monounsaturated fatty acids (MUSFAs) ($p < 0.05$). The ANOVA results showed that the seasonal effect had more impact ($p < 0.001$) on the variation in the FA contents than the altitude effect. In order to develop a better view of our results, we performed principal component analysis (Figure 2).

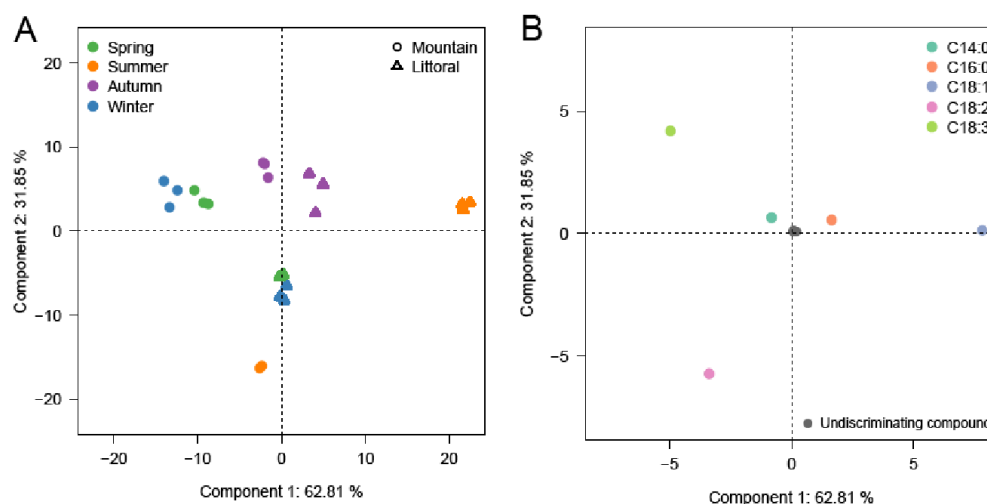


Figure 2. PCA analysis of the FAMES from the leaf acyl lipids of *Pistacia lentiscus* leaf samples collected at the two altitude sites (Δ for littoral versus \circ for mountain) and 4 consecutive seasons: (A) score plot of the PC1 versus PC2 scores; (B) loading plot of PC1- and PC2-contributing FAME compounds (the most discriminating compounds are represented in color).

2.6. Multivariate Analysis of the Fatty Acids Detected (as Methyl Ester: FAMES) in the Leaf Acyl-Lipids

The two axes of the PCA analysis of the FAMES show 94.7% of the variance of the data. The score plot (Figure 2A) separates the samples from mountain and littoral, principally, for the most extreme conditions (i.e., mountain in winter and littoral in summer) in the first dimension of the PCA. The second dimension of the PCA mainly separates the mountain samples in the summer. The loading plot (Figure 2B) shows that unsaturated fatty acids

(i.e., C18:1, C18:2, and C18:3) were the main markers of seasonal and altitudinal variations within the analyzed samples. In the case of monounsaturated fatty acids, the oleic acid content increased significantly during the summer months and decreased again strongly in winter at both sites. However, the levels of this acid were higher at the lowest altitude (i.e., littoral). Polyunsaturated fatty acids (PUFAs) decreased during the warm season and increased in winter at the two growing areas. Linolenic acid (a triunsaturated PUFA, also qualified as an omega 6 fatty acid) reached its maximum (35%) of total FAs during the winter months at high altitude. In contrast, linoleic acid was characteristic of the winter samples at low altitude. During the winter months, PUFAs generally increased [62]. This can be attributed to a higher expression of delta12 and delta15 desaturases genes that drive the respective transformation of (i) oleic acid to linoleic acid and (ii) linoleic acid to linolenic acid [63]. This corresponds to an acclimatization of the plant to lower temperatures [59,62]. At the level of cell membranes, it allows a maintenance of phospholipid fluidity and, thus, of membrane activity. Such an increase in the level of polyunsaturated fatty acyls in plant cell membranes was associated with a progressive decrease in the daily temperatures at the end of autumn and the beginning of winter. It also includes the effects of decreasing temperature during the night. This phenomenon was amplified at higher altitudes, where night temperatures are usually colder (and earlier) compared to littoral areas.

The correlation coefficients between the main FAs in leaves and the main terpenoids from the EOs (Table 5) showed that β -caryophyllene and bisabolol were oppositely associated with linolenic (C18:3) and oleic (C18:1) acyls. When colder conditions occurred, C18:3 and bisabolol levels increased. When medium temperatures occurred, C18:1 and β -caryophyllene levels were higher. There was no direct link established between the biosynthetic pathway of fatty acids and terpenoids, but this correlation clearly demonstrated that the variation in temperatures from the mountain versus littoral site explained most of the variations in the EO compositions. Finally, two main EO chemotypes could be distinguished: a “cold winter chemotype”, characterized by relatively high levels of bisabolol (Group 3 in Figure 2); and a “temperate chemotype” characterized by high levels of β -caryophyllene. The chemotype of temperate samples can be subdivided into three sub-chemotypes: Groups 1, 4, and 5 as discussed above in Figure 2.

Table 5. Correlation coefficients between major components of EOs and FAMES from the leaves of *Pistacia lentiscus*.

Compounds	C18:3	C18:2	C18:1	C16:0	C14:0
β -Caryophyllene	−0.58 *	−0.19	0.77 ***	0.48 *	0.31
β -Myrcene	0.15	−0.55 ***	0.49 *	0.76 ***	0.08
α -Pinene	0.10	−0.08	−0.18	0.21	0.009
Bornyl acetate	0.29	0.44 *	−0.57 *	−0.68 **	0.3
δ -Cadinene	0.15	0.36	−0.42 *	−0.34	0.39
Bisabolol	0.81 ***	0.29	−0.83 ***	−0.37	0.80 ***
β -Pinene	−0.26 *	−0.10	0.18	0.21	−0.41 *
Caryophyllene oxide	−0.14	0.40	0.09	−0.46 *	−0.32
α -Cadinol	−0.30	0.34	0.21	−0.33	−0.48 *

Significant correlation (in bold): *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

3. Materials and Methods

3.1. Sampling and Botanical Identification

Samples from *Pistacia lentiscus* were collected from plants in the Algerian region of Tizi-Ouzou. Locally called “Wilaya of Tizi-Ouzou”, this region covers approximately 3000 km² (100 km EW; 50 km NS) and is limited at the south side by the Djurdjura Mountains and to the north by the Mediterranean Sea. Its relatively contrasted climatic regimes are representative of those of the larger zone of the north of Algeria in between the coast and the mountains. For this study, we selected two geographical sites in the region of Tizi-Ouzou: The first site was located in Ait-Irane, on a mountain in the Tizi-Ouzou

region (longitude 36°29'58.3" N, latitude 4°04'43.4" E, altitude 876 m). Its Mediterranean climate is characterized by a dry summer and relatively low temperatures during the winter (Table S1; [64], see Supplementary Materials). The second sampling site was selected in Tizirt, on the littoral of the Tizi-Ouzou region (longitude 36°53'43.0" N, latitude 4°11'00.4" E, altitude 13 m). Its summer and winter temperatures are less extreme (Table S2; [65]) and more humidity is present all year long (Table S2; [65]). The contrast between the night/day temperatures is also lower (Table S2; [65]). At each site, a set of three individual female trees were chosen for all the different samplings. The average height of each tree was between 3.1 and 3.7 m. The leaves were collected at human height (1.6–2 m, i.e., halfway up the crown of each shrub) in order to present a homogeneous set of leaves. Each leaf sample was taken from 4 branches located at four opposite sites all around each tree. Each sample was taken in a staggered manner so as not to resample areas that had already been taken in the previous–following season. Sampling was conducted in the morning at sunrise (6–8 a.m.). Samples were taken at both sites on the same plants over four seasons in 2019 at four different vegetative stages: vegetative overwintering (mid-February), full flowering (mid-April), early fruiting (mid-August), and late fruiting (mid-October).

The plant species, *Pistacia lentiscus*, was identified using the Flora of Quézel and Santa (1962–1963). Furthermore, samples of lentisk were deposited at the ENSA (National High School of Agronomy in Algeria) herbarium. This identification was confirmed by Benhouhou and this led to the delivery of a certificate (ref: 18112020/22:09/ENSA24112020) attesting that this plant was, indeed, *Pistacia lentiscus*.

3.2. Extraction of Essential Oils

Leaf 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 a hydrodistillation process using a Clevenger-type apparatus. Then, the EOs were stored in opaque glass bottles at 4 °C until further use. According to the period and altitude of the harvest, eight groups of essential oils were obtained: winter mountain, spring mountain, summer mountain, autumn mountain, winter littoral, spring littoral, summer littoral, and autumn littoral.

3.3. 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 (single-quadrupole) mass spectrometer supplied by Thermo Fisher Scientific (Asnières-sur-Seine, France). 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 (4.18 scan per second). 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. Comparison of experimental RIs (RI_{exp}) was conducted with RIs from the literature [2,16,18,19,23,29,31,37,44,66] and with those of authentic compounds available in the authors' laboratory. 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 [67]. 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.

3.4. Antioxidant Activity

The antioxidant activity of 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 (see below) 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 vitamin C.

3.4.1. Radical Scavenging Test (DPPH)

An essential oil diluted in absolute ethanol (50 μ L; 2 mg/mL) was added to 950 μ L of freshly prepared ethanolic (EtOH 100%) solution of DPPH (100 mM) and diluted to 10 mM on the test day. The mixture was shaken and kept in the dark at room temperature for 20 min. The absorbance was measured at 517 nm. A blank solution was prepared in parallel: 50 μ L of absolute ethanol and 950 μ L of DPPH 10 mM. The Trolox standard range (i.e., 0, 5, 12.5, 20, and 25 μ M) was prepared in absolute ethanol [19,68].

3.4.2. Ferric Reducing Power Test (FRAP)

The FRAP test was prepared by mixing a 10 mM solution of tripyridyltriazine (TPTZ) in hydrochloric acid (HCl 40 mM), a 20 mM ferric chloride (FeCl_3) solution, and an acetate buffer (300 mM, pH: 3.6), the proportions of 1:1:10, respectively. The reaction was set up by mixing 900 μ L of the above FRAP solution with 50 μ L of the ethanol (100%)-diluted essential oil sample (2 mg/mL). The reaction mixture was incubated at 37 $^\circ$ C for 30 min. The absorbance was measured at 593 nm. A blank consisted of 50 μ L of absolute ethanol and 950 μ L of FRAP solution. The Trolox standard range (i.e., 0, 50, 100, 200 μ M, and 400 μ M) was prepared in absolute ethanol [19,69].

3.4.3. Radical Cation Reduction Test (ABTS)

One hundred microliters of essential oil diluted in absolute ethanol (2 mg/mL) was added to 900 μ L of the (100%) ethanolic solution of ABTS. ABTS⁺ was produced by the reaction of 7 mM of ABTS with 2.5 mM of sodium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) in distilled water for 16 h in the dark. The concentration of ABTS⁺ was then adjusted by dilution to an absorbance of 0.7 ± 0.02 (at 734 nm). A blank consisted of 100 μ L of absolute ethanol and 900 μ L of ABTS solution. The Trolox standard range (i.e., 0, 4, 10, 16, and 20 μ M) was prepared in absolute ethanol. After incubation in the dark for 6 min, the absorbance of each sample was measured at 734 nm [70].

3.5. Preparation of Lipidic Extracts from Lentisk Leaves and GC-MS Analysis

The lipid extracts from leaves were prepared for four harvest periods and from both sites. The extraction of leaf lipids was performed mainly according to Akdemir et al. [60]. Powdered air-dried leaves (300 mg) were extracted in 1.5 mL of methanol–chloroform (1–2 V/V) for 30 min under stirring (tripentadecanoin was also added at 1 mg/mL as an internal standard (Sigma-Aldrich, Saint-Quentin-Fallavier, France) for further GC-MS analysis). After a 5 min centrifugation at $1100 \times g$, the supernatant was treated by adding an equivalent volume of hexane. After stirring the mixture, 8 μ L of tetramethylammonium hydroxide was added (TMAH; Sigma-Aldrich). The use of tetramethylammonium hydroxide (TMAH) allowed for the preconversion of fatty acids into fatty acid methyl esters (FAMES) in order to increase their volatility for GC-MS analysis [58]. After resting, the supernatant was recovered and put in vials for GC-MS analysis. For each sample, the separation of the different compounds was performed on a TR-5 MS (5% phenyl and 95% polysiloxane) capillary GC column (30 m \times 0.25 mm \times 0.25 μ m) from Thermo (Asnières-sur-Seine, France). One microliter of each GC-MS sample was injected in the splitless mode

at 230 °C. The detector temperature was 250 °C. A gradient of oven temperatures from 230 °C to 290 °C was used (230 to 245 °C at 15 °C·min⁻¹; 245 to 290 °C at 90 °C·min⁻¹; 290 °C for 2 min). Helium was used as the carrier gas at a flow rate of 1 mL/min. The ionization in the electron impact mode was conducted with an energy of 70 eV and full-scan detection for masses (m/z) between 35 and 350 atomic mass unit (AMU). The retention indices were calculated according to a series of n-alkanes (C10–C40). The identification was performed by comparison of their mass spectra with those stored in the NIST 05 library (protocol mainly issuing from [71]).

3.6. Statistical Analysis

All determinations were performed in triplicate. The results were then expressed as the mean \pm standard deviation of three measurements. Statistical analyses were performed in R (R core team, 2021) [72]. The data were analyzed by comparison of the means using a two-factor analysis of variance ANOVA model of Type III, followed by a Tukey test for the determination of significant differences in concentrations of all the detected compounds in the different samples: EOs (with monoterpenes, sesquiterpenes, and others), trans-esterified lipid extracts (fatty acid methyl esters). For each analyzed compound, the assumptions of the ANOVA model (normal distribution and homoscedasticity of the error terms) were checked using the diagnostic plots generated by the plot() function in R, and, if necessary, square root transformation was applied.

The same procedure was used to assess the variation in the antioxidant activities among samples. The correlations between components of the essential oils and antioxidant activities were estimated using Spearman's correlation coefficient (rs). The same correlation analysis was conducted for major components of the essential oils and acyl composition. Differentiation between populations (based on their EO and acyl compositions) was assessed by principal component analysis (PCA) using R library FactoMiner R (R Core Team 2020) [72,73].

4. Conclusions

This paper showed a large variability in the terpenoid (i.e., essential oils) and fatty acid compositions of leaves from *Pistacia lentiscus* collected at two contrasting natural sites in the Algerian region of Tizi-Ouzou. Wild plants naturally growing at high altitudes (mountain site at 876 m) and at low altitudes (littoral site at 13 m) were selected, and some of their leaves were consecutively sampled four times during their different vegetative–reproductive stages over one year. The extraction of essential oils (EOs) from the leaves and the further gas chromatography analysis allowed for the identification and relative quantification of 47 compounds. Based on the main constituents of the EOs, different chemotypes could be described. EOs from the coastal site corresponded to four harvest periods: winter, spring, summer, and autumn. They were characterized by high amounts of β -caryophyllene (C26). EOs from the mountain site were seasonally variable. In winter, EOs contained high amounts of δ -cadinene (C36), bisabolol (C46), and bornyl acetate (C20). Samples collected in spring and summer were characterized by high amounts of α -pinene (C2) and β -pinene (C5). Samples collected in autumn had a high proportion of β -myrcene (C6) followed by α -pinene (C2). The chemical class of monoterpene hydrocarbons was higher in the mountains. In addition, the highest rate of this class was observed in autumn for both sites. Contrary to monoterpene hydrocarbons, sesquiterpene hydrocarbons were present at higher concentrations in the EOs issuing from the coastal area. The antioxidant (AOx) activity of the different EOs varied according to the harvesting period and the altitude. The highest antiradical effects were observed with the FRAP test. The variation in the AOx activities can partially be correlated to the composition of the essential oils. This study thus showed that the change in altitude and harvesting period affected the chemotypic nature of the lentisk EOs as well as their AOx potential, especially at the highest altitudes. The *Pistacia lentiscus* natural population of the Tizi-Ouzou littoral can thus be used for the production of a quite constant chemotyped EO. Oppositely, because of the

seasonal variability, EOs from high-altitude populations may be better suited for obtaining dedicated original chemotypes and for the further cultivation and exploitation of their (potentially therapeutic) potential.

The fatty acyl (FA) profile of the leaf acyl lipids was analyzed by GC-MS after total lipid extraction and transesterification into FA methyl esters (FAMES). The resulting composition was characterized by its richness in polyunsaturated fatty acids (PUFAs) especially in winter in the mountain. PUFA levels were followed by saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs). The amounts of FAs decreased in the order PUFA > SFA > MUFA. The major FAs detected in the leaves were linolenic acid, palmitic acid, linoleic acid, and oleic acid. Unsaturated fatty acids (i.e., C18:1, C18:2, and C18:3) levels significantly varied with season and altitude. The colder the climatic conditions the higher the relative ratio of PUFAs. The variability in the EOs' chemotypes can be associated with this physiological response of plants to temperatures. The leaves harvested at the coldest season were characterized by the highest levels of bisabolol. The EOs from other sites and seasons were characterized by three other chemotypes in which β -caryophyllene was always the main compound. From these results, a planification of leaf harvest can be conducted for producing EOs with specific chemotypes. It is the first step in the further sustainable and rational valorization of natural resources from the region of Tizi-Ouzou. It is also an opportunity to more deeply study the impact of climatic changes on the resulting terpenoid biosynthesis using correlation networks and fluxomic analysis.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules27134148/s1>: Table S1: Weather data collected between 1999 and 2019 in Ait Irane, Algeria; Table S2: Weather data collected between 1999 and 2019 in Tizirt, Algeria.

Author Contributions: The individual contribution of authors was as follows. C.S. was in charge of all phases (i.e., design, experimentation, extractions, analyses, statistical evaluations, interpretation of results, and writing of the article) of the work that is included in her PhD based on an Algeria–France collaboration. N.J. was in charge of part of the supervision of that work and contributed to the writing and revision of the manuscript. E.C. supervised the experiments devoted to antioxidant analysis and contributed to the specific interpretation and the revision of these aspects in the manuscript. R.D. supervised the statistical analysis and contributed to the interpretation and the revision of the manuscript. J.X.F. supervised the statistical analysis and contributed to the interpretation and the revision of the manuscript. R.M. supervised the statistical analysis and contributed to the interpretation and the revision of the manuscript. F.A. contributed to the initiation of the project with her process engineering expertise. F.F. contributed to the whole project through supervision of the dedicated sample collection, essential oil production, and participation in the interpretation of the results, the writing, and the improvement of this article. E.G. contributed to the whole project through supervision of and participation to the analytical work, the interpretation of results, the writing, and the improvement of this article. Conceptualization, C.S., F.F. and E.G.; methodology, C.S., N.J., E.C., R.D., J.X.F., R.M., F.A., F.F. and E.G.; software, C.S., R.D., J.X.F. and R.M.; validation, C.S., R.D., J.X.F., R.M., F.F. and E.G.; formal analysis, C.S., F.F. and E.G.; investigation, C.S., F.F. and E.G.; resources, F.F. and E.G.; data curation, C.S. and E.G.; writing—original draft preparation, C.S., F.F. and E.G.; writing—review and editing, C.S., F.F. and E.G.; visualization, C.S., N.J., E.C., R.D., J.X.F., R.M., F.A., F.F. and E.G.; supervision, N.J., F.A., F.F. and E.G.; project administration, F.F. and E.G.; funding acquisition, F.F. and E.G. Authorship here is limited to those who have contributed substantially to the work reported. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by (1) the French Ministry of Foreign Affairs through Campus France (file no. 973496A); (2) the “Conseil Régional de Picardie” through the MTV (Métabo-Typage Végétal) project; (3) the University of Picardie Jules Verne through the French Ministry of Higher Education and Research, region “Hauts de France”, the European FEDER funds within the so-called CPER programs devoted to equipment acquisitions. This work was also funded by Algerian authorities through the financial support of the Tizi-Ouzou UMMTO University and a social grant attributed to Chabha SEHAKI for her PhD.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article or Supplementary Materials.

Acknowledgments: The authors thank Salima Benhouhou (Ecole Nationale Supérieure Agronomique, ENSA, Algiers, Algeria) for the identification of the *Pistacia lentiscus* plants, the recording of samples, and the delivery of a certificate.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

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Données supplémentaires de l'article 2 (conditions climatiques; échantillonnage),
quelques chromatogrammes, la méthode d'identification détaillée)

1. Echantillonnage

1.1. Cartographie (site d'échantillonnage de la plante)



Cartographie en mode satellite

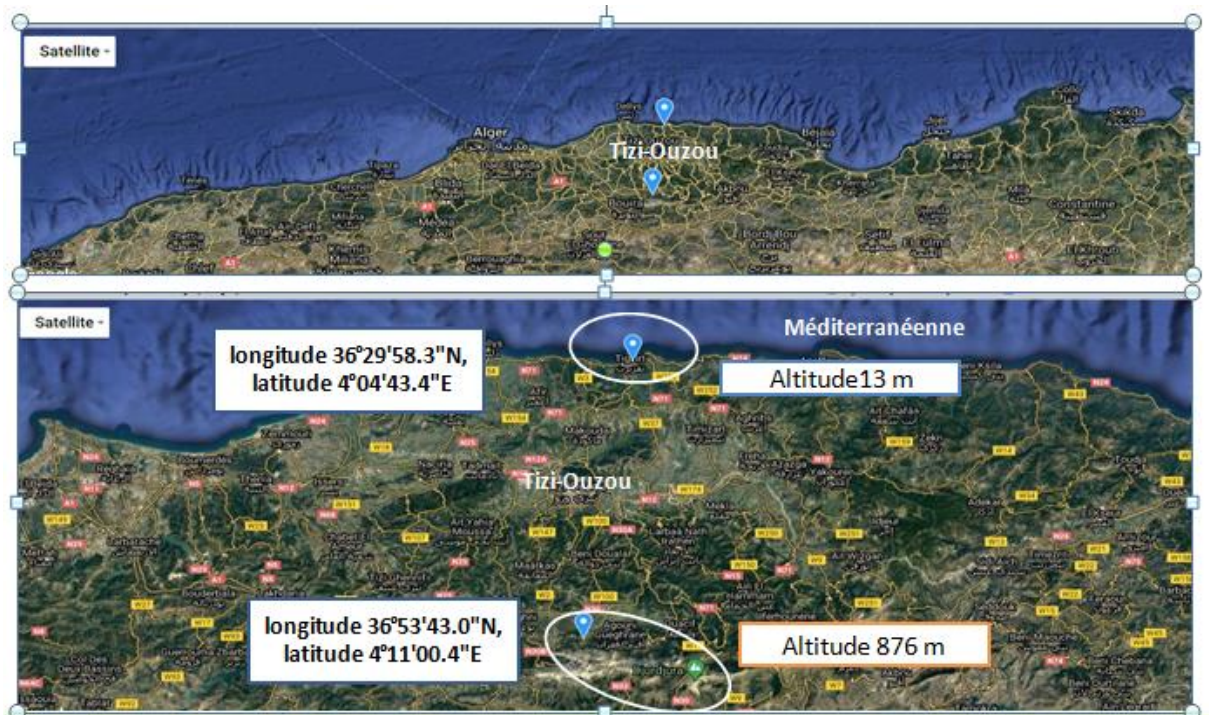


Figure S1: Cartographie présentant les deux sites d'échantillonnage de la plante

[Ait-Iran (876 m) et Tizirt (13 m)]

1.2. Données météorologiques

Tableau S1: Données météorologiques recueillies entre 1999 et 2019 à Ait Irane, Algérie

(<https://fr.climate-data.org/afrique/algerie/tizi-ouzou/ait-irane-693594/>)

	January	February	March	April	May	June	July	August	September	October	November	December
Meantemperature (°C)	5.2	5.3	8.5	11.6	15.6	20.8	24.8	24.5	20	15.9	9.4	6.2
Mean minimum temperature (°C)	0.8	0.5	3.1	5.8	9.4	13.9	17.5	17.8	14.5	10.7	5.2	2.1
Maximum temperature (°C)	10.4	10.7	14.3	17.5	21.6	27.4	31.9	31.6	26.3	21.9	14.5	11.2
Precipitation (mm)	88	78	84	86	74	21	8	19	53	60	81	82
Humidity (%)	79	77	73	70	67	55	45	47	59	64	75	79
Rainydays (jree)	9	8	8	8	7	3	2	4	7	7	9	8
Hours of sunshine (h)	5.7	6.4	7.6	9	10.3	12.3	12.6	11.7	9.9	8.4	6.4	5.7

Tableau S2: Données météorologiques recueillies entre 1999 et 2019 à Tizirt, Algérie

(<https://fr.climate-data.org/afrique/algerie/tizi-ouzou/tigirt-321206/>)

	January	February	March	April	May	June	July	August	September	October	November	December
Meantemperature (°C)	10.2	10	12.1	14.2	17.1	21.1	24.4	25	22.3	19.3	14.1	11.3
Mean minimum temperature (°C)	7.7	7.4	9.3	11.3	14.1	17.9	21.2	22	19.6	16.5	11.7	8.9
Maximum temperature (°C)	12.8	12.7	14.8	16.8	19.7	23.9	27.3	27.9	25	22.2	16.8	13.9
Precipitation (mm)	142	107	86	68	51	9	2	6	36	73	140	146
Humidity(%)	75	74	75	77	77	72	69	69	71	71	73	74
Rainydays (jree)	10	8	7	6	4	1	0	1	4	6	10	10
Hours of sunshine (h)	7	7.8	9	10.2	11.1	12.2	12.3	11.3	10.1	9.1	7.6	7

2. Caractérisation du profil chimique des huiles essentielles

2.1. Chromatogrammes

L'analyse GC-MS de 24 huiles essentielles de lentisque provenant de la montagne et du littoral sur les 4 saisons a généré 72 chromatogrammes (répétitions techniques et biologiques).

Ci-dessous un exemple de chromatogramme pour chaque groupe; Montagne-Hiver (MH), Montagne-Printemps (MP), Montagne-Eté (ME), Montagne-Automne (MA), Littoral-Hiver (LH), Littoral-Printemps (LP), Littoral-Eté (LE), Littoral-Automne (LA).

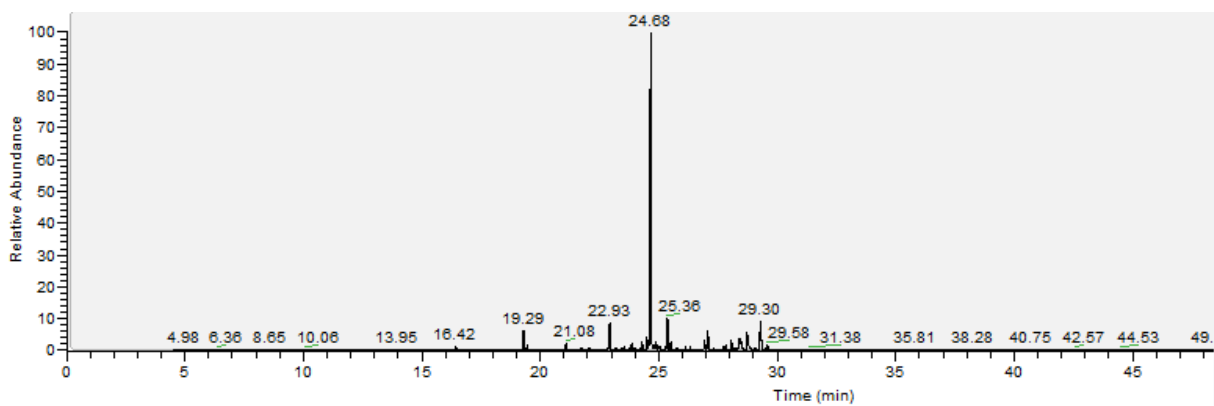


Figure S2: Profil chimique de l'une des huiles essentielles de lentisque récoltée en montagne en hiver

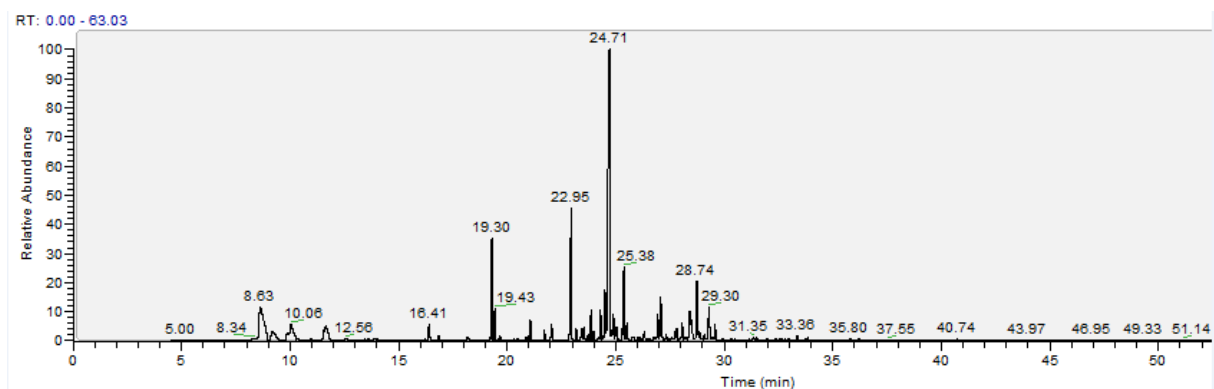


Figure S3: Profil chimique de l'une des huiles essentielles de lentisque récoltée en montagne au printemps

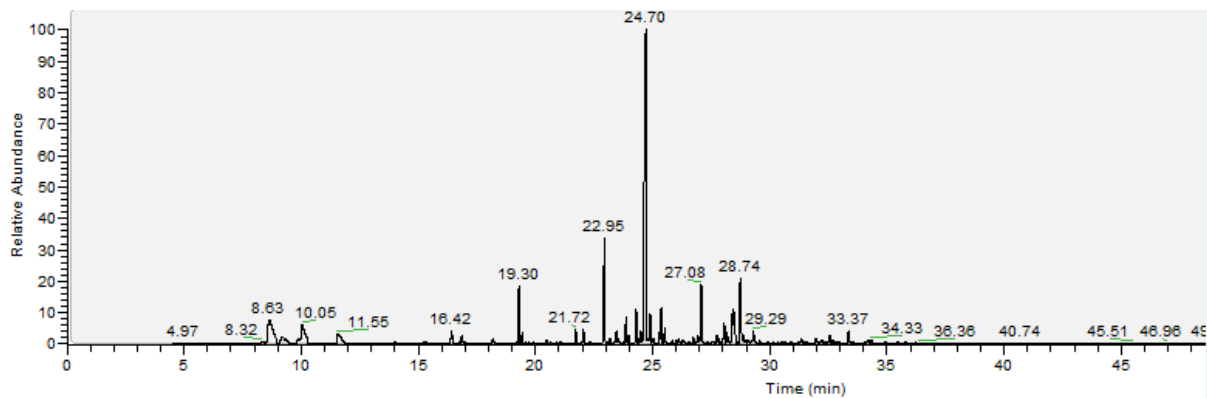


Figure S4: Profil chimique de l'une des huiles essentielles de lentisque récolté en montagne en été

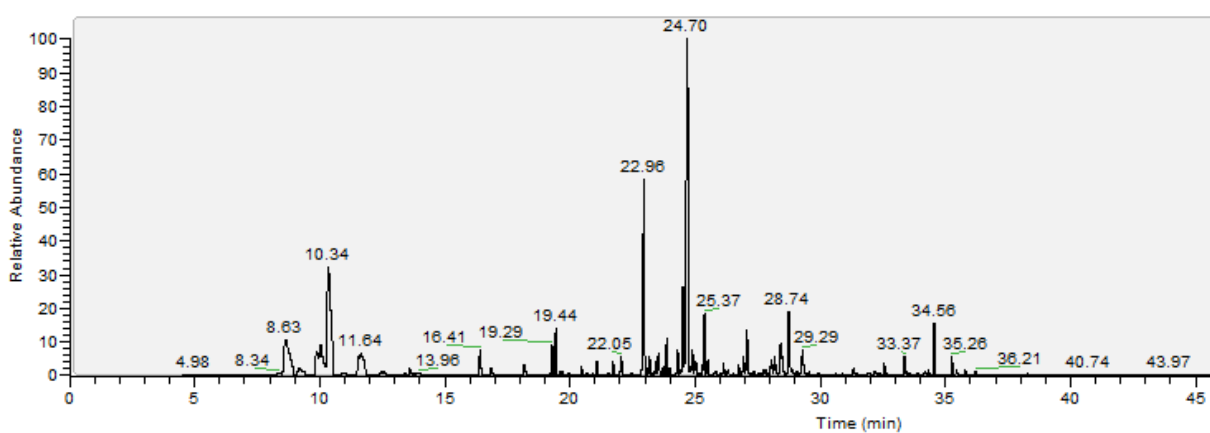


Figure S5: Profil chimique de l'une des huiles essentielles de lentisque récolté en montagne en automne

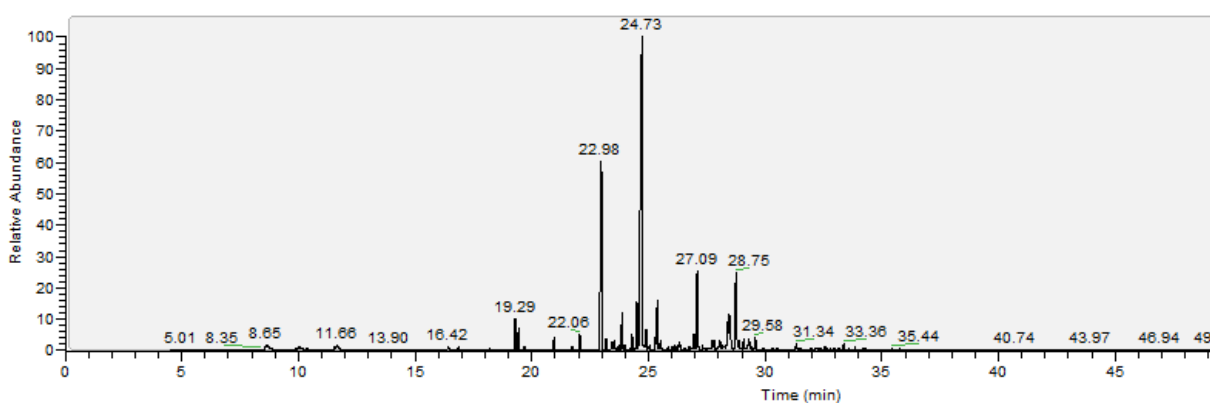


Figure S6: Profil chimique de l'une des huiles essentielles de lentisque récolté au littoral en hiver

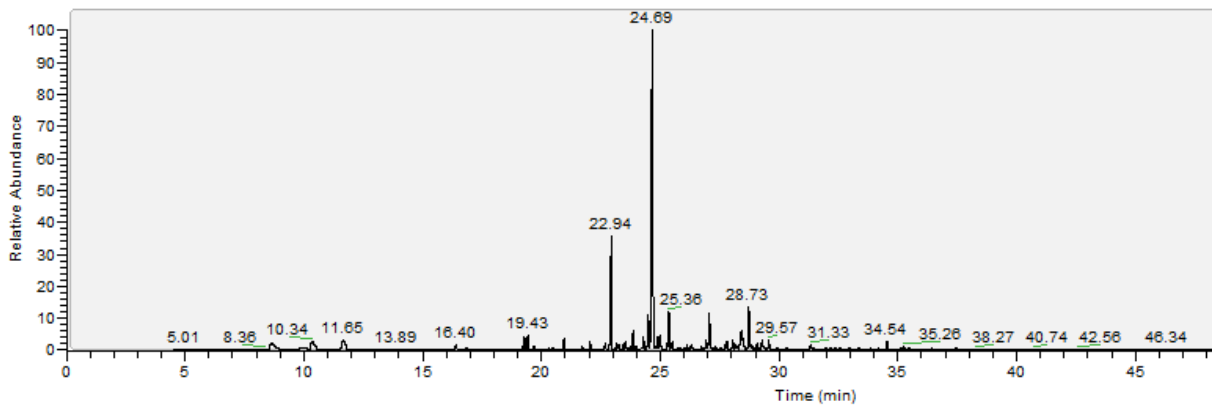


Figure S7: Profil chimique de l'une des huiles essentielles de lentisque récolté au littoral au printemps

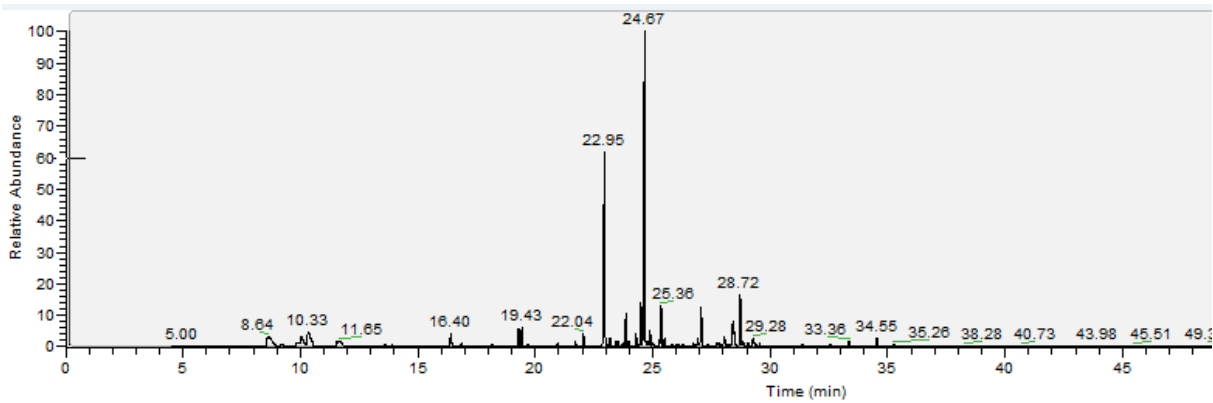


Figure S8: Profil chimique de l'une des huiles essentielles de lentisque récolté au littoral en été

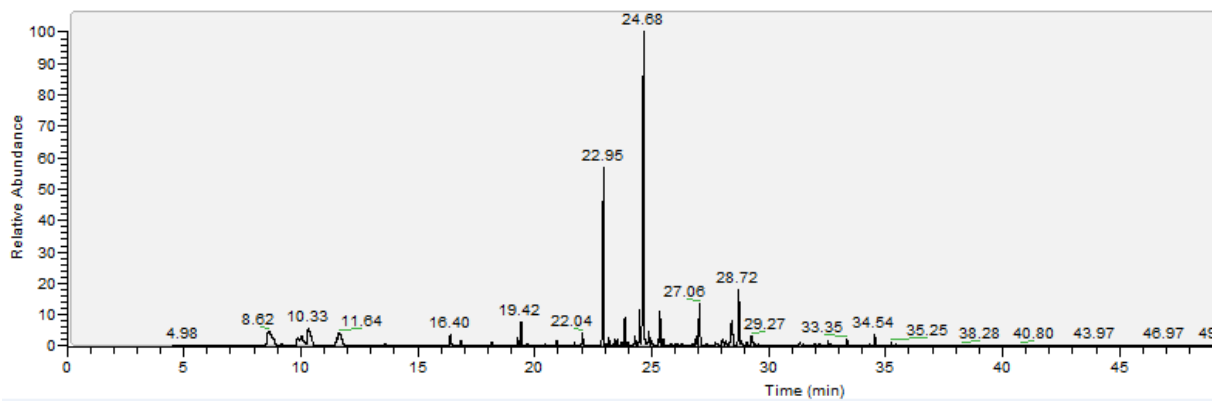


Figure S9: Profil chimique de l'une des huiles essentielles de lentisque récolté au littoral en automne

2.2. Annotation des composés détectés dans nos huiles essentielles de *Pistacia lentiscus*

Suggested name	RT (min)	IR exp	Ion Pic Nist 05	10 ions (largest) (Nist 05)	(Match and R MATCH) Nist 05		Adams (Book)	IR literature Means (10 recent articles)
					Match	R Match		
Tricyclene	8.25	923	93	93 999 91 333 92 261 79 224 77 212 41 185 121 179 136 136 39 110 43 110	889	909	926	918
α -Pinene	8.59	935	93	93 999 91 477 92 400 77 306 79 248 41 148 39 118 121 111 105 100 94 96	952	953	939	934
Camphen	9.12	952	93	93 999 121 593 79 408 91 337 67 290 41 258 77 252 107 227 94 175 39 173	935	956		949
Sabinene	9.81	975	93	93 999 91 564 77 425 79 302 41 256 92 142 94 140 39 131 80 129 136 125	905	936	975	971
β -Pinene	10.01	981	93	93 999 41 324 91 305 77 271 79 254 69 207 92 131 94 129 39 126 80 109	938	942	979	976
β -Myrcene	10.36	993	41	41 999 93 954 69 683 91 256 39 198 77 176 79 174 67 135 92 135 53 122	868	871	990	989
α -Phellandrene	10.89	1011	93	93 999 91 633 77 391 92 332 136 178 41 169 44 161 43 121 39 104 57 93	848	905	1002	1004
o-Cymene	11.55	1032	119	119 999 91 289 134 270 117 148 120 90 77 86 115 71 65 65 41 64 39 60	925	935	1022	1019

p-Cymene	11.58	1033	119	119 999 91 283 134 265 117 155 77 92 115 77 120 72 65 70 41 60 39 51	923	944	1024	1024
Limonene	11.66	1036	119	119 999 68 767 67 650 93 626 91 495 79 363 134 259 77 257 94 252 92 248	781	817	1029	1027
γ -Terpinene	12.55	1066	93	43 999 71 983 70 923 55 474 41 358 42 239 44 221 89 179 39 140 69 110	856	920	1088	1059
α -Terpinolene	13.41	1094	93	93 999 121 865 91 663 136 661 79 484 77 396 41 285 43 276 105 265 39 225	881	911	1090	1086
2-Nonanone	13.56	1099	43	43 999 58 903 57 349 71 243 44 223 59 195 41 145 42 86 55 70 39 69	816	886	1098	1092
Nonanol	13.87	1109	45	45 999 43 264 41 221 69 220 55 207 57 135 56 120 44 117 71 105 70 90	851	920	1100	1100
Nonanal	14.03	1114	57	45 999 43 264 41 221 69 220 55 207 57 135 56 120 44 117 71 105 70 90	790	809		1108
Menta-2-en-1-ol <E-p>	14.71	1137	43	43 999 44 419 93 399 41 398 79 392 71 271 121 271 81 257 139 253 55 251	851	894	1169	1135
Borneol	16.22	1187	95	95 999 110 194 41 180 43 127 67 116 55 110 93 94 79 83 96 74 39 70	907	912	1177	1160
Terpinen-4-ol	16.82	1207	71	59 999 93 638 43 424 121 399 81 395 136 338 92 249 67 246 41 209 91 208	924	934	1188	1180
α -Terpineol	16.86	1209	59	59 999 93 534 121 382 81 376 43 339 136 297 79 278 92 194 67 188 41 173	888	910	1288	1188

Bornyl acetate	19.29	1297	95	95 999 43 623 93 477 121 347 136 328 80 160 92 148 108 144 41 139 55 138	951	951	1294	1285
2-Undecanone	19.4	1301	58	58 999 43 813 59 297 71 258 41 201 57 143 55 103 85 76 42 62 39 47	931	946	1349	1291
α -Terpinyl acetate	20.95	1358	121	121 999 43 899 93 841 136 487 81 215 67 205 59 198 41 196 92 177 91 173	916	925	1376	1331
α -Copaene	21.71	1386	105	105 999 119 889 161 760 93 538 91 500 92 289 41 277 81 236 120 231 55 213	931	935	1387	1376
β -Bourbonene	21.95	1395	81	81 999 80 682 123 544 79 367 41 207 161 197 91 182 105 157 77 155 43 128	871	885	1390	1386
β -Elemene	22.05	1398	81	81 999 93 948 79 659 67 616 107 590 68 559 41 518 91 506 105 431 55 379	907	908	1419	1390
β -Caryophyllene	22.95	1435	93	93 999 41 867 91 855 69 840 133 769 79 704 105 601 77 414 107 410 67 397	947	947		1427
β -Cubebene	23.17	1444	161	161 999 105 585 91 550 119 338 41 310 79 294 81 280 93 258 77 231 120 221	912	927	1435	1391
Isoamyl benzoate	23.45	1455	105	105 999 70 889 77 485 55 281 123 271 51 145 43 139 41 117 42 97 106 75	884	919		1437
α -Caryophyllene	23.86	1472	93	93 999 80 328 121 267 91 206 92 196 79 193 41 186 77 153 107 140 147 136	949	952	1460	1452

Allo-aromadendrene	23.98	1477	91	91 999 105 816 41 758 161 737 93 707 79 691 107 548 133 540 119 524 81 508	915	919	1479	1461
γ -Muuroolene	24.29	1489	161	161 999 105 687 119 586 91 576 93 556 79 423 81 315 41 313 133 279 77 267	946	947	1485	1477
Germacrene D	24.5	1498	161	161 999 105 785 91 685 81 442 119 439 79 397 41 344 77 330 93 328 120 240	922	945	1500	1482
α -Muuroolene	24.89	1514	57	57 999 71 659 43 658 85 314 41 308 55 185 56 170 70 131 99 106 42 84	940	949	1505	1500
β -Bisabolene	25.04	1520	105	105 999 93 392 161 376 91 332 94 288 81 262 41 247 119 240 79 226 204 182	907	920	1513	1505
γ -Cadinene	25.29	1530	69	69 999 93 983 41 884 79 449 67 422 91 414 94 355 109 294 107 277 119 273	929	937		1514
δ -Cadinene	25.38	1534	161	161 999 105 619 91 539 119 438 93 403 79 376 41 320 81 314 133 300 77 248	897	909	1529	1520
Calamenene	25.52	1540	161	161 999 119 619 105 575 134 488 204 401 91 381 81 263 41 217 162 180 133 178	813	868	1549	1525
Elemol	26.14	1565	59	59 999 93 857 43 551 107 479 81 470 41 440 79 400 67 378 161 375 91 355	902	933	1583	1546

Caryophyllene oxide	26.33	1573	123	123 999 91 886 105 682 109 648 43 644 41 637 93 625 131 555 79 554 81 526	801	819	1608	1580
Humulene epoxide II	27.73	1633	43	43 999 67 920 109 865 96 801 41 600 138 580 93 520 95 490 81 484 55 477	859	902	1646	1595
Cubenol	28.06	1648	119	119 999 161 636 105 540 43 300 55 295 41 291 82 291 95 273 93 268 81 266	881	898	1640	1622
Epi-Cadinol	28.4	1663	161	161 999 43 404 105 390 119 376 81 334 204 293 95 254 93 231 79 210 41 204	895	907		1636
δ -Cadinol	28.51	1668	161	161 999 43 951 119 894 105 881 95 741 81 612 79 557 93 556 41 471 121 454	878	923		1640
α -Cadinol	28.74	1679	95	95 999 121 695 43 657 93 364 161 363 105 358 81 341 79 330 109 328 41 286	936	947		1652
Aromadendreneoxide	28.86	1684	159	159 999 91 924 43 875 81 846 105 678 93 671 41 598 117 558 67 550 79 548	815	838	1675	1648
Bisabolol	29.3	1704	43	43 999 69 868 109 850 119 795 41 489 93 489 95 339 67 316 55 267 71 260	918	953	1760	1654
Benzyl Benzoate	31.35	1797	105	105 999 91 539 77 321 79 136 51 133 212 130 65 118 106 101 107 100 43 94	733	923		1760

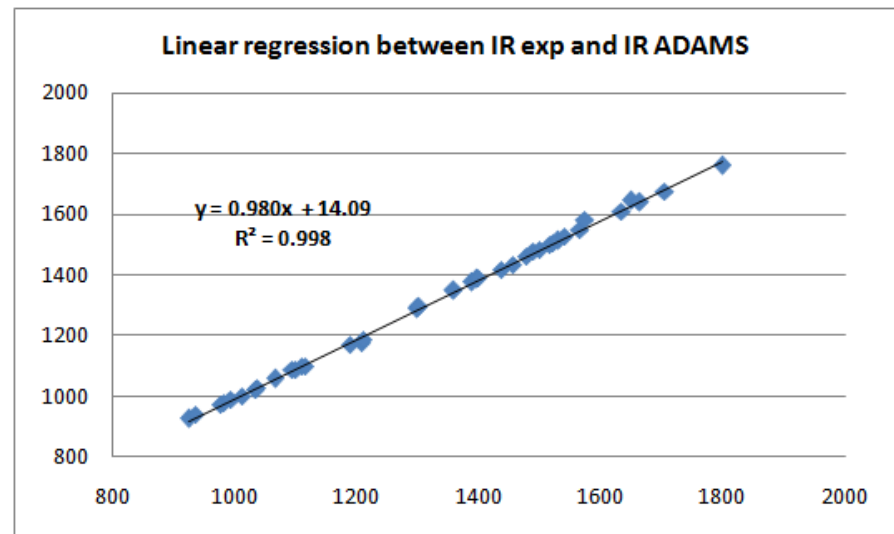
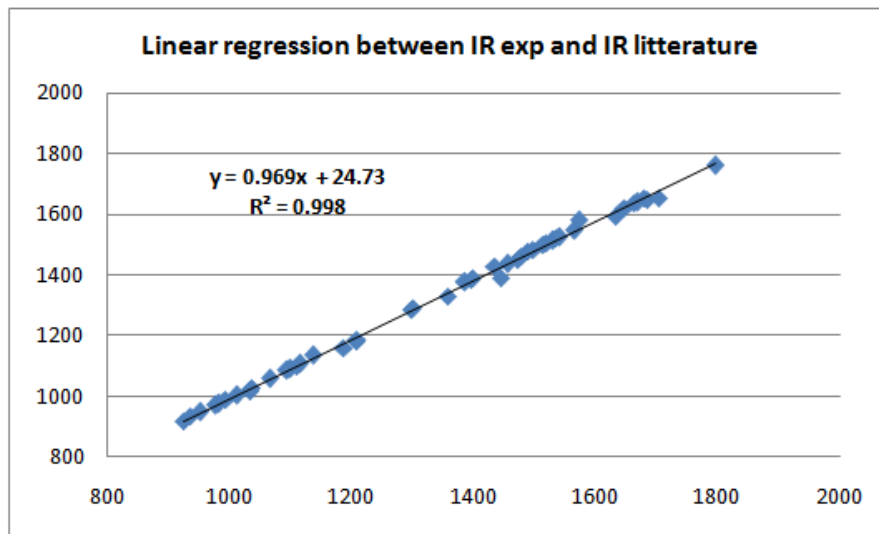


Figure S10: Régression linéaire entre les indices de rétentions expérimentaux et ceux publiés (10 articles récents et livre Adams)

Chapitre III:
**Revue bibliographique sur les polyphénols chez le
lentisque; analyses phytochimiques et potentiel
pharmacologique**

Résumé de l'étude

Introduction

Les polyphénols sont l'une des familles de métabolites secondaires des plantes les plus connues. Ces composés contiennent un ou plusieurs cycles aromatiques liés à un ou plusieurs groupes hydroxyle dans leur structure de base et sont divisés en différentes classes, telles que les acides phénoliques, les flavonoïdes, les tannins, les stilbènes et les lignanes (Sagar et *al.*, 2018). Ils sont caractérisés par un éventail large et diversifié de propriétés bioactives uniques, ce qui les rend très appréciés pour leurs effets bénéfiques sur (les plantes et) les humains (Belščak-Cvitanović et *al.*, 2018). Ces métabolites peuvent contribuer à diminuer l'incidence des maladies cardiovasculaires (McSweeney et *al.*, 2015), du diabète de type 2 (Scalbert et *al.*, 2005), des troubles hépatiques (Bose et *al.*, 2008), de l'obésité (Lu et *al.*, 2012 ; Aviram et *al.*, 2000), ou du cancer du côlon (Yang et *al.*, 2000). Ces propriétés favorables à la santé de composés issus du lentisque ont été attribuées à la présence de divers composés chimiques biologiquement actifs tels que les composés phénoliques (Bampouli et *al.*, 2015).

Plusieurs travaux ont été réalisés sur l'identification et la quantification des polyphénols de *Pistacia lentiscus*, et sur leurs propriétés biologiques. Il n'existe pas de revue résumant les investigations détaillées réalisées sur les polyphénols de lentisque. Par conséquent, dans ce travail de synthèse nous avons synthétisé trois parties principales. Une partie porte sur le contenu des classes phénoliques dans les différentes parties du lentisque. La deuxième partie vise à examiner la variation de la composition et du rendement en polyphénols sous l'influence de l'origine géographique, de la partie de la plante utilisée et du type de solvant d'extraction. La dernière partie illustre une synthèse détaillée sur les propriétés pharmacologiques des différents extraits phénoliques.

2. Les composés phénoliques chez le lentisque

2.1. Analyse phytochimique (Dosage colorimétrique)

Des études récentes ont montré que toutes les parties de *Pistacia lentiscus L.* sont riches en composés chimiques bioactifs. Parmi ceux-ci, les flavonoïdes, les acides phénoliques et les tanins étaient les composés les plus abondants dans les feuilles de *P. lentiscus* (Tableau 1 de l'article 3). Les analyses phytochimiques ont été basées sur l'application de méthodes de dosage colorimétriques en utilisant des réactifs spécifiques pour chaque famille chimique.

Selon les résultats présentés dans le Tableau 1 (article 3), le contenu phénolique total (exprimé en Gallic Acid Equivalent (GAE) per gram of dry matter (g DM)) variait de 10 mg GAE/g DM, à 955 mg GAE/g DM dans les extraits de *P. lentiscus* (Yosr et al., 2018 ; Belhachat et al., 2017). Pour les autres classes polyphénoliques, les concentrations en flavonoïdes dans les extraits de lentisque (exprimées en Rutin Equivalents (RE)) variaient de 2 mg RE/g DM à 278 mg RE/g DM (Yosr et al, 2018; Bakli et al, 2020). La teneur en tanins condensés était estimée à 7 mg de Catéchine Equivalent/g DM (Zitouni et al., 2016), et à 997 mg d'acide tannique Equivalent/g d'extrait (Atmani et al., 2008) (voir Tableau 1, art.3). D'autres composés comme la myricétine rhamnoside (3,6 g/kg de poids sec) et/ou la quercétine rhamnoside (1,6 g/kg de poids sec) (Azib et al., 2019) ont été décrits dans l'extrait de lentisque. Des flavonols (7,2 mg QE/g DW) et des proanthocyanidines (39,2 mg CE/g DW) ont également été détectés (Amessis et al., 2014).

Ces études (Tableau 1, art.3) ont montré que les extraits de feuilles de *P. lentiscus* contiennent des niveaux élevés de composés phénoliques totaux. Par conséquent, les résultats obtenus sont utiles pour des recherches ultérieures telles que l'identification de composés phénoliques spécifiques. Des méthodes analytiques ont été développées pour l'analyse fine de la composition complexe des extraits phénoliques. Nous détaillerons ce point dans la section suivante.

La séparation, l'identification et la quantification des polyphénols ont été effectuées sur des extraits de *Pistacia lentiscus* L. provenant de plusieurs pays méditerranéens. Ceci a été réalisé en utilisant plusieurs techniques chromatographiques, y compris la chromatographie liquide haute performance (HPLC) semi-préparative, la détection « HPLC-photodiodearray » par comparaison avec un standard authentique. La chromatographie liquide haute performance (HPLC) à détection par barrette de diodes (DAD)-couplée à la spectrométrie de masse (MS) (HPLC-MS) équipée d'une interface d'ionisation par électro-nébulisation (ESI), et la RMN ^1H et ^{13}C sont également utilisées. Ces techniques ont permis d'obtenir des informations qualitatives et quantitatives sur la constitution biochimique des extraits de *Pistacia lentiscus*.

2.b. Identification et/ou quantification des polyphénols chez le lentisque

Une synthèse détaillée a été réalisée sur les différents composés phénoliques identifiés chez le lentisque. Les résultats de cette synthèse sont présentés dans le Tableau 2 (article 3). L'analyse des extraits de différentes parties de lentisque a permis d'identifier plusieurs métabolites. On trouve les flavonoïdes (flavonols, flavanols, flavones, glycosides flavonoïdes, dérivés de la myricétine, anthocyanines, catéchines, et les différents acides phénoliques ainsi que leurs dérivés).

Acides phénoliques : Plusieurs acides phénoliques ont été détectés, parmi lesquels figure l'acide gallique mentionné par plusieurs auteurs (El Bisbishy et *al.*, 2020; Umadevi et *al.*, 1988; Romani et *al.*, 2002; Yemmen et *al.*, 2017; Meheni et *al.*, 2016; Garofuli'c et *al.*, 2020). Les dérivés de l'acide quinique, les dérivés galloyl du glucose et de l'acide quinique, comme l'acide 5-O-digalloyl quinique, l'acide trigalloylquinique bêta-glucogalline ont été détectés (Landau et *al.*, 2014; El Bisbishy et *al.*, 2020; Garofuli'c et *al.*, 2020; Bozorgi et *al.*, 2013).

Flavonol et glycosides de flavonol: les plus importants sont la myricétine, les glycosides de myricétine, les glycosides de quercétine (Romanai et *al.*, 2002).

Flavanols: des flavanols comme la procyanidine B1, la procyanidine B2, l'épicatéchine, la catéchine, le gallate d'épigallocatechine et le gallate d'épicatéchine sont présents dans les extraits décrits. La catéchine était le flavanol le plus représentatif des feuilles de *Pistacia lentiscus* L. (Zitouni et *al.*, 2016 ; Mehenni et *al.*, 2016).

Flavones: on retrouve de la lutéoline et l'apigénine (Bampouli et *al.*, 2014). On peut observer que la concentration de lutéoline était significativement plus élevée que celle de l'apigénine dans les extraits de feuilles et de fruits. Alors que Mehenni et *al.*, 2016 ont trouvé que la lutéoline était le deuxième polyphénol le plus abondant dans les fruits.

Anthocyanines: des Anthocyanines telles que la delphinidine 3-O-glucoside et la cyanidine 3-O-glucoside (Romanai et *al.*, 2002) et la cyanidine 3-O-arabinoside ont été mises en évidence (Longo et *al.*, 2007).

3. Variabilité de la composition en polyphénols de *Pistacia lentiscus*

Les données rassemblées dans les deux Tableaux 1 et 2 (art.3), présentent des informations détaillées sur la composition en polyphénols de lentisque. Ces détails sont relatifs à l'origine géographique, la partie de la plante extraite, la méthode et le solvant d'extraction. Ainsi que les classes et les composés phénoliques identifiés. Cette façon de présenter permet d'observer la diversité de la composition en polyphénols chez le lentisque et de discuter la variabilité de cette dernière.

Une grande variabilité dans la composition et la quantité de molécules phénoliques pourrait être attribuée à l'origine de la plante, aux conditions environnementales, au stade phénologique, aux méthodes d'extraction et à la polarité des solvants organiques utilisés.

4. Activités pharmacologiques

Plusieurs activités pharmacologiques de *Pistacia lentiscus* ont été révélées. La plupart de ces activités sont liées à la composition phénolique de cette plante. Un certain nombre de ces activités sont décrites dans le Tableau 3 (art.3). Ce Tableau contient des données sur les activités pharmacologiques étudiées sur les différents extraits phénoliques des organes de *Pistacia lentiscus*. Les résultats de ces études confirment que la plante *Pistacia lentiscus* se caractérise par un potentiel bioactif varié et intéressant. Les extraits de *P. lentiscus* présentent principalement des propriétés antioxydantes, anti-inflammatoires, anticancéreuses et antimicrobiennes, en accord avec les utilisations traditionnelles de cette plante.

A Review of *Pistacia lentiscus* Polyphenols: Chemical Diversity and Pharmacological Activities

Chabha Sehaki ^{1,2}, Nathalie Jullian ¹, Fadila Ayati ², Farida Fernane ² and Eric Gontier ^{1,*} 

¹ BIOPI-UPJV Laboratory UMRT BioEcoAgro INRAE1158, SFR Condorcet FR CNRS 3417, UFR of Sciences, University of Picardie Jules Verne, 33 Rue Saint Leu, 80000 Amiens, France

² Laboratory of Natural Resources, University Mouloud Mammeri of Tizi-Ouzou, Tizi Ouzou 15000, Algeria

* Correspondence: eric.gontier@u-picardie.fr; Tel.: +33-6-59-21-81-08

Abstract: *Pistacia lentiscus* (lentisk) is a plant species of the Anacardiaceae family. It is a medicinal plant that grows wild in the Mediterranean region. This review aims to update the existing knowledge regarding *P. lentiscus* polyphenols by consulting references dated from 1996 to 2022. The data are organized and analyzed as follows: (i) to show the chemical diversity of phenolic products from *P. lentiscus*; (ii) to summarize the variability in phenolic composition and quantity; this could be attributed to plant origin, environmental conditions, phenological stage, and the polarity of the extraction solvents; (iii) to present the pharmacological properties in agreement with the traditional uses of this plant; and (iv) to demonstrate the correlation between the chemical profile and the pharmacological effect. Various compositions were observed, including phenolic acids, flavonoid glycosides, anthocyanins, catechins, and their derivatives. The biological and therapeutic potentials of lentisk extracts have been evaluated in terms of antioxidant, antimicrobial, and anti-inflammatory activities. Most of these activities are related to the phenolic composition of this plant. The content of this review will undoubtedly contribute to the choice of techniques for isolating the different bioactive molecules contained in the *P. lentiscus*. It is also of significance for the potential development of a micro-industrial sector based on the valorization of lentisk polyphenols.

Keywords: *Pistacia lentiscus*; polyphenols; phenolic acids; flavonoids; yields; pharmacological properties



Citation: Sehaki, C.; Jullian, N.; Ayati, F.; Fernane, F.; Gontier, E. A Review of *Pistacia lentiscus* Polyphenols: Chemical Diversity and Pharmacological Activities. *Plants* **2023**, *12*, 279. <https://doi.org/10.3390/plants12020279>

Academic Editors: Ivayla Dincheva, Ilian Badjakov and Bistra Galunska

Received: 29 November 2022

Revised: 3 January 2023

Accepted: 5 January 2023

Published: 7 January 2023



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1. Introduction

P. lentiscus L. is an evergreen shrub of the Anacardiaceae family that is widely distributed in Mediterranean countries [1]. This plant is also called lentisk [2]. It has been used in traditional medicine for the treatment of several diseases, such as for gastrointestinal diseases, eczema, and throat infections [1,2], due to its potent antioxidant, anti-inflammatory, and antimicrobial effects [3]. The aerial part helps in the treatment of hypertension and has diuretic properties [4]. It also acts as an antioxidant [5], antiproliferative [6], and antimicrobial agent [7]. It has been reported to be effective in the treatment of scabies and rheumatism [8]. Its different parts contain a variety of chemical compounds that are medically important such as resin, essential oils, gallic acid, anthocyanins, flavonol glycosides, triterpenoids, tocopherol, and arabinogalactan proteins [9]. These health-promoting properties of lentisk have also been attributed to the presence of various biologically active compounds (BACs) such as phenolic compounds [10].

Polyphenols represent a class of the most well-known secondary plant metabolites [11]. This category of compounds includes more than 8000 molecules [12]. These compounds contain one or more aromatic rings linked to one or more hydroxyl groups in their basic structure and are divided into different classes, such as phenolic acids, flavonoids, tannins, stilbenes, and lignans [13]. Polyphenols are known for their health-promoting effects, such as their antioxidant and anti-inflammatory activities [14,15]. Polyphenols contribute to decreasing the incidence of cardiovascular diseases [16], liver disorders [17], obesity [18,19],

and colon cancer [20]. These compounds have received more attention in recent years, and many aspects of their chemical and biological activities have been identified and evaluated [21,22].

Many studies have been carried out regarding the identification and quantification of the chemical composition of *P. lentiscus* secondary metabolites as well as their biological and pharmacological properties. However, there are no reviews summarizing the detailed investigations that have been carried out on lentisk polyphenols. Therefore, this review aims to (i) illustrate the composition and content of phenolic compounds in the different parts of lentisk; (ii) examine the variation of its composition and polyphenol yield under the influence of geographical origin, the part of the plant used, and the type of extraction solvents; and (iii) illustrate the pharmacological properties of the different phenolic extracts. The different parts of this review are linked to establish a scientific basis for the curative capacities of this plant by explaining the relationship between the phytochemistry and pharmacology of lentisk phenolic compounds.

2. Research Methodology

To prepare a review article on the chemical diversity and pharmacological activities of polyphenols from the plant *P. lentiscus*, existing knowledge was integrated. The corresponding data were collected by reviewing articles dating from 1996 to 2022 published in Elsevier, Google Scholar, MDPI, Taylor and Francis, and Springer databases.

3. Quantitative Analyses of *P. lentiscus* Phenolic Classes

Polyphenols are reactive metabolites abundant in plant-derived foods, particularly fruits, seeds, and leaves. Polyphenolic compounds are specialized plant metabolites found in numerous plant species. Recent phytochemical analyses have shown that all the parts (leaf, stem, fruit, and root) of *P. lentiscus* are rich in bioactive phenolic components. The results of these analyses are shown in Table 1. Lentisk extracts were found to be composed of total polyphenols, flavonoids, flavonols, tannins, anthocyanins, and proanthocyanidins. Depending on the type of extracts, as well as the part of the plant used, one can observe a variation in the yield of these metabolites. Classically, phytochemical analyses were based on the use of specific colorimetric methods for each chemical family. Their contents were determined by spectrophotometry. The total phenolic content was evaluated using the Folin–Ciocalteu reagent with gallic acid (GA) as standard. The flavonoid content was determined using the aluminum trichloride method with quercetin (Q) as standard. The total tannin (TA) content was estimated based on their precipitation using bovine serum albumin (BSA) protein. The concentration of proanthocyanidins was determined using a butanol-HCl test (Table 1).

Table 1. Lentisk phenolic classes.

Plant Part	Origin	Type of Extract	Classes Detected	Content	Analysis Method	Ref
Leaves	Algeria	Ethanol extract	Total phenols	0.90 mg pyrocatechol Eq/g DM	FC (Folin–Ciocalteu method) (Pyrocatechol as standard (Std), Eq: equivalent)	[23]
Leaves	Algeria	Aqueous extracts obtained from chloroform partition (solvent fractionation)	Total flavonoids	44.25 mg Q Eq/g dry matter (DM)	ATC (Aluminum Trichloride Colorimetric method: quercetin; Std)	[24]
			Tannins	997.8 mg TA Eq/g DM	BSA (Bovine Serum Albumin method) (Tannic acid: Std)	
Fruits	Algeria	Acetonic extract	Total phenols Total flavonoids	250.6 mg GA Eq/g DM 20.6 mg of Q Eq/g DM	FC (GA: gallic acid; Std) ATC (Quercetine (Q): Std)	[25]

Table 1. Cont.

Plant Part	Origin	Type of Extract	Classes Detected	Content	Analysis Method	Ref
Leaves	Algeria	Chloroform extract	Total phenols	207 mg GA Eq/g DM	FC	[26]
		Methanolic extract		390 mg GA Eq/g DM		
		Aqueous extract	Total flavonoids	13 mg Q Eq/g DM	ATC	
		Ethyl acetate extract		82.3 mg Q Eq/g DM		
Leaves	Algeria	Aqueous extract (decoction)	Total flavonoids	3.1 mg Q Eq/g DM 8.2 mg Q Eq/g DM	ATC	[27]
leaves	Algeria	Ethanollic extract				
		Aqueous extract (infusion)	Total phenols	119.7 mg GAE/g DM	FC	[28]
Leaves Stems Fruits Roots	Algeria	Methanolic extract	Total phenols	216.2 mg GA Eq/g DM 121.4 mg GA Eq/g DM 103.3 mg GA Eq/g DM 30.1 mg GA Eq/g DM	FC	[29]
Leaves Stems Fruits Roots	Algeria	Methanolic extract	Total flavonoids	19.1 mg C Eq/g DM 16.7 mg C Eq/g DM 4.7 mg C Eq/g DM 4.2 mg C Eq/g DM	ATC (Catechine: standard; Std)	
Leaves Stems Fruits Roots	Algeria	Methanolic extract	Tannins	121.5 mg C Eq/g DM 80.2 mg C Eq/g DM 7.8 mg C Eq/g DM 7.1 mg C Eq/g DM	Vanillin method	
Leaves	Algeria	Methanolic extract (maceration)	Total phenols	238.3 mg GA Eq/g DM	FC	[30]
			Total flavonoids	19.5 mg Q Eq/g DM	ATC	
			Flavonols	7.2 mg Q Eq/g DM	ATC and sodium acetate method	
			Proanthocyanidins	39.2 mg C Eq/g DM	Butanol–HCl method	
			Total tannins	100.6 mg TA Eq/g DM	BSA (Tannic acid: Std)	
Leaves	Algeria	Methanolic extract (maceration)	Total phenols	286 mg GA Eq/g DM	FC	[31]
		Aqueous extract (decoction)		227 mg GA Eq/g DM		
		Methanolic extract (maceration)	Total flavonoids	15 mg Q Eq/g DM	ATC	
		Aqueous extract (decoction)	11 mg Q Eq/g DM			
Berries	Algeria	Ethanollic extract Solid–liquid “soxhlet” extraction	Total phenols	955.2 mg GA Eq/g DM	FC	[32]
			Total flavonoids	13.4 mg Q Eq/g DM	ATC	
Leaves	Algeria	Ethanollic extract	Total phenols	95.8 mg GA Eq/g DM	FC	[33]
			Total flavonoids	5.1 mg Q Eq/g DM	ATC	
			Myricetin-rhamnoside Quercetin-rhamnoside	3.6 mg/g DM 1.6 mg/g DM	Preparative-HPLC purification ¹ H-NMR analysis	
Leaves	Algeria	Ethyl acetate fraction from ethanollic extract	Total flavonoids	278.5 mg R Eq/g DM	ATC (Rutin: Std)	[34]
Aerial parts February May August	Greece	Methanolic extracts	Total phenols	483 mg GA Eq/g DM 588 mg GA Eq/g 5 DM 81 mg GA Eq/g DM	FC	[35]

Table 1. Cont.

Plant Part	Origin	Type of Extract	Classes Detected	Content			Analysis Method	Ref
Vegetable oil	Morocco		Total phenols	122.7 mg GA Eq/g DM			FC	[36]
			Total flavonoids	18.4 mg Q Eq/g DM			ATC	
Leaves	Morocco	Soxhlet extraction Solvents: [(hexane, ethyl acetate, methanol, ethanol) and water (infusion)]	Total phenols	67.8–345.9 mg GA Eq/g DM			FC	[37]
Twigs				48.7–302 mg GA Eq/g DM				
Fruits				36.4–192.6 mg GA Eq/g DM				
Femal and mal shrubs	Tunisia	Aqueous acetone (80:20; v/v) extracts Maceration under magnetic stirring	TPC FVD TAN	TPC	FVD	TAN	TPC: total phenols (mg GA Eq/g DM) FC	[38]
Leaves				193.8	47.5	68.4		
DP				125.8	28.1	57.3		
FS				160.4	40.9	31.8		
EF				96.8	24.4	30.7		
LF								
Stems				49.6	7.8	41.6	FVD: Total flavonoids (mg R Eq/g DM) Aluminum ATC	
DP				22.9	2.6	33.8		
FS				43.4	6.7	30.7		
EF				17.4	1.7	20.4	TAN: Tannins (mg C Eq/g DM) Vanillin method	
LF								
Flowers				55.0	28.1	26.6		
Fruits (Female)								
FS	59.4	9.1	22.1					
EF	27.1	4.5	11.6					
LF								

DP: Dormancy period; FS: flowering stage; EF: early fruiting stages; LF: late fruiting stage.

Variability in Phenolic Classes in *P. lentiscus*

Variable levels of total phenolic contents, flavonoids, and condensed tannins were found in all parts of *P. lentiscus*. According to the results shown in Table 1, the total phenolic content varied from 17 mg gallic acid equivalent/g dry matter (DM) to 955 mg gallic acid equivalent/g DM in the *P. lentiscus* extracts [32,38]. For the other polyphenolic classes, the low concentration of flavonoids in the lentisk extracts was 0.9 µg quercetin equivalent/g DM and the higher concentration was 278 mg rutin equivalent/g DM [34,36]. For the contents of condensed tannins, according to the Table 1, the minimum value was 7 mg catechin equivalent/g DM and the maximum value was 997 mg tannic acid equivalent/g DM [24,29]. Other compounds were also determined in lentisk extract, such as myricetin-rhamnoside (3.6 mg/g DM) and quercetin-rhamnoside, (1.6 mg/g DM) [33]. Flavonols (7.2 mg quercetin eq/g DM) and proanthocyanidins (39.2 mg catechin equivalent/g DM) were also detected [30].

The data gathered in Table 1 show that the amount and nature of polyphenolic compounds in the different lentisk extracts largely depend on the geographical origin, the sampling period [35,39], the plant part [29,37,38], and the type of solvent used during the extraction [26,27,31].

According of the plant part, the total phenolic content in the leaves was significantly higher than that revealed in the stems and fruits in most of the studies described in Table 1. Root extracts have a relatively low content of phenolic compounds [29]. The highest concentrations of flavonoids and condensed tannins were recorded in the leaves and stems of *P. lentiscus*.

The solvent used during extraction plays a key role in the variation in the amount of polyphenols [26,27,31]. The determination of the amount of polyphenols for the same plant part extracted with different solvents revealed quantitative differences in the lentisk phenolic contents. Studies have shown that the total phenolic content and flavonoids in the methanolic extract from leaves were higher than those in the aqueous extracts [31]. This result is in agreement with previously studies [29,35,39–41] that revealed that methanol is an interesting and suitable solvent for extracting bioactive chemicals from plants, such as phenolic and flavonoid components. Other works aimed to quantify the polyphenolic content according to the solvent used for extraction. Salhi et al. (2019) [42] prepared extracts

using dichloromethane, ethyl acetate, methanol, ethanol, or water as solvents. The results of this study, listed above in Table 1, illustrate that the total phenols, total flavonoids, and total flavonols decreased in the following order according to the solvent extracts used: ethanol > water > methanol > ethylacetate > dichloromethane. The results obtained also indicate that amongst the five extracts, the ethanol extract contained the highest amount. The quantitative analyses performed prove that the whole plant contains a high amount of total phenols. This amount increases as the polarity of the extraction solvents used increases. Other factors can impact the polyphenolic content in lentisk, such as the sampling period of the plant [35,38]. Yosr et al. (2018) [38] studied the variation in polyphenolic content in acetone extracts of different lentisk parts during four vegetative stages of female and male shrubs. For all the growth cycle periods of the plants, the male vegetative organs had significantly higher phenolic contents than the females. In both sexes, the leaves showed the maximum contents in contrast to the stems, flowers, and fruits. Flavonoid and tannin contents were also consistently higher in the males than in the females for all the tested organs. Flavonoids in leaf and flower extracts were higher than those in fruits and stems. The tannin content was in the following order (highest to lowest): leaves > stems > flowers > fruits.

The total phenolic and flavonoid contents in the vegetative organs of both genders varied between the harvesting times. A decrease in the content of compounds depending on plant part was observed from the vegetative dormancy stage (December) to the late ripening stage [38].

Therefore, we can state that the great variability in phenolic composition and quantity could be attributed to plant origin, environmental conditions, phenological stage, extraction methods, and the polarity of the organic solvents used.

These studies showed that leaf extracts of *P. lentiscus* contained high levels of total phenolic compounds. Therefore, the results obtained are useful for further research such as in the identification of specific phenolic compounds. Analytical methods for the determination of the composition have been developed. We discuss this point in more detail in the next section.

4. Identification and Quantification of Individual Phenolic Compounds

The separation, identification, and quantification of polyphenols were performed on several extracts of *P. lentiscus* from Mediterranean countries. Several chromatographic techniques, including semi-preparative high-performance liquid chromatography (HPLC) and HPLC–photodiode array detection by comparison with authentic standards. High-performance liquid chromatography (HPLC)–photodiode array detection (DAD)–mass spectrometry (MS) (HPLC-MS) equipped with an electrospray ionization (ESI) interface and ¹H and ¹³C NMR was used. These techniques allowed us to obtain qualitative and quantitative information on the biochemical constitution of *P. lentiscus*. Several metabolites including flavonoids, flavonols, flavanols, flavones, flavonoid glycosides, myricetin derivatives, anthocyanins, catechins, and different phenolic acids and their derivatives were identified (Table 2).

Table 2. Individual phenolic compounds identified in lentisk extracts.

Compounds Detected	Plant Part	Type of Extract	Analysis Method	Provenance of the Plant	Ref
Catechin β-glucogallin Quercitrin gallate	Leaves	Methanolic extract	HPLC-ESI-QTOF-MS	Algeria	[3]
Glucogallin, gallic acid, galloyl quinic acid digalloyl quinic acid, trigalloyl quinic acid	Leaves	Ethanol extract (maceration under stirring)	UHPLC-MS	Algeria	[43]

Table 2. Cont.

Compounds Detected	Plant Part	Type of Extract	Analysis Method	Provenance of the Plant	Ref
Gallic acid Catechin Syringic acid Ellagic acid Quercetin 3-O-rhamnoside Luteolin	Leaves	Ethanollic extracts (maceration)	Fourier transform infrared spectroscopy HPLC-DAD	Algeria	[44]
Myricetin-3-O-glucuronide Myricetin-3-O-glucoside Myricetin-3-O-rhamnoside Quercetin-3-O-rhamnoside Catechine	Leaves	Hydro-methanolic extract (80%)	HPLC UV/Vis PDA	Croatia	[1]
Hydroxybenzoic acid derivatives; Hydroxycinnamic acid derivatives; Hydroxy cyclohexane carboxylic acid derivatives	Leaves	Ethanollic extracts	LC-ESI-MS/MS	Egypt	[45]
Quinic acid derivatives 3,5-O-digalloyl quinic acid 3,4,5-O-trigalloyl quinic acid Luteolin-3-O-rutinoside Epicatechin-3-gallate Quercetin-3-O-glucuronide 5,7-dihydroxyflavone	Leaves	Methanolic extract Solid-liquid "soxhlet" extraction	UHPLC-ESI-MS	Egypt	[46]
Kaempferol Myricetin Quercetin Luteolin Genistein Taxifolin	Leaves	Ethanollic extract	Reverse phase (HPLC)-UV/VIS array electrospray ionization (ESI)-mass spectrometry (MS)	Israel	[47]
Galloyl derivatives Catechin	Leaves	Hydro-ethanollic extract (7:3 v/v)	HPLC	Israel	[48]
Gallic acid and galloyl derivatives of glucose Galloyl derivatives of quinic acid Myricetin Quercetin glycosides: delphinidin 3-O-glucoside and cyanidin 3-O-glucoside	Leaves	Ethanollic extract	Semi-preparative HPLC HPLC-MS analysis with 1H- and 13C NMR.	Italy	[49]
Cyanidin 3-O-glucoside Delphinidin 3-O-glucoside Cyanidin 3-O-arabinoside	Fruits	Methanolic extract	HPLC-DAD mass spectrometry (MS)	Italy	[50]
Shikimic acid, quinic acid, acid derivatives, gallic acid, Glabrol, proanthocyanidin prodelfinidin B-4, (dimer of gallo catechin and epigallo catechin), catechin or epicatechin, (epi)gallo catechin, (epi)catechin gallate, myricetin glycosides, myricetin derivatives, quercetin glycosides, quercetin O-galloylpentoside, kaempferol, luteolin	Leaves	Methanolic fraction -from chlorformic extract by sonication	LC-ESI-MS/MS	Italy	[51]
Myricetin glucopyranoside Myricetin-3-O-rutinoside Quercetin-3,4-diglucoside Quercetin-3-O-rutinoside Quercetin-3-O-galattoside Myricetin-3-O-rhamnoside Myricetin-3-O-xylopyranoside Quercetin-3-O-glucoside	Leaves/fruits	Aqueous extract (reflux heating)	LC-MS	Italy	[52]

Table 2. Cont.

Compounds Detected	Plant Part	Type of Extract	Analysis Method	Provenance of the Plant	Ref
Gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid isomer, caffeic acid, syringic acid isomer, p-coumaric acid, ferulic acid, rutin, quercetin 3-O-glucoside	Edible fixed oil from fruits	Hydro-methanolic extract (methanol 80%, v/v)	UHPLC-ESI-MS/MS and HPLC-DAD	Italy	[53]
Gallic acid, tyrosol, hydroxyphenylacetic acid, Vanillic acid, p-coumaric acid, methoxycinnamic acid, carnosic acid, salicylic acid, luteolin, kaempferol, naphtoresorcinol	Edible oil from fruits	Hydro-methanolic extract	HPLC-DAD/MSD	Tunisia	[54]
1.5 or 3.5 Dicafeoylquinic acid Ellagic acid Catechin Luteolin-7-glucosid Isoquercetin Kaempferol rutinoside Rutin	Leaves	Aqueous extract (maceration under magnetic agitation)	HPLC-MS	Tunisia	[55]

HPLC: High-performance liquid chromatography; HPLC-DAD-MS: high-performance liquid chromatography (HPLC)–diode array detection (DAD)–mass spectrometry (MS) analysis; HPLC-ESI-QTOF-MS: high-performance liquid chromatography with diode array coupled to electrospray ionization mass spectrometry; HPLC-DAD: high-performance liquid chromatography with diode array detection analysis; HPLC UV/Vis PDA: high-performance liquid chromatography (HPLC) coupled with UV/Vis PDA detector. ND: Not determined.

Several phenolic acids were detected, among which gallic acid was recovered by several authors [44,46,49,56,57]. Quinic acid derivatives and galloyl derivatives of both glucose and quinic acid, such as 3,5-O-digalloyl quinic acid and 3,4,5-tri-O-galloyl quinic acid (Figure 1), beta-glucogallin, were detected [46,57–59]. Some other studies also reported the presence of tannic acid, chlorogenic acid, caffeic acid, vanillic acid, p-coumaric acid [60], ferulic acid, 3,4-dihydroxybenzoic acid, and trans-cinnamic acid [44,60].

The analysis of the different parts of the *P. lentiscus* extracts using UHPLC-ESI-MS identified different metabolites including flavonoid glycosides, anthocyanins, and catechins and their derivatives. Among flavonoids, flavonol glycosides were found to be the most abundant class of flavonoids in the leaves and fruits of *P. lentiscus* [57]. Myricetin-3-O-rhamnoside (Figure 1) was found to be the predominant flavonol glycoside, followed by myricetin-3-O-glucoside, myricetin-3-O-glucuronide, and quercetin-3-O-rhamnoside [1]. Myricetin derivatives were also determined to account for 20% of the total amount of polyphenols in *P. lentiscus* leaves [47,49,57]. Bozorgi et al. (2013) [59] reported that quercetin-3-glucoside is the major flavonol in *P. lentiscus* as well as in other *Pistacia* species. Flavanols assigned as procyanidin B1, procyanidin B2, epicatechin, catechin, epigallocatechin gallate, and epicatechin gallate were recovered in several studies. Catechin was the most representative flavanol in *P. lentiscus* leaves [29,44], while procyanidin B1 (Figure 1) was the most abundant flavanol in fruit extracts [57]. Belonging to the class of flavones, luteolin and apigenin were previously reported by Bampouli et al. (2014) [10] in *P. lentiscus* leaves. It can be observed that the concentration of luteolin was significantly higher than apigenin in both leaves and fruit extracts. Mehenni et al. (2016) [44] found luteolin to be the second most abundant polyphenol in fruit. Anthocyanins, namely, delphinidin-3-O-glucoside, cyanidin-3-O-glucoside [49], and cyanidin 3-O-arabinoside [50] were detected in *P. lentiscus* berries and leaves. The major anthocyanins in *P. lentiscus* berries were identified to be cyanidin-3-O-glucoside (Figure 1). Delphinidin-3-O-glucoside and cyanidin 3-O-arabinoside were also found but in minor quantities [50].

Table 2 completes Table 1 by identifying the phenolic molecules belonging to the different polyphenolic classes noted in Table 1. The data presented in Table 2 also show the variability in the nature of polyphenols in lentisk under the influence of the factors described above (the part of the plant used, the extraction method, and the extracting

solvent and geographical origin). It is reported that lentisk leaves are a rich source of polyphenolic compounds (5–7% of the dry matter of the leaf), especially galloyl derivatives such as mono-, di-, and tri-O-galloyl quinic acid and mono-galloyl glucose [49]. Siano et al. (2020) [53] reported the presence of other phenolic acids such as vanillic and syringic acid derivatives, hydroxycinnamic acid, and flavonoids in lentisk seed oil extracted from fruits. The digalloyl quinic acid derivative was detected as the main phenolic component in the three parts of the plant (leaf, fruit, and stem), at percentages of 61.38%, 13.5%, and 45.11% in the stem, fruit, and leaf, respectively [60]. Dragović et al. (2020) studied the effect of the extraction solvent (ethanol or methanol) on the polyphenol composition of *P. lentiscus* leaf extracts from four locations at three different phenological stages. In both ethanolic and methanolic leaf extracts, phenolic acids and flavonol glycosides were quantified, whereas catechin was quantified only in methanolic extracts. The mass concentration of these compounds varied significantly according to phenological stages, cultivation sites, and extraction solvents used [1].

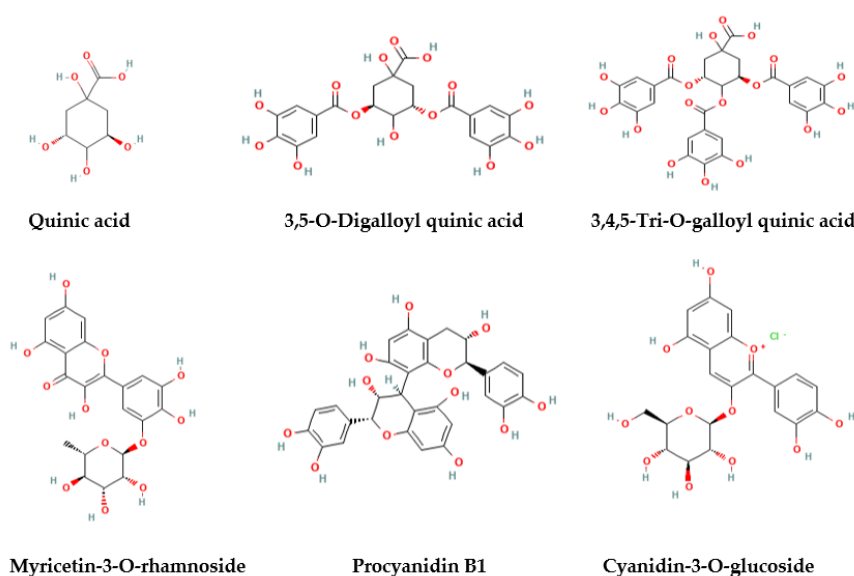


Figure 1. Structure of major phenolic acids and flavonoids detected in *P. lentiscus* extracts.

5. Prominent Pharmacological Activities

The plant *P. lentiscus*, which is used in traditional medicine, possesses pharmacological attributes and may offer significant potential as a therapeutic agent [28–30,34,42,43,51,53]. As shown in Table 3, several studies have therefore been undertaken to justify its traditional use and to establish a scientific basis for the curative capacities of this plant. The most marked pharmacological properties are its antioxidant [28–30,43], antimicrobial [28,34,42], anticancer [51,53], and anti-inflammatory [31,43] activities. The phytochemistry of the lentisk extracts tested for the curative properties described above is also discussed in these same studies (Tables 1 and 2). We are interested in summarizing and highlighting the correlation between the chemical composition and the pharmacological effect of this plant, particularly for its antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. To explain this relationship, we drew on data based on the phytochemical compositions presented in Tables 1 and 2 and the pharmacological effects illustrated in Table 3, and also on other studies in the literature that aimed at explaining the link between the phytochemistry and pharmacology of the plant.

5.1. Antioxidant Activity

Different methods were used to evaluate the antioxidant capacity of phenolic extracts of *P. lentiscus*: DPPH (2,2-diphenyl-1-picrylhydrazyl), i.e., free radical scavenging activity; free hydroxyl radicals (HO); ferric-reducing power (FRAP); the carotene bleaching (CB)

assay; the oxygen radical absorbance capacity (ORAC) test; hydrogen peroxide scavenging activity (H_2O_2); the phosphomolybdenum (TAC) assay; and the ABTS·+: 2,2'-azino-bis-(3 ethylbenzthiazoline-6-sulphonic acid) assay were the most used (Table 3). These methods have different modes of action. Electron transfer, proton transfer, and iron reduction are the main mechanisms involved. The observations noted after the evaluation of the antioxidant potential of this plant are illustrated in Table 3. Investigations (Table 3) were conducted regarding the evaluation of antioxidant activity by comparing to reference antioxidants (ascorbic acid, gallic acid, trolox, rutin, and quercetin). The results of these investigations revealed that lentisk's antioxidant properties are comparable to those of the reference antioxidants. The extracts of *P. lentiscus* remain a considerable source of natural antioxidants.

The ability of the *P. lentiscus* extracts to prevent free radicals could be attributed to the high content of phenolic compounds [23,29,30,34]. There is a significant positive correlation between the antioxidant test results and the amount of total phenols [37]. The antioxidant capacity of *P. lentiscus* extract has been shown to be due primarily to gallic acids and their galloyl derivatives (5-Ogalloyl; 3,5-O-digalloyl; 3,4,5-tri-O-galloyl) [23]. The trapping of DPPH increases accordingly with the number of galloyl groups [23]. Quercetin and gallic acid are powerful natural antioxidants [23], and monophenols are less effective than polyphenols [61,62]. With gallic acid, the inductive effect of these three hydroxyl groups is a significant factor influencing the increase in antioxidant activity [61]. This suggests that they are partly responsible for the antiradical potential observed in the lentisk extracts [43]. In addition, the antioxidant activity also depends on the polarity of the solvent, the solubility of the phenolic compounds, and the hydrophobic nature of the reaction medium [42]. A high polarity of the extract solvent increases the antioxidant capacity (DPPH and FRAP) of the lentisk extracts. Polar extracts such as ethanol, methanol, and aqueous extracts were found to be rich in polar compounds, which are either hydrogen atom donors or singular atom transfer agents [42]. In addition to the solvent polarity, the solubility of the phenolic compounds is governed by the degree of polymerization of the phenols, the part of the plant used, and the variability of soil and climatic conditions [63,64]. The flavonol glycosides found in lentisk [1,43,57] are known for their antioxidant attributes, which are strongly related to their structural characteristics, i.e., the hydrogen donor substituents (OH groups) and the presence of a 2,3 double bond that increases their scavenging capacity and inhibition of pro-oxidant enzymes [24]. It has been shown that the main compound present in lentisk extracts, i.e., myricetin-rhamnoside [1,43], has a DPPH scavenging capacity comparable to that of vitamin C [65].

Table 3. Pharmacological activities of lentisk phenolic extracts.

Pharmacological Activity	Plant Part	Product	Method	Significant Results)	Ref
Antioxidant activity	Leaves	Ethanol extract from leaves	DPPH (2,2-diphenyl-1-picrylhydrazyl): free radical scavenging activity	The extract (3 g/L) inhibited 95.69% of the activity of the DPPH radicals	[23]
	Leaves	Dichloromethane extract	DPPH free radical scavenging activity	IC ₅₀ = 05.44 (µg/mL) (Ethanol extract)	[42]
		Ethylacetate extract	Ferric-reducing activity power (FRAP)	309.60 mg ascorbic acid equivalent/g extract (methanolic extract)	
		Ethanol extract Methanol extract Aqueous extract	Carotene bleaching (CB) assay	Inhibition = 90.32% per 2 g/L of extract (dichloromethane extract)	
Leaves	Ethanol extract	Oxygen radical absorbance capacity (ORAC) test	Antioxydant capacity = 5865 µmol TE/100 gE	[43]	

Table 3. Cont.

Pharmacological Activity	Plant Part	Product	Method	Significant Results)	Ref
	Leaves	Aqueous extract	Free radical scavenging activity (DPPH assay) Hydrogen peroxide scavenging activity (H ₂ O ₂) Ferric-reducing power (FRAP) assay Antioxidant assay by phosphomolybdate method	IC ₅₀ (aqueous extract) = 9.86 µg/mL	[28]
	Berries	Ethanol extract	DPPH assay (ABTS·+: 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid)) assay Reducing power activity assay	IC ₅₀ = 8.60 mg/mL IC ₅₀ = 8.65 mg/mL IC ₅₀ = 12.21 mg/L	[32]
	Leaves	Methanolic extract	DPPH assay β-carotene bleaching test	IC ₅₀ = 0.008 mg/mL IC ₅₀ = 0.12 mg/mL	[30]
	Leaves	Aqueous fraction obtained from chloroformic extract	Reducing power assay Scavenging ability against DPPH radical Activity against linoleic acid peroxidation	IC ₅₀ = 50.03 µg/mL IC ₅₀ = 4.24 µg/mL IC ₅₀ = 0.82 µg/mL	[33]
	Leaves	Methanolic fraction from chloroformic extract	DPPH assay ABTS assay	inhibition of 50% of DPPH radicals (2.9 µg/mL extract) inhibition of 50% of ABTS•+ (0.6 µg/mL extract)	[51]
	Fruits	Aqueous extract Ethyl acetate extract Butanol extract	Free radical scavenging (DPPH assay)	100 mg/mL of aqueous extract inhibited 86.13% of DPPH radicals	[66]
	Leaves	Ethyl acetate fraction from ethanolic extract	Ferric-reducing power assay (FRAP)	IC ₅₀ = 15.0 µg/mL (FRAP)	[34]
	Fruits	Phenolic extract from vegetable oil	DPPH assay	Significant antioxidant power (IC ₅₀ = 37.38 mg/mL)	[36]
	Fruits Twigs Leaves	Aqueous extract Hexane extract Ethyl acetate extract Methanol extract Ethanol extract	Phosphomolybdenum (TAC) assay	The aqueous extract of <i>P. lentiscus</i> leaves showed the highest TAC with 488.16 mg AA/g of extract.	[37]
	Leaves Fruits	Methanolic extracts	Free radical DPPH assay	EC 50 = 0.121 mg/mL for leaves and EC 50 = 0.26 mg/mL for fruits	[67]
	Aerial parts	Methanolic extract	FRAP	reducing power = 84.6–131.4 mmol Fe ²⁺ /L plant extract	[35]
Antibacterial activity	Leaves	Dichloromethane extract Ethylacetate extract Ethanolic extract Methanolic extract Aqueous extract	The disk diffusion method on Muller–Hinton agar (MHA).	All these extracts had efficient antimicrobial activity against: Gram-positive bacteria: <i>Micrococcus luteus</i> , <i>Bacillus subtilis</i> , and <i>Listeria innocua</i> Gram-negative bacteria: <i>Escherichia coli</i> The activity was almost the same for all the extracts against each bacterium	[42]

Table 3. Cont.

Pharmacological Activity	Plant Part	Product	Method	Significant Results)	Ref
	Leaves	Aqueous extract	The disc diffusion method	A maximum inhibition zone of 12 mm was observed on <i>Pseudomonas aeruginosae</i> , while moderate activity was obtained against all strains	[28]
	Leaves	Decoction Petroleum ether extract Ethanol extract Maceration Infusion	The minimal inhibitory concentration (MIC) was determined by a microdilution assay in microtiter plates The minimal bactericidal concentration (MBC) was determined by carrying out a subculture of the tubes showing no growth on plates	Antimicrobial activity against: <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> Decoction showed the best activity (MIC = 312 mg/L for all the three bacterial strains). MIC and MBC values were the same, so the substances should possess bactericidal activity	[68]
	Leaves	Ethyl acetate fraction from ethanolic extract	The MIC of the extract was determined using the agar dilution method The MBC was determined by taking samples from the nutrient agar plates that showed no visible growth after 24 h incubation and subculturing them in tubes containing nutrient broth	Moderate inhibitory activities against: <i>Staphylococcus aureus</i> , <i>Listeria innocua</i> , <i>Bacillus cereus</i> , <i>Escherichia coli</i> , <i>Salmonella typhi</i> , <i>Salmonella enterica</i> , <i>Pseudomonas aeruginosa</i> , <i>Proteus mirabilis</i> , <i>Vibrio cholerae</i> , and <i>Enterococcus faecalis</i> There was remarkable activity against <i>Vibrio cholerae</i> with an MBC value of 0.3 mg/mL	[34]
	Leaves	Methanolic extract Aqueous extract	The disk diffusion method	Antibacterial activity against: <i>Staphylococcus aureus</i> , <i>Staphylococcus haemolyticus</i> , <i>Pseudomonas aeruginosa</i> , and <i>Proteus mirabilis</i> Methanol extract showed a significant inhibitory effect on the growth of all tested bacterial isolates, with 33 mm and 27 mm against <i>S. aurous</i> and <i>S. haemolyticus</i> , respectively	[69]
Antifungal activity	Leaves	Dichloromethane extract Ethylacetate extract Ethanolic extract Methanolic extract Aqueous extract	The disk diffusion method on Muller–Hinton agar (MHA).	Significant antifungal activity against: <i>Candida pelliculosa</i> and <i>fusarium oxysporum albidinis</i> The ethanolic extract was the most active	[42]
	Leaves	Hydro-methanolic extract (70/30 v/v)	Diffusion using solid medium method	The extract was more active against <i>Trichophyton mentagrophyte</i> and <i>Microsporium canis</i> , with growth inhibition: <i>Trichophyton mentagrophyte</i> (17 mm) <i>Microsporium canis</i> (16.7 mm)	[70]

Table 3. Cont.

Pharmacological Activity	Plant Part	Product	Method	Significant Results)	Ref
	Leaves	Aqueous extract	The minimal inhibitory concentration (MIC) was determined by a microdilution assay in microtiter plates	Antifungal activity against: <i>Candida albicans</i> , <i>Candida parapsilosis</i> and <i>Cryptococcus neoformans</i> The highest activity of <i>P.lentiscus</i> was against <i>T. glabrata</i> (MIC = 39–156 mg/L)	[68]
	Leaves	Ethyl acetate fraction from ethanolic extract	The MIC of the extract was determined using the agar dilution method	Good antifungal activity against <i>Candida albicans</i> with CMI 0.1 mg/mL	[34]
Anticancer activity	Leaves	Ethanolic extract	The in vitro cytotoxicity of the extract was determined by sulforhodamine B (SRB) assay	Moderate cytotoxic activity against lung cancer A549, breast cancer MCF7, prostate cancer PC3, and HepG2 liver cancer	[45]
	Leaves	Ethanolic extract	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay	Anticancer potential against melanoma (B16F10) cell lines	[43]
	Leaves	Methanolic fraction from chloroformic extract (sonication)	MTT, SRB, and LDH assays for SH-5YSY, and SK-N-BE(2)-C human, and neuronal cell lines, and also on C6 mouse glial cell line	Significant cytoprotective response in both the oxidized cell systems	[51]
	Edible fixed oil (fruits)	Hydro-methanolic extract (methanol 80%, v/v)	A crystal violet viability assay with increasing concentrations was carried out	The extract induced clear dose-dependent effects on the growth of the HT-29 cell line derived from human colorectal adenocarcinoma	[53]
	Leaves	Hydro-methanolic extract (8:2 v/v)	Cell viability by MTT assay	The extract showed activity on: the SK-N-BE(2)C cell line with an IC50 value of $100.4 \pm 1.6 \mu\text{g/mL}$ the SH-SY5Y cell line with IC50 value of $56.4 \pm 1.1 \mu\text{g/mL}$	[60]
Anti-inflammatory activity	Leaves	Ethanolic extract	The measurement of the secretion of interleukin-1 by macrophages exposed to ATP or H ₂ O ₂ on the THP-1 monocytic cell line	Significant anti-inflammatory activity	[42]
	Leaves	Methanolic extract	Albumin denaturation inhibition method in human red blood cell suspension	Apparent anti-inflammatory activity	[46]
	Leaves	Chloroformic extract Ethyl acetate extract Methanolic extract	The carrageenan-induced paw edema assay	MeOH extract presented the best anti-inflammatory activity Dose of 200 mg/kg showed 68% edema inhibition	[71]

Table 3. Cont.

Pharmacological Activity	Plant Part	Product	Method	Significant Results)	Ref
	Leaves	Methanolic extract (maceration)	Three inflammation models: Croton oil-induced ear edema in mice	Local treatment with 2 mg/ear of: alcoholic extract significantly decreased ear edema (65%) aqueous extract exerted a lower inhibitory effect (51%).	[31]
		Aqueous extract (decotion)	Carrageenan induced-pleurisy in rats Acetic acid-induced vascular permeability in mice	methanolic and aqueous extracts: at (400 mg/kg) inhibited neutrophil migration by 29% and 38%, respectively; at methanolic and aqueous extracts (100 µg/mL) inhibited neutrophil chemotaxis by 81% and 71%, respectively	
	Fruits	Acetonic extract	The ear edema model induced by Croton oil and the airpouche model induced by lambda carrageenan	Oral administration dose of 300 mg/Kg of extract decreased ear edema by 80% Dose of 1 mg of extract/pouche decreased pouch edema by 34%	[25]

MTT: 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2Htetrazoliumbromide. SH-SY5Y: neuroblastoma cells were purchased. SK-N-BE(2)-C: human bone marrow neuroblastoma cells. SRB: sulforhodamine B.

5.2. Antimicrobial Activity

Researchers evaluated the antimicrobial and antifungal activities of lentisk phenolic extracts (Table 3). The evaluation of the antimicrobial activity was based on the comparison of the antimicrobial effects of lentisk extracts and those of the different antibiotics (such as amphotericin, amoxicillin, and ciprofloxacin) used in the studies presented in Table 3. The results of these studies revealed that the extracts from leaves show an interesting antimicrobial potential against Gram-positive and Gram-negative bacteria, but also against different fungi. The disk diffusion method and/or the dilution method were the most useful methods. Lentisk leaf extracts prepared in different solvents (dichloromethane, methanol, ethanol, ethyl acetate, and water) were all found to be effective against the bacteria *Micrococcus luteus*, *Bacillus subtilis*, *Listeria innocua*, and *Escherichia coli* [42]. The same extracts also have an inhibiting effect against fungal strains as *Candida pelliculosa* and *Fusarium oxysporum albidini* [42]. Lauk et al. (1996) [68] showed that lentisk leaf decoctions have good antibacterial activity against *Sarcina lutea*, *Staphylococcus aureus*, and *Escherichia coli* (with MIC = 312 mg/L for the three bacteria tested). The activity against fungal cells, *Candida albicans*, *Candida parapsilosis*, *Torulopsis glabrata*, and *Cryptococcus neoformans* seems to be much more interesting [64]. Bakli et al. (2020) [34] showed that the ethyl acetate fraction from an ethanolic leaf extract had remarkable activity against *V. cholerae* with a value of MBC 0.3 mg/mL [34]. Another study showed that methanol extracts had a significant inhibitory effect on the growth of bacterial isolates (*Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*). This methanolic extract showed a higher zone of inhibition than that of the aqueous extract. The maximum zone of inhibition was observed in the methanolic extract at 100% concentration with 33 mm and 27 mm against *S. aureus* and *S. haemolyticus*, respectively [69]. *P. aeruginosa* was of particular interest, as this bacterium was inhibited by the methanol extract to a greater extent than with the use of the reference antibiotic levofloxacin [69]. In most of these studies, Gram-negative bacteria were found to be less sensitive to the extracts than Gram-positive bacteria.

This can be explained due to the presence of hydrophobic lipopolysaccharides in the outer membrane, which provide protection against different agents [72], in addition to

enzymes in the periplasm that destroy foreign molecules introduced from the outside [73]. The antimicrobial activity observed in this study could be due to the presence of flavonoid compounds (kaempferol, myricetin, quercetin, and rutin) in the lentisk extracts (see Table 2). Flavonoids are known for their antimicrobial activity against a wide range of microorganisms [34]. They have multiple cellular targets and can be applied to different components and functions in the bacterial cell [74,75]. These kinds of molecules promote antimicrobial activity against human pathogenic microorganisms [76].

5.3. Anticancer Potential

Some studies (Table 3) aimed to evaluate the anticancer potential of different phenolic extracts of this plant in vitro. The ethanolic leaf extracts showed anticancer potential against lung cancer A549, breast cancer MCF7, prostate cancer PC3, and HepG2 liver cancer in vitro [45]. Yemmen et al. (2017) [60] revealed the anti-proliferative activities of leaf hydro-methanolic extracts against two human neuroblastoma cell lines (SK-N-BE(2) C and SH-SY5Y) with IC₅₀ values of 100 µg/mL and 56 µg/mL, respectively. The anticancer potential of the crude extracts against melanoma (B16F10) and breast (EMT6) cell lines was also evaluated. The leaf and fruit extracts inhibited the growth of B16F10 cells (IC₅₀ = 56 and 58 µg/mL, respectively) [42].

Phytochemical studies have indicated the presence of significant amounts of flavonoids, tannins, and phenolic compounds in *P. lentiscus* extracts (see Tables 1 and 2) [24], which may be responsible for the anticancer activity of lentisk extracts. It has been shown that the presence of a 2,3-double bond and three adjacent hydroxyl groups in the structure could confer a higher anticancer potential to a flavonoid [77]. One example of the flavonoids found in lentisk extracts is myricetin (see Tables 1 and 2); this molecule was found to have significant cytotoxic activity against B16F10 melanoma cell cultures [77].

5.4. Anti-Inflammatory Activity

P. lentiscus is used in traditional medicine for the treatment of inflammation, burns, and gastrointestinal disorders. Anti-inflammatory activity has been the focus of many recent investigations (Table 3). Dellai et al. (2013) [71] examined the efficacy of aqueous and organic extracts of *P. lentiscus* leaves in vivo for their anti-inflammatory and anti-ulcerogenic activities using the carrageenan-induced paw edema assay and HCl/ethanol-induced gastric injury in rats, respectively. Aqueous (AQ), chloroformic (CHCl₃), ethyl acetate (EtOAc), and methanolic (MeOH) leaf extracts administered intraperitoneally showed a dose-dependent anti-inflammatory effect. Leaf extracts of CHCl₃, EtOAc, and MeOH, administered orally, showed concentration-dependent inhibition of gastric lesions. The effect of all the extracts in both activities is comparable to the reference drugs: cimetidine and acetylsalicylate of lysine, respectively [71]. The pharmacological evaluation of the *P. lentiscus* leaf extracts showed the anti-inflammatory potential of this plant and that its activity is unlike non-steroidal anti-inflammatory drugs and corticosteroids. The extracts did not cause damage to the stomach mucosa but showed an inhibition of lesion formation. The work carried out by Bouriche et al. (2016) [31] concerns the measurement of the anti-inflammatory activity of alcoholic and aqueous extracts of *P. lentiscus* leaves. Croton oil-induced ear edema in mice was used as a model of acute inflammation. The results showed that a local treatment with 2 mg/ear of alcoholic extract significantly decreased ear edema (65%), while the aqueous extract exerted a weaker inhibitory effect (51%). The anti-inflammatory activity of lentisk was otherwise examined by measuring the secretion of interleukin-1β by macrophages exposed to ATP or H₂O₂. The leaf extract (100 µg/mL) showed significant anti-inflammatory activity compared to acetylsalicylic acid (ASA) [31].

Constituents such as quercetin derivatives (quercetin 3-O-rhamnoside, see Table 2) [44] participate in the promotion of anti-inflammatory effects such as the attenuation of swelling [44]. This has been proven in mice [78].

The study conducted by Bouriche et al. (2016) [31] (Tables 1 and 3) proved that the anti-inflammatory effect of the acetone extract of *P. lentiscus* fruit was similar to that of

indomethacin, which is used as a standard anti-inflammatory agent. The mechanism of action of indomethacin on inflammation is based on the inhibition of pro-inflammatory prostaglandin synthesis [79]. The anti-inflammatory effect of the acetone extract of *P. lentiscus* fruit is probably attributed to the lipophilic soluble substances that are able to penetrate through the skin barrier [80] and can thereby exert their anti-inflammatory effects. Likely candidates for these anti-inflammatory substances are flavonoids and polyphenols, which have been isolated from *P. lentiscus*. Phenolic compounds are known to interact with and penetrate through lipid bilayers [81]. The observed anti-inflammatory effect is also likely due to the presence of antioxidant compounds in the extract.

The anti-inflammatory results [25,31,43,46,71] provide valuable evidence regarding the anti-inflammatory potential of *P. lentiscus* leaves, suggesting that this plant can be exploited as a natural source of anti-inflammatory agents.

The results obtained in all the studies illustrated above indicate that the extracts of *P. lentiscus* present antioxidant, anti-inflammatory, anticancer, and antimicrobial properties in agreement with the traditional uses of the plant.

6. Other Activities

6.1. Antidiabetic Activity

The antidiabetic potential of mastic preparations (decoctions and infusions) is already known among people in rural areas. Scientific investigations have been carried out on the evaluation of the antidiabetic activity of lentisk extracts in vitro and in vivo by measuring the inhibition of α -amylase and α -glucosidase enzymes (digestive enzyme that hydrolyzes polysaccharides). The results of the study carried out by Mehenni et al. (2016) [44] showed that the leaf and fruit ethanolic extracts possessed promising antidiabetic activity in streptozotocin-induced diabetic rats, similar to that of the reference drug glibenclamide (0.91 g/L). This result was confirmed by in vitro tests for the inhibition of α -amylase [44]. Further investigations confirmed that aqueous extracts of the leaves were identified as strong and effective dual inhibitors of α -amylase and glucosidase in vitro [52].

6.2. Hepatoprotective Activity

The hepatoprotective effect of lentisk extracts has also been reported. Mehenni et al. (2016) [44] performed a histological examination of the liver of mice pretreated with the same dose of leaf and fruit extract (125 mg/kg body weight) against paracetamol-induced liver damage (165 mg/kg body weight). Ethanolic leaf and fruit extracts were found to provide significant protection against paracetamol-induced hepatic necrosis. The leaf extracts show better hepatoprotective potential as compared to the fruit extracts.

6.3. Hypocholesterolemic Activity

Chourfa et al. (2015) [27] studied the hypocholesterolemic activity of alcoholic and aqueous extracts of *P. lentiscus* leaves. Lipid parameters such as total cholesterol, triacylglyceride, low-density lipoprotein, very-low-density lipoprotein, and high-density lipoprotein were measured in the plasma. The administration of *P. lentiscus* extracts (200 mg/kg) produced in the ethanolic extract resulted in a significant decrease in total cholesterol, triacylglycerides, and low-density lipoprotein cholesterol (154 and 99 mg/dL, respectively). The aqueous extract produced a significant decrease in total cholesterol and triacylglycerides (203 and 97 mg/dL, respectively).

6.4. Anti-Ulcer Activity

Dellai et al. (2013) [71] compared the intensity of the anti-ulcer effects of lentisk leaf extracts prepared in different solvents. (chloroformic, ethyl acetate, methanolic, and aqueous extracts). The aqueous extract of *P. lentiscus* leaves showed an excellent protective effect against HCl/ethanol-induced gastric lesions in rats. The effect of lentisk leaf extract on behavioral, histological, and biochemical alterations caused by a metal neurotoxin (aluminum) in male albino mice was examined. The ethanolic extract and its isolated

bioactive compounds were found to have a dose-dependent neuroprotective effect, with the highest effect obtained with myricetin rhamnoside (IC₅₀ = 0.04 mM) [33].

7. Conclusions and Perspectives

Several phenolic acids were detected, among which gallic acid and galloyl derivatives were the major compounds. As for flavonoids, myricetin-3-O-rhamnoside was found to be the predominant flavonol glycoside and quercetin-3-glucoside was found to be the major flavonol in *P. lentiscus*. Flavanols assigned as procyanidin B1, procyanidin B2, epicatechin, catechin, epigallocatechin gallate, and epicatechin gallate were recovered. This review also covers investigations conducted on the biological potential of *P. lentiscus* phenolic extracts. The results gathered in these studies indicate that the *P. lentiscus* extracts have antioxidant, anti-inflammatory, anticancer, and antimicrobial properties in agreement with the traditional uses of this plant.

The observed anti-inflammatory effect is likely due to the presence of antioxidant compounds in the extract. There is a significant positive correlation between the antioxidant activity results and the amount of total phenols. The antioxidant capacity of *P. lentiscus* extract has been shown to be due primarily to gallic acids and their galloyl derivatives (5-Ogalloyl, 3,5-O-digalloyl, and 3,4,5-tri-O-galloyl). The anticancer potential could be due to flavonoids such as myricetin. The antimicrobial activity observed could be due to the presence of flavonoid compounds (kaempferol, myricetin, quercetin, and rutin) in the lentisk extracts.

To better analyze the correlation between the chemical composition and the pharmacological effect in relation to the traditional use of this plant, it would be interesting to use chemometric tools by applying metabolomics. Moreover, additional investigations on its pharmacological potential in vivo biological models are strongly recommended.

These results should be supported by future studies regarding the possible toxic effects of lentisk extract so as to confirm the use of the plant in the treatment of various diseases.

P. lentiscus extracts remain a considerable source of natural antioxidants. This plant could be a promising source of natural antioxidants in food and pharmaceutical industries as alternatives to certain synthetic antioxidant additives.

This review can help in moving towards improving the production of phenolic molecules responsible for a given pharmacological potential by focusing on the various factors that can influence the composition and yield. It can therefore also help the potential development of a micro-industrial sector based on the valorization of lentisk polyphenols.

Author Contributions: Conceptualization, C.S.; methodology, C.S.; validation, E.G., F.F., N.J. and F.A.; formal analysis, C.S.; investigation, C.S., E.G., F.F., N.J. and F.A.; resources, C.S.; data curation, C.S.; writing—original draft preparation, C.S., E.G. and F.F.; writing—review and editing, C.S., E.G. and F.F.; visualization, E.G., F.F., N.J. and F.A.; supervision, E.G., F.F. and N.J.; project administration, C.S., E.G. and F.F.; funding acquisition, C.S., E.G. and F.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by (1) the French Ministry of Foreign Affairs through Campus France (file no. 973496A); (2) the “Conseil Régional de Picardie” through the MTV (Métabo-Typage Végétal 2007-2022) project; and (3) the University of Picardie Jules Verne through the French Ministry of Higher Education and Research, the “Hauts de France” region, and the European FEDER funds within the so-called CPER programs devoted to equipment acquisition. This work was also funded by Algerian authorities through the financial support of the Tizi-Ouzou UMMTO University and the social grant attributed to Chabha Sehaki for her PhD.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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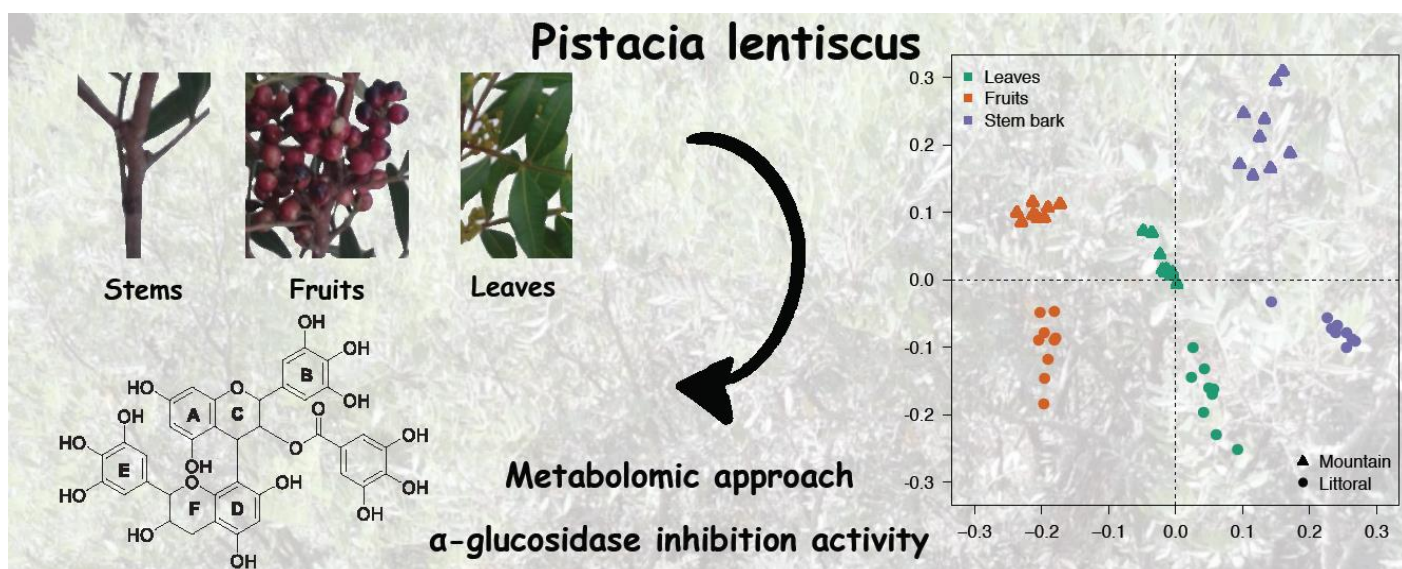
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Chapitre IV:

Analyse UHPLC-ESI-HRMS des extraits phénoliques de lentisque et étude de l'inhibition de l' α -glucosidase *in vitro*: profilage métabolomique.



Graphical abstract (<https://doi.org/10.3390/metabo13020275>)

Résumé de l'étude

Introduction

L'incidence du diabète de type 2 est beaucoup plus élevée que celle du diabète de type 1. Le diabète est une maladie métabolique résultant d'une interaction dynamique entre les défauts de la sécrétion d'insuline et l'action de l'insuline (Tundis et *al.*, 2010). Plusieurs médicaments sont utilisés pour faire baisser la glycémie chez les diabétiques de type 2. Parmi ces médicaments, il existe des inhibiteurs de l' α -glucosidase; une enzyme responsable de l'hydrolyse des glucides pour permettre le passage du glucose dans le sang (Vinholes et *al.*, 2017). Actuellement, l'acarbose, le miglitol et le voglibose sont les médicaments qui inhibent l'activité enzymatique de l' α -glucosidase dans l'intestin (Hedrington et *al.*, 2019). Cependant, l'utilisation de ces médicaments a montré de nombreux effets secondaires et une toxicité accrue (Nakhaee et *al.*, 2013). Des recherches scientifiques récentes montrent que les produits naturels ont un effet hypoglycémiant significatif et pourraient être des alternatives pour le traitement du diabète de type 2. *Pistacia lentiscus* figure parmi les plantes utilisées traditionnellement pour traiter ce type de diabète (Bouyahia et *al.*, 2021).

L'enquête ethnobotanique sur les plantes antidiabétiques menée au Maroc par Bouyahya et *al.* 2021 a révélé que les feuilles de lentisque étaient la partie la plus utilisée et que la décoction était le principal mode de préparation, suivi de l'infusion. L'administration de l'extrait de lentisque se fait par voie orale pour le traitement du diabète en médecine traditionnelle. Pour mieux caractériser le profil phytochimique de *Pistacia lentiscus* en lien avec son utilisation comme agent hypoglycémiant en médecine traditionnelle, notre travail expérimental s'est basé sur deux principales parties. Une partie consiste à évaluer l'effet inhibiteur de l' α -glucosidase (activité antidiabétique *in vitro*) des feuilles, écorces de tige et fruits de *Pistacia lentiscus* récoltés en montagnes et au littoral de Tizi-Ouzou en Algérie (54 échantillons). Une autre partie porte sur le profilage phytochimique de ces extraits par UHPLC-ESI-HRMS. Dans cette étude nous avons appliqué le profilage métabolomique pour caractériser les inhibiteurs potentiels de l' α -glucosidase dans le lentisque et pour élucider les différences entre les populations de *Pistacia lentiscus* trouvées naturellement dans ces deux endroits.

Matériel et méthodes

Collecte des échantillons de plantes, extraction, et analyse UHPLC-ESI-HRMS

Pour cette étude, nous avons réalisé l'échantillonnage de lentisque sur les mêmes sites sélectionnés que pour l'étude précédente sur les huiles essentielles (Ait-Irane, en montagne //Tigzirt, au littoral). Sur chaque site un ensemble de neuf arbres individuels femelles a été sélectionné pour l'ensemble des échantillons. Des échantillons de feuilles, de tiges et de fruits de *Pistacia lentiscus* ont été collectés séparément sur ces plantes. Ce qui donne 54 échantillons différents (9arbres*2sites*3organes). La collecte a été effectuée en automne de l'année 2019, au lever du soleil (6-8h) en 2019.

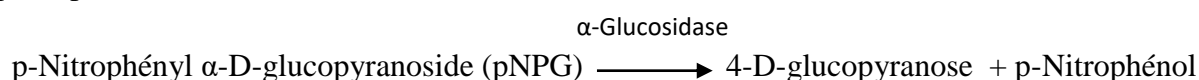
Après extraction au méthanol et fractionnement (cf détails dans l'article 4), la fraction aqueuse de chaque échantillon a été lyophilisée et stockée à -20 C° pour l'analyse UHPLC-ESI-HRMS et pour l'évaluation de l'activité biologique (le protocole d'extraction est détaillé dans l'article 4 ci-après).

Chaque extrait aqueux lyophilisé a été dissout dans 2 ml d'un mélange eau/méthanol 50:50 (v/v). Après agitation et sonication pendant une minute, les solutions ont été diluées à 1:10 dans un mélange eau/méthanol 50:50 (v/v) pour l'analyse UHPLC-ESI-HRMS [chromatographie liquide à ultra haute performance (UHPLC), couplée à un spectromètre de masse hybride haute résolution (HRMS), équipé d'une source d'ionisation par électro-nébulisation (ESI)]. Les conditions d'analyse UHPLC-ESI-HRMS ont été réalisées conformément au protocole de Tchoumtchoua et *al.*, 2019 (voir les précisions dans l'article 4).

Activité inhibitrice de l' α -Glucosidase : principe de la mesure

L'effet de chaque extrait sur l'activité catalytique de l' α -glucosidase a été évalué selon la méthode de Bachhawat et *al.*, 2011 avec une légère modification, et dans une plaque à 96 puits.

Le test d'inhibition d' α -glucosidase a été effectué *in vitro*, par dosage colorimétrique, selon le principe de la réaction suivante :



L'activité inhibitrice des extraits de lentisque (et du témoin acarbose) a été quantifiée en mesurant le p-nitrophénol libéré dans le milieu réactionnel (Kee et *al.*, 2013). Le substrat 4-Nitrophényl- α -D-glucopyranoside (pNPG) est hydrolysé par l' α -glucosidase en libérant le p-nitrophenol de coloration jaune, dont l'absorbance a été mesurée par spectrophotométrie UV/Visible à une longueur d'onde de 405 nm.

Analyse statistique

a) Analyse multivariée

L'analyse multivariée OPLS (moindres carrés partiels orthogonaux consensuels) a été utilisée comme outil chimiométrique pour modéliser les données chimiques et biologiques élaborées dans cette étude. Cette analyse OPLS a été réalisée en utilisant la dernière version de Matlab 2020b (Boccard et *al.*, 2013).

L'OPLS a montré le lien entre les réponses (surfaces d'aires) des métabolites détectés via les chromatogrammes UHPLC-ESI-HRMS des extraits analysés, et l'intensité de l'effet inhibiteur de l' α -glucosidase observé. Cette analyse nous a ainsi permis d'identifier les variables (métabolites) ayant les plus grands effets sur l'activité inhibitrice de l' α -glucosidase.

b) Analyse univariée

L'objectif de l'analyse univariée est d'étudier la variation i) de l'inhibition de l'enzyme α -glucosidase et ii) de l'intensité des métabolites les plus probablement responsables de cette inhibition en fonction de la partie du lentisque utilisée et de son origine géographique.

Ces analyses statistiques ont été réalisées à l'aide du test Kruskal-Wallis ajusté avec Bonferroni en utilisant le package PMCMR-plus pour les analyses de variances et le package MulticompView pour l'analyse des groupes. Les différences significatives ont été considérées à un niveau de confiance de 95%. Ces analyses sont réalisées avec R version 3.5.

Résultats et discussion

Inhibition de l' α -glucosidase

L'effet inhibiteur sur l' α -glucosidase a été évalué en calculant le pourcentage d'inhibition de cette enzyme dans une plaque à 96 puits. Les résultats de cette évaluation sont présentés dans la figure 1 (article 4). Les extraits d'écorces de tiges ont montré une activité inhibitrice plus forte. La valeur moyenne est de 84,7 % et 69,9 % respectivement pour les

échantillons de lentisque récoltés au littoral et à la montagne. Les extraits de fruits montrent un effet plus faible (13,6 %) aux deux endroits. Selon la localisation de la plante, la variation de l'effet inhibiteur n'est pas significative pour chaque type d'organe. Par contre, pour un site donné, selon la partie de la plante utilisée, la différence de cet effet est significative entre les écorces de tiges et les fruits, et entre les feuilles et les fruits.

Nous avons également testé l'acarbose (témoin positif), un médicament (inhibiteur d' α -glucosidase) utilisé pour le traitement du diabète de type 2. L'inhibition de l'acarbose sur l' α -glucosidase est d'environ 100% (voir figure 1). En comparant l'effet de ces extraits avec celui de l'acarbose, nous pouvons dire que les effets inhibiteurs de l'acarbose et des extraits d'écorce de tige sur l' α -glucosidase sont similaires. L'inhibition de l' α -glucosidase est significativement différente entre l'acarbose et les extraits des fruits.

Analyse multi variée des données (MVDA)

Les résultats de l'analyse multi variée OPLS sont présentés dans la figure 2 de l'article 4 sous forme de score plot et loading plot.

Le score plot et le loading plot sont deux graphiques complémentaires. Le score plot permet de séparer les 54 échantillons (feuilles, écorces des tiges et fruits) selon la partie de la plante utilisée et le lieu d'échantillonnage (littoral ou montagne) en 6 groupes différents. Le loading plot montre les métabolites détectés dans ces extraits en lien avec l'activité inhibitrice de l' α -glucosidase. Ces deux plots (représentations graphiques de l'analyse) montrent que les métabolites ayant la plus forte activité inhibitrice sur l' α -glucosidase caractérisent principalement les extraits d'écorces des tiges.

Par conséquent, sur la base des signaux de molécules candidates identifiées ci-dessus, nous nous sommes intéressés à l'identification formelle de quelques métabolites ayant l'effet inhibiteur le plus significatif sur l'activité de l' α -glucosidase. Ces métabolites sont très probablement responsables de l'activité inhibitrice de l' α -glucosidase de la plante, d'où leur nom " marqueurs". Ces marqueurs se trouvent à l'extrémité droite du graphique de charge avec des coordonnées supérieures à 5 sur la composante prédictive (voir les valeurs de ces coordonnées dans le Tableau S6 des données supplémentaires). Ces marqueurs sont présentés en couleurs dans la figure 2.B.

Identification des marqueurs de l'inhibition de l' α -glucosidase

L'identification de ces marqueurs a été basée sur les données issues de l'analyse UHPLC-QTOF-MS (variables mesurées: Tr, M/Z, formule moléculaire), voir Tableau 2 (art.4). Grâce aux fragments caractéristiques obtenus après analyse MS dans notre étude, nous avons pu préparer les schémas de fragmentation (Figures 3-6) identifiant chaque molécule. Cette identification a également été confirmée à l'aide des comparaisons basées sur les bases de données disponibles, notamment la base de données métabolomique KnaPSacK, MassBank, Kyoto Encyclopedia of Genes and Genomes (KEGG) et Met frag ainsi que la bibliothèque UNIFI et sur des comparaisons basées sur les bases de données disponibles. En suivant cette méthodologie d'identification, nous avons identifié 6 marqueurs présentés en couleurs dans la figure 2.B.

Cette identification a également été confirmée à l'aide de publications antérieures sur les mêmes molécules. De plus, grâce aux fragments caractéristiques obtenus après analyse MSe (spectrométrie de masse avec mobilité ionique) dans notre étude, nous avons pu préparer les schémas de fragmentation identifiant chaque molécule. En suivant cette méthodologie d'identification, nous avons identifié 6 marqueurs présentés en couleurs dans la figure 2.B: épigallocatechine(4a>8)épigallocatechine (2 isomères),(epi)gallocatechine-3'-o-galloyl-(epi)gallocatechine (2 isomères), acide 3,5-O- digalloylquinique et pentoside de l'acide dihydroxy benzoïque.

Discussion

Dans notre travail, *Pistacia lentiscus* a été étudiée pour son utilisation comme agent hypoglycémiant dans la médecine traditionnelle. C'est la première étude qui rapporte le criblage phytochimique des composés inhibiteurs de l' α -glucosidase dans différentes parties de *Pistacia lentiscus* en utilisant une étude chimiométrique qui relie l'activité biologique et les données UHPLC-ESI-HRMS. En outre, ce travail s'intéresse également à l'évaluation de la variation de l'intensité de l'inhibition de l'activité alpha glucosidase en fonction de l'habitat de la plante (montagne et littoral). Par ailleurs, il n'existe qu'une seule étude décrivant le profil phytochimique de la tige entière (Yemmen et al., 2017). Par conséquent, notre étude est le premier rapport sur la composition phytochimique des écorces de tige de lentisque. Un total de 6 composés a été identifié, dont 4 flavonoïdes dérivés de l'épigallocatechine. A notre connaissance, et selon notre étude, toutes les molécules identifiées comme marqueurs d'activité biologique [(épigallocatechine(4a->8)épigallocatechine (2 isomères),



(épi)gallocatéchine-3'-Ogalloyl-(épi)gallocatéchine (2 isomères), pentoside de l'acide dihydroxy benzoïque) sauf l'acide 3,5-O-digalloylquinique] sont identifiées pour la première fois dans les différents extraits (feuilles, écorces de tiges et fruits) de *Pistacia lentiscus* (bien que le monomère épigallocatéchine, la structure de base des 4 molécules, ait déjà été identifié précédemment). La plupart de ces marqueurs ont été rapportés pour leur capacité à inhiber l' α -glucosidase. Des études antérieures ont déterminé plusieurs caractéristiques structurales clés nécessaires aux flavonoïdes monomères pour inhiber l'activité α -glucosidase (Kamiyama et al., 2010; Gamberucci et al., 2006; Tadera et al., 2006). Selon les résultats de notre présente étude, les composés dérivés de l'épigallocatéchine; épigallocatéchine-(4 α →8)-épigallocatéchine (2 isomères), (épi)gallocatéchine-3'-Ogalloyl-(épi)gallocatéchine (2 isomères), identifiés dans *Pistacia lentiscus* ont très probablement un rôle majeur dans l'activité inhibitrice de l' α -glucosidase en raison de la configuration structurale des doubles liaisons conjuguées aux groupes 4-oxo et hydroxyle (mécanisme évoqué par Tadera et al., 2006). De plus, la présence d'un groupe gallate estérifié en position 3 du cycle C a été suggérée comme étant critique pour l'interaction des flavan-3-ols avec l'enzyme α -glucosidase (Kamiyama et al., 2010; Gamberucci et al., 2006); c'est le cas pour la molécule (épi)gallocatéchine-3'-Ogalloyl-(épi)gallocatéchine.

Conclusion

Les résultats de ce travail révèlent que l'écorce des tiges récoltées sur la côte présente la plus forte activité inhibitrice sur l' α -glucosidase. Cette activité est comparable à celle de l'acarbose. L'OPLS a été utilisée pour définir les molécules qui marquent l'effet inhibiteur de l' α -glucosidase. L'analyse UHPLC-ESI-HRMS a permis d'identifier 6 marqueurs: épigallocatéchine(4 α ->8)épigallocatéchine (2 isomères), (épi)gallocatéchine-3'-Ogalloyl-(épi)gallocatéchine (2 isomères), acide 3,5-O-digalloyl quinique, pentoside de l'acide dihydroxy benzoïque. L'intensité de ces marqueurs varie en fonction de l'organe du lentisque et aussi de son habitat. Il serait intéressant d'isoler et de purifier ces molécules et de les quantifier pour un éventuel développement pharmaceutique à partir de cette plante. Il est également important d'envisager des essais précliniques et cliniques sur ces extraits de lentisque, afin de déterminer leurs mécanismes d'inhibition, leur pharmacocinétique et leur toxicologie.

Article

Metabolomics-Based Profiling via a Chemometric Approach to Investigate the Antidiabetic Property of Different Parts and Origins of *Pistacia lentiscus* L.

Chabha Sehaki ^{1,2}, Roland Molinie ¹ , David Mathiron ³ , Jean-Xavier Fontaine ¹ , Nathalie Jullian ¹, Fadila Ayati ², Farida Fernane ²  and Eric Gontier ^{1,*} 

¹ BIOPI-UPJV Laboratory UMRT BioEcoAgro INRAE1158, SFR Condorcet FR CNRS 3417, UFR of Sciences, University of Picardie Jules Verne, 33 Rue Saint Leu, 80000 Amiens, France

² Laboratory of Natural Resources, University Mouloud Mammeri of Tizi-Ouzou, Tizi-Ouzou 15000, Algeria

³ Analytical Platform, UFR of Sciences, University of Picardie Jules Verne, 33 Rue Saint Leu, 80000 Amiens, France

* Correspondence: eric.gontier@u-picardie.fr; Tel.: +33-6-59-21-81-08

Abstract: *Pistacia lentiscus* L. is a medicinal plant that grows spontaneously throughout the Mediterranean basin and is traditionally used to treat diseases, including diabetes. The aim of this work consists of the evaluation of the α -glucosidase inhibitory effect (i.e., antidiabetic activity in vitro) of different extracts from the leaves, stem barks and fruits of *P. lentiscus* harvested on mountains and the littoral of Tizi-Ouzou in Algeria. Metabolomic profiling combined with a chemometric approach highlighted the variation of the antidiabetic properties of *P. lentiscus* according to the plant's part and origin. A multiblock OPLS analysis showed that the metabolites most involved in α -glucosidase inhibition activity were mainly found in the stem bark extracts. The highest inhibitory activity was found for the stem bark extracts, with averaged inhibition percentage values of 84.7% and 69.9% for the harvested samples from the littoral and mountain, respectively. On the other hand, the fruit extracts showed a lower effect (13.6%) at both locations. The UHPLC-ESI-HRMS characterization of the metabolites most likely responsible for the α -glucosidase-inhibitory activity allowed the identification of six compounds: epigallocatechin(4a>8)epigallocatechin (two isomers), (epi)gallocatechin-3'-O-galloyl-(epi)gallocatechin (two isomers), 3,5-O-digalloylquinic acid and dihydroxy benzoic acid pentoside.

Keywords: *P. lentiscus*; α -glucosidase; phytochemical profiling; UHPLC-ESI-HRMS; metabolomic approach; antidiabetic



Citation: Sehaki, C.; Molinie, R.; Mathiron, D.; Fontaine, J.-X.; Jullian, N.; Ayati, F.; Fernane, F.; Gontier, E. Metabolomics-Based Profiling via a Chemometric Approach to Investigate the Antidiabetic Property of Different Parts and Origins of *Pistacia lentiscus* L. *Metabolites* **2023**, *13*, 275. <https://doi.org/10.3390/metabo13020275>

Academic Editor: Markus R. Meyer

Received: 18 December 2022

Revised: 3 February 2023

Accepted: 7 February 2023

Published: 14 February 2023



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1. Introduction

Statistics on people diagnosed with diabetes reveal that diabetes is a worldwide disease [1]. This disease affects approximately 5% of the world's population [2]. Diabetes mellitus (type 1) is characterized by chronic hyperglycemia caused by insufficient insulin secretion [3], whereas diabetes (type 2) is a metabolic disease resulting from the dynamic interaction between defects in insulin secretion and insulin action [2]. This insulin deficiency due to the dysfunction of pancreatic β cells [4] leads to increased glucose concentrations in the blood. Several drugs, such as sulfonylureas and biguanides, are used to lower blood glucose levels in diabetics. Among these classes, there are inhibitors of α -glucosidase, an enzyme responsible for the hydrolysis of carbohydrates to allow the passage of glucose into the bloodstream [5]. Currently, acarbose, miglitol and voglibose belong to classes of competitive α -glucosidase inhibitors in the intestine [6], reducing postprandial hyperglycemia. However, the use of these drugs has shown many side effects and toxicities [7]. It causes diarrhea [8] and adverse digestive disorders [8,9]. It has also been reported that acarbose can induce hepatitis [10,11] and increase liver enzyme levels [12].

Recent scientific research has shown that natural products could have an important hypoglycemic effect and could be alternatives for the treatment of type 2 diabetes. The plant families, which enclose the species, studied the most as inhibitors of α -glucosidase, based on a review by Benalla et al. (2010) [1], are Fabaceae, Crassulaceae, Hippocrateaceae, Lamiaceae and Myrtaceae. These studies have revealed the importance of medicinal plants as natural sources of antidiabetic bioactive agents. *P. lentiscus* (lentisk or mastic tree), a medicinal plant growing in the wild in the Mediterranean area [13,14] and belonging to the Anacardiaceae family, is traditionally used to treat hypertension, cough, sore throat, eczema, stomach ache, toothache, kidney disorders, jaundice [15], skin problems and diarrhea [16,17], but also diabetes [18]. In the ethnobotanical investigation on antidiabetic plants conducted in Morocco by Bouyahya et al. (2021), it was revealed that lentisk leaves were the most used part. In traditional medicine, for the treatment of diabetes, the administration of lentisk extract is conducted orally; leaf decoction is the main mode of preparation, followed by infusion. The phytochemistry of lentisk has been extensively studied using high-performance liquid chromatography–mass spectrometry (HPLC-MS). Several phenolic acids have been detected, such as gallic acid, quinic acid and galloyl derivatives [19–23], as well as flavonoid glycoside compounds [21].

Recent investigations have focused on the study of phytochemical profiling and the multibiological potential of plant extracts via integrated approaches [24,25]. The researchers in these studies were interested in metabolomic analyses to characterize metabolites in plants that may be responsible for particular bioactive activities, such as antioxidant and in vitro antidiabetic activities [26–28].

Even though there are many ethnobotanical pharmaceutical uses of *P. lentiscus* [29], very few studies [23,30] have been carried out to explore the possibilities of using this species as an antidiabetic agent. These studies were carried out by Foddai et al. [30] on aqueous leaf and fruits extract and Mehenni et al. [23] on ethanolic extracts. Most of the research on *P. lentiscus* has been conducted to find out its antioxidant [31–37], antimicrobial [38–41], anticancer [22,42–44] and anti-inflammatory potential [19,45–47]. So far, no study has reported a metabolomic analysis to identify inhibitors of antidiabetic potential in different lentisk organs. It is known that different geographical regions can affect the composition and biological activity of plants [48]. Therefore, our study is the first work that aimed to achieve the following:

- (i) Evaluate α -glucosidase inhibition in *P. lentiscus* samples and identify compounds more positively correlated to α -glucosidase inhibition using a metabolomic approach;
- (ii) Study the variation of this inhibition according to the organ (i.e., leaf, stem bark and fruit) and the geographical origin (i.e., mountain and littoral) of the plant *P. lentiscus*. This metabolomic method was based on the link between phytochemical profiling by ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-ESI-HRMS) and α -glucosidase dosage data.

This study could contribute to the valorization of the antidiabetic potential of this resource in the pharmaceutical field through, for example, the elaboration of traditionally improved drugs (TID) in developing countries. The antidiabetic potential of this plant could also be exploited in the field of food supplements.

2. Materials and Methods

2.1. 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, $\geq 99.0\%$) and acarbose (PHR1253-Pharmaceutical Secondary Standard; Certified Reference Material). The analytical standards for the UHPLC-ESI-HRMS analysis were (–)-epigallocatechin (EGC) ($\geq 90\%$, 08108-supeplo), myricitrin ($\geq 99.0\%$, 91255, Sigma-Aldrich, Saint Quentin Fallavier, France), apigenin 7-glucoside ($\geq 97.0\%$, 44692, Supelco) and tiliroside ($\geq 95.0\%$, PHL89809-phyproof® Reference Sub-

stance). These analytical-grade chemicals used were purchased from Supelco and Sigma-Aldrich, Saint Quentin Fallavier, France.

2.2. Plant Collection

The plant species *P. lentiscus* was identified using the flora of Quézel and Santa (1962–1963). In addition, the lentisk samples were deposited in the herbarium of the National Superior School of Agronomy of Algeria. This identification was confirmed by Benhouhou and resulted in the issuance of a certificate attesting that this plant was indeed *P. lentiscus*. The samples of leaves, stems and fruits of *P. lentiscus* were collected separately from plants in the Algerian region of Tizi-Ouzou. Its relatively contrasted climatic regimes are representative of those of the large area of northern Algeria between the littoral and mountains. For this study, we selected two geographical sites in the Tizi-Ouzou region: Ait-Irane on the mountain and Tizirt on the littoral. In all, 54 samples were collected. The information of the 54 samples, such as the collected site, collected date, GPS of each collection site and morphological variation in the characters of each sample, is provided in Table 1. The lentisk samples harvested from the two sites (mountain and littoral) do not show remarkable morphological differences according to the difference in altitude of the harvesting sites (Table 1).

Table 1. Information on lentisk samples harvested on the mountain and on the littoral.

Sites	Collected Date	54 Samples (9 Trees × 3 Organs × 2 Sites)	Morphological Variations	Longitude	Latitude	Altitude	Annual Temperature	
Site 1 Ait-Irane Mountain	Mid-October 2019	9 lentisk trees (3 organs: leaves, stem bark and fruit) = 27 samples	Unremarkable for the same organ type	36°29'58.3" N	4°04'43.4" E	876 m	Min 0.5 °C	Max 31.9 °C
Site 2 Tizirt Littoral	Mid-October 2019	9 lentisk trees (3 organs: leaves, stem bark and fruit) = 27 samples	Unremarkable for the same organ type	36°53'43.0" N	4°11'00.4" E	13 m	Min 7.4 °C	Max 27.9 °C

No site-specific morphological variations for the same organ type. More information on the climatic conditions at these two sites is presented in Tables S1 and S2.

The Mediterranean climate in Ait-Irane is characterized by a dry summer and relatively low temperatures during winter (Table S1). On the other hand, the summer and winter temperatures are less extreme, and the humidity is higher throughout the year in Tizirt (Table S2). At each site, a set of nine individual female trees was selected for all of the different samples. The collection was conducted at sunrise in autumn (6–8 a.m.) in October 2019.

2.3. Drying and Extraction

The samples from each organ were cleared of debris and air-dried in the dark for 7 days at 25–30 °C. The samples were ground using a ball mill. Fifty-four powdered samples were obtained (9arbusts*2sites*3organs). A methanol extraction of each powder (200 mg) from each organ was performed under heating at 70 °C for 30 min in an Eppendorf Thermo Mixer. The resulting mixture was centrifuged at 1100× *g* for 5 min. The recovered supernatant was evaporated under nitrogen. The dry crude extract recovered after the evaporation of methanol was dissolved in a 50:50 (*v/v*) water/cyclohexane mixture. After centrifugation, the aqueous phase was collected and lyophilized [49]. The lyophilized aqueous extract was stored at −20 °C for LC-MS analysis and biological activity evaluation.

2.4. UHPLC-ESI-HRMS Analysis of the Extracts

Each lyophilized aqueous extract (54 extracts) was dissolved in 2 mL of a 50:50 (*v/v*) water/methanol mixture. After shaking and sonication for one minute, the solutions were diluted to 1:10 in a 50:50 (*v/v*) water/methanol mixture for UHPLC-ESI-HRMS analysis. The UHPLC-ESI-HRMS analysis conditions were set according to Tchoumtchoua et al. (2019) [50], with some slight modifications.

The UHPLC-ESI-HRMS analyses were performed on an ACQUITY UPLC I-Class system coupled to a high-resolution hybrid Vion IMS QTOF (ion mobility quadrupole

time-of-flight) mass spectrometer equipped with an electrospray ionization (ESI) source (Waters, Manchester, UK). One microliter of each sample was injected, and the chromatographic separation was performed on a Kinetex Biphenyl column (100 × 2.1 mm, 1.7 μm) (Phenomenex, Torrance, CA, USA) maintained at 55 °C. The mobile phase flow rate was set at 0.5 mL/min, and a gradient elution from water with 0.1% formic acid (A) to methanol with 0.1% formic acid (B) was programmed as follows (A:B): 80:20 (t = 0 min), 80:20 (t = 0.5 min), 40:60 (t = 5 min), 10:90 (t = 6 min), 10:90 (t = 7 min), 80:20 (t = 7.5 min) and 80:20 (t = 10 min). The ESI source was set to a capillary voltage of 2.4 kV in the negative and positive ionization modes, with a sample cone voltage of 20 V. The source and desolvation temperatures were set to 120 and 450 °C, respectively. Nitrogen was used as the desolvation and cone gas at flow rates of 1000 and 50 L/h, respectively. For accurate mass measurements, lockmass correction was applied using the [M-H][−] ion at an *m/z* of 554.2615 from a solution of leu-enkephalin (100 pg/L in H₂O/CH₃CN (50:50, *v/v*) with 0.1% formic acid. The TOF was operated in the sensitivity mode, providing an average resolving power of 50,000 (FWHM). The HDMS^E spectra were recorded in the profile mode over a mass range of 50 to 2000 *m/z*, with the scan time set to 0.2 s. HDMS^E acquisition works by alternating two functions, a so-called LE (low-energy) one with a collision energy set at 6 eV to obtain non-fragmented spectra with information on precursor ions and a HE (high-energy) one with a ramp of collision energy values from 25 to 50 eV to obtain fragmented ion spectra thanks to an alignment by ion mobility of the fragments that had the same drift time as the precursors they came from. The analytical standards for the UHPLC-ESI-HRMS analysis were injected.

2.5. Glucosidase-Inhibitory Activity

The measurement of effect of the extract on the catalytic activity of α-glucosidase was performed according to the method of Bachhawat et al. (2011) [51], with a slight modification. In a 96-well plate, a reaction mixture containing 50 μL of phosphate buffer (50 mM, pH = 6.8), 10 μL of α-glucosidase (1 U/mL) (α-glucosidase from *Bacillus stearothermophilus*) 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 (with A corresponding to the absorbance):

$$\text{Inhibition(\%)} = \frac{(A_{\text{negative control}} - A_{\text{sample}})}{A_{\text{negative control}}} \times 100$$

where $A_{\text{negative control}}$ 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.

2.6. Metabolomic and Chemometric Analysis

Data processing was performed with the UNIFI software V1.9.4 (Waters, Manchester, UK). The multivariate matrix consisted of the ions detected in the samples after the UHPLC-QTOF-MS analysis in the negative (neg) and positive (pos) ionization modes (see Tables S3 and S4) in the retention time range (0.5–5 min). Only the ions with a response greater than 2000 (peak area) were retained in at least one condition (i.e., leaves, stem bark and fruit). Among these ions, those correlated with each other ($r \geq 0.80$) at the same retention time

were removed. Consensus orthogonal partial least squares (multiblock OPLS) was used as the chemometric tool to model the information in the rich chemical and biological data elaborated in this study [52]. This multiblock OPLS analysis was performed using the latest version of MATLAB 2020b. The UHPLC-QTOF-MS chromatograms in the negative and positive ion modes and the percentage of α -glucosidase inhibition were used for untargeted analysis. The multiblock OPLS showed the relationship between the responses (i.e., peak area) of the metabolites detected via the UHPLC-QTOF-MS chromatograms of the extracts analyzed and the intensity of the observed α -glucosidase inhibitory effect.

2.7. Intensity Variation of the Metabolites Identified

The objective of the univariate analysis was to study the variation of (i) the inhibition of the α -glucosidase enzyme and (ii) the intensity of the metabolites most likely responsible for this inhibition according to the part of the lentisk used and its geographical origin. These statistical analyses were performed by pairwise comparison through a post hoc Kruskal–Wallis test adjusted with Bonferroni correction using the package PMCMRplus version 1.9.4 [53] for analysis of the variances and the package MulticompView version 0.1–8 for analysis of the groups [54]. Significant differences were considered at the 95% confidence level. These analyses were performed with the R software version R ($\geq 3.5.0$).

3. Results

In the present work, the different aerial parts (i.e., leaves, stem barks and fruits) of the plant *P. lentiscus* were collected from two sites (i.e., mountain and littoral) in Tizi-Ouzou (Algeria). The 54 samples obtained were characterized phytochemically and biologically. Firstly, the evaluation of the α -glucosidase inhibition property in vitro of all of the extracts was performed. Secondly, metabolite profiling was performed using an up-to-date analytical technique: UHPLC-ESI-HRMS. Finally, a multivariate metabolomic analysis (multiblock OPLS) was performed in the following order:

- (i) Study of the α -glucosidase inhibition differences between the aerial parts and between the geographical origins of *P. lentiscus*;
- (ii) Characterization of the metabolites most involved in the inhibition of α -glucosidase.

3.1. α -Glucosidase-Inhibitory Activity

The inhibitory effect on α -glucosidase was estimated by evaluating the percentage of inhibition of this enzyme in a 96-well plate. Figure 1 presents the percentages of inhibition of α -glucosidase by the different lentisk extracts (i.e., leaves, stem barks and fruits at a concentration of 10 $\mu\text{g}/\text{mL}$) harvested from the two locations in Tizi-Ouzou (i.e., littoral and mountain). The stem bark extracts showed stronger inhibitory activity, and the average values were $84.7 \pm 5.9\%$ ($\text{IC}_{50} = 5.8 \pm 0.4$) and $69.9 \pm 19.9\%$ ($\text{IC}_{50} = 7.9 \pm 3.3$) at the littoral and mountain, respectively. The fruit extracts showed a lower effect ($13.6 \pm 6.2\%$) at both locations. The pairwise comparison through the Kruskal–Wallis test adjusted with Bonferroni correction [53,54] allowed for the study of the significance of the inhibition variation according to the organ and also the location. Depending on the location of the plant, the variation of the inhibitory effect was not significant for each type of organ. On the other hand, depending on the part of the plant used, the inhibition difference was significant between the stem barks and fruits and between the leaves and fruits.

We also tested acarbose (reference control; α -glucosidase inhibitor), a drug used for the treatment of type 2 diabetes. The inhibition by acarbose of α -glucosidase was approximately 100% (see Figure 1). Comparing the effect of the lentisk extracts with acarbose, we noticed that the inhibitory effects of acarbose and stem bark extracts on α -glucosidase were comparable. The difference between the two effects was not significant (p -value > 0.05) according to the Kruskal–Wallis test following pairwise comparisons using Dunn's many-to-one test [53]. This same test revealed that the difference in α -glucosidase inhibition was not significant between acarbose and leaf extracts, but it was significant

between acarbose and fruit extracts (p -value < 0.05). A figure showing the same results by an IC₅₀ calculation is included in the Supplementary Materials (Figure S1).

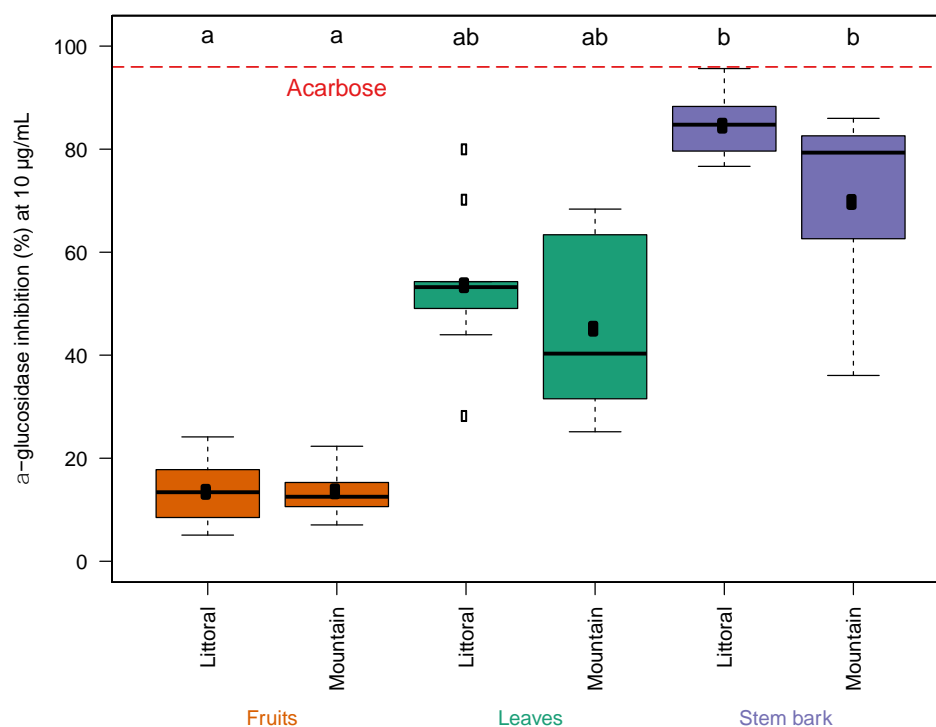


Figure 1. Boxplot showing α -glucosidase-inhibitory effect of lentisk leaves, stems barks and fruits from the mountain and littoral. (The means and medians are presented as black dots in the center of each box plot and by bars, respectively. The significance of the α -glucosidase inhibition variation is represented by letters (groups, a, b and ab) according to pairwise comparison through the Kruskal–Wallis test adjusted with Bonferroni correction).

3.2. Metabolomic and Chemometric Analysis

In order to objectively assess the differences in the α -glucosidase inhibition and chemical profile depending on the plant part and its geographical origin, the supervised approach, consensus orthogonal partial least squares (multiblock OPLS) based on UHPLC-ESI-HRMS profiling and in vitro enzyme inhibitory bioactivities, was performed. This chemometric approach also aimed to characterize the metabolites positively correlated with α -glucosidase inhibition.

The UHPLC-QTOF-MS data of 54 extracts were analyzed, and the values of the α -glucosidase-inhibitory activities were processed using multivariate data analysis (multiblock OPLS). The final matrix that was used for the multiblock OPLS analysis was composed of 198 ions in the negative ionization mode and 160 ions in the positive one. The quality parameters of the multiblock OPLS model ($R^2 = 0.74$ and $Q^2 = 0.72$) demonstrate the good fit of this model. The two ion blocks (i.e., negative ionization mode (neg) and positive ionization mode (pos)) contributed in the same way to the constitution of the multiblock OPLS model (see Figure S2). The multiblock OPLS analysis generated two graphs: a score plot (Figure 2A) and a loading plot (Figure 2B). The two components (i.e., predictive and orthogonal) of the score plot showed a total variance of 81.5%, with 75% for the predictive component and 6.5% for the orthogonal component. This score plot separated the 54 samples (i.e., leaves, stem barks and fruits) according to the part of the plant used and the sampling location (i.e., littoral or mountain) into six different groups. The loading plot shows the metabolites detected in these extracts. The inhibitory effect increases going to the right of the predictive component of the loading plot. Therefore, we were interested in identifying metabolites located on the right end of the loading plot, with coordinates greater than five on the predictive component (see the values of these coordinates in

Table S5 in the Supplementary Materials). These metabolites were the ones most involved in the inhibition of α -glucosidase in the different lentisk extracts (Figure 2B). These metabolites were present in intense concentrations in the stem barks (Figure 2A,B).

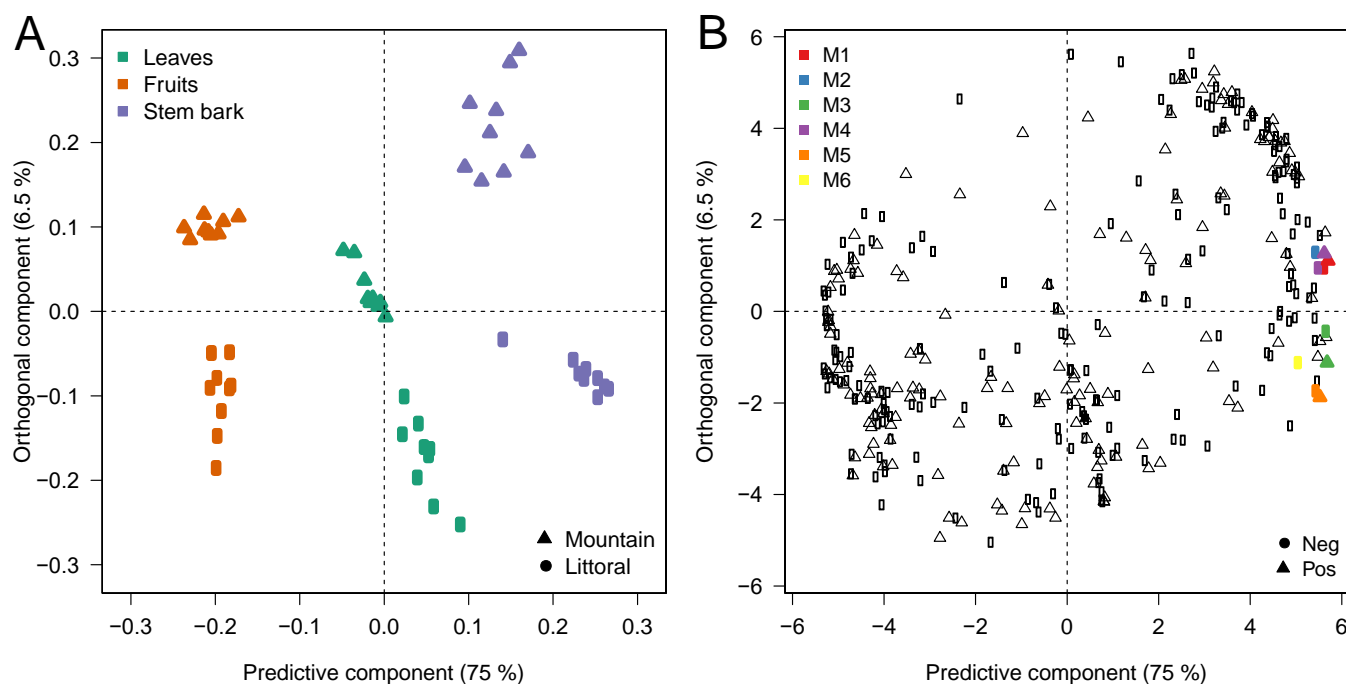


Figure 2. Multiblock OPLS analysis performed on the UHPLC-ESI-HRMS and α -glucosidase inhibition data: (A) score plot—lentisk samples: ■ Littoral/ Δ Mountain; (B) loading plot—metabolites detected: ■ ions with negative ionization/ Δ ions with positive ionization: metabolites; M1: epigallocatechin(4a->8)epigallocatechin (1); M2: 3,5-O-digalloylquinic acid; M3: (epi)gallocatechin-3'-O-galloyl-(epi)gallocatechin (1); M4: epigallocatechin (4a->8)epigallocatechin (2); M5: (epi)gallocatechin-3'-O-galloyl-(epi)gallocatechin (2); M6: dihydroxy benzoic acid pentoside.

The tentative Identification of the metabolites most positively correlated with α -glucosidase inhibition was based on UHPLC-QTOF-MS (RT, m/z , molecular formula) data. Using the characteristic fragments obtained after the HDMS^E analysis, we were able to propose fragmentation patterns identifying each molecule. The identification process was also based on comparisons using on the available databases, including the KnaPSack metabolome database, MassBank, Kyoto Encyclopedia of Genes and Genomes (KEGG) and MetFrag, as well as the UNIFI library. This identification was also confirmed using previous publications on the same molecules. Following this identification methodology, we identified four metabolites, shown in color in Figure 2B, in the negative and positive ionization modes and two other metabolites in the negative ionization mode.

3.3. UHPLC-ESI-HRMS Identification of α -Glucosidase Inhibitory Metabolites

The *P. lentiscus* extracts were found to have an α -glucosidase inhibition effect that was more or less impactful according to the plant's living environment (i.e., mountain or littoral) and the considered organ (i.e., leaves, stem barks or fruits). With the aim of obtaining a better understanding of this effect, UHPLC-ESI-HRMS analyses were performed to characterize the phytochemical content that could be responsible for α -glucosidase inhibition. Statistical analyses (i.e., multiblock OPLS) were used for comparing the data of the different extracts and to highlight those for which the metabolic content could be correlated with strong α -glucosidase inhibition activity. This approach led to a total of six compounds. These compounds were putatively identified as phenolic compounds by ESI-HRMS in the HDMS^E mode based on accurate mass measurements of the corre-

sponding ions and their fragments. The UHPLC-ESI-HRMS data results are summarized in Tables 2 and S5. Indeed, these tables include the retention time (RT), observed m/z , mass error value (ppm), molecular formula, characteristic fragment ions used for identification with their intensities and the suggested identity of each compound in agreement with previous studies. Table S5 in the Supplementary Materials contains more information than Table 2.

Table 2. Phenolic compounds identified in *P. lentiscus* extracts by UHPLC-ESI-HRMS in the negative mode. (The equivalent information for the positive mode is presented in Supplementary Materials Table S6).

N° Metabolite	RT (min)	m/z (obs) [M-H] [−]	Error (ppm)	HDMS ^E Fragment Ions (Intensity, %)	Molecular Formula	Suggested Compound	Ref
1	0.45	609.1242	−1.26	441.0825 (2.99), 423.0722 (100), 305.0667 (29.49), 261.0397 (15.71), 219.0659 (14.46), 177.0186 (26.94), 125.0241(18.09)	C ₃₀ H ₂₆ O ₁₄	Epigallocatechin(4a>8)epigallocatechin	[55–57]
2	0.55	495.0774	−2.03	343.0661(100), 325.0550(15.74), 191.0551(87.47), 169.0136(37.71)	C ₂₁ H ₂₀ O ₁₄	3,5-O-Digalloylquinic acid	[43]
3	0.56	761.1339	−3.46	609.1228(12.42), 591.1134(5.98), 465.0820 (12.76), 423.0820(100), 305.0658(46.73)	C ₃₇ H ₃₀ O ₁₈	(Epi)gallocatechin-3'-Ogalloyl-(epi)gallocatechin	[58]
4	0.61	609.1233	−1.51	423.0716 (100)	C ₃₀ H ₂₆ O ₁₄	Epigallocatechin (4a->8)epigallocatechin (isomer)	[57–59]
5	0.72	761.1344	−2.28	423.0715 (100)	C ₃₇ H ₃₀ O ₁₈	(Epi)gallocatechin-3'-Ogalloyl-(epi)gallocatechin (isomer)	[58]
6	0.73	285.0608	−2.76	108.0209 (100)	C ₁₂ H ₁₄ O ₈	Dihydroxy benzoic acid pentoside	[60]

According to these HDMS^E data, the six different metabolites have been widely identified as:

Epigallocatechin(4a->8)epigallocatechin, or a dimer of (epi)gallocatechin (2×EGC): The HDMS^E spectrum of metabolite 1 (RT 0.45 min) revealed a deprotonated pseudomolecular ion [M-H][−] at m/z 609.1242, allowing us to access the molecular formula C₃₀H₂₆O₁₄. All of the fragment ions obtained were found to be typical of the proanthocyanidin family. Heterocyclic ring fission (HRF), retro-Diels–Alder (RDA) and quinone-methide (QM) were the characteristic fragmentation mechanisms observed for this class of molecule. Indeed, RDA and HRF fragmentation provide information on bond hydroxylation following ring cleavage, while interflavan bond cleavage, also called quinone methide (QM) fragmentation, can reveal information on the nature of monomeric units. A proposed fragmentation pathway describing the formation of the characteristic fragment ions for this molecule is depicted in Figure 3. First, the fragmentation of the [M-H][−] ion at m/z 609.1242 occurred after a neutral loss of 168 Da to the low-intensity fragment ion at m/z 441.0825 by the RDA mechanism in the C ring. Thereafter, the loss of a water molecule (−18 Da) from the ion at m/z 441.0825 was observed to result in the ion at m/z 423.0722, which was the base peak in the spectrum. The fragment ion at m/z 305.0667 was obtained from m/z 609.1242 by an interflavan bond cleavage (QM) between the C and D rings, evidencing the nature of this compound as one composed of two units having the same mass, supporting the

hypothesis of a dimer of (epi)gallocatechin [56,61]. The presence of the fragment ion at m/z 125.0241 can be explained by the HRF mechanism from the corresponding ion of (epi)gallocatechin at m/z 305 releasing phloroglucinol from the A ring [62]. The other fragments detected at m/z 177, 219, 261 and 285 were characteristic ions produced from the fragmentation of m/z 305 [43,62]. It should be noted that epigallocatechine dimers composed of two (epi)gallocatechin units have been previously identified in grape canes and red wine [56,61,63] but never in *P. lentiscus* extracts so far.

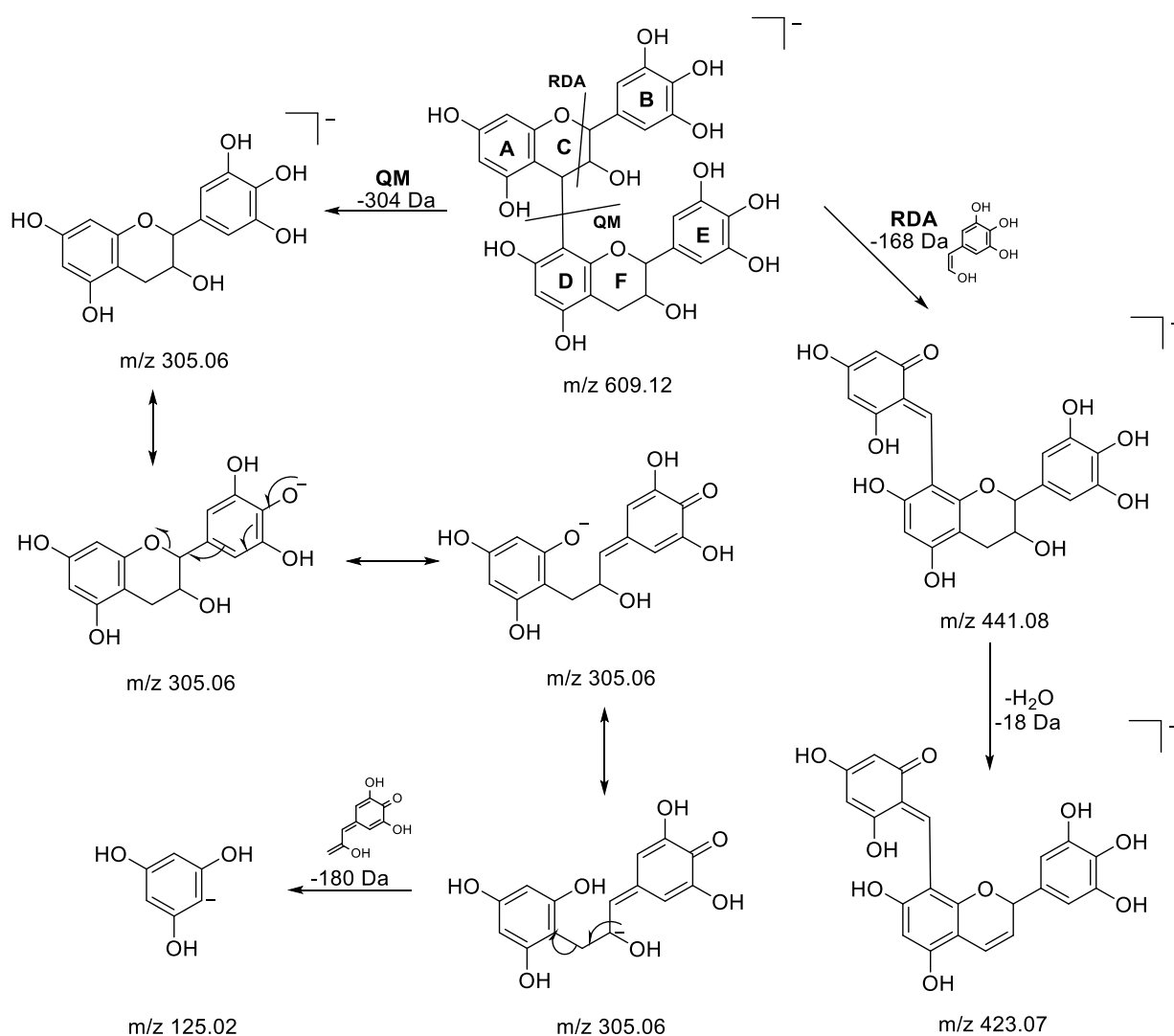


Figure 3. Proposed fragmentation pathways for epigallocatechin(4a->8)epigallocatechin.

Epigallocatechin(4a->8)epigallocatechin (isomer of metabolite 1): Metabolite 4 (RT 0.61 min) was putatively identified by HDMS^E as an isomer of metabolite 1 (epigallocatechin(4a->8)epigallocatechin, a dimer of epigallocatechine), as the same molecular formula, $C_{30}H_{26}O_{14}$, was found from the corresponding $[M-H]^-$ ion at m/z 609.1233. Moreover, the same characteristic fragment at m/z 423.0716 was obtained from the precursor ion at m/z 609.1233 by the loss of an RDA fragment (-168 Da) followed by the loss of water (-18 Da) (Figure 3).

(Epi)gallocatechin-3'-O-galloyl-(epi)gallocatechin: Metabolite 3 (RT 0.56 min), detected at m/z 761.1339 with the corresponding molecular formula $C_{37}H_{30}O_{18}$, was identified as (epi)gallocatechin-3'-O-galloyl-(epi)gallocatechin. Firstly, the fragmentation of the $[M-H]^-$ ion at m/z 761.1339 resulted in the fragment at m/z 609.1228 by the loss of 152 Da corresponding to a galloyl residue. For this last fragment ion, the molecular formula

$C_{30}H_{26}O_{14}$ was obtained, revealing that this metabolite could have an epigallocatechin dimer structure type. Indeed, the typical fragment ions at m/z 423.0820 and 305.0568, as previously described for epigallocatechin dimers, were observed. In addition, two other fragments were observed on the fragmented HDMS^E spectrum in agreement with the structure proposed for metabolite 3, namely, the ion at m/z 591.1134, which was produced by the loss of a water molecule (-18 Da) from the m/z 609.1228, and the ion at m/z 465.0820 obtained by the loss of a phloroglucinol (-126 Da) molecule at m/z 591.1134. The details of the fragmentation mechanism and of the characteristic fragmentation structures discussed above can be found in Figure 4a,b.

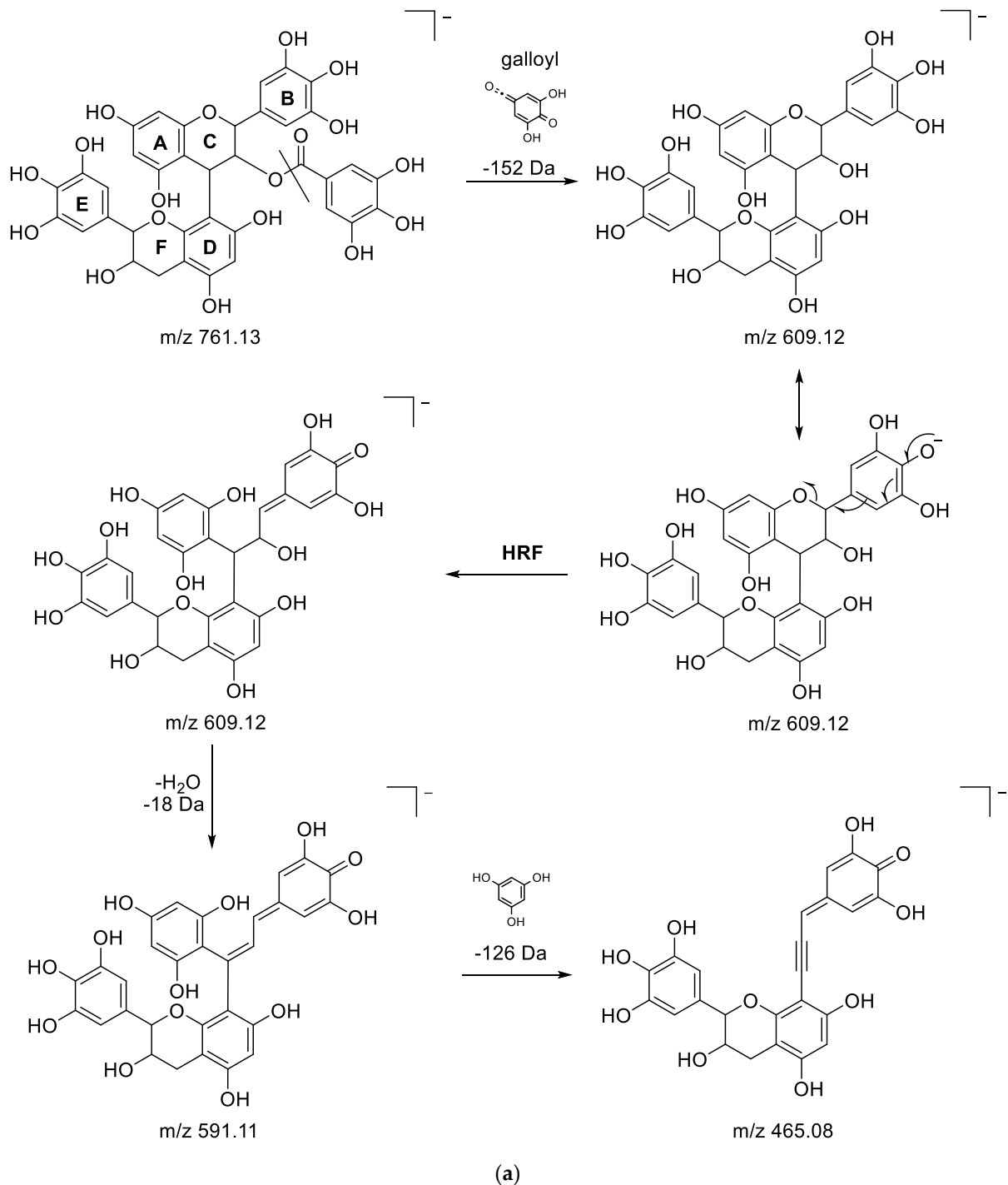


Figure 4. Cont.

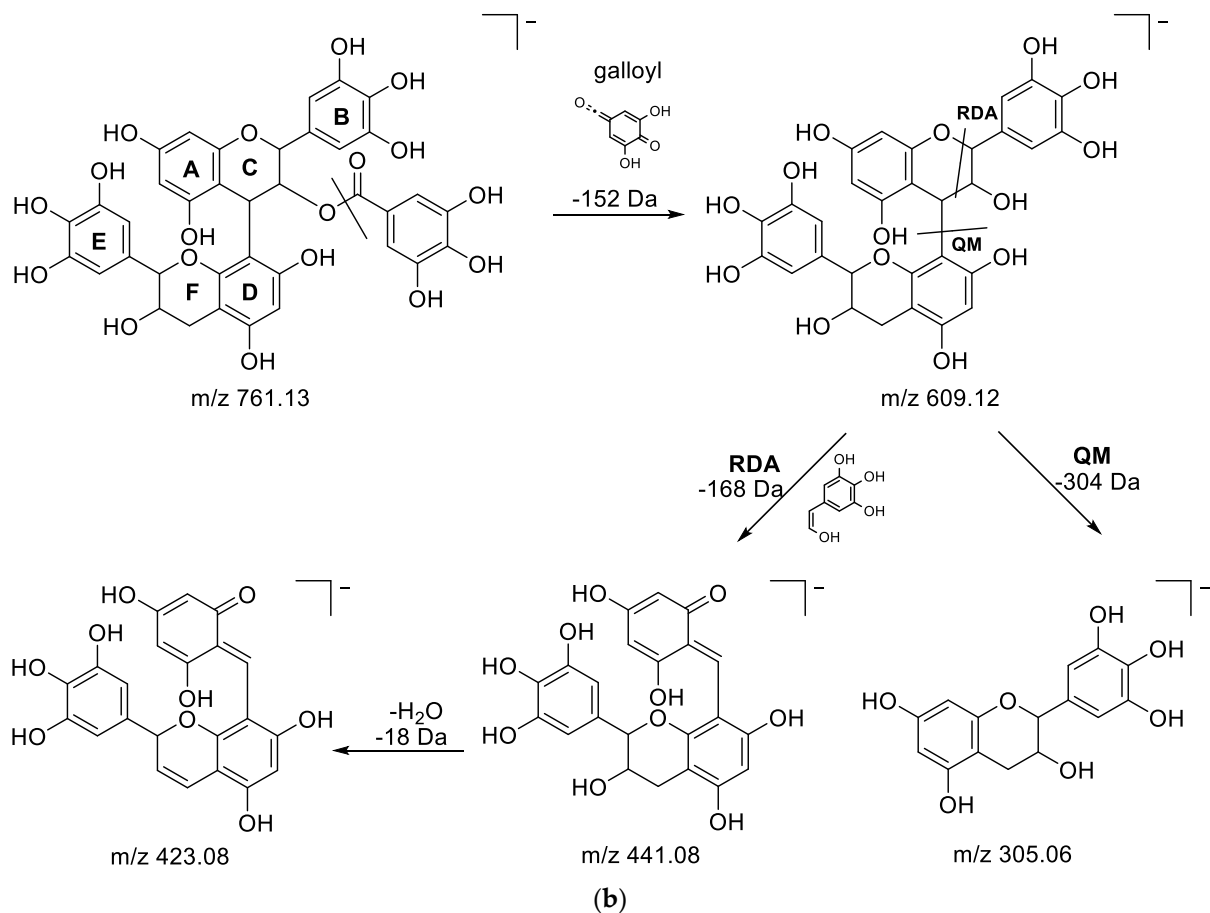


Figure 4. (a) Fragmentation pathways of (epi)galloocatechin-3'-O-galloyl-(epi)galloocatechin showing the formation of the fragmentations at m/z 609.12, 591.11 and 465.08. (b) Fragmentation pathways of (epi)galloocatechin-3'-O-galloyl-(epi)galloocatechin showing the formation of the fragment ions at m/z 423.08 and 305.06.

From the preceding points, this compound was identified as (epi)galloocatechin-(4,8')-3'-O-galloyl-(epi)galloocatechin [58]. This compound has never been detected in *P. lentiscus* extracts before.

(Epi)galloocatechin-3'-O-galloyl-(epi)galloocatechin (isomer of metabolite 3): Metabolite 5 (RT 0.72 min) was putatively identified by HDMS^E as an isomer of metabolite 3 ((epi)galloocatechin-3'-O-galloyl-(epi)galloocatechin), as the same molecular formula, C₃₇H₃₀O₁₈, was found from the corresponding [M-H][−] ion at m/z 761.1344. Moreover, the same characteristic fragment ion at m/z 423.0716 was obtained from the precursor ion at m/z 609.1233 by the loss of an RDA fragment (−168 Da) followed by the loss of water (−18 Da) (Figure 4b). All of these observations are in agreement with a structure that could be an isomer of (epi)galloocatechin-3'-O-galloyl-(epi)galloocatechin.

3,5-O-digalloylquinic acid: Metabolite 2 (RT 0.55 min) was putatively identified as 3,5-O-digalloylquinic acid. It was characterized by an [M-H][−] ion at m/z 495.0774, enabling us to find the molecular formula C₂₁H₂₀O₁₄. Under fragmented HDMS^E conditions, the [M-H][−] ion at m/z 495.0774 underwent two successive losses of a galloyl moiety (−152 Da), leading to fragment ions at m/z 343.0661 and 191.0551. The accurate mass measurement for the fragment at m/z 191.0551, with the determination of its molecular formula as C₇H₁₁O₆, confirmed the quinate structure, and for the fragment ion at m/z 169.0136 with the corresponding molecular formula C₇H₅O₅, a gallate structure was determined [42,43,64]. A proposed fragmentation pathway is described in Figure 5.

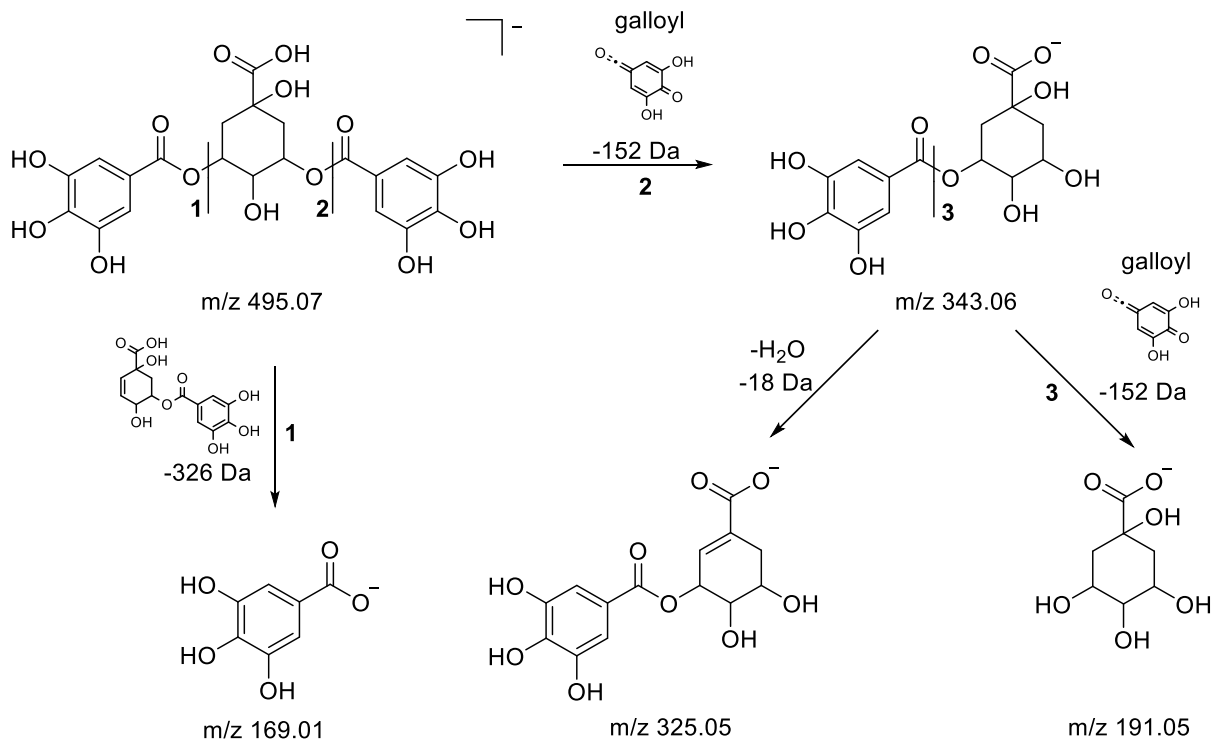


Figure 5. Fragmentation pathway of 3,5-O-digalloylquinic acid.

This compound, labeled metabolite 2, was recently identified in *P. lentiscus* leaves [43,64].

Dihydroxy benzoic acid pentoside: Metabolite 6 (RT 0.73 min) was characterized by an $[M-H]^-$ ion at m/z 285.0608, enabling us to find the molecular formula $C_{12}H_{14}O_8$ for this compound. Under fragmented HDMS^E conditions, the $[M-H]^-$ ion at m/z 285.0608 first underwent a loss of dehydrated pentose (-132 Da) to provide hydroxybenzoate, which was immediately followed by the loss of a radical species, CO_2H (-45 Da), leading to the radical anion at m/z 108.0209 [60]. All of the structures of the fragments and the losses are presented in Figure 6. Thus, metabolite 6 was putatively identified as dihydroxy benzoic acid pentoside.

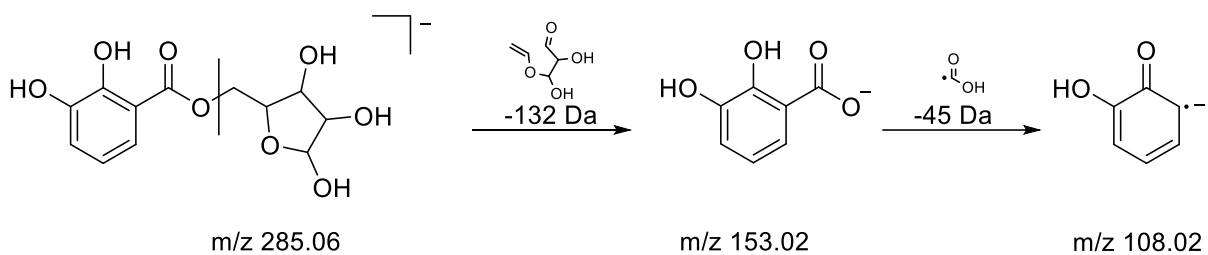


Figure 6. Fragmentation pathways of dihydroxy benzoic acid pentoside.

3.4. Intensity Variation of the Metabolites Identified

The univariate analysis generated box plots of the metabolites identified (in negative ionization, Figure 7). Each box plot presents the contents of a metabolite in the different lentisk parts from the mountain and littoral. The metabolite intensities were expressed as the area of the chromatographic peak corresponding to each metabolite in the extracts. The different letters indicate that the intensities were significantly dissimilar after a post hoc Kruskal–Wallis test (p -value < 0.05) for each compound. This statistical test showed that the location of the plant had no significant effect on the variation of the intensity of the metabolites detected in the lentisk's different parts.

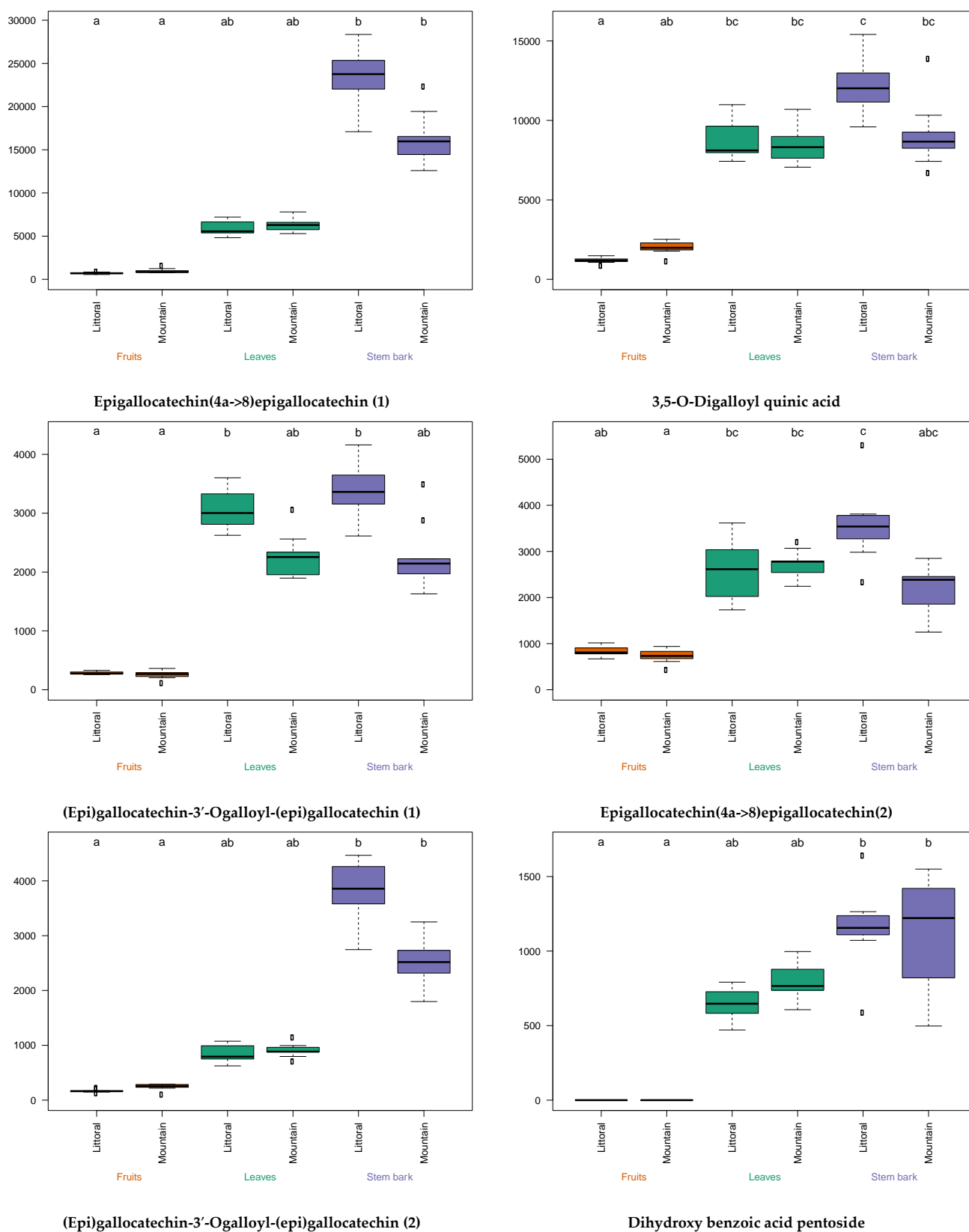


Figure 7. Box plots of the metabolite contents in different organs—leaves, stem barks and fruits—from the mountain and littoral. (The significance of the metabolite intensity variation is presented by letters (groups, a, b, c, ab, bc and abc) according to pairwise comparison through the Kruskal-Wallis test adjusted with Bonferroni correction).

According to the intensities of the identified metabolites (i.e., chromatographic peak areas), we can say that the samples from the littoral had higher concentrations of α -glucosidase-inhibitory compounds, especially the stem barks. These results are consistent with those of the multiblock OPLS analysis.

4. Discussion

In the present work, *P. lentiscus* was studied for its use as a hypoglycemic agent in traditional medicine. This is the first study that reports the phytochemical screening of α -glucosidase-inhibiting compounds. These compounds were present in the different parts of *P. lentiscus* harvested in two different regions. The work was based on a chemometric study that linked biologic activity to data from a UHPLC-ESI-HRMS analysis.

Our results show the variability of the inhibition of α -glucosidase in regards to the organ and the geographical origin of Algerian lentisk. The α -glucosidase inhibition of lentisk stem bark was measured in this study. Compared to the previous studies in the literature, the fruit extracts (IC₅₀ = 42 μ g/mL) showed a greater inhibitory potency than did Sardinian *P. lentiscus* fruit aqueous extracts, with IC₅₀ = 230 μ g/mL [30]. The inhibition percentages of α -glucosidase of the leaves and fruit extracts (49% and 13%, respectively) were comparable to the inhibition percentages of α -amylase of ethanolic leaf (55%) and fruit (54%) extracts [23]. The activity of α -glucosidase inhibition has been tested in several other plants [65–67]. Plant extracts including *Tamarix nilotica* (IC₅₀ = 33.3 μ g/mL) [65], *Tetracera scandens* (IC₅₀ = 3.4 μ g/mL) [66] and *Cosmos caudatus* (IC₅₀ = 39.1 μ g/mL) [67] have shown promising α -glucosidase inhibition. These activities are comparable to those of acarbose and to the results found in the present study. In our case, the geographical origin did not have a significant effect on the variation of the α -glucosidase inhibition. A previous study revealed that *Parkia speciosa* pods collected from different locations in Malaysia show different levels of α -glucosidase-inhibitory activity [68]. Our results indicate that the change in the α -glucosidase inhibition activity was more significant according to the part of the plant than its habitat. This is explained by the variation in the chemical compositions of the different extracts. Multivariate models can show the predictive power of metabolomic analysis for α -glucosidase inhibition and can indicate which groups of metabolites contribute most to this activity [24,25]. Therefore, the metabolomic approach applied in our study allowed us to characterize the metabolites that contributed more to the inhibition of α -glucosidase enzymatic activity. A total of six compounds were identified, of which four were flavonoids derived from epigallocatechin. To the best of our knowledge, epigallocatechin(4a→8)epigallocatechin (two isomers), (epi)gallocatechin-3'-O-galloyl-(epi)gallocatechin (two isomers) and dihydroxy benzoic acid pentoside, but not 3,5-O-digalloylquinic acid, were identified in the different extracts (i.e., leaves, stem barks and fruits) of *P. lentiscus*. The monomer epigallocatechin, the basic structure of four molecules among these metabolites, has been identified previously in lentisk leaves [19,64]. Epigallocatechin, analyzed by UHPLC-ESI-HRMS as a standard, was detected even in our lentisk extracts. In addition, this compound had a better α -glucosidase-inhibitory activity (81.7%) compared to the other standards tested. Therefore, it is quite coherent that the metabolites derived from epigallocatechin (M1, M3, M4 and M5) contribute to the inhibition of α -glucosidase. Previous work has established statistical models of the relationship between the phytochemical profiles obtained via LC-MS/MS and the inhibition of α -glucosidase by plant extracts [66,67,69]. The results of this work revealed that α -glucosidase-inhibitory activity was probably due to the presence of terpenoids, fatty acids, phenolic, flavonoids (epigallocatechin-3-O-gallate) and flavonoid glycosides (quercetin 3-O-glucoside) [65,67]. (-)-Epigallocatechin-3-O-gallate offers a promising hypoglycemic effect [65,70].

Most of these metabolites, especially epigallocatechin derivatives, have been reported for their ability to inhibit α -glucosidase, isolated from other plants [24,26]. Flavanol compounds (i.e., epigallocatechin, epigallocatechin gallate isomers, asinensin A, aflavin-3-gallate isomers, gallocatechin-(4 α →8)-epigallocatechin and herbacetin) identified in

P. speciosa most likely have a major role in α -glucosidase-inhibitory activity [68]. Previous studies determined several key structural features needed for monomeric flavonoids to inhibit α -glucosidase activity [71–73]. The compounds derived from epigallocatechin (i.e., epigallocatechin-(4 α →8)-epigallocatechin (two isomers) and(epi)gallocatechin-3'-O-galloyl-(epi)gallocatechin (two isomers)) and identified in *P. lentiscus* have been described as having a major role in α -glucosidase-inhibitory activity because of the structural configuration of the double bonds conjugated to the 4-oxo and hydroxyl groups [73]. In addition, the presence of an esterified gallate group at the 3 position of the C ring has been suggested to be critical for the interaction of flavan-3-ols with the α -glucosidase enzyme [71,72]; this is the case for the molecule (epi)gallocatechin-3'-O-galloyl-(epi)gallocatechin. In addition, the substitution of sugar units or a hydroxyl group by a galloyl increases the efficacy of flavonoids against α -glucosidase [74]. The molecule 3,5-O-digalloylquinic acid probably has an important role in the inhibition of α -glucosidase. An ethnomedicinal study carried out on the hypoglycemic effects of medicinal plants showed that the leaves and fruits of *Arbutus unedo*, an antidiabetic medicinal plant, are rich in galloyl quinic acid [18]. A study by Quaresma et al.(2020) [75] reported that dihydroxy pentoside acid is among the phenolic compounds that can influence the inhibition of the active site of enzymes [75]. In this study, the butanol fraction of *Banisteriopsis argyrophylla* leaves rich in dihydroxy pentoside acid showed good inhibitory activity of α -glucosidase.

Examining the literature, in terms of lentisk phytochemistry composition, the leaf is the most studied. Shikimic acid [43], myricetin and its derivatives, including myricetin galactosides, as well as quercetin and its derivatives, were detected [64]. Luteolin-7-O-glycuronide, luteolin-3-O-rutinoside and kaempferol-3-O-di-hexose-O-pentose [19] were identified in the methanolic extracts. Other researchers have reported the presence of pentoside derivatives including quercetin-O-galloylpentoside in hydromethanolic leaf extracts [43]. Phenolic acids including gallic acid, 3-galloyl quinic acid, 5-galloyl quinic acid, 3,5-di-O-galloyl quinic acid and 1,5-di-O-galloyl quinic acid were detected in the aqueous extracts of leaves and fruits [30]. A digalloylquinic acid derivative was detected in the lentisk fruit and stem [22]. Catechin was the most representative flavanol in *P. lentiscus* leaves [23,76], while procyanidin B1 was the most abundant flavanol in the fruit extracts [77].The study conducted by Aissat et al. (2022) [78] resulted in the identification of seven flavanols, such as procyanidin B1 and B3, epigallocatechin gallate and gallocatechin and epicatechin gallate, in five different physiological stages of lentisk fruit from Algeria. There is only one study describing the phytochemical profile of the whole stem [22]. Therefore, our study is the first report on the phytochemical composition of lentisk stem barks.

Studies have shown that a plant's geographical location has a significant influence on the composition and content of secondary metabolites [48,68]. According to the boxplots presented in Figure 7, the intensity of the identified metabolites was relatively stable (letters a, b, ab) according to the geographical origin (i.e., littoral and mountain). The slight variations were due to the many climatic and abiotic factors which can affect the biosynthesis of secondary metabolites in plants [68,79]. Colder temperatures at higher altitudes (mountains) can cause overproduction of phytochemicals [79]. Previous research has suggested that increasing altitude and consequent changes in solar radiation and temperature in plant habitats (as in Tables S1 and S2) may be strongly correlated with the contents of secondary metabolites, especially phenolics, due to their defensive function against oxidative damage [79]. On the other hand, the variation in the contents of metabolites (α -glucosidase inhibitors) depending on the part of the *P. lentiscus* plant used was more significant. The plant part plays a key role in the variation of the chemical composition of the plant extract [22]. The stem barks were characterized by an abundance of these α -glucosidase inhibitors compared to the other lentisk organs (i.e., leaves and fruits) at both sites (i.e., littoral and mountain). The results of this study suggest that lentisk stem bark from the littoral area is a good source of α -glucosidase inhibitors.

5. Conclusions

The results of this work revealed that the stem bark and leaves from *P. 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. A multi-block OPLS analysis was used to define the molecules that exhibited α -glucosidase inhibitory effects. The UHPLC-ESI-HRMS analysis enabled the identification of six metabolites: epigallocatechin(4a->8)-epigallocatechin (two isomers), (epi)gallocatechin-3'-O-galloyl-(epi)gallocatechin (two isomers), 3,5-O-digalloylquinic acid and dihydroxy benzoic acid pentoside. The intensities of these metabolites varied depending on the lentisk organ and habitat. The results of our study justify scientifically and consolidate the ethnopharmacological reports on the traditional use of lentisk leaves for the treatment of type 2 diabetes. According to these results, the use of stem barks could be better adapted to the treatment of type 2 diabetes by traditional means—for example, the elaboration of traditionally improved drugs (TID) in developing countries. The antidiabetic potential of this plant could also be exploited in the field of food supplements.

Further studies on the identification of other active metabolites and their synergistic effects on α -glucosidase-inhibitory activity are still needed.

It is also important to consider preclinical and clinical trials of these lentisk extracts to determine their mechanisms of inhibition, their pharmacokinetics and their toxicology. Our results on the antidiabetic activity carried out *in vitro* show that the variation was quite stable according to the habitat. The same was true for the intensity variation of the metabolites that contributed most to this activity. Therefore, it would be interesting to extend the sampling to several geographical sites to better evaluate the antidiabetic activity and phytochemistry of lentisk as they relate to the environmental conditions of its habitat.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/metabo13020275/s1>, Figure S1: Boxplot showing the α -glucosidase-inhibitory effect of lentisk leaves, stems barks and fruits from the mountain and littoral; Figure S2: Contribution of the two blocks (neg and pos) to the constitution of the OPLS model; Table S1: Weather data collected between 1999 and 2019 in Ait-Irane, Algeria; Table S2: Weather data collected between 1999 and 2019 in Tizirt, Algeria; Table S3: Metabolomic data (OPLS, matrix-negative ionization); Table S4: Metabolomic data (OPLS, matrix-positive ionization); Table S5: UHPLC-ESI-HRMS data results and OPLS coordinates; Table S6: Phenolic compounds identified in *P. lentiscus* extracts by UHPLC-ESI-HRMS in the positive mode.

Author Contributions: Conceptualization, C.S. and R.M.; methodology, C.S. and R.M.; software, J.-X.F., C.S. and R.M.; validation, R.M., D.M., J.-X.F., N.J., F.A., F.F. and E.G.; formal analysis, C.S., R.M. and J.-X.F.; investigation, C.S., R.M., J.-X.F. and D.M.; resources, C.S., E.G. and F.F.; data curation, C.S., R.M., J.-X.F. and D.M.; writing—original draft preparation, C.S., R.M., D.M., J.-X.F., F.F., N.J., E.G. and F.A.; visualization, C.S., R.M., D.M., J.-X.F., F.F., N.J., E.G. and F.A.; supervision, E.G., F.F. and N.J.; project administration, C.S., E.G., F.F. and N.J.; funding acquisition, C.S., E.G., F.F., R.M. and D.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by (1) the French Ministry of Foreign Affairs through Campus France (No.973496A); (2) the “Conseil Régional de Picardie” through the MTV (Métabo-Typage Végétal) project; (3) the University of Picardie Jules Verne through the French Ministry of Higher Education and Research; the region “Hauts de France”; and the European FEDER fund within the so-called CPER programs devoted to equipment acquisitions. This work was also funded by Algerian authorities through the financial support of the Tizi-Ouzou UMMTO University and a social grant attributed to Chabha Sehaki for her PhD.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article or the Supplementary Materials.

Acknowledgments: The authors thank Salima Benhouhou (Ecole Nationale Supérieure Agronomique, ENSA, Algiers, Algeria) for the identification of the *P. lentiscus* plants, the recording of samples and the delivery of a certificate.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Données supplémentaires (article 4)

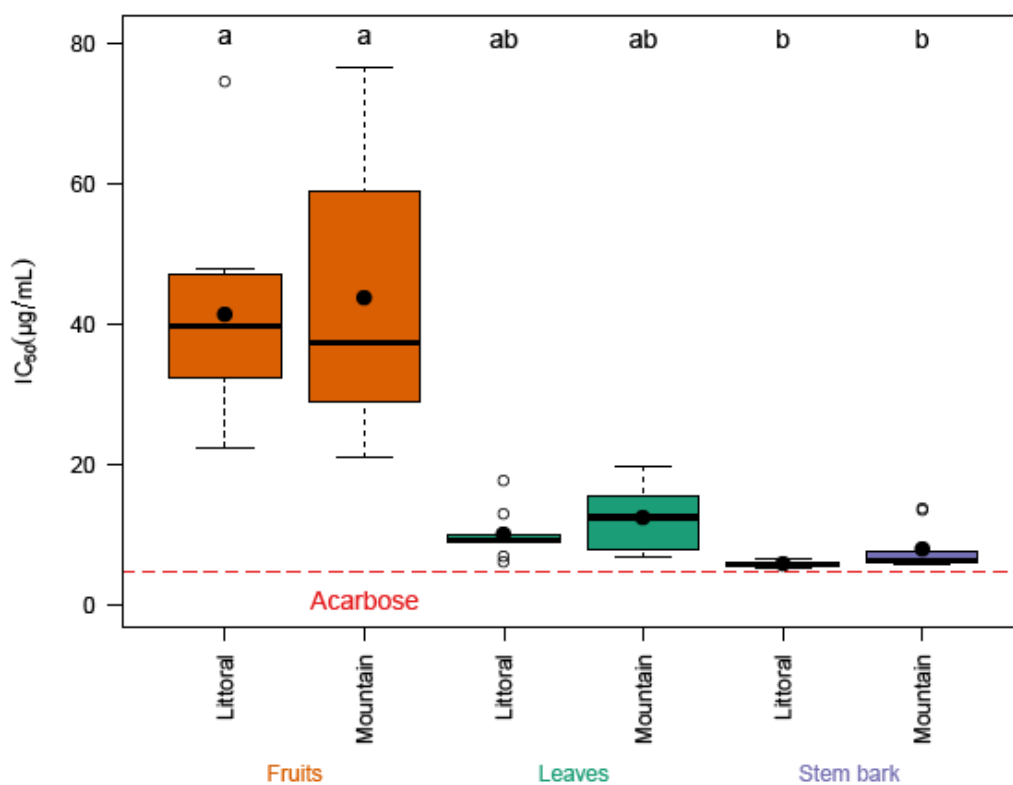


Figure S1: Boxplot showing α -glucosidase inhibitory effect of lentisk leaves, stems barks and fruits from mountain and littoral

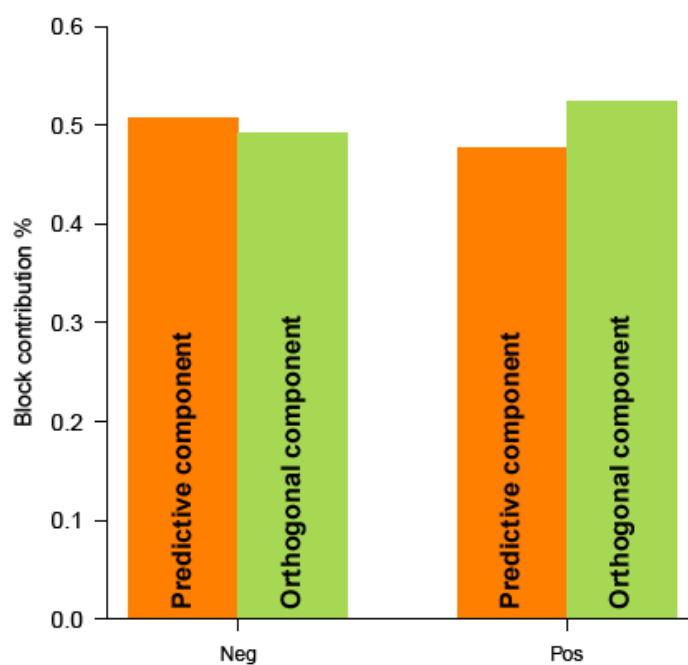


Figure S2: Contribution of the two blocks (neg and pos) to the constitution of the OPLS model

Table S1: Weather data collected between 1999 and 2019 in Ait-Irane, Algeria (from <https://fr.climate-data.org/afrique/algerie/tizi-ouzou/ait-irane-693594/>)

	January	February	March	April	May	June	July	August	September	October	November	December
Meantemperature(°C)	5.2	5.3	8.5	11.6	15.6	20.8	24.8	24.5	20	15.9	9.4	6.2
Mean minimum temperature(°C)	0.8	0.5	3.1	5.8	9.4	13.9	17.5	17.8	14.5	10.7	5.2	2.1
Maximum temperature(°C)	10.4	10.7	14.3	17.5	21.6	27.4	31.9	31.6	26.3	21.9	14.5	11.2
Precipitation (mm)	88	78	84	86	74	21	8	19	53	60	81	82
Humidity (%)	79	77	73	70	67	55	45	47	59	64	75	79
Rainydays (jree)	9	8	8	8	7	3	2	4	7	7	9	8
Hours of sunshine (h)	5.7	6.4	7.6	9	10.3	12.3	12.6	11.7	9.9	8.4	6.4	5.7

Table S2: Weather data collected between 1999 and 2019 in Tizirt, Algeria (from <https://fr.climate-data.org/afrique/algerie/tizi-ouzou/tizirt-321206/>)

	January	February	March	April	May	June	July	August	September	October	November	December
Meantemperature(°C)	10.2	10	12.1	14.2	17.1	21.1	24.4	25	22.3	19.3	14.1	11.3
Mean minimum temperature(°C)	7.7	7.4	9.3	11.3	14.1	17.9	21.2	22	19.6	16.5	11.7	8.9
Maximum temperature(°C)	12.8	12.7	14.8	16.8	19.7	23.9	27.3	27.9	25	22.2	16.8	13.9
Precipitation (mm)	142	107	86	68	51	9	2	6	36	73	140	146
Humidity(%)	75	74	75	77	77	72	69	69	71	71	73	74
Rainydays (jree)	10	8	7	6	4	1	0	1	4	6	10	10
Hours of sunshine (h)	7	7.8	9	10.2	11.1	12.2	12.3	11.3	10.1	9.1	7.6	7

Table S6: Phenolic compounds identified in *Pistacia lentiscus* extracts by UHPLC-ESI-HRMS in positive mode

N° Metabolite	RT (min)	m/z (obs) (M-H) ⁻	Error (ppm)	HDMS ^E Fragment Ions (Intensity, %)	Molecular Formula	Suggested Compound
1	0.45	611.1391	-0.59	425.08572 (42.86), 299.05412 (66.25), 263.05419 (43.44), 175.03792 (20.42)	C ₃₀ H ₂₆ O ₁₄	Epigallocatechin(4a>8)epigallocatechin
3	0.56	763.1505	-0.29	425.08596 (28.64), 299.054 76 (23.31),	C ₃₇ H ₃₀ O ₁₈	(Epi)gallocatechin-3'-Ogalloyl-(epi)gallocatechin
4	0.61	611.1383	-1.99	425.08572 (42.86), 299.0542 (66.25), 263.05419 (43.44)	C ₃₀ H ₂₆ O ₁₄	Epigallocatechin(4a->8)epigallocatechin (isomer)
5	0.72	763.1504	0.31	/	C ₃₇ H ₃₀ O ₁₈	(Epi)gallocatechin-3'-Ogalloyl-(epi)gallocatechin (isomer)

Les données supplémentaires sur les données brutes de l'approche métabolomique (Table S3, S4 et S5) sont disponibles sur <https://www.mdpi.com/article/10.3390/metabo13020275/s1>

Discussion générale, conclusions et perspectives de la Thèse

Les résultats du travail de synthèse réalisé ici, illustrent que la composition chimique de la plante *Pistacia lentiscus* est caractérisée à la fois par des traits constants et par des différences (qualitatives et quantitatives) en fonction des origines (potentiellement génétiques) de la plante et des facteurs environnementaux, biotique et abiotiques (géographie, température, durée du jour, sols, partie de la plante utilisée... etc).

Un large spectre de propriétés médicinales avérées ou potentielles a déjà été démontré pour différents produits de *Pistacia lentiscus*. Dans ce contexte, nos travaux de recherche visaient l'approfondissement de l'étude des substances bioactives de cette plante. Il s'agit à la fois de compléter des analyses phytochimiques, évaluer-confirmer des propriétés bioactives, en s'appuyant sur la variabilité spontanée trouvée dans l'espace naturel algérien de la région de Tizi-Ouzou.

La première partie de ce projet de thèse menée sur la caractérisation des huiles essentielles de lentisque de Tizi-Ouzou a abouti à de nouveaux résultats. Notre étude a ainsi montré que le changement d'altitude et la période de récolte affectent la nature chimiotypique des huiles essentielles de lentisque ainsi que leur potentiel antioxydant; en particulier aux altitudes les plus élevées. La population naturelle de *Pistacia lentiscus* du littoral de Tizi-Ouzou peut être recommandée pour la production d'une HE chémotypée assez constante. A l'inverse, en raison de la variabilité saisonnière, les HE des populations de haute altitude pourraient être mieux adaptées à l'obtention de chémotypes originaux dédiés (voire à la maîtrise de leur culture) pour l'exploitation de leur potentiel thérapeutique.

Ces observations montrent également qu'il est possible d'étudier et de connaître les variations de la composition chimique de l'huile essentielle au fil des saisons et ainsi de définir le meilleur moment pour la récolte par le meilleur équilibre entre rendement et composition chimique. Il est important de connaître les facteurs influençant la composition de l'huile essentielle. Cela permet de contrôler ceux qui sont possibles (période de récolte, altitude de récolte, méthode d'extraction, sexe de la plante) et d'anticiper (c'est-à-dire de prédire partiellement) la composition de l'HE obtenue. Cela intéresse les aromathérapeutes industriels qui recherchent de bons rendements dans l'extraction de molécules spécifiques ou une composition chimique stable.

La seconde partie de la thèse portant sur le profilage métabolomique des extraits phénoliques a permis d'identifier pour la première fois les marqueurs de l'inhibition de l' α -glucosidase chez le lentisque. L'activité antidiabétique *in vitro* réalisée par l'étude de l'inhibition de l' α -glucosidase varie en fonction de la partie de la plante et de son origine géographique. Les écorces des tiges récoltées du littoral présentent la plus forte activité inhibitrice sur l' α -glucosidase. Cette activité est comparable à celle du médicament de référence « l'acarbose ». Ceci montre que le potentiel antidiabétique de cette plante est intéressant et justifie son usage en médecine traditionnelle pour le traitement du diabète.

Des études complémentaires sur l'identification d'autres métabolites actifs dans ces tiges et leurs effets synergiques sur l'activité inhibitrice de l' α -glucosidase sont encore nécessaires.

Il est également important d'envisager des essais précliniques et cliniques de ces extraits de lentisque afin de déterminer leurs mécanismes d'inhibition, leur pharmacocinétique et leur toxicologie. Nos résultats sur l'activité antidiabétique réalisée *in vitro* montrent que la variation est assez stable en fonction de l'habitat. Il en est de même pour la variation d'intensité des métabolites qui contribuent le plus à cette activité. Il serait donc intéressant d'étendre l'échantillonnage à plusieurs sites géographiques pour mieux évaluer l'activité antidiabétique et la phytochimie du lentisque en fonction des conditions environnementales de son habitat.

L'ensemble des travaux réalisés dans cette thèse met en évidence ce qui est connu, ce qui est commun, et ce qui varie entre les différentes occurrences de *P.l.*. Autrement dit, cette thèse constitue une phase initiale qui pourrait contribuer à la valorisation du lentisque algérien. En s'appuyant sur les différents facteurs ayant un impact sur la variabilité de la composition chimique et du rendement de ces métabolites étudiés, il serait possible d'optimiser leur production. Cela permettrait de développer des applications scientifiques -et donc, rationnelles- pour la médecine, la pharmacie ou d'autres utilisations industrielles basées sur les activités biologiques d'extraits de plantes isolées à partir des ressources de *Pistacia lentiscus*. Par exemple, développer une micro-filière de valorisation de la partie aérienne de lentisque en appliquant des pratiques de récoltes respectueuses à cette ressource naturelle. Cela permettra la valorisation de l'huile essentielle des feuilles, en fonction de la nature des chémotypes détectés. Selon nos résultats, l'huile essentielle de lentisque de Tizi-Ouzou pourrait être utilisée pour les propriétés antimicrobiennes de ses chémotypes pour la conservation des aliments. Il est important de noter la présence de sabinène (0.3-1.4 %) dans

nos huiles essentielles, une molécule neuro-toxique. Pour un éventuel usage de cette huile comme additif pour la conservation des aliments, il serait nécessaire d'isoler le sabinène de cette huile. Quant aux extraits phénoliques, les résultats de notre étude justifient scientifiquement et consolident les rapports ethnopharmacologiques sur l'utilisation traditionnelle de lentisque pour le traitement du diabète de type 2. L'utilisation des écorces de tiges pourrait être mieux adaptée au traitement du diabète de type 2 et valorisée par des moyens traditionnels - par exemple, l'élaboration de médicaments traditionnellement améliorés (MTI) dans les pays en voie de développement. Le potentiel antidiabétique de cette plante pourrait également être exploité dans le domaine des compléments alimentaires.

Références (pour la partie rédigée en Français et par chapitres)

Introduction

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Chapitre I

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Annexes

Communications et publications scientifiques

- Publications:

Profiling of Essential Oils from the Leaves of *Pistacia lentiscus* Collected in the Algerian Region of Tizi-Ouzou: Evidence of Chemical Variations Associated with Climatic Contrasts between Littoral and Mountain Samples. <https://doi.org/10.3390/molecules27134148>

A Review of *Pistacia lentiscus* Polyphenols: Chemical Diversity and Pharmacological Activities <https://doi.org/10.3390/plants12020279>

Metabolomics-Based Profiling via a Chemometric Approach to Investigate the Antidiabetic Property of Different Parts and Origins of *Pistacia lentiscus* L. <https://doi.org/10.3390/metabo13020275>

- Communications :

Communications par affiche:

1/ SEHAKI C., FERNANE F., AYATI F., GONTIER E. (2019) Evaluation de la composition chimique de l'huile essentielle de *Pistacia lentiscus* de Tizirt. Journée Scientifique de l'Univ. De Batna, 27 Novembre 2019, Batna, **Algérie**.

2/ SEHAKI C., FERNANE F., AYATI F., GONTIER E. (2020) Phytochemical tests and chemical composition of extracts *Pistacia lentiscus* leaves of Tizi-Ouzou. Third International Symposium: Medicinal Plants and Materials (MPM-2020), 25-27 February 2020, Tebessa, **Algeria**.

3/ SEHAKI C., GONTIER E., FERNANE F., CHOQUE E., MEGHZI R. (2020) Chemical composition and antioxidant activity of essential oil of *Pistacia lentiscus* from Algeria. Sixth international Mediterranean symposium on medicinal and Aromatic Plants, 15-17 October 2020, Izmir, **Turkey**.

4/ SEHAKI C., FERNANE F., GONTIER E., BERGIA T., AYATI F., MEGHZI R. (2021) Effet de la technique d'extraction sur le rendement et la composition chimique de l'huile essentielle de *Pistacia lentiscus* de Tizi-ouzou. Les journées jeunes chercheurs Condorcet, SFR Condorcet FR CNRS 3417, 14-15 Janvier 2021, Amiens, **France**.

Communications orales:

1/ SEHAKI C., FERNANE F., BERGIA T., GONTIER E. (2021) Effet de paramètres environnementaux sur la composition chimique de l'huile essentielle de *Pistacia lentiscus* de Tizi-Ouzou. Les journées jeunes chercheurs Condorcet, SFR Condorcet FR CNRS 3417. 14-15 Janvier 2021, Amiens, **France**.

2/ SEHAKI C., GONTIER E., FERNANE F., PAWLICKI-JULLIAN N., CHOQUE E., MEGHZI R. (2021) Caractérisation CG-MS d'un extrait de la plante méditerranéenne « *Pistacia lentiscus* » et étude de son potentiel antioxydant par les trois tests ABTS, DPPH et FRAP. Journée Scientifique MétaSP (Réseau métabolisme spécialisé des plantes). 15 Octobre 2020, Amiens, **France**.

3/ SEHAKI C., GONTIER E., FERNANE F., PAWLICKI-JULLIAN N., CHOQUE E., DAUWE R., MOLINIE R., FONTAINE J-X. (2021) Essential oils from *Pistacia lentiscus* leaves: variability of chemotypes and antioxidant activities depending on altitude and sampling period. Présentation au sein de l'UMR transfrontalière Bio Eco Agro. 6 décembre 2021, Amiens, **France**

4/ SEHAKI C., GONTIER E., FERNANE F., PAWLICKI-JULLIAN N., CHOQUE E., DAUWE R., MOLINIE R., FONTAINE J-X. (2021) Variabilité des chémotypes et des activités antioxydantes des huiles essentielles de feuilles de *Pistacia lentiscus* selon l'altitude et la période d'échantillonnage. Présentation au sein de l'équipe 5 de l'UMR transfrontalière Bio Eco Agro. 7 décembre 2021, Amiens, **France**

- **Autres publications:**

SEHAKI C. (2020) « Les plantes médicinales ; un avenir prometteur ». <https://theconversation.com/les-plantes-medicinales-un-avenir-prometteur-147955>, fête de la science UPJV 2020.

Etude de la composition chimique et du potentiel biologique de *Pistacia lentiscus*, plante médicinale traditionnelle d'Algérie

Cette thèse vise à caractériser et à évaluer la composition chimique de *Pistacia lentiscus* provenant de la région algérienne de Tizi-Ouzou. Le travail est construit à partir de prélèvements réalisés en montagne et en plaine littorale au cours des quatre saisons de l'année 2019. En parallèle, ce travail de recherche met en évidence des propriétés biologiques potentiellement valorisables ; en particulier des activités antioxydantes et une activité antidiabétique *in vitro* (effet inhibiteur de l' α -glucosidase).

Une étude bibliographique exhaustive a été effectuée sur la phytochimie, les propriétés pharmacologiques et l'usage traditionnel de la plante *Pistacia lentiscus*. Les données extraites ont conduit au développement de la partie expérimentale visant à répondre à la problématique de cette thèse.

Les travaux expérimentaux sont basés sur deux principaux volets:

- Le premier volet porte sur l'étude de la variabilité de la composition et du potentiel antioxydant de l'huile essentielle sous l'effet de l'interaction entre l'altitude et la saison de récolte de lentisque. L'extraction des huiles essentielles a été faite par hydrodistillation Clevenger. La caractérisation de leur composition chimique a été effectuée par GC-MS. L'activité antioxydante de ces huiles a été évaluée avec les trois tests ABTS, DPPH, et FRAP. Les résultats montrent que la saison et l'altitude de récolte ont un effet significatif sur la variation chémotypique et le potentiel antioxydant des huiles essentielles de lentisque.

- Le second volet a été consacré au criblage phytochimique des composés inhibiteurs de l' α -glucosidase dans différentes parties de *Pistacia lentiscus* en utilisant une étude chimiométrique qui relie l'activité biologique et les données UHPLC-ESI-HRMS. En outre, ce travail s'intéresse également à l'évaluation de la variation de l'intensité de l'inhibition de l'activité de l' α -glucosidase en fonction de l'habitat de la plante (montagne et littoral) et de l'organe (feuilles, écorces de tige, fruits). Les résultats de cette partie révèlent que l'écorce des tiges récoltées au littoral présente la plus forte activité inhibitrice sur l' α -glucosidase. Cette activité est comparable à celle du médicament de référence « l'acarbose ». L'analyse UHPLC-ESI-HRMS a permis d'identifier 6 métabolites inhibiteurs de l' α -glucosidase dont les dérivés d'epigallocatechine.

Cette thèse constitue une phase initiale qui doit contribuer à la valorisation de lentisque. En s'appuyant sur les différents facteurs ayant un impact sur la variabilité de la composition chimique et du rendement de ces métabolites étudiés, il devient possible d'optimiser leur production. Cela permettra de développer des applications scientifiques-technologiques industrielles basées sur les activités biologiques d'extraits de plantes isolées à partir des ressources de *Pistacia lentiscus*.

Mots-clés: *Pistacia lentiscus*, littoral, montagne, huile essentielle, GC-MC, chémotypes, activité antioxydante, polyphénols, UHPLC-ESI-HRMS, α -glucosidase.

Study of the chemical composition and biological potential of *Pistacia lentiscus*, traditional medicinal plant of Algeria

This thesis aims to characterize and evaluate the variation of the chemical composition of *Pistacia lentiscus* from Tizi-Ouzou region (Algeria) from samples collected in the mountains and coastal plain during the four seasons of the year 2019. In parallel, it aims to highlight potentially valuable biological properties, in particular antioxidant activities and *in-vitro* anti-diabetic activity (the inhibitory effect of α -glucosidase).

An extensive bibliographic study was carried out on the phytochemistry, pharmacological properties and traditional use of the plant *Pistacia lentiscus*. The extracted knowledge led to the development of the experimental part aiming to answer the problematic of this thesis.

The experimental work carried out in this thesis is based on two main components:

- The first part concerns the study of the variability of the composition and the antioxidant potential of the essential oil under the effect of the interaction between the altitude and the harvesting season of lentisk. The extraction of the essential oils was done by Clevenger hydrodistillation. The characterization of their chemical composition was performed by GC-MS. The antioxidant activity of these oils was evaluated with the three tests ABTS, DPPH, and FRAP. The results of this work showed that the season and the altitude of harvest have a significant effect on the chemotypic variation and the antioxidant potential of the essential oils of lentisque.

- The second part was devoted to the phytochemical screening of α -glucosidase inhibitory compounds in different parts of *Pistacia lentiscus* using a chemometric study that links biological activity and UHPLC-ESI HRMS data. In addition, this work is also interested in evaluating the variation in the intensity of inhibition of α -glucosidase activity as a function of plant habitat (mountain and coastal) and organ (leaves, stem bark, fruit). The results of this section reveal that stem bark harvested from the coastline has the strongest inhibitory activity on α -glucosidase. This activity is comparable to that of the reference drug "acarbose". The UHPLC-ESI-HRMS analysis identified 6 α -glucosidase inhibitory metabolites including epigallocatechin derivatives.

This thesis constitutes an initial phase that could contribute to the valorization of lentisk. Based on the different factors that have an impact on the variability of the chemical composition and yield of these studied metabolites, it would be possible to optimize their production. This would allow the development of industrial scientific and technological applications based on the biological activities of plant extracts isolated from the resources of *Pistacia lentiscus*.

Keywords: *Pistacia lentiscus*, littoral, mountain, essential oil, GC-MC, chemotypes, antioxidant activity, polyphenols, UHPLC-ESI-HRMS, α -glucosidase.