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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *PISTACIA LENTISCUS L.*

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Abstract

This work aims at the study of the chemical composition, and the evaluation of the antimicrobial activity of the essential oil of *Pistacia lentiscus L.* The essential oil of the aerial part was extracted with a device of the modified Clevenger type, then analyzed by gas chromatography coupled with mass spectrometry (GC/MS). The results showed that the essential oil *of Pistacia lentiscus L.* is characterized by the presence of Linalool, formate (28.86%), α-Longipinene (16.13%), Chamigrene (7.34%), Longifolene-(V4) (5.63%), Propanoic acid, 2-methyl-, 2-methylbutyl ester (5.17%), and n-Amyl isovalerate (4.85%). The antimicrobial power has been studied in vitro against Gram-positive bacteria: *Enterococcus faecium (CECT 4932), Bacillus subtilis (CECT 4071),* and *Staphylococcus aureus (CECT 976),* Gram-negative bacteria: *Escherichia coli (CECT 431), Yersinia enterocolitica (CECT 4315)* and *Pseudomonas aeruginosa (CECT 4080).* The in vitro antibacterial tests performed revealed significant bacterial activity against all the strains studied. The gasoline showed strong activity, this bioactivity is mainly due to the richness of this gasoline in major molecules known for its effectiveness against microbial agents.

Keywords: *Pistacia lentiscus L., Extraction, GC/MS; Essential oil, Anti-microbial activities.*

Introduction

Since antiquity, aromatic and medicinal plants have formed a booming niche at the global level, where in recent years there has been a resurgence of interest in its cultivation both in industrialized countries and in developing countries. development, and consequently the return to natural products which offers a new expanding market [1]. Plants still play a very important role in medical traditions they cover the primary health care needs of 80% of the population of developing countries, but the rules for their use are sometimes lacking in rigor and do not take into account the new requirements of modern therapy [2-4]. In industrialized countries, they are interested in aromatic and medicinal plants as substitute crops for modern intensive agriculture suffering from overproduction on a global scale (legumes or cereals). This type of agriculture is often considered to be a crop well suited to disadvantaged regions (mountainous regions) [5]. In developing countries, the cultivation of aromatic and medicinal plants is seen as a means of diversifying agricultural activity [6]. It is also considered to be a highly attractive activity for disadvantaged regions thanks to the job opportunities it offers. This is the case of Morocco which, by virtue of its geographical situation, has a flora rich in its physiographic and bioclimatic diversity which constitutes an explanatory variable of the richness and of the floristic diversity, in particular in terms of medicinal and aromatic plants, and therefore it has about 4200 species of which only a hundred are currently exploited [7-9].The studies carried out on this topic are essentially aimed at the valuation of essential oils, in particular by the valuation of their active ingredients via various synthesis techniques, either by verifying their pharmacological, toxicological, antifungal, antiviral, antibacterial or insecticide powers.

In the other hand, *Pistacia lentiscus L.*, the lentiscus pistachio or mastic tree is a small tree belonging to the Anacardiaceae family, a relative of the pistachio tree. The lentisque pistachio is part of our French flora, since it is abundant in our scrubland. It is distributed more widely all around the Mediterranean, on a strip of land that follows the coast. The mastic tree is a beautiful ornamental shrub with evergreen, very tough foliage, particularly adapted to heat and drought [10]. *Pistacia lentiscus L.* develops a dense branch, it is generally wider (3 m) than high (2 m), but its shape ultimately depends on its growing conditions: it is low, almost crawling in a stale area, but stands tall. protected area. Its alternate buds make its branches elegant. Its trunk produces an orange-gray bark that contains the channels from which the famous mastic resin can be extracted [11].

Material and methods

Plant Material

In this study Samples of the aerial part (stems, leaves and flowers) of *Pistacia lentiscus L.* was collected from the region of Khenifra-Morocco. These species were verified by a botanist at the Khenifra Forest Research Center - Morocco.

Essential oil extraction

The essential oils are extracted by hydrodistillation of the aerial parts (stems, leaves and flowers) of *Pistacia lentiscus L.* for 3 hours using a Clevenger type extractor. The operation is repeated several times for each sample of the dry plant material. The essential oil yield is determined in ml / kg of dry matter. The essential oil is then stored at 4 ° C protected from light [12]. The "Y" yield of essential oil is the ratio of the volume V of the essential oil extracted to the weight W of the plant material. It is expressed in mL of distillate per 100 g of dry matter (percentage volume / mass: v / w%) [13].

$$Y(\%) = \frac{V}{W} \times 100$$

Chemical compositions of essential oil

The chromatographic analysis of essential oils was carried out by gas chromatography coupled to a mass spectrometer of the Clarus 600 D MS type (Perkin Elmer USA). The capillary column used is of the RESTEK Rtx® -5MS type 30 meters in length, 0.25 mm internal diameter, film thickness 0.25 µm, the stationary phase. The injections were made in splitless mode. The temperatures of the injector and the transfer line were raised to 250 ° C. Helium was used as carrier gas at a flow rate of 1mL / min. The initial temperature was set at 60 ° C and held for 1 minute, then increased by 3 ° C / min to 200 ° C and

kept isothermal for 13 minutes. The acquisition is made in electronic impact at 70 eV, with a source at 250 ° C in Scan mode (from 40 to 600). The identification of the compounds was carried out by comparison of the mass spectra with those given by the WILEY and NIST libraries. The essential oils were diluted in absolute ethanol to a concentration of 1 g / L. The percentage content of the constituents of essential oils is determined by the internal standardization method.

Antimicrobial activities

The evaluations of the essential oil properties of *Pistacia lentiscus L.* were carried out in vitro against Gram-positive bacteria: *Enterococcus faecium* (CECT 4932), *Bacillus subtilis* (CECT 4071) and *Staphylococcus aureus* (CECT 976), Gram-negative bacteria: *Escherichia coli* (CECT 431), *Yersinia enterocolitica* (CECT 4315) and *Pseudomonas aeruginosa* (CECT 4080).

The preparation of bacterial inoculum is usually done after several steps. Initially, samples kept frozen or refrigerated should be activated in liquid HD medium. After 6-8 hours at 35 ° C, an aliquot is transferred to MH medium. After 24 h at 35 °C, the axenic culture colonies can be suspended in 5 mL of sterile saline (8.5 g / L NaCl) and measured using a 0.5 McFarland densitometer (corresponding to 1-2 x 10^8 CFU / mL).

The culture medium consists of liquid Muller Hinton with 0.5% tween 80. The technique is generally carried out in U-shaped plates with 96 wells and presents variations compared to the original method described by Eloff. 20 µL of essential oil is added to the first well which contains 170 µL of Mueller-Hinton broth (tween 80: 0.5%) the other wells already contain 95 µl Mueller-Hinton (tween 80: 0.5%) After homogenization of the first well 95 µl of the mixture from the first well is transferred to the second well and so on, the 95 µl from the last well are removed. At the end of 5 µL of a bacterial suspension of 3.5 × 10^7 CFU / mL are added to each well. The results are read after an incubation period of 24/35 ° C. The MIC value is the lowest concentration of the natural product that visually inhibits microbial growth. From the tubes without visible growth, 10 µL of solution are removed and spread on Mueller-Hinton agar and incubated for a further 24h/35 ° C to determine the minimum bactericidal concentration (CMB). The absence of colony-forming units (or a growth of less than 0.1% of the initial inoculum) indicates that the essential oils are bactericidal. The percentage of inhibition of bacterial growth by essential oils of the two plants can also be calculated using a spectrophotometer in comparison with positive control wells (culture medium without extracts and free of microorganisms) and negative control wells (antibiotic plus microorganism).

Result and discussion

Essential oil

The average yield of essential oil of *Pistacia lentiscus L.* was calculated according to the dry vegetable matter of the aerial part of the plant. The *Pistacia lentiscus L.* species sample yielded a rate of approximately 0.17%. Few studies have been carried out on *Pistacia lentiscus L.* According to Bammou et al. (2015) [14], the yield of essential oils from the leaves and small branches of Pistacia lentiscus L. is low; it is around 0.16%. This corroborates the results found by Dob et al. (2006) [15], which were in the order of 0.11 to 0.20% and that of 0.14% obtained by Amhamdi et al. (2009) [16]. The results obtained by Benhammou et al. (2007) [17], show that the essential oil of *Pistacia lentiscus L.* from two stations in the region of Tlemcen, Ain Fezza and Oum El Alou (Algeria) are of the order of 0.05% and 0.07 % respectively. So, we can conclude that the influencing factors on the yield are numerous. The variability can be explained by the differences in environmental conditions: climate, altitude, geographic location, harvest season, the parts of the plant used and the distillation technique or extraction process. It should also be noted that the production of essential and aromatic oils from the plant results from a series of physiological, biochemical, metabolic and genetic regulations.

The analysis of the results of the chemical composition carried out by gas chromatography coupled with mass spectrometry of essential oil of *Pistacia lentiscus L.* studied is mentioned in Table 1. Analysis of the results of this table showed the chemical composition of the essential oil of *Pistacia lentiscus L.*, The main majority compounds identified are: Linalool, formate (28.86%), α-Longipinene

(16.13%), Chamigrene (7.34%), Longifolene- (V4) (5.63%), Propanoic acid, 2-methyl-, 2-methylbutyl ester (5.17%), and n-Amyl isovalerate (4.85%). The majority compounds were obtained with a percentage of (67.98%). The plant contains many biologically active compounds. The oil has been little studied, its composition is quite variable according to the geographical region of production, the harvest season and the duration of the distillation. The results obtained by Koutsoudaki et al. (2005) [18], show that the main constituents of the essential oil of *Pistacia lentiscus* cultivated in Zakynthos (western Greece) were R-pinene (63%), α-pinene (3.3%), α- myrcene (25%), limonene (1.5%) and α-caryophyllene (1%). The results obtained by Barra et al. (2007) [19], carried out on the essential oil of the aerial parts of *Pistacia lentiscus L.* growing in the wild in five localities of Sardinia (Italy) extracted by steam distillation show a total of 45 compounds representing 97.5-98.4% of total HE was identified, and the major compounds were R-pinene (14.8-22.6%), α-myrcene (1-19.4%), p- cymene (1.6-16.2%), and terpinene-4-ol (14.2-28.3%). The results obtained by Zrira et al. (2003) [20], carried out on the essential oil of Moroccan *Pistacia lentiscus L.* collected on three zones: Mehdia, Oulmes and Chaouen, respectively. Forty-five compounds, representing 92% of the oil, have been identified. The main components of the oil from Oulmes were α-pinene (16.5 to 38.5%), β-myrcene (10.2 to 11.5%) and limonene (6.8 to 9 , 8%), while terpinene-4-ol (32.7 to 43.8%), α-pinene (7.1 to 13.5%) and bornyl acetate (6.8 to 10 , 3%) were the main constituents of Chaouen oil. For *P. lentiscus* of Mehdia, terpinene-4-ol (14.5–19.3%), caryophyllene oxide (6.5–10.3%) and limonene (6.7–8.1 %) were the main components. Analysis of the marker data in the essential oil of Tunisian *Pistacia lentiscus* obtained by Douissa et al. (2005) [21], shows that α-pinene (17%), γ-terpinene (9%) and terpinene-4-ol (12%) are the major constituents, they define a different profile compared to that of essential oils of Egyptian origin extracted with the same process. The Egyptian species was characterized by δ-3-carene (65%), bisabolene (4%) and β-bourbonene (4%) as main components De Pooter et al. (1991) [22].

Antimicrobial activities

The results of the antibacterial evaluations of all the methods applied to the essential oil of *Pistacia lentiscus L.* are expressed in terms of diameters (Φ) of zones of inhibition measured around the discs, of the minimum inhibitory concentration (MIC), of the minimum bactericidal concentration (CMB) and ratio (CMB / MIC). All the values obtained are listed in Table 2. From the results obtained, it can be concluded that the essential oil of *Pistacia lentiscus L.* has interesting antimicrobial activities against the strains studied. The diameters of zones of inhibition Φ obtained from the disk diffusion method vary from 7.11 mm to 18.18, The strains *Enterococcus faecium (CECT 4932), Bacillus subtilis (CECT 4071), Yersinia enterocolitica (CECT 4315)* and *Pseudomonas aeruginosa (CECT 4080)* have strong activity, While *Staphylococcus aureus (CECT 976)* and *Escherichia coli (CECT 431)* have moderate acceptable activities. The minimum MIC inhibitory concentrations vary from 36.34 to 96.49 µl/ ml and the minimum bactericidal CMB concentrations vary from 36.59 to 97.83 µl/ml. So in general, the essential oil of *Pistacia lentiscus L.* exhibits remarkable activities with respect to all the strains studied. According to the CMB / MIC ratio, the essential oil *Pistacia lentiscus* L. has bactericidal effects against all the strains studied (Table 3). In general, it has also been established in numerous studies that the activity of an essential oil is related to the majority compounds and the possible synergistic effects between the constituents.

Conclusion

In this work, we characterized the chemical composition of the essence of Pistacia lentiscus L. collected from the Khenifra-Morocco region. The identification of constituents was carried out based on their mass spectrum obtained by gas chromatography coupled with mass spectrometry. Chromatographic analysis identified 52 constituents with a codominance of Linalool, formate (28.86%), α-Longipinene (16.13%), Chamigrene (7.34%), Longifolene- (V4) (5.63%), Propanoic acid, 2-methyl-, 2-methylbutyl ester (5.17%), and n-Amyl isovalerate (4.85%). The antimicrobial efficacy of *Pistacia lentiscus L.* essential oil has been demonstrated against six bacteria. The results we obtained confirm that the essential oil of this species has significant antimicrobial power, this activity is mainly due to the major constituents. All of its results

suggest prospects for formulation research based on the essences of *Pistacia lentiscus L.* in place of certain synthetic preservatives in the field of the food, pharmaceutical and cosmetics industry.

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Table 1. Chemical composition of the essential oil of *Pistacia lentiscus L.*

Peak	RT	Composants	(%)
1	7.74	Propanoic acid, 2-methyl-, 2-methylpropyl ester	0.35
2	9.26	Cyclohexene, 4-methylene-1-(1-methylethyl)-	0.12
3	10.44	Butyl 2-methylbutanoate	1.91
4	10.53	Isobutyl isovalerate	0.85
5	10.75	Butanoic acid, 3-methylbutyl ester	0.52
6	10.93	Propanoic acid, 2-methyl-, 2-methylbutyl ester	5.17
7	11.06	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	0.28
8	11.13	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, acetate	0.15
9	11.60	Eucalyptol	2.51
10	12.26	Butanoic acid, 2-pentenyl ester, (Z)-	0.19
11	13.20	α-Methyl-α-[4-methyl-3-pentenyl]oxiranemethanol	0.20
12	13.77	Butanoic acid, 2-methyl-, 3-methylbutyl ester	0.12
13	14.13	n-Amyl isovalerate	4.85
14	14.37	Linalool, formate	28.86
15	14.48	Butanoic acid, 2-methyl-, 2-methylbutyl ester	0.07
16	15.12	Bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl-	0.25
17	15.24	4-Acetyl-1-methylcyclohexene	0.15
18	15.45	2,2-Dimethylpropanoic acid, 3-methylbut-2-enyl ester	0.94
19	15.62	Valeric acid, 3-methylbut-2-enyl ester	0.32
20	15.89	(+)-2-Bornanone	0.52
21	16.83	Terpinen-4-ol	0.65
22	17.38	α-Terpineol	0.87
23	19.26	Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	0.17
24	20.66	(R)-lavandulyl acetate	0.08
25	23.60	α-Copaene	0.22
26	23.97	(-)-α-Bourbonene	0.88
27	24.12	Butanoic acid, 2-methyl-, phenylmethyl ester	0.92
28	24.55	1H-Cyclopropa[a]naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-, [1aR-(1aα,3aα,7bα)]-	0.22
29	24.77	Longifolene	0.12
30	25.24	1,6-Octadien-3-ol, 3,7-dimethyl-, formate	1.57
31	26.18	1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-, (4aS-cis)-	0.09
32	26.35	Chamigrene	7.34
33	27.26	Longifolene-(V4)	5.63
34	27.65	Butanoic acid, 3-methyl-, 2-phenylethyl ester	0.17
35	27.92	1H-Benzocycloheptene, 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-, (R)-	0.61
36	28.16	α-Longipinene	16.13
37	28.36	Isolongifolene, 4,5,9,10-dehydro-	1.05
38	28.57	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1α,4aα,8aα)]-	1.73
39	28.75	4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane	0.37
40	28.86	2H-Benz[e]inden-3-ol, 3,3a,4,5-tetrahydro-3a-methyl-, (3S-cis)-	0.99
41	29.03	trans-Sesquisabinene hydrate	0.53
42	29.11	Cycloisolongifolene, 9,10-dehydro-	0.15

43	29.27	α-Calacorene	0.22
44	29.98	6-Octen-1-ol, 3,7-dimethyl-, propanoate	0.21
45	30.66	3-Isobutyl-4,5-dimethyl-3H-isobenzofuran-1-one	0.17
46	31.81	Longipinocarveol, trans-	0.49
47	32.78	Himachala-2,4-diene	0.28
48	33.20	5α,7αH,10α-Eudesm-11-en-1α-ol	0.42
49	34.23	6-(1,3-Dimethyl-buta-1,3-dienyl)-1,5,5-trimethyl-7-oxa-bicyclo[4.1.0]hept-2-ene	1.11
50	34.48	Murolan-3,9(11)-diene-10-peroxy	0.38
51	34.96	Propanoic acid, 2-methyl-, dodecyl ester	0.16
52	36.30	6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	2.91
Total			**99.9**

Table 2. Parameters of antibacterial activity of essential oil of *Pistacia lentiscus L.*

Strains	*Applied methods*	*Results*
***Enterococcus faecium* (CECT 4932)**	Φ (mm)	17.68 ± 0.76
	CMI (µL/mL)	36.34
	CMB (µL/mL)	36.59
	CMB/CMI	1.00
***Bacillus subtilis* (CECT 4071)**	Φ (mm)	18.18 ± 0.71
	CMI (µL/mL)	54.64
	CMB (µL/mL)	63.47
	CMB/CMI	1.16
***Staphylococcus aureus* (CECT 976)**	Φ (mm)	8.68 ± 0.74
	CMI (µL/mL)	83.33
	CMB (µL/mL)	85.51
	CMB/CMI	1.02
***Escherichia coli* (CECT 431)**	Φ (mm)	7.11 ± 0.21
	CMI (µL/mL)	96.49
	CMB (µL/mL)	97.83
	CMB/CMI	1.01
***Yersinia enterocolitica* (CECT 4315)**	Φ (mm)	10.64 ± 0.31
	CMI (µL/mL)	55.87
	CMB (µL/mL)	55.88
	CMB/CMI	1.00
***Pseudomonas aeruginosa* (CECT 4080)**	Φ (mm)	14 ± 0.91
	CMI (µL/mL)	66.10
	CMB (µL/mL)	67.77
	CMB/CMI	1.02

Table 3. Bactericidal or bacteriostatic nature of the essential oil of *Pistacia lentiscus L.*

Strains	***Effect***
Enterococcus faecium (CECT 4932)	Bactericidal
Bacillus subtilis (CECT 4071)	Bactericidal
Staphylococcus aureus (CECT 976)	Bactericidal
Escherichia coli (CECT 431)	Bactericidal
Yersinia enterocolitica (CECT 4315)	Bactericidal
Pseudomonas aeruginosa (CECT 4080)	Bactericidal