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REGULAR ARTICLE

Gas chromatography-Mass spectrometry analysis and antibacterial evaluation of essential oils of *Pistacia lentiscus* from Wilaya of Tissemsilt in Algeria

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ABSTRACT

The botanic genera *Pistacia* are groups around fifteen species of shrubs that belong to the *Anacardiaceae* family which is believed to have originated in Asian or Mediterranean region. *P. lentiscus* is abundant species of the *Pistacia* genus encountered in the forest region of Ouarsenis mount (Northwest of Algeria), exactly in the national park of Thniet El Had in the Wilaya of Tissemsilt in Algeria. In the present study, chemical composition and antibacterial activities of essential oil (EO) of *P. lentiscus* were evaluated. The EO was isolated and analyzed by gas chromatography-mass spectrometry (GC-MS). The minimum inhibitory concentration (MIC) was determined using 6 strains of Gram-positive and negative bacteria. The antibiogram was made following the gliosis-environment diffusion method and which makes it possible to determinate bacteria's sensibility to antibiotics. From a exponential culture (18 to 24 hours), a bacterial suspension was prepared and well-homogenized afterwards. The opacity was adjusted equivalent to 0.5 McF (McFarland). Based on the GC-MS analysis, thirty-five compounds representing 100 % of the total oil composition were identified. The essential oils could be explored to test their antimicrobial activity, especially against some bacteria that cause alimentary intoxications. The results revealed that the essential oil exhibited strong levels of antibacterial activity against the tested microorganism regarding the MIC values, *Salmonella sp* was found to be the most sensitive strain (inhibition zone 23cm MIC 1.25%). Based on the findings of the present study, new antibacterial agents could be developed, and the use of *P. lentiscus* should be promoted in the traditional treatment of ailments.

1. Introduction

Natural products can presently be the source of new molecules of medical usage (Balouiriet al., 2016.). One of the main sources of natural active substances are essential oils (EO) used in ethno-medical, alimentary, and cosmetic processes (F. Bakkali et al., 2008).

P.lentiscus (Anacardiaceae) is known for its presence in folkloric medicine since the ancient Greece, it is very abundant in the Mediterranean basin. It can be found in its wild form in the fog and the scrubland in all ground types, although it prefers siliceous soils **(**More et White, 2005). The essential oil and the Goma of the mentioned plant have been largely used as alimentary additives and drinks in the Mediterranean region without any toxic effect (Loutrari et al., 2006). The essential oil of *P. lentiscu*s also used in cosmetics, perfumery and as an aromatic agent in alimentary preparations (Daferera et al., 2002).

Despite its worthwhile importance, data on chemical composition, antibacterial activity of essential oils from *P. lentiscus* from Algeria are scanty. Hence, in the present study we aimed to investigate chemical composition, antibacterial activity of essential oils from *P. lentiscus.*

2. Materials and Methods

2.1. Plant material

P. lentiscus leaves have been harvested in Thniet El Had national Parc in the Wilaya of Tissemsilt (extreme south east peripheral of the Park) in 10/04/2017, at an altitude of 1100 à 1300m, geographic coordinates: 35° 49'41" and 35°54' 04" North latitude and 01°52'45" and 02°02'04" East longitude.

Isolation of essential oil

Leaves and branches have been cleaned, purified from any foreign elements, put in bags and transported to the laboratory. A sample of 250g of fresh material from *P.lentiscus* has been submitted to hydro distillation in a Clevenger apparatus during 3h with 2000ml of distilled water (Duru et al., 2003). The obtained essential oil was dried using anhydrous sodium sulfate (Na_2SO_4). The oil's yield was calculated with the equation below (Kusuma and Mahfud, 2017). Then the essential oil was stocked in the dark at 4°C (Gardeli et al., 2008).

$$y = \frac{V}{W} \times 100$$

Where: y is the yield in essential oil (%, p/p),

V in the weight of the extracted oil (g)

W is the weight of the fresh vegetal parts (g)

2.2. GC – MS Analysis

GC – MS Analysis was done in a MASTER GC DANI device and a mass spectrometer of the TOFMS DANI type with a HP-5 MS column (30m × 0.25mm of diameter, 0,25 µm film thickness). The other analysis parameters were the following : oven temperature [60 ° C (8 min), 50 ° C à 240 ° C (2 ° C / min), 240 ° C (10 min)]; injector temperature , 250 ° C, injection volume : 0,2 µL; carrier gas: pure helium with a 0.5 ml per minute rate; filament intensity: 70 ev; identification of individual compounds was done through a comparing their mass spectrums and their retention indices (RI) with those of authentic samples and those provided in literature (Adams, 2007). Quantification of the relative quantity of individual compounds was done in function of surface percentage, without consideration of calibration factor.

2.3. Determination antibacterial activity

EO of P. lentiscus leaves was examined against 2 Gram-positives (Staphylococcus aureus ATCC 29213, Bacillus cereus ATCC 14579) et 4 Gram negatives (Pseudomonas aeruginosa ATCC 27853, Salmonella ATCC 14028, Acinetobacter ATCC 49139; Proteus mirabilis ATCC 35659). First, for antibacterial tests, all bacterial stains were subjected of a growth culture during 24 h at 37 ° C; a bacterial suspension was prepared and then well-homogenized. Opacity must be equal to 0.5McF (McFarland).

2.4. Determination of minimum inhibitory concentration (MIC)

Inhibition tests of P.lenticus EO against tested bacteria were done using Mueller-Hinton agar described in previous studies (Bouyahya et al., 2017). All inhibition tests of bacterial growth were done three times. The minimal inhibition concentrations (MIC) were determined through du two-fold serial dilution method following the method reported by Bouhdid et al. (2009).

3. Results and Discussions

3.1. Extraction of P.lentiscus essential oils

After 3 hours of hydro distillation of 250g of vegetal matter, we obtained the quantities of essential oils and thus the yield (R) of the extraction was calculated with the formula:

$$R = \frac{EssentialOilMass\ \ (g)}{UsedVegetalMatterMass\ (g)} \text{x100}$$

The average yield of the essential oil extracted from *P.lentiscus* leaves and branches was equal to 0.42%.

This value corresponds of the results of Arab et al. (2014) in the region of Boumerdes in Algeria, Zrira et al. (2003). However, our values were higher than those reported in Tunisia (Amri et al., 2012) and in Greece (Tsokou et al., 2007).

3.2. GC-MS Composition of P.lentiscus oil

Table 1 presents the individual components identified in the oil extract with their respective relative percentages. In total, thirty-five components have been identified.

The main identified components were β -pinene (16.33%), γ-terpinene(12.78%), β-phellandrene (9.12%), Terpineol (8.7%), alpha-terpinene (7.78%), and para-mentha-2.8-diene (3.78%).On the other hand, l'alpha-pinène was the main component of EO originated from du Morocco (Oulmes) (16.1-38.5% by) (Zrira. 2003;, Algeria (20.0-34.2% and 19% (by Mecherara-Idjeri, 2008 and Dob, 2006 respectively, in Tunisia 16.8%) by Ben Douissa et al., 2005 ; in Greece 24.9-9.4% by Chryssavgi, 2008 ; in Italy 14.8-22.6% and 18%by Barra et al., 2007 andLo Presti et al., 2008 respectively ;in Spain 13.0%by Fernandez et al., 2000 and in France 31.9% by Castola et al., 2000). Moreover, terpinène-4-ol was mainly present in Morocco samples (Chaoun, Mehdia) (14.5-19.3%; (Zrira,2003),, Algeria (17.3-34.7%; Benyoussef et al., 2005 ; Turkey30.0% and (29.2% (Duru et al., 2003 ; Kivçak et al., 2004 respectively) and France (25.6%; Castola et al., 2000). Other chimotypes have also been reported: longifolen (16.4-12.8% in Algeria by Dob et al., 2006 ; limonen 47.0% in France (Corse) et 44 à 29% in Algeriaby Castola et al., ; 2000 ;Mecherara-Idjeri, 2008); β-caryophellen (19.3-13.1% in Algeria by Mecherara-Idjeri,2008); and 31.5% in Italy (Sardaigne)) (by Congiu et al., 2002). Finally, egyptian oil contains great quantitiesof α-3-caren (65.3%) (Congiu et al., 2002). Our results do not conform to those of the study made by Rezaie et al. (2015). The EO of the Bene hull (P. atlantica subsp. mutica) contain a high percentage of[hydrocarbons monoterpene (75.7%), followed by Oxygenated monoterpenes (13.4%), sesquiterpene hydrocarbons (6.0%) and Oxygenated sesquiterpenes (1.4%) (Rezaie et al., 2015).

Composés[a]	RT (min)	RI	%
Tricyclene	10.27	923	0.09
α-Thujene	10.85	933	0.25
Camphene	11.51	943	3.25
Sabinene	13.14	970	12.7
β-Pinene	13.35	973	16.33
α-Phellandrene	15.02	1000	1.21
β-Phellandrene	16.81	1025	9.12
E-β-Ocimene	18.18	1045	1.32
Z-β-Ocimene	17.42	1034	0.12
γ-Terpinene	19.00	1056	12.78
2-Nonanone	21.98	1089	1.6
Nonanol 2	22.043	1099	0.54
iso-Amyl isovalerate	32.5	1247	0.41
Terpinen-4-ol	27.57	1176	8.7
α-Terpineol	28.52	1189	3.66
Bornyl acetate	32.88	1281	1.23
2-Undecanone	35.56	1291	3.11
Isoamyl benzoate	32.5	1247	0.41
α-Humulene	45.66	1447	0.4
α-Muurolol	57.13	1643	0.04
γ-Cadinene	49.99	1518	0.49
E Caryophyllene	43.64	1415	3.57
Epi- α -Cadinol	56.91	1639	0.65
α-Cadinol	57.69	1653	0.96
Santolinatriene	9.93	918	0.25
Cumene	10.7	930	0.18
Meta-cis-Mentha-2,8-diene	14.21	987	2.77
Alpha-terpinene	15.95	1013	7.78
Para-cynene	16.45	1020	0.75
Para-Mentha-2,8-diene	20.98	1084	3.78
Cis-thujone	22.18	1101	0.03
Isopentyl-isovalerate	22.29	1102	0.11
E-tagetone	24.87	1138	0.02
Isoamylhexanoate	32.5	1247	0.41
Trans-Cadina 1(6),4,diene	47.43	1476	1.42

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

The other EO from tissues of many species and varieties of pistachio have approved our constitute (Pirbalouti et al., 2011;, Darvish-Tafvizi et al., 2005; Dragull et al.,2010).

3.3. Antibacterial assay and MIC

Antibacterial activity of EO against pathogen agents are of great importance and interest for researchers. In this study, antibacterial activity of the P.lentiscus EO has been determined. The evaluation of antibacterial results against six bacterial stains using the MIC method are presented in table

Bacteriea	Diameter	CMI %
Salmonella sp	23.00	1.25
Pseudomonas.a	16.71	0.625
Bacillus.c.	19.56	2.5
Acinetobacter sp	20.07	0.625
Proteus mirabilis	12.00	0.625
Staphylococcus aureus	19.00	2.5

Table 2: Inhibition zones diameters of microbial growth and MIC obtained for pure EO.

2. In this regard, the most sensitive strains were *Salmonella sp.*

The MIC (Minimal Inhibition Concentration) has been defined as the lowest concentration of tested samples for which an absence of growth has been registered (Ponce et al., 2003). *P. lentiscus* essential oil's MIC has been tested in concentration varying from 5% to 0.156%., *P.lentiscus* essential oil has revealed a strong inhibition activity against all tested germs (Although the studied microorganisms did not show the same sensitivity to the oil). Our data indicate that *P. aeruginosa; Acinetobacter sp; P. mirabilis* were inhibited at a concentration of 0.625% while *Salmonella sp* has been inhibited at a concentration of 1.25% However, a greater value of the MIC 2.5% has been obtained with *Staphylococcus. a* and *Bacillus*.

According to Hafse et al. (2017), the MIC for *M. aurum*, *Bacillus sp*. And *S. aureus* was equal to 1/250 (v/v), while the most resistant strains were *P. aeruginosa, E. faecalis* and *Salmonella sp.* with MIC equal to 1/125 (v/v).

Tahiri (2008) has demonstrated that de *P. lentiscus* leaves (Ethanolic extract, [Éthyleacétate, Chloroform, Hexane, Aqueux de l'hexane) have a remarkable antimicrobial effect on Salmonella enteritidis with inhibition zones of 8.6 ± 0.9, 16.5 ± 1.3, 14.6±0.2, 4.7±0.4 and 13.8±0.4 respectively at 5 µg/ml).

4. Conclusion

Most of the oil extracted from leaves is characterized by the presence of monoterpens as main components. However, all of our study and the precedent ones show clearly differences as other chemotypes were observed. It seems that environmental factors like geography, temperature, daytime duration, nutrients play an important role in the chemical composition of the *P. lentiscus* oil. The plant's genetic characteristics may also affect the chemotype of the *P. lentiscus* essential oil. These factors affect the biosynthesis and, as a consequence, the main characteristic components and their respective percentages. These phenomena induce the existence of different chemotypes that distinguish *P. lentiscus* oil extracted from different origins. Subsequent researches need to be performed in order to define the genotype of *P. lentiscus.* Our study has led us to conclude that the oil had interesting antibacterial properties and can then be useful as a conservator in food and pharmaceutical industry.

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