CHEMICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF 12 *PISTACIA LENTISCUS*L. OIL GROWING IN ALGERIA. CHARACTERIZATION OF ALGERIAN LENTISK OIL.

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Pistacia lentiscus oil is used in traditional medicine. The purpose of the present study was to determine if the *Pistacia lentiscus* oil is of any interest for human nutrition and pharmaceutical industry. For this purpose, the physicochemical properties of the oils of *P. lentiscus*from 12 localities of Northern Algeria were studied and compared as well as their compositions in free fatty acids, total polyphenols, flavonoids and chlorophylls.The agar well diffusion method and broth microdilution assay method are used to evaluate the antimicrobialand the antifungal activity of the 12 oils against 8 referenced microorganisms. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)were also calculated. Results showed that *Pistacia lentiscus*oilhas similar physicochemical properties as virgin olive oil with an acid index of around3,5, a peroxide index of around 6 and a saponification index between 179 and 200mg/g oil. And these oils were rich in chlorophyll, flavonoids and total polyphenols. *Micrococcus luteus* and *Staphylococcus aureus* have a sensibility against *Pistacia lentiscus* oils. An antibacterial effect was also noted against *Micrococcus luteus* with inhibition zone diameters reaching18,5 mm. The Minimum Inhibitory Concentration attains 2,5mg/mL for *Aspergillus niger,* in Bouira locality. Statistical analysis could not differentiate groups according to the origin. Due to the *Pistacia lentiscus* oil being rich in unsaturated fatty acids, this oil can be used for human nutrition, agro-industrial processing and for pharmaceutical industry.

KEYWORDS: FATTY ACIDS, PISTACIA LENTISCUS L., VEGETABLE OILS,TRADITIONAL USE, BIOCHEMISTRY, DIVERSITY.

ABBREVIATIONS:IR; REFRACTION INDEX; AC: ACIDITY; AI: ACID INDEX; IP: PEROXIDE INDEX; IS: SAPONIFICATION INDEX; INSAPO: UNSAPONIFIABLE; II: IODINE INDEX;MBC: MINIMUM BACTERICIDAL CONCENTRATION; MIC: MINIMUM INHIBITORY CONCENTRATION.

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INTRODUCTION

The genus *Pistacia* belongs to the *Anacardiaceae* family. *Pistacia lentiscus* L. is a sclerophyllous dioecious shrub that forms bushes of up to 3 m height, sometimes attaining a tree growth form (Munne-Bosch et *al*., 2003). It is a low altitude species which grows in several Mediterranean regions (Bonnier and Douin, 1990). In Algeria, *Pistacia lentiscus*plants are called “Darw”, “Thidakth” or “amadagh”. It is commonly dispersed over the entire littoral, from El Kalla to Tlemcen in the west and from Algiers to Biskra in the south. Oil is the most used form of the plant. This oil is used in the culinary, cosmetic, aromatic fields and it has been extensively used in Algerian folk medicine as an astringent, expectorant and healing agent (Dob et *al*., 2006).

Vegetable oils are highly recommended for their health benefits due to their richness in unsaturated fatty acids and their great availability, unlike animal oils and fats which are rich in saturated fatty acids (Novidzro et *al*., 2019). Fruits of *P. lentiscus* give edible oil that is rich in oleic, linoleic, and other unsaturated fatty acids (Charef et *al*., 2008). Lentisk oil, also known as mastic tree oil, possesses great potential for nutritional, curative and cosmetic purposes (Djedaia, 2016). This interest comes from the effectiveness of the oil known in traditional medicine as well as its low toxicity proven by several studies (Bekeloua et al., 2012).

Few studies characterized mastic tree oil forits chemical composition. These researches mainly focused on oils extracted from berries harvested in a small area in Eastern Algeria, and are only interested in a few properties reported by traditional medicine, cited above. Thus, these present studies aims to add supplementary knowledge on this plant, enhance the importance of the oleaginous seeds used in traditional medicine, shed light on the potentialities of this oil and classify it in comparison with other vegetable oils.

The main goal of this study is to characterize and evaluate the quality of *Pistacia lentiscus* oils from 12 Algerian localities by analyzing fatty acid composition and physicochemical properties (acid index, saponification index, index of iodine, etc.), which are the main criteria for the characterization of any oil, as well as the study of polyphenols, flavonoids and chlorophylls. Although the latter constituents are minor, they have an important role in the biological activity of oils (Lecerf, 2011). Oils compositions are compared according to the respective studied regions.Finally, antimicrobial activity on 8 referenced strains was studied to highlight other potential properties of the oils.

MATERIALS AND METHODS

2.1 Plant material

2.1.1 Collection site

We made sampling in four provinces.In each , three municipalities were chosen.

- ✓ Eastern region:
  - Province of Guelma: municipalities of AinMakhlouf, Ain Ben Beida, and Ben Djerrah;
  - Province of Mila: municipalities of Terrai Bainen, Bouhatem, and Teleghma;
- ✓ Central region
  - Province of Bouira: municipalities ofLakhdaria, Taguedit, and Tikadjda;
  - Province of Ain Defla: municipalities ofMiliana, Ain Bouyahia, and Tarek ibn Ziyad.
- ✓ Western region

Other sites in western Algeria have been the subject of field trips to collect berry samples.

However, a phenomenon in which the fruits fall off prematurely at the red fruit stage has been observed for 3 years in a row. This led us to eliminate, for this study, the sampling sites of Ain Timouchant, Sidi Bel Abbes, Tlemcen, Oran, and Mostaganem. Different hypothesis can explain this phenomenon: the temperature gradient existing from East to West, the rainfall gradient, or the salinity of the soils existing in the western region.

On some sites of the Western province of Tlemcen, after having questioned the local population, it was documented that the gathering of the fruits is done as soon as the first black fruits are spotted. A more thorough study of the climatic conditions of the sites and a physiological study of the plant are still necessary to explain the premature fall of the fruits.

GPS data from sites located on GoogleMaps was documented sorted by province; the first site has low altitude (110-250m), the second has medium altitude (700-900m), and the last has high altitude (840-1400m). Precipitations and temperatures are annual averages by province:

- ✓ Bouira: 659mL – 16,2°C
- ✓ Mila: 742mL – 16,2°C
- ✓ Guelma: 554 mL- 17,2°C
- ✓ Ain Defla: 534 mL- 18,6°C.

TABLE 1. GPS DATA OF THE SITES STUDIED.

Area (Province)	Location	GPS data (Latitude Longitude)
Bouira	Lakhdaria	36° 37’ 0” N 3° 34’ 60” E
	Taguedit	36° 01’ 03” N 3° 59’ 41” E
	Tikadjda	36° 15’ 53” N 4° 04’ 26” E
Mila	Terrai Bainen	36° 31’ 51” N 6° 07’ 20” E
	Bouhatem	36° 18’ 14” N 6° 00’ 51” E
	Teleghma	36° 06’ 55” N 6° 21’ 51” E
Guelma	Ain Makhlouf	36°14’36’’ N 7°15’03’’ E
	Ain Ben Beida	36° 37’04’’ N 7°41’ 43’’ E
	Ben Djerrah	36°25’56’’N 7°22’7’’ E
Ain defla	Miliana	36°19’12”N 2°13’37”E
	Ain Bouyahia	36°14’43”N 1°44’20”E
	Tarek ibn Ziyad	35°58’06”N 2°09’36”E

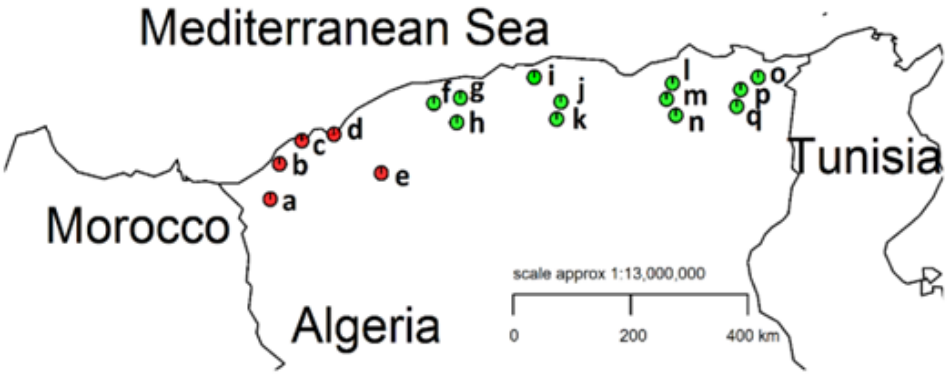

***FIGURE 1. DISTRIBUTION OF* P. LENTISCUS *COLLECTION SITES IN NORTHERN ALGERIA*.**

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2.1.2 Collection method

Fruits have been harvested at full maturity in the four regions starting from Mila and Guelma in the east, followed by Bouira and Ain Deflain the west. In each locality, three perimeters were marked; each one included 10 lentisk shrubs. Fruits of the 3 perimeters were harvested and mixed so as to have, at the end, one sample bylocality. The harvested berries were then dried in the shade for 7 days.

2.2 Oil pressing

2.2.1 Traditional pressing method

The traditional pressing method reported by women is similar in all regions studied. Four steps are followed: 1. Sorting and washing; 2. Grinding; 3. Pressing; 4. Recovering theoil.

However, some differences are noted regarding the pressing tools. In the East (Guelma and Mila), pressing becomes mechanized by using a small handcrafted press fitted with a heavy washer and placed in a perforated case filled with the berry paste. The whole press is placed on a stainless-steel tray. Under the pressure of the washer and the presence of hot water, the dough is pressed. The oil flows from the holes and is collected in the tray.

In Bouira, jute bags and a stone are used. In Ain Defla, the same traditional pressing method of olives is used. The pressing is done into stone containers using workers' feet and cold water.
After pressing, the recovered oil contains a percentage of water, which is removed either by settling or by heating the water-oil mixture. By this last method, some of the water is evaporated and then the oil is collected. Currently, heating is avoided to preserve the oil's qualities.

2.2.2 Oil pressing methods used in laboratory

Pressing of oils was conducted in the laboratory using the traditional pressing method, following the steps listed below:

- Sorting and washing the black fruits;
- Putting the fruits in hot water for a few minutes and grinding them;
- Putting the berry paste in a traditional press;
- Pressing the paste to extract the oil by adding, gradually, water to facilitate infiltration;
- Collecting oil in a stainless-steel tray placed under the press and then in containers;

- Allowing it to settlebefore collecting the supernatant oil;
- Storing the extracted oils in glass bottles inside a refrigerating unit, at the temperature of -4 °C.

The pressing of samples and analysis were done in January 2017 at the regional laboratory of the Algerian Center for Quality Control and Packaging CACQE Algiers.

2.3 P. lentiscus oil analysis

2.3.1 Physicochemical properties of the oils

Physicochemical properties of *P. lentiscus* oils were determined according to the methods described by international ISO and European standards.

Acid index (AI) was determined according to NF EN ISO 660 official test method 2009 (ISO, 2009). Acidity was expressed as oleic acid percentage. The peroxide value (PI) was determined according to NF ISO 3657 2017 official test method (ISO, 2017).The saponification index (SI) was expressed in mg of KOH per gram of fatty substance (ISO, 1990).Iodine values of the *P. lentiscus* oils were determined as described by ISO 3961 1996 Standard protocol (ISO, 1996). Relative density was measured by UICPA protocol 1999 (UICPA, 1999).Refractive index at 26 °C was determined using ABBE refractometer. Finally, unsaponifiable matters were calculated according to ISO standard method 186092000 (ISO, 2000).

TABLE 2 PHYSICOCHEMICAL PROPERTIES OF 12 VEGETAL OILS OF PISTACIA LENTISCUS L. GROWING IN ALGERIA.

Each value represents the average of three measures (n-3) ± standard deviation. a: Guelma, b: Mila, c: Bouira, d: Ain Defla.

Location	**IR**	**Ac%**	**IA** mg/g	**IP** meqO_2/kg	**IS** mg KOH/ g d'huile	**Insapo%**	**Ii**
Ain Makhlouf[a]	1,47±0,00	2,06±3,06	4,33±0,38	6,03±0,70	194,33±1,53	0,45±0,04	43,10±0,21
Ain Ben Beida[a]	1,45±0,01	3,17±1,53	6,67±0,75	5,20±1,00	194,17±1,78	0,47±0,01	43,41±0,50
Ben Djerrah[a]	1,47±0,01	2,15±2,75	4,52±2,93	6,80±0,46	195,63±0,67	0,76±0,25	42,79±1,47
Terrai Bainen[b]	1,47±0,01	2,2±0,08	4,62±1,01	8,33±1,68	186,58±0,50	0,97±0,02	43,46±0,37
Bouhatem[b]	1,48±0,01	2,38±0,72	5,01±1,01	6,53±0,55	190,41±1,11	0,86±0,13	43,63±0,47
Teleghma[b]	1,48±0,02	1,91±2,12	4,02±1,22	5,03±2,25	188,31±0,25	0,73±0,15	44,12±2,10
Lakhdaria[c]	1,47±0,01	1,68±0,10	3,54±0,26	6,67±0,40	182,80±4,59	0,56±0,09	43,89±0,37
Taguedit[c]	1,47±0,01	1,77±0,32	3,73±0,21	6,43±0,45	180,57±1,35	0,72±0,09	44,57±0,19
Tikadjda[c]	1,47±0,02	1,87±0,35	3,93±0,31	4,10±0,62	179,50±2,77	0,59±0,05	44,47±0,57
Miliana[d]	1,45±0,02	3,65±1,07	7,68±0,44	7,73±0,38	200,50±0,78	0,54±0,04	42,77±1,32
Ain Bouyahia [d]	1,47±0,01	2,01±0,01	4,24±0,26	6,10±1,56	198,23±2,78	0,58±0,02	44,01±2,14
Tarek Ibn Ziyad [d]	1,46±0,01	3,02±0,11	6,32±0,21	9,17±0,81	200,03±1,40	0,52±0,02	41,22±0,03

2.3.2 Fatty acid composition

The fatty acid composition in *P. lentiscus* oil was determined by conversion of the oil to fatty acid methyl esters (FAME) according to the method of Cocks and Van Rede (1966). FAME wasprepared by adding 5 mL of hexane added to 100 mg of oil, followedby 250 µLof sodium methanolate. 5 mL of saturated sodium chloride solution were added; the mixtures were vortex for 1 mn and let rest for 10

min. The upper phase was injected into a gas ChromatographChrompack(CP 9002) provided with a flame-ionization detector (280°C), a SPLITinjector1/100 (250 °C)and a polar capillary column (CP Sil 5 CB-Agilent Technologies, USA), with 30cm, 0,32 mm*0,25µm, 0,25µmlength, internal diameter and thickness, respectively,to get individual FAME peaks. These were later identified by comparing their retention time and those of standards. Relative percentage of each fatty acid represents the product of retention time and peak height (Nyama et *al*., 2009).

TABLE 3. COMPOSITION, IN FATTY ACIDS, OF ALGERIAN *P. LENTISCUS* BERRY OILS COLLECTED IN 12 LOCALITIES.

Each value represents the average of three measures (n-3) ± standard deviation. a: Guelma, b: Mila, c: Bouira, d: Ain Defla. C16_0: palmitic acid; C16_1w7: Palmitoleic acid; C18_0: stearic acid; C18_1w9: oleic acid; C18_2w6: linoleic acid; C18_3w3: alpha-linolenic acid; C20_1w9: Gondoic acid.

Location	C16_0	C16_1w7	C18_0	C18_1w9	C18_2w6	C18_3w3	C20_1w9
Ain Makhlouf[a]	26,54±0,56	1,21±0,42	1,03±0,05	53,17±0,74	17,63±0,74	0,52±0,09	0,15±0,07
Ain Ben Beida[a]	26,07±0,80	1,04±0,17	1,02±0,07	52,78±2,23	17,27±2,17	0,48±0,06	0,27±0,10
Ben Djerrah[a]	23,43±2,14	2,53±0,59	1,28±0,02	52,37±0,71	15,67±0,68	0,44±0,21	0,18±0,07
Terrai Bainen[b]	24,47±1,34	0,93±0,08	1,38±0,17	54,08±1,97	15,2±0,19	0,33±0,04	0,21±0,06
Bouhatem[b]	25,68±4,00	1,37±0,07	1,32±0,13	52,68±0,55	16,47±1,03	0,37±0,01	0,21±0,05
Teleghma[b]	25,45±2,06	1,07±0,13	1,21±0,12	51,78±0,50	16,7±0,44	0,42±0,05	0,18±0,02
Lakhdaria[c]	21,13±0,78	4,88±0,60	1,67±0,51	51,44±1,16	21,71±0,46	0,36±0,01	0,15±0,01
Taguedit[c]	22,71±0,45	3,24±0,07	1,06±0,12	52,27±0,96	23,78±0,99	0,35±0,01	0,18±0,02
Tikadjda[c]	21,15±0,70	3,86±0,16	1,38±0,42	52,26±0,16	23,5±0,18	0,27±0,09	0,13±0,02
unsaponifiable	unsaponifiable	unsaponifiable	unsaponifiable	unsaponifiable	unsaponifiable	unsaponifiable	unsaponifiable
Ain Bouyahia[d]	21,77±0,21	0,96±0,06	1,07±0,03	54,67±1,39	17,82±0,54	0,63±0,05	0,21±0,02
Tarek ibn Ziyad[d]	20,16±0,73	1,7±0,10	1,25±0,22	54,67±1,55	19,03±0,15	0,63±0,13	0,29±0,07

2.4 Determination of total phenols

2.4.1 Samples preparation and biochemical characterization

Two grams of *P. lentiscus* oil were put in an Eppendorf, then 1 mL of n-hexane and 2 mL of methanol/distilled water (v/v, 60/40) were added. After vortexing, the mixture was centrifuged at 3000 rpm for 5 min, and then the upper phase was collected after centrifugation. The extraction was done twice. The upper phases were mixed with 2 mL of n-hexane (Pirisi et *al.*, 2000).The extract obtained was stored at -20 °C until further use.

2.4.2 Determination of total polyphenol contents

Total polyphenol content was determined by using the Folin-Ciocalteu reagent as described by Singleton et *al.*(1999). The absorption was measured at 760nm using gallic acid as standard.

2.5 Determination of flavonoids and chlorophyll contents

Flavonoids content was determined as described by Bouaziz et al. (2010), based on the formation of complexes between phenolic compounds and aluminum trichloride. Catechin was used as a standard for the calibration curve. The total flavonoids content of the extract was expressed as mg catechin equivalents per gram of sample (mg/g). Finally, the chlorophyll content was determined

by using the protocol described by Pokorny et *al.*(1995). The absorbance was measured at 630, 670 and 710 nm.Results were expressed as mg pheophytin a/kg oil.

2.6. Antimicrobial activity and antifungal activity

2.6.1. The Microorganisms

The microorganisms used in the study were: *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Pseudomonas fluorescens* ATCC 27853, *Staphylococcus aureus* ATCC29213, *Micrococcus luteus* ATCC 4698, *Aspergillus niger* ATCC9029, *Candida albicans* ATCC 20027 and *Bacillus subtilis* M23. These microorganisms were provided by the Pasteur Institute of Algeria and the Microbiology Laboratory of the Higher Normal School, Kouba (Algiers).

2.6.2. The Assay

The agar well diffusion method (Tagg and McGiven, 1971) and the broth microdilution method were used for the determination of antibacterial activity and antifungal activity in *P. lentiscus* L. vegetable oil.

The obtained *P.lentiscus* L. oils were dissolved in 1:5 (v/v) DMSO (Khémiri et *al.,* 2019) and then deposited in the wells. Muller-Hinton agar was used for the antibacterial test and potato dextrose agar was used for the antifungal test. After inoculation of the microorganisms, a 6mm diameter well was made in the agar using a drill. The wells received either 50µL of dissolved oil at different concentrations (0.04, 0.16, 0.24, 0.4mg/mL), 50µL of DMSO, or 50µL of positive control. The diameter of the inhibition zone was measured in mm.

Bacteria plates were incubated at 37°C for 24h. The fungi plates were incubated at 28°C for 48h for *Bacillus subtilis* M23 and *Candida albicans* ATCC 20027 and for 3-4 days for *Aspergillus niger*ATCC9029.

Positive controls were prepared in solutions and incorporated into the culture medium. Gentamicin (600mg/L) and rifampicin (200mg/L) were used as antibiotics and voriconazole (20mg/L) was used as an antifungal agent. 50µl of DMSO wasused as a Negative control.

2.6.3. Determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) against the studied microorganisms was visually evaluated. Three measurements were conducted for each test. The MIC is the smallest concentration in which there is no visible growth (Khémiri et *al.,* 2019), and it is determined by the broth microdilution method (Celiktas et *al.,* 2007). The lowest dose which shows a complete absence of microorganism growth is considered as the minimum bactericidal concentration (MBC) (Celiktas et *al.*, 2007).

2.7. Statistical analysis

The tests were conducted with three repetitions. The results obtained are means ± standard deviation of which the significance has been analyzed by the ANOVA procedure with the R program version R*64 3.4.1. The principal component analysis (PCA) was applied to the separate growing regions according to all the investigated parameters.

RESULTS

3.1. Physicochemical characteristics of *P. lentiscus* L. oils from the 12 localities

3.1.1. Refractive index (IR), iodine value (Ii) and density (D)

Mastic oil from the different regions had a similar refractive index measured at 20°C of 1.47. The iodine index values rangedbetween 41.22 and 44.47 iodine/100g oil(Table 2).No significant differences were revealed in the statistical analysis.

3.1.2 Acid Index (AI)

The acidity values presented in Table 2 were found to be around 3 and 4, except for one station in Guelma and one station in Ain Defla, where the Acid Index AI reached 7. There was a very significant inter-region effect ($p<0.001$) and the Newman keuls test revealed 3 homogeneous groups. Group a with the Ain Defla province, b for Bouira and c for Mila and Guelma. The AI decreases following this order: Ain Defla (6.08); Guelma (5.17); Mila (4.55); Bouira (3.73).

TABLE 4. THE SUM OF MUFA MONOUNSATURATED FATTY ACIDS, PUFA POLYUNSATURATED FATTY ACIDS AND SFA SATURATED FATTY ACIDS FOUND IN ALGERIAN *P. LENTISCUS* BERRY OILS COLLECTED IN 12 LOCALITIES.

MUFA combines C16:1w7, C18:1w9 and C20:1w9; PUFA combines C18:2w6 and C18:3w3 acids; SFA includes C18:0, C16:0, C17:0, C20:0. a: Guelma, b: Mila, c: Bouira, d: Ain Defla.

Location	MUFA %	PUFA %	SFA %
Ain Makhlouf[a]	54,61	18,3	28
Ain Ben Beida[a]	53,48	17,4	27
Ben Djerrah[a]	55,31	16,4	27,2
Terrai Bainen[b]	54,06	15,8	27,2
Bouhatem[b]	54,23	17,8	28,1
Teleghma[b]	52,91	16,8	28,1
Lakhdaria[c]	54,52	22	23,2
Taguedit[c]	54,54	23	23,3
Tikadjda[c]	56,25	23	20,8
Miliana[d]	53,95	17,5	22,6
Ain Bouyahia[d]	54,71	18,5	22,6
Tarek ibn Ziyad[d]	56,42	19,5	22,1

The dots on the map correspond to the presence of populations of P. lentiscus. Green dots are populations whose fruit is maturing (black fruit stage). Red dots indicate the presence of shrubs that are unable to finalize the maturation of thefruit (stopped at the red fruit stage). The collection sites are: a:Tlemcen; b: Ain Timouchant; c: Oran; d: Mostaganem; e: Sidi Bel Abbes. Ain Defla with: f: Ain Bouyahia; g: Miliana; h: Tarek ibn Ziyad. Bouira with: i: Lakhdaria; j: Tikadjda; k: Taghedit. Mila with: l: TerraiBainen; m: Bouhatem; n: Teleghma. Guelma with: o: Ain Ben Beida; p: Bendjerrah; q: Ain Makhlouf.

3.1.3 Peroxide value (PI)

The values of the peroxide index were seen between 4 and 9. The ANOVA analyses revealed a highly significant effect ($p<0.001$) within and between regions.

3.1.4 Saponification index (SI)

The saponification index SI of the samples presented in Table 2 rangedbetween 179 and 200 mgKOH/ g oil. The region of Ain Defla had the highest saponification index, with 200.05 mgKOH/g oil. There was a very significant inter-region effect ($p<0.001$) and the Newman keuls test revealed 3 homogeneous groups. Group ain Ain Defla, b in Bouira and c in Mila and Guelma.

3.1.5 Unsaponifiable matter (Insapo)

The unsaponifiable fraction representedvalues between 0.4 and 1% of the oil (Table 2). The highest value was recorded at Terrai Bainen and the lowest at Ain Makhlouf.

3.2 Fatty acid composition of *P. lentiscus* L. oil

According to the results present in Table 3, the most abundant fatty acid was oleic acid C18: 1w9 (51-54%). Unsaturated fatty acids in *P. lentiscus*L. oil represent 72-75% of the fatty acids in this oil. These unsaturated fatty acids are divided, approximately, in54% of monounsaturated fatty acids (MUFA) and between 18 and 23% of polyunsaturated fatty acids (PUFA) (Table 4). Saturated fatty acids (SFA) represent between 22 and 27% of the total fatty acid composition of the oil. According to statistical analysis, the composition and distribution of fatty acids are not affected by geographical origin. The Ain Defla province recorded the highest rate of monounsaturated fatty acidsandthe lowest rate of saturated fatty acids (Table 4).

III.3 Composition of *P. lentiscus* L. oil in chlorophyll, flavonoids and polyphenols

According to the results presented in Figure 4, the highest rate of polyphenol was recorded in the province of Mila, and the highest rate of flavonoids and chlorophylls was recorded in Bouira with its three localities. The province of Ain Defla recordedthe lowest rate of chlorophyll and flavonoids. The Kruskal-Wallis test showed a very significant inter-region effect for the three parameters.

III.4. Principal Components Analysis (PCA)

To examine the geographical location effect on the fatty acid profile, physicochemical analysis and microelements present in lentisk oil, the principal components analysis was performed to get more details on the degree of variability between the different parameters studied in 12 sites and to get a general overview of the data distribution. The PCA revealed that 65% of the variability is expressed by axes 1 and 2, which explains the interpretation being limited only to these two components. Subsequently, plotting the scores of the samples in the sub-spaces PC1 vs. PC2 (Table5, Figure 2A.) showed a clear grouping based on the geographical origin. Table 5. Principal component analysis (PCA) based on physic-chemical parameters, fatty acids compositions and microelements of ***Pistacia lentiscus*** vegetal oil collected on 12 localities.

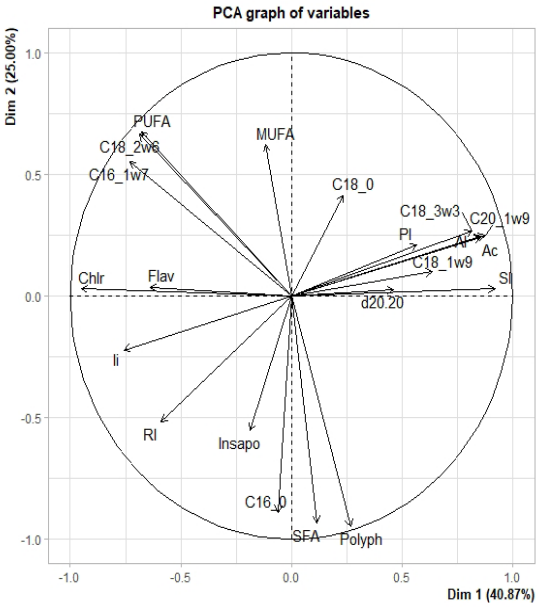

FIGURE 2A. PROJECTION OF THE VARIABLES ON THE FACTOR PLAN (1-2)

TABLE 5. PRINCIPAL COMPONENT ANALYSIS (PCA) BASED ON PHYSIC-CHEMICAL PARAMETERS, FATTY ACIDS COMPOSITIONS AND MICROELEMENTS OF *PISTACIA LENTISCUS* VEGETAL OIL COLLECTED ON 12 LOCALITIES.

Parameters	Component 1	Component 2
Eigenvalues	8.584	5.250
% of variance	40.875	25.000
Cumulative % of var.	40.875	65.875
Variables correlations		
RI	0.590	0.517
Ac	0.857	0.244
AI	0.856	0.243
PI	0.568	0.210
SI	0.921	0.029
d20.20	0.464	0.025
Insapo	-0.185	-0.554
Ii	-0.758	-0.222
C16_0	-0.062	-0.889
C16_1w7	-0.731	0.551
C18_0	0.235	0.414
C18_1w9	0.638	0.100
C18_2w6	-0.686	0.665
C18_3w3	0.816	0.267
C20_1w9	0.877	0.250
MUFA	-0.116	0.623
PUFA	-0.679	0.675
SFA	0.117	-0.932
Polyphenols	0.268	-0.946
Flavonoids	-0.637	0.036
Chlrophylls	-0.950	0.030

MICmg/L	Terrai Bainen	Bouhatem	Teleghma	Miliana	Ain Bouyahia	Tarek ibn Ziyad	Lakhdaria	Taghedit	Tikadjda	Ain Makhlouf	Ain Ben Beida	Ben Djerrah
E. coli ATCC 25922	10	10	10	10	10	10	10	10	10	10	10	10
P. aeruginosa ATCC 27853	10	10	10	10	10	10	10	10	10	10	10	10
P. fluorescens ATCC 27853	/	/	/	/	/	/	/	/	/	/	/	/
S. aureus ATCC 29213	10	10	10	5	5	5	10	10	10	10	10	10
M. luteus ATCC4698	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5
B. subtilis M23	10	10	10	10	10	10	10	10	10	10	10	10
A. niger ATCC 9029	10	10	10	10	10	10	5	5	5	10	10	10
C. albicans ATCC 20027	10	10	10	10	10	10	10	10	10	10	10	10

Axis 1 is positively correlated with Ac, AI, PI, SI, C18_1w9, C18_3w3 and C20_1w9; and negatively correlated with Ii, C18_2w6, C16_1w7, PUFA, flavonoids and chlrophylls. While axis 1 provides 40.87% of the variability, axis 2 describes 25.00% of the variability and is correlated positively with C16_1w7, C18_2w6, MUFA and PUFA; and negatively with Insapo, C16_0, SFA and Polyphenols (Table 5, Figure 2A). The opposition of the variable AI, AC, PI and SI to chlorophylls and flavonoids and its components on axis 1 shows that the acidity is negatively correlated to chlorophylls and flavonoids, which indicates that when acidity, PI and SI increase, chlorophylls contents and flavonoids decrease.

The projection of the populations on the 1-2 plan (Figure 2B) shows a clear grouping based on the geographical origin,with an opposition between the Bouira and Ain Defla localities in axis 2.

As shown in Figure 2B, four distinctive groups could be distinguished independently of the sites studied. The first group encompassed three sites of the province of Bouira: Tikadjda, Lakhdaria and Taghedit. These sites are characterized by their high content on polyunsaturated fatty acids, C18_2w6 and C16_1w7. The second group includesthree sites in Ain Defla: Tarek Ibn Ziyad, Miliana and Ain Ben Beida, which have average values in PUFA, SFA and polyphenols. The third group hasthree sitesin the province ofGuelma: Ain Makhlouf, Ben Djerrah and Ain Ben Beida, which are characterized by a balanced content of oleic acid, MUFA and PUFA. The last group comprised three localities, Terrai Bainen, Bouhatem, Teleghma, which are characterized by having the highest content ofunsaponifiable matter, C16_0, SFA and polyphenols (Figure 2A, Figure 2B).

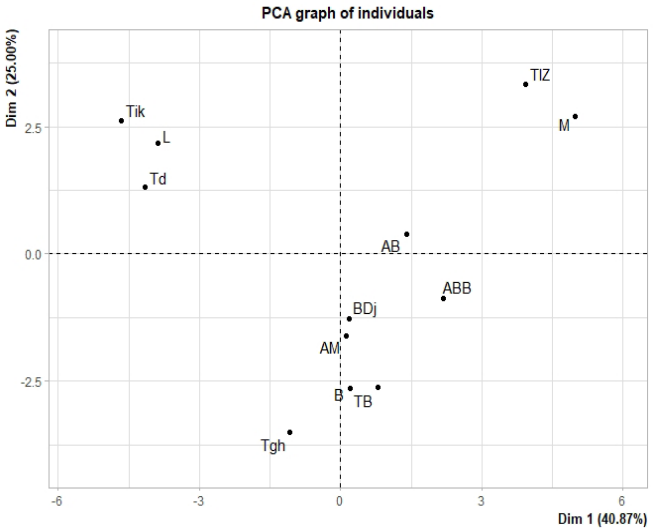

Figure 2B.*Projection of the individuals on the factor plan (1-2)*

III.5. Antimicrobial and antifungal activity

For the bacteria *S. aureus* and *M.luteus*, the inhibition zones diameter of the different oil concentrations were between 7 and 18.5 mm. The bacteria*S. aureus* and *M. luteus* showed sensitivity to the *P. lentiscus* oil, which shows an antimicrobial effect similar to the rifampicin used as a positive control.

B. subtilis and *A. niger* showed an intermediate sensitivity with inhibition zone diameters of 6 to 12 mm. *E. coli*, *P. aerogenosa* and *P. fluoresecens* showed resistance to *P. lentiscus* oil; these bacteria also showed intermediate resistance to the antibiotic Gentamicin.

C. albicans also showed resistance, except in the Ain Defla site, where an intermediate sensitivity was noted.

The negative control DMSO shows zones of inhibition from 0.1 to 0.5 mm. The inhibition effect was observed in some localities with the concentration of 0.04 mg/ml, but the most effective concentrations were found to be 0.24 and 0.4 mg/ml. A small increase in the inhibition zones diameter was observed above the 0.24mg/ml concentration. In several localities, there was stagnation in the evolution of the inhibition at thisconcentration.

The best inhibition results were recorded in the following locations: Ain Makhlouf, Lakhdaria, Miliana, and Terrai Bainen.The broth microdilution method revealed antibacterial and antifungal activity against all bacteria and fungi tested, except *P. fluorescence,* which is resistant.

Mastic oil recorded the lowest MIC against *M. luteus,* with a value of 2.5mg/mL for all sites studied (Table 6). MIC of 5mg/mLwas recorded. Assay system for bacteriocins. *Applied microbiology*, *21*(5) : 943inBouira against *A. niger* and inAin Defla, against *S.aureus*. All other localities had a MIC of 10mg/mL. No activity was noted against *P. fluoresecens* (Table 6).

TABLE 6. DIAMETER OF INHIBITION ZONES (MM) WITH DIFFERENT CONCENTRATIONS OF *P. LENTISCUS L* OILS.

/ :Development of the bacteria in all tubes: no effect of the oil on the bacteria. The first part of the table represents the minimum inhibitory concentrations and the second part represents the minimum bactericidal concentrations.

MBC mg/L	Terrai Bainen	Bouhatem	Teleghma	Miliana	Ain Bouyahia	Tarek ibn Ziyad	Lakhdaria	Taghedit	Tikadjda	Ain Makhlouf	Ain Ben Beida	Ben Djerrah
E. coli ATCC 25922	> 20	> 20	> 20	20	20	20	20	20	20	20	20	20
P. aeruginosa ATCC 27853	> 20	> 20	> 20	> 20	20	20	20	> 20	> 20	> 20	20	20
P. fluorescens ATCC 27853	/	/	/	/	/	/	/	/	/	/	/	/
S. aureus ATCC 29213	20	20	> 20	20	20	20	20	20	20	20	20	20
M. luteus ATCC4698	10	10	10	10	10	10	10	10	10	5	5	5
B. subtilis M23	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20
A. niger ATCC 9029	> 20	> 20	> 20	> 20	> 20	> 20	5	5	5	> 20	> 20	> 20
C. albicans ATCC 20027	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20

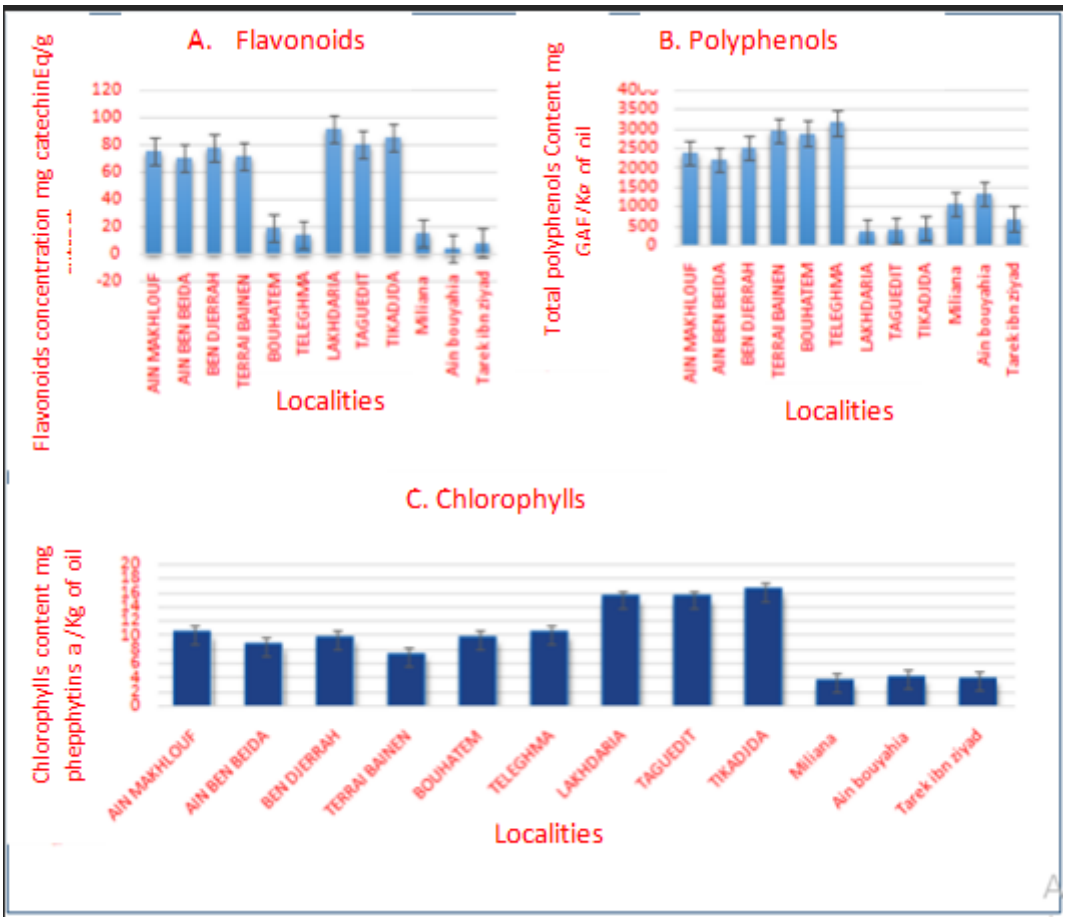

FIGURE 3.THE OIL CONTENT OF THE 12 LOCALITIES IN CHLOROPHYLLS, FLAVONOIDS AND POLYPHEN

DISCUSSION

4.1. Physicochemical characteristics of *P. lentiscus* oils

The parameters acidity, peroxide value, fatty acid compositionwere used to evaluate oil quality andthe methods used are carried out according to UICPA and ISO standards.

4.1.1. Refractive Index (RI), Iodine Index (Ii) and Density (D)

The refractive index depends on the degree of lipid saturation and the molecular weight of the most abundant fatty acid (FAO.,1993). RI values presented in Table 2 (1.47) of the studied *P. lentiscus*are close to the values of olive (1.461-1.469) and sunflower oils (1.461-1.468) (Cerchiara et *al.,* 2010). Ano et *al*. (2019) report that with an RI between 1.468 and 1.472, the most abundant fatty acid is oleic acid. This was confirmed by our results.

The Codex Alimentarius classifies oils with an Ii of <100 and a RI between 1.467-1.472 as a non-drying oil. The density of the studied oil ranges between 0.87 and 0.91, which confirms that *P. lentiscus* is a non-drying oil (Djedaia, 2016). The iodine value provides information on the degree of unsaturation of fatty acids contained in the oil,which is directly related to the degree of oxidation;oil with a low Ii is less exposed to the risk of oxidation.Lentisk oils have a lower iodine value than olive (75-94 iodine/100g oil), corn (110-128iodine/100g oil) and sunflower oils (120-136 iodine/100g oil), which makes it less oxidizing than the oils aforementioned.

4.1.2. Acidity Index (AI)

The most important parameters that define the oil quality are acidity and peroxide index. Based on the acidity index, we can classify the oils in different categories (Grossi et *al.,* 2014). The acidity index in our study (Table 2) is close to the resultspresented byCharef et *al*. (2008) for the same species, but remains lower than in other vegetable oils, such as palm kernel and palm oils, in which AI are 4.49 and 29.17mg KOH/g, respectively (Djedaia, 2016). High AIvalues could be due to incomplete fruit ripening or poor storage (Charef et *al*., 2008). Therefore, the high acidity level found in the samples from Ain Defla can be explained by the higher rate of red (immature) fruits. Thus, the best harvesting stageis the black fruit stage, which corresponds to complete maturity. To classify the oils studied, we have comparedtheiracidity with olive oil. The acidity of the tested oils allows us toclassify them as virgin oil and current virgin oil (Table 2).

In order to have virgin or extra virgin oil, a better control of the extraction process is recommended. The low value in acidity index shows that the fruit did not undergo oxidization and hydrolytic deterioration during the storage. This conclusion confirms the resultsfromDjedaia(2016).

4.1.3. Peroxide Index (PI)

The peroxide value indicates the degree of oxidation of oil (Tchiégang and Aissatou, 2004). The PI of the oils in this study was less than 9meqO_2/kg (Table 2), which indicates that the oils tested were not rancid. According to Nehdi(2011),oils are considered to be rancid when the IP values are between 20 and 40 meq O_2/kg. European regulation indicates that oils with an IP lower than 20 meq O_2/kgand an oleic acidity level less than 0.8% are to be classified as extra virgin oils (Lagardere et *al*.,2004). The PI of the studied oils was lower than several edible oils such as linseed and sunflower oils,in which the PI is 11.28 and 12.87 meqO_2/kg, respectively (Cerchiara et *al*., 2010).

4.1.4. Saponification index (SI)

The SI values in Table 2 remain within the standards for virgin olive oil (184-196) and palm oil (190-196) defined by the Codex Alimentarius (Djedaia, 2016). Samples from Ain Defla had the highest saponification indexcompared to the other three samples.This high value is due to a higher rate of red fruits in the berry sample. Such a hypothesis is confirmed by Charef et *al*.(2008). A saponification index around 196 mg KOH/gindicates the richness of the oil in C16 and C18 (Sbihi et *al*.,2013);this is confirmed by our GC profile. The SI values recorded indicate that these oils are intended for consumption rather than soap making (Haile et *al*., 2019).

4.1.5. Unsaponifiable contents (Unsap)

The unsaponifiable fraction represents less than 1% of the oil and is present in all samples (Table 2). This percentage is more or less representative of several vegetable oils such as olive oil (2%), peanut oil (1%) and sunflower oil (1-5%) (Belfadel, 2009). The unsaponifiable fraction is one of the minor compounds of oil;however,its nutritional value can be major. Research conducted byLecerf (2011) confirms that the biological properties of olive oil are mainly due to the unsaponifiable fraction. This fraction includes sterols, tocopherols, tocotrienols, polyphenols and other minor compounds (Covas et *al*., 2006). Polyphenols and tocopherols (vitamin E) are known for their antioxidant power (10 times greater than vitamin C for the polyphenols).Sterols are bile acid sequestrants and Acetyl-Coenzyme A Cholesterol-acyltransferase inhibitors. The consumption of foods rich in sterols leads to lower plasma cholesterol levels (Covas et *al*., 2006). Tocotrienols have neuroprotective, anticancer effects and lower blood cholesterol levels (Sen et *al*., 2006).

4.2. Fatty acids composition of the *P. lentiscus* oil

P. lentiscus oil is rich in oleic and palmitic acids (Table 3). The fatty acids composition of the studied oils is similar to the Algerian Lentisk oil presented by Djerrou et *al*. (2010), Dhifi et *al*. (2013) and Belyagoubi−Benhammouet *al*. (2018). Our results are in accordance with the description of Ucciani (1995) in his dictionary.

With 75% unsaturated fatty acids and 23% polyunsaturated fatty acids (Table 4), lentisk oil has a similar composition as other edible oils such as cotton, peanuts, olive, and avocado, but is less oxidative (Cuvelier et Maillard, 2012). The oleic acidcontributes in maintaining oil quality and stability (Qureshi et *al*., 2020), has a fundamental role in the prevention of cardiovascular disease (Nehdi et *al.,* 2010) and works effectively in lowering total cholesterol and LDL levels in the blood.Oils rich in oleic acid are recommended for healthy diets (Ghouila et *al*., 2019). PUFAs are very important for human nutrition due to their contents in Omega 6 and Omega 3 fatty acids, which are essential for the proper functioning of the body. These two fatty acids are not synthesized by the human body and must be provided in food. The lentisk oil has between 15 and 23% of polyunsaturated fatty acids(Table 4). Among these acids are Linoleic acid (C18: 2w6) and Linolenic acid (C18: 3W3), which are precursors of the n-6 series and the n-3 series, respectively(Ghouila et *al*., 2019).

Vegetable oil with a high level of oleic acid (> 40%) and linoleic acid (> 14%) is considered as cooking oil, in addition to being involved in the manufacturing of other products such as margarine (Tan et *al*., 2017). The levels of oleic and linoleic acids recorded for the studied oils (Table 3) correspond to the values cited by the previous study, which means that lentisk oil is suitable as a cooking and processing oil.

The statistical analysis showed an effect of geographical origin on the composition of fatty acids. This result is in agreement with other studies such as Hilali et al. (2005) for argan oil and Mezni et *al*. (2012) for Tunisian lentisk.

Generally speaking, fatty acids are essential in human nutrition as a source of energy in addition to their role in certain structural and metabolic functions (FAO., 2008).In the absence of the erucic acid in the oil, this oil is considered non-toxic and suitable for human consumption.

4.3. Composition of *P. lentiscus* oil in chlorophyll, flavonoids and polyphenols

The phenolic compounds present in the oil determine the quality and biological activities of the oil. Phenolic acids are also able to inhibit the propagation of the autooxidation reactions of unsaturated fatty acids (Hlima et al., 2017). It has been pointed out that the stability of virgin olive oil against oxidation is due to the presence of phenolic substance (Qureshi et *al*., 2020).

Our results are in accordance with Brahmi et *al*. (2020), which states that*P. lentiscus* seed oil showed a significantly higher content of chlorophyll (16.66 ± 1.20 mg/ kg) than the *Opuntia Ficus Indica* seed oil (5.66 ± 0.60 mg/kg), but this chlorophyll content (Figure 3) is low compared to other edible oils (Ben Tekaya & Hassouna, 2006).

The highest rate of chlorophylls was noted in the province of Bouira (Figure 3), which enjoys a milder climate than the other provinces: less frost, and the minimum temperatures in winter and the maximum temperatures in summer are lower than the sites of other provinces; especially, those of the province of Ain Defla, which presents the most important temperature variation of all the sites studied.

According to the work of Charef(2011), the oil extracted from the red fruits of the*P. Lentiscus*had fewer flavonoids than the oil extracted from the black fruits. This is in line with our results,as the province of Ain Defla had the lowest level of flavonoids and the highest quantity of red fruits during extraction.

The total phenol concentrations found in *P. lentiscus* by Charef(2011) andBrahmi et *al*. (2020) are very similar to our results: 3000 mg GAE/kg and 2000 mg GAE/kg respectively. In Brahmi et *al*. (2020),only protocatechuic acid was identified. Charef (2011) identified mainlyphenolic acids (hydroxybenzoic and hydroxycinnamic acids).

4.4.Principal Components Analysis (PCA)

The acidity increases with the degradation of unsaturated fatty acids and therefore with the increase of saturated fatty acids (Hlima et al., 2017). The degradation of fatty acids is often linked to poor storage conditions and extraction conditions. Poor storage conditions can be due to increased or decreased temperatures, since chlorophylls are sensitive to temperatures and can degrade. Kiritsakis et al.(1998) has proven that the chlorophylls present in olive oil degrade if the temperature rises or falls by 5°C, which explains the negative correlation on axis 1 between the parameters related to oil acidity and chlorophyll (Table 5, Figure 2A).

A negative correlation on axis 2 between SFA on one side and MUFA, PUFA, C18_2w6, C16_1w7 in the other side was also noted (Table 5, Figure 2A). It is explained by the fact that the degradation of MUFA and PUFA generates saturated fatty acids. Thus, with the decrease of MUFA and PUFA, the SFA increases. The level of polyphenols in oils is related to the origin of the oils and the conditions of extraction and storage (Abramovič et al., 2007).

PI, AC, IA are positively correlated as seen in axis 1 (Table 5, Figure 2A). IA increases with the deterioration of free fatty acids.However, the higher the PI is, more the oil is oxidized. The groups three and four had similarities as they are located in the same region (east).

4.5. Antimicrobial and antifungal activity

The oil of the*P. lentiscus* has an antibacterial activity against *M. luteus* and *S. aureus.* The MIC and CMB tests revealed a weak antifungal and antibacterial activity against the other microorganisms studied (Table 6). In spite of this, its richness in compounds havea proven antibacterial effect (fatty acids, polyphenols, flavonoids). Our results in Table 6 are supported by those of Mezni et *al*.(2014) and Abdeldjelil et *al.*(2014). However, Brahmi et *al.* (2020) andBeldi et *al*.(2020) showed no inhibitory

effect on all the tested strains. They specify that these results may be related to bacterial strains used,which were considered as resistant pathogens; the low content of phenolic compounds found in the studied oils could be also responsible for their inefficiency.

We deduct that the largest zone of inhibition does not always correspond to the lowest MIC value (Table 6). This can be explained by the fact that the diameter of the inhibition zone is affected by the solubility and volatility of the extract and its degree of diffusion in the agar (Mezni et *al.,* 2014).Gram positive bacteria were more sensitive to the effect of the oils tested when compared to gram negative bacteria. This agrees with the results of d'Ortega-Ortega et *al.* (2017) for the oil extracted from *Opuntia ficus indica* seeds,which explains this phenomenon with the fact that the outer membrane is more permeable in grampositive bacteria. The most efficient oil concentrations were 0.24 and 0.4 mg/mL.

Worldwide, the most widely used part of the mastic tree is still its mastic (Pachi et *al.,* 2020). However, in Algeria, a high consumption of the oil is noted in the region of Mila and Guelma. In addition to the consumption of the oil, the consumption of the black fruits, the use of lentisk leaves for the disinfection of wounds in compresses, and the disinfection of containers like water jars are common in the central regions of Bouira and Ain Defla.

CONCLUSION

The present study brings new informationabout the physicochemical proprieties of the *P. lentiscus oil*: the fatty acids, polyphenols,flavonoids contents and antimicrobial activity. The most used part of this plant is the oil. The acidity of theoil is low, but it is likely to be high if the fruit is not ripe, the pressing is poorly done, or the storage conditions are not appropriate. Oil of *P.lentiscus*berries is rich in unsaturated fatty acids with a dominance of oleic acid and it contains a relevant quantity of Omega 3 and Omega 6, which are not synthesized by the human body and are essential to its metabolism. The rate of unsaturated fatty acids for the oils of the 12 localities is between 69 and 79% is intereseting; especially with the presence of polyphenols and flavonoids. This composition confers to the oil a dietetic and nutritional interest with a low risk of toxicity, which places this oil as a possible new source of vegetable lipids. Thus, the properties of Lentisk oil make it suitable to be used as an oil or to be transformed into margarine or cream.

The geographical origin influences the composition of the oils in FA, in chlorophyll and flavonoids. The highest levels of the latter were recorded in the central region (Bouira). As for the polyphenols, the highest levels were recorded in sites in the eastern region of Algeria (Mila and Guelma). The chlorophyll level was low for all studied regions.

However, unlikethe leaf extracts and essential oils of lentisk, the edible oil has only a weak antimicrobial effect. In future studies, other activities can be tested, toxicity can be evaluated and the pressing method can be standardized for better oil samples.

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