OCL 2024, 31, 26
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https://doi.org/10.1051/ocl/2024025

OCL Oilseeds & fats Crops and Lipids
Available online at:
www.ocl-journal.org

RESEARCH ARTICLE OPEN ACCESS

Valorization of *Pistacia Lentiscus* L. oil: antifungal activity in an emulsion formula☆

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Received 9 May 2024 – Accepted 9 October 2024

Abstract – This study was carried out with a view to replacing the synthetic active ingredient with a natural antioxidant, and thus contributing to the development of vegetable oil extracted from the Pistacia lentiscus, a plant that is abundant in Algeria.
Lentiscus oil was extracted from seeds collected in two regions (Boumerdes and Tizi Ouzou). Extraction by cold pressing yielded a vegetable oil that was better in terms of quantity and quality, while avoiding the use of solvents.
The study was completed by a formulation trial for an antifungal emulsion based on Pistacia lentiscus oil extracted at different percentages (0.5, 1.5 and 2%). Physico-chemical characterizations of the oil and the emulsions produced were carried out, as well as a study of the antifungal and antibacterial activities.
The results show that the Pistacia lentiscus oil analyzed has significant antioxidant activity due to its polyphenol content (843.55 and 499.26 meq g gallic acid /ml oil from the Boumerdes and Tizi-Ouzou regions, respectively) and flavonoids (39.15 and 17.85 mg Eq / mg oil extract from the Boumerdes and Tizi-Ouzou regions respectively), considered to be secondary metabolites and antioxidants.
The study of antifungal and antibacterial activity showed that both the vegetable oil and the emulsion had encouraging antibacterial and antifungal effects. These could contribute to the development of new antimicrobial agents.
Sensory analysis of the emulsions produced showed that they were moisturizing, creamy, homogeneous and easy to apply and incorporate into the skin. There were no side effects such as skin allergies.
Based on the results obtained, it may be possible to replace lentisk oil synthetic active ingredients in the formulation of an antifungal cream.

Keywords: Antioxidant activity / antimicrobial activity / formulation / *Pistacia lentiscus* / vegetable oil

Résumé – Valorisation de l'huile de lentisque : activité antifongique d'une émulsion. Cette étude a été menée afin de substituer le principe actif synthétique par un antioxydant naturel, contribuant ainsi, à la valorisation de l'huile végétale extraite du fruit du lentisque, plante abondante en Algérie.
L'huile de lentisque a été extraite à partir de graines récoltées dans deux régions (Boumerdes et Tizi Ouzou). L'extraction par pression à froid a permis d'obtenir une huile végétale meilleure en termes de quantité et de qualité, tout en évitant l'utilisation de solvant.
L'étude a été complétée par un essai de formulation d'une émulsion antifongique à base d'huile de lentisque extraite à différents pourcentages (0.5, 1.5 et 2g). Des caractérisations physico-chimiques de l'huile et des émulsions produites ont été réalisées ainsi qu'une étude des activités antifongique et antibactérienne.
Les résultats montrent que l'huile de lentisque analysée possède une activité antioxydante appréciable grâce à sa teneur en polyphénols (843,55 et 499,26 meq g d'acide gallique/ml d'huile issue de la région de

☆ Contribution to the Topical Issue: "Lipids and Cosmetics / Lipides et cosmétiques".
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Boumerdes et Tizi Ouzou, respectivement) et en flavonoïdes (39,15 et 17,85 mg Eq/mg d'extrait d'huile issue de la région de Boumerdes et Tizi Ouzou, respectivement), considérés comme des métabolites secondaires et des antioxydants.
L'étude de l'activité antifongique et antibactérienne a permis de constater que l'huile végétale et l'émulsion présentent des effets antibactériens et antifongiques encourageants.
L'analyse sensorielle des émulsions élaborées, a permis de constater qu'elles sont hydratantes, onctueuses, homogènes et facile à étaler et à incorporer dans la peau. Il n'y a eu aucun effet secondaire tel que les allergies cutanées.
D'après les résultats obtenus on peut dire qu'il est possible de remplacer les principes actifs synthétiques par l'huile de lentisque dans la préparation d'une crème antifongique

Mots-clés : Activité antioxydante / activité antimicrobienne / formulation / *Pistacia lentiscus* / huile végétale

Highlights

Integrating oilseed crops and essential oils, such as Pistacia lentiscus, into the cosmetics and pharmaceutical industries can reduce the use of synthetic chemicals, which are often non-biodegradable, thus reducing the load of pollutants in freshwater and aquatic ecosystems in general.

1 Introduction

Lentisk, *Pistacia lentiscus* L is an evergreen shrub or tree of the *Anacardiaceae* family, mainly known as Darou, dherou or Drou in North Africa (Amara *et al*., 2019), Listincu or Chessa in Sardinia; or Mastiha tree in Greece. Its distribution covers the whole Mediterranean area, from the Iberic Peninsula to the Middle East (Benalia *et al*., 2020). The use of *Pistacia lentiscus* berries, galls, resin, and leaves in folk medicine to treat a wide range of diseases dates back to Greek antiquity (Siano *et al.,* 2020). Lentisk fruits, galls, resin, and leaves have been identified in almost all parts of the P. lentisk a long tradition in folk medicine dating from the times of the ancient Greeks (Dhifi *et al.,* 2013).

The essential oil of *Pistacia lentiscus* is considered a promising source of natural cytotoxic compounds with anticancer properties. Phytoconstituents with pharmacological properties have been identified in almost all parts of the *Pistacia lentiscus* plant (Bozorgi *et al.,* 2013). For example, the essential oil of the aerial parts (leaves, twigs and berries) of *Pistacia lentiscus* exhibited antibacterial and anti-inflammatory effects. Lentisk berries can be considered oleaginous drupes, as they contain high average quantities of an edible oil exceeding 40% (w/w) on a dry weight basis (Siano *et al*., 2020). Numerous studies have investigated the phytochemical composition of the resin, the leaves and the galls of Lentisk, as well as its essential oils (Castola *et al*., 2000), but the composition of the fruit oil has been little studied.

The oil extracted from ripe fruits is commonly used in Algerian traditional medicine as an anti-ulcer, healing and antiseptic agent (Nahida and Siddiqui, 2012; Ait Mohand *et al*., 2020). Lentisk berry oil can improve diabetes, gastric ulcers, asthma and possesses antidiarrheal, anti-helminthic, anti-inflammatory properties, as well as preventing wound infections.

The aim of this study is to evaluate the physicochemical properties and biological activities (antibacterial and antifungal) of *Pistacia lentiscus* oil harvested from two stations in Algeria (Boumerdes and Tizi ouzou), and to test the formulation of an antifungal emulsion based on extracted lentisk oil.

2 Materials and methods

2.1 Oil extraction

Pistacia lentiscus L. fruit were harvested from two regions in Algeria (Boumerdes 36° 46′ 00″ Nord, 3° 28′ 00″ East, and TiziOuzou 36° 43′ 30″ North, 4° 03′ 30″ East) in the second half of December 2022.

In order to obtain the best oil in terms of quantity and quality, a cold extraction press has been used to avoid any traces of the solvent. The traditionally extracted virgin fatty oil was stored in a well-filled, tightly-sealed glass bottle. It was kept cool and protect from light until use.

2.2 Methods for assessing physico-chemical parameters

The aim of this study was to determine certain physico-chemical properties of two samples of lentisk oil from two different regions. This is a qualitative study with the purpose of identifying the differences between these samples, influenced by a number of factors (geographical). The absence of standards for this oil has prompted us to compare our results with those of other vegetable oils.

2.3 Chemical analysis

This is a qualitative study to determine the main physico-chemical parameters of the extracted oils. Free acidity (ISO 660), peroxide values (ISO 6320), and UV absorption indices (K_{232}, K_{270}) were determined according to commercial standard methods for olive oil (ISO 3656).

Acidity is the percentage of free fatty acids expressed as a percentage of the molecular weight of oleic acid, palmitic acid or auric acid: 282, 256, 200 g, respectively. The acid number is the number of milligrams of potassium (KOH) needed to neutralize the acidity of 1 g of fat.

$$\%\text{Acidity} = 28.2 \times V \times N/P$$

Where V the volume of KOH solution used (ml); N: normality of KOH solution (0.1 N); 282.2, molecular weight of oleic acid (mol/L); P weight of oil aliquot (g).

To obtain the percentage of oleic acid, simply divide the acid number by 2.

$$\%\text{Oleic Acid} = I_a/2$$

The peroxide index measures a fundamental phenomenon that occurs in all fats, namely oxidation. The chemical alteration of unsaturated fats by oxygen in the air begins with the formation of peroxide by odometry. This is either the number of micrograms of peroxide per kg of fat, or the milliequivalents of active oxygen per kg of fat.

The principles are potassium iodide oxygenation and the titration of iodine released by sodium thiosulfate.

The peroxide value is calculated according to the following formula:

$$IP = (V_1 - V_0) \times N \times 10/P$$

Where IP, peroxide value (meq/kg); V_1, is the volume of Na thiosulfate in the sample; V_0, is the volume required to titrate the white; *N*, is the exact title of Na thiosulfate used and P, oil sample in grams.

The saponification index is the number of milligrams of KOH required to neutralize the free acidity and saponify the esters of 1 g of lipid. The value of the saponification index allows us to estimate the carbon chain lengths of the fatty acids that make up the oil (Azizi *et al.*, 2022).

Fourier Transform Infrared (FTIR) analysis of the specimen was carried out the 400–4000 cm^{-1} rang.

2.4 Total phenol contents

Total phenol contents of samples were determined according to the method of Singleton and Rossi (1965), based on the principle of a colorimetric reaction using the Folin–Cicalteu (FC) reagent.

Folin–Ciocalteu (0.5 ml) was added to the sample (0.5 ml) and the solution mixed for five minutes. Next, 0.5 ml to 10% Na_2CO_3 was added to the solution tubes, and the final volume was made up to 5 ml with distilled water. The absorbance for total phenolic content was measured at 750 nm using a spectrophotometer, taking gallic acid (0–2 mg/ml) as the standard for the calibration curve.

2.5 Flavonoids assay

Flavonoids were quantified using the method of Zhishen *et al.* (1999), a calibration curve produced by a standard flavonoid, quercetin. Flavonoid content is expressed in milligrams of quercetin equivalent per gram of extract.

In a 100 mL volumetric flask, 1 mL of oil is added to 1 mL 20% $AlCl_3$ and 100 mL pure ethanol, then the flask is topped up with distilled water. Incubated in the shade at room temperature for 30 min. After incubation, absorbance is read at 430 nm on a blank.

2.6 Determination of anti-radical activity

The anti-radical activity was determined by the DPPH test using 2,2- diphenyl-1-picrylhydrazyl reagents.

A solution of DPPH- (0.004%) was freshly prepared. A 1300 µL volume of this solution was mixed with 100 µL of ethanolic extract prepared by dissolving 1 ml of lentisk oil in 5 ml of ethanol to recover as much of the active ingredient as possible. The solutions were shaken well and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm. A control was prepared by replacing the ethanolic extract with the same volume of ethanol. The percentage of radical inhibition due to the antioxidant properties of the extracts was calculated using the following formula:

$$\%\text{inhibition} = [(Abs_{\text{control}} - Abs_{\text{extract}})/Abs_{\text{control}}] \times 100$$

Where Abs_{contro} absorbance of control; Abs_{extract} absorbance of sample.

3 Microbial strains

A number of four microbial strains, provided by the microbiology laboratory of the SAIDAL group at El-Harrach (Algiers), were tested, including one Gram-positive bacterial strain (*Staphylococcus aureus* ATCC6538), two Gram-negative (*Escherichia coli* ATCC839 and *Pseudomonas aeruginosa* ATCC 27853) and one yeast (*Candida albicans* ATCC 10231).

3.1 Evaluation of the antibacterial activity of lentiscus oil

The method adopted for the determination of antibacterial activity is that of disc diffusion (Bammou *et al.,* 2015; Debbabi *et al.,* 2017) This method consists of using Wattman paper discs 6 mm in diameter, impregnated with a known volume of the oil to be tested, placed on the surface of an agar medium (Muller Hinton Agar "AMH"), previously inoculated by swabbing. On the surface by the bacterial suspension and the oil diffuses radially from the disc into the agar, thus forming a concentration gradient. After incubation for 24 h at 37 °C, the results were read by measuring the diameter of the inhibition zone in (mm) produced around the discs containing the oil following the National Committee for Clinical Laboratory Standards (NCCLS, 2006).

Antibacterial activity, when observed, appears as a halo of inhibition around the disc. The measured diameter gives qualitative data on inhibition, with the rule: "The greater the inhibition diameter, the greater the antibacterial activity the tested sample presents". The discs were prepared in two different ways according to Fontanay *et al.* (2015): At 10 µL of an HV/DMSO mixture (V/V 50/50) per disc; At 10 µL of pure HV per disc. The aim of these two different conditions is to evaluate the impact of the dispersant (here DMSO) on the diffusion of the HV in the agar culture medium. This test was carried out to determine the resistance or sensitivity of bacteria to the two types of lentisk oil. The results are read by

Table 1. Composition of different emulsions.

		Emulsions 1		Emulsions 2		Emulsions 3	
		In mg	In %	In mg	In %	In mg	In %
Bioactive additive	lentiscus Oil	500	0.97	1500	2.85	2000	3.77
Oil phase	Isopropyl myristate	1500	2.91	1500	2.85	1500	2.83
	Stearic acid	2500	4.85	2500	4.76	2500	4.71
	polyoxy 40 sterate	2500	4.85	2500	4.76	2500	4.71
	cetyl alcohol	2500	4.85	2500	4.76	2500	4.71
Aqueous phase	Sodium loryl sulphate	75	0.14	75	0.14	75	0.14
	Purified water	41925	81.4	41925	79.85	41925	79.1
Total		51000	100	51000	100	51000	100

measuring the diameter of the inhibition zone according to the method of Ponce *et al.* (2003).

From 18 h of bacteria young culture, we marked suspensions by taking 3 to 5 well-isolated, identical colonies and placing them in 5 ml sterile distilled water. We vortexed for a few seconds. We used a spectrophotometer to read the optical density at a wavelength of 620 nm, which should be between (0.8 − 1) for *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*, corresponding to a concentration of 10^7-10^8 germs/ml (Fontanay *et al.,* 2015). From of yeast young culture of 48 h, we marked suspensions by taking 3 to 5 well-isolated and identical colonies and placing them in 5 ml of sterile physiological water, then we vortexed for a few seconds and we used a spectrophotometer to read the optical density, which should be between (0.8- 1) for *Candida albicans*, corresponding to a concentration.

4 Analysis by Fourier Transform Infrared Spectroscopy (FTIR)

Analysis by infrared spectrophotometry makes it possible to distinguish the bands characteristic of the main chemical functions of a food; it is an essential tool for monitoring induced chemical modifications. Thus, a sample irradiated by an infrared beam undergoes molecular vibrations and absorbs part of the incident energy. Its infrared spectrum therefore presents absorption bands whose frequencies are characteristic of the chemical nature of the compound. The device used is an Alpha brand Fourier Transform spectrophotometer from Bruker. The analysis of the spectrum is carried out in the 4000–400 cm^{-1} wave number zone (resolution 1 cm^{-1}).

5 Formulation experimental protocol

The emulsion (W/O) was prepared according to the protocol used for the preparation of pharmaceutical emulsion (W/O) (Phanazol) in the physicochemical laboratory of the SAIDAL unit in Dar el Beida, Algiers. The formulation prepared is a water-in-oil (W/O) type emulsion.

5.1 Preparation of the emulsion (W/O)

The oil phase mixture was obtained by using acetyl alcohol as a stabilizer, stearic acid and isopropyl myristate as an oil phase constituent and stabilizer, polyoxy 40 stearates as an emulsifier. The mixture was heated at 70 °C for 05 min.

In parallel, we prepared the aqueous phase by dispersing the sodium lauroyl sulfate in purified water and heated for 05 min it at 70 °C. Then, the oily phase is dispersed and mixed with the aqueous phase at 70 °C for 15 min until an emulsion is obtained. The emulsion is transferred in a cold bath for 15 min until it is stabilized in an emulsion texture. Finally, the active ingredient is added (Tab. 1).

5.2 Physicochemical characterization of emulsion (W/O)

Emulsion characterization (W/O) is based on pH and electrical conductivity measurements, organoleptic analyzes, stability, emulsion direction (W/O) and viscosity study.

5.3 Sensory analysis

In our study, we chose to carry out a sensory analysis to evaluate the quality criteria of the emulsion (W/O) produced, by obtaining the levels of appreciation of the emulsion (W/O) by consumers. Evaluation sheets were prepared and distributed to the 20 examiners for possible sensory testing. We chose the following quality criteria: emulsibility, color, odor, viscosity, dermal allergy, homogeneity, easy to incorporate into the skin, itching, and ease of spreading on the skin.

6 Results and discussion

6.1 Physicochemical parameters

Table 2 shows the Physico-chemical characteristics of oils extracted from *Pistacia lentiscus* fruits in two regions.

We can observe from data on Table 2 that the peroxide index for the analyzed oil from Tizi Ouzou is 0.012 mg/kg, while that for the oil from Boumerdes is about 0.0150 mg/kg. These values comply with Codex Alimentarius standards,

Table 2. Physicochemical characteristics of *Pistacia lentiscus* oil.

	Tizi −Ouzou lentisk oil	Boumerdes lentisk oil
Density	0.920	0.917
Refractive index	1.472	1.4421
Peroxide index mg/kg	0.0125 ± 0.0025	0.015 ± 0.005
Acide index mg KOH / g	0.954 ± 0.353	1.136 ± 0.300
Acidity %	30.399 ± 7.212	26.736 ± 2.316
Saponification index mg KOH/g	204.195	199.78
Ester index	203.241	198.644
K_{232}	2.241 ± 0.002	2.2 ± 0.006
K_{270}	1.064 ± 0.015	1.019 ± 0.001

Table 3. The total phenol contents of lentisk oil from the two regions (Tizi- Ouzou and Boumerdes).

Samples	Oil extract meq g of gallic acid/ml for Tizi Ouzou oil	Oil extract meq g of gallic acid/ml for Boumerdes oil
total phenol contents	843.5583	499.2683

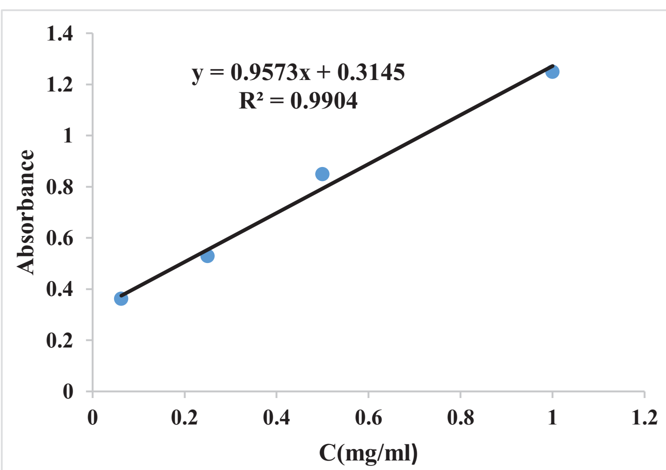

Fig. 1. Gallic acid calibration curve.

which sets the value below 20 meq of peroxides/kg of oil. Our values are significantly lower than those found by Siano *et al.* (2020), which are 6 mg/kg. The low values found confirm that the samples are not exposed to air or oxidation during storage and processing. Fatty substances can oxidize in the presence of oxygen and certain contributing factors (high temperature, water, enzymes, trace metals: Cu, Fe, etc.). Indeed, good hygiene and manufacturing practices will have a positive impact on peroxide content immediately after extraction.

The acidity indices obtained varies from 1.136 to 0.954 (mg KOH / g) for lentisk oil from the Tizi Ouzou and Boumerdes regions respectively. These values are in line with Codex Alimentarius standards (0.8-3.3), indicating that this oil contains low levels of free fatty acids since it is freshly extracted. This low content may be due to the fruit being well preserved from harvesting to extraction and analysis, and thus to complete ripening. Our values are significantly lower than those found by Beldi *et al.* (2020), which are 2,24 to 8,36.

Lentiscus oil analysed from Boumerdes had a saponification index of 199.78 mg KOH/g oil and that from Tizi Ouzou had an index of 204.195 mg KOH/g oil. The values for both regions are lower than those found by Kechidi *et al.* (2020) which are 296.28.

Our values are below those found by Beldi *et al.* (2020), with saponification indices for Algerian varieties ranging from 197.75 to 213.45 mg KOH/g. On the other hand, Charef *et al.* (2008) (recorded a low index compared with that of the current study, where the index for oil extracted from black *Pistacia lentiscus* fruits is 147.8 ± 0.2 mg KOH /g of oil, while for red fruits, this exponent is 154.6 ±. 0.1 mg KOH/g oil.

The variation in the saponification index can be attributed to climatic factors and fruit maturity.

The ultraviolet (UV) absorbance values of the coefficients (K_{232}, K_{270}) are respectively (2.241, 1.064) for Tizi Ouzou oil and (2.2, 1.019) for Boumerdes oil. These values are close to those found by Karoui *et al.* (2020).

The UV absorbance results (Tab. 1) show that the samples analyzed have UV absorbances that comply with the values recommended by the COI standard: K232 $\leq$ 2.5; around 230 nm, while the values recorded are higher than the standard around 270 nm (K270 $\leq$ 0.25), indicating the presence of secondary oxidation products.

6.2 Total phenolic content

The concentration of total phenolic compounds is determined by reference to the calibration curve obtained using gallic acid as the calibration standard.

The total phenol contents of the oils from the Tizi Ouzou and Boumerdes regions are extremely different, 843.5583 meq g of gallic acid/ml for Boumerdes oil and 499.2683 meq g of gallic acid/ml for Tizi-Ouzou oil (Tab. 3). Siano *et al.* (2020) obtained a value of 339. Mezni *et al.* (2018) gave a value higher than ours, equal to 3358.08 mg.g^{-1}.

Variations in observed values may be due to difference in the degree of maturity of the lentisk seeds before crushing

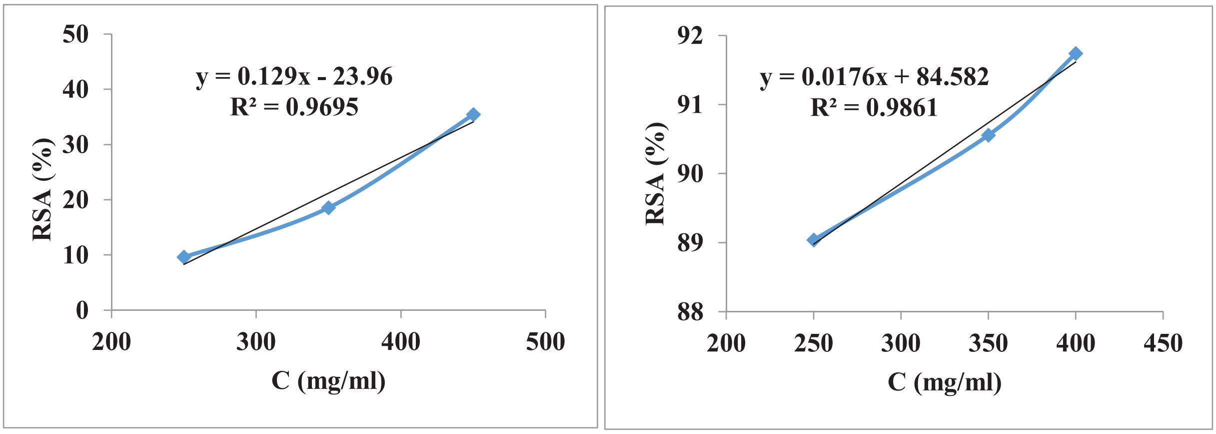

Fig. 2. Percentage of DPPH free radical inhibition as a function of the different concentrations used for the two oils.

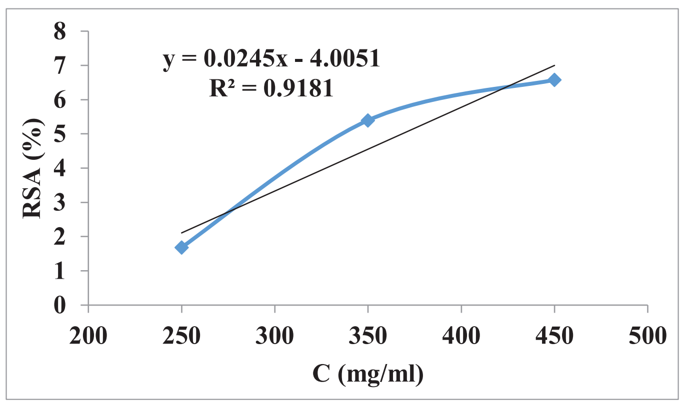

Fig. 3. Percentage of DPPH free radical inhibition as a function of the different ascorbic acid concentrations.

(early harvesting lentisk) but may also depend on the variety grown and the geographical area (García *et al.,* 2003). The variations in content observed may also depend on the difference in the part of the plant studied (Fig. 1).

6.3 Flavonoid dosage

The quercetin calibration curve was used ($Y = 0.0259X - 0.0978, R2 = 0.9958$) for flavonoid determination, and results are expressed in mg quercetin equivalent per mg of extract (mg/ mg extract). The results show variability in total flavonoid contents (from 17,854 mg Eq/mg extract to 39,150 mg Eq/mg oil extract) depending on the type of oil. High levels of total flavonoids were observed in Boumerdes lentisk oil extracts, while the lowest values were recorded in Tizi Ouzou oils (Fig. 1).

6.4 Antioxidant activity

DPPH, initially violet, discolors when the single electron pairs. This discoloration is representative of the phenolic compounds that can scavenge these free radicals. This test therefore provides information on the direct anti-radical power of the various phenolics present in the extracts. In our work, we chose to test the different extracts of each plant oil. The values obtained were used to draw exponential curves (Figs. 2 and 3).

From these curves, we can determine the inhibition percentages obtained as a function of the concentration used as well as the IC_{50} value of each extract.

6.5 Determination of antioxidant capacity (IC50)

The antioxidant capacity of the different extracts was determined from the IC50. This index corresponds to the concentration required to reduce 50% of the DPPH radical. The smaller the IC50 value, the greater the activity of the extract tested. The IC50 values found for all the extracts tested are shown in Table 4.

Table 4 shows the IC50 values of extracts from the two plant oils studied, compared with those of the reference antioxidant.

Antioxidant tests of extracts by $DPPH^{\cdot}$ method showed that extract from Tizi ouzou oil has a greater activity than extract from Boumerdes oil. Inhibitory concentrations at 50% (IC50) were respectively 919.405 mg/ml and 948.06 mg/ml for extract from Tizi ouzou and Boumerdes oil while ascorbic acid used as standard showed an IC50 value equal to 4,777 g/ ml. The results thus discussed confirm that these results point that this oil is a promising source of new bioactive compounds source.

Table 4. IC50 values of lentisk oil from the two regions (Tizi Ouzou and Boumerdes) compared to the reference antioxidant.

Samples	Tizi Ouzou oil extract mg/ml	Boumerdes oil extract mg/ml	Acide ascorbique mg/ml
IC_{50}	919.405	948.06	4.777

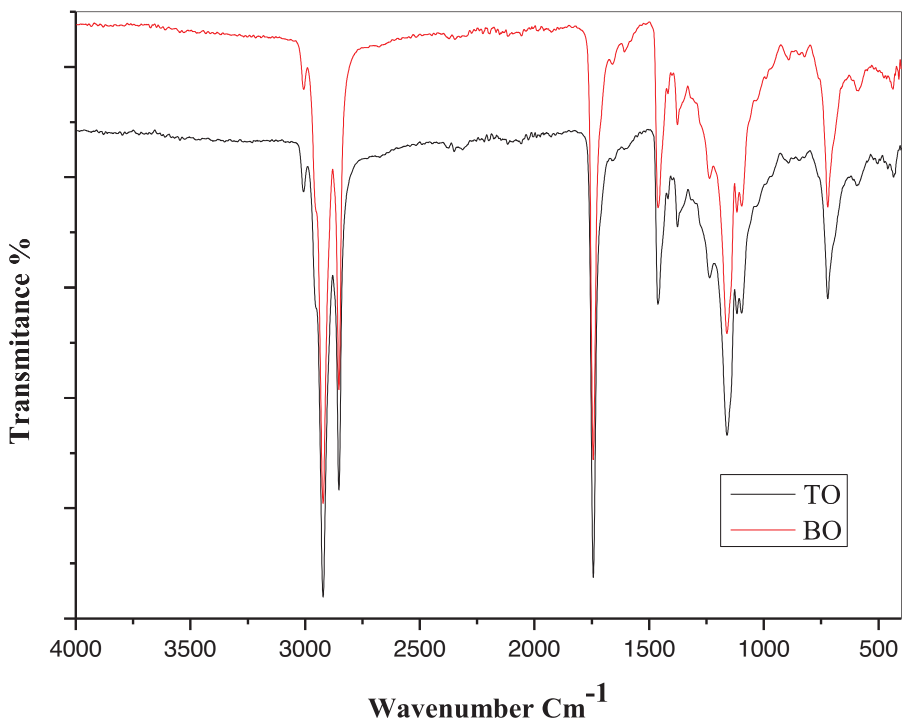

Fig. 4. FTIR spectra of TiziOuzou lentisk oil (TO) and Boumerdes lentisk oil (BO).

Several recent studies have proven that *pistacia lentiscus* fruits have been shown *in vitro* radical scavenging properties by using DPPH. As reported by Belyagoubi Benhammou *et al.* (2018), the results of Antioxidant activity; showed that oil of fruits has a height capacity to scavenge DPPH radical (EC50 = 20.619 ± 0.312 mg/mL).

6.6 Infrared analysis

Fourier transform infrared spectroscopy was carried out to identify the functional groups of lentisk oils. Figure 4 represents the comparison of the FTIR spectra of lentisk oils from Tizi Ouzou lentisk oil (TO) and Boumerdès lentisk oil (BO), (two national regions).

The spectra obtained for the two lentisk oil samples are similar in shape, but slightly different in peak intensities; the peaks observed on the infrared spectra of lentisk vegetable oils show good agreement with the literature (Souza *et al.,* 2007). Indeed, by referring to existing infrared spectrometry databases and considering the absorption bands of the peaks obtained, it was possible to identify the characteristic groups and bonds of lentisk oil as follows:

- The carbonyl groups (C=O) present at the ends of the fatty acid ester chains show an absorption band around 1740 cm^{-1};
- The double bond (C=C) that characterizes the establishment of oleic acid C18:1 and linoleic acid C18:2, contained in lentisk oil, shows an absorption band around 1464 cm^{-1};
- The (C-O-C) groups present at the fatty acid/glycerol function show a corresponding peak at 1160 cm^{-1};
- The $(-CH2-)_n$ groups characterizing the long aliphatic chains of fatty acids show a band with an average intensity of 722 cm^{-1};
- The $(-CH_2-)$ groups in the adsorption band [2922–2850 cm^{-1}].

7 Physicochemical characterization of emulsion

7.1 Macroscopic analysis

The examination of macroscopic characteristics constitutes the first approach to the quality of the preparation, and must be the subject of an in depth study by the manufacturer, so that any anomalies can be observed. Indeed, changes recorded at the level of visual characteristics are possible indicators of degradation of the emulsion depending on various causes and factors (variation in pH, conductivity, viscosity, appearance, color, odor, texture). In this work, macroscopic analysis,

Table 5. Macroscopic appearance, stability and pH of various emulsions produced.

	Emulsions formulas	B_1	B_2	B_3	T_1	T_2	T_3	FR
Stability	In day (D_0) of formulation	stable	stable	stable	stable	stable	stable	stable
	After 60 days	stable	stable	stable	stable	stable	stable	stable
pH		4.458	4.178	4.531	4.534	4.626	4.225	2.89
	Texture	Emulsiony	Emulsiony	Emulsiony	Emulsiony	Emulsiony	Emulsiony	Emulsiony
	Order	Aroma of lentisk	Aroma of lentisk	Aroma of lentisk	Aroma of lentisk	Aroma of lentisk	Aroma of lentisk	/
	Color	Pale yellow	Pale yellow	Dark yellow	Pale yellow	Pale yellow	Dark yellow	White

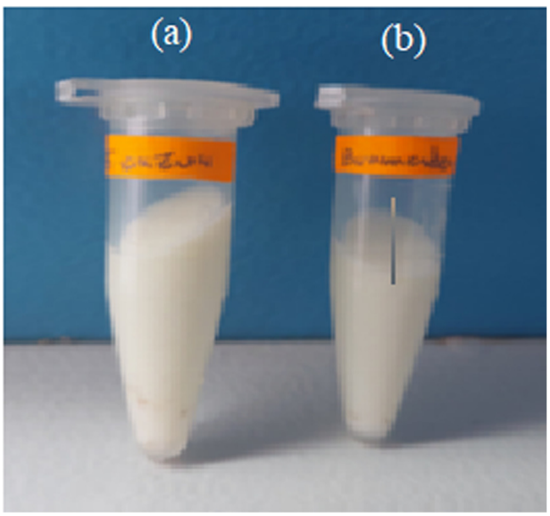

Fig. 5. Stability of emulsion after centrifugation (2000 rpm for 15 min) with Pistacia lentiscus L. vegetable oil from the two regions of Algeria (Tizi Ouzou (a) and Boumerdes (b)).

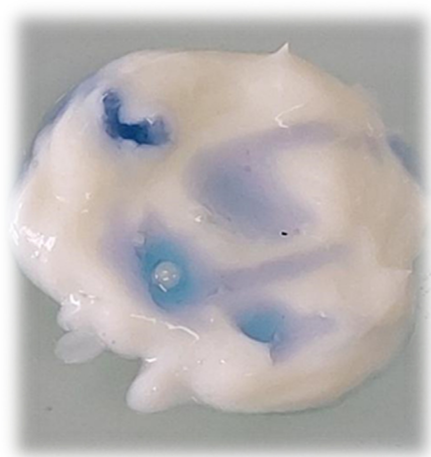

Fig. 6. Colorimetric reaction of methylene blue on the antifungal emulsion formulated with Pistacia lentiscus L. vegetable oil.

stability and pH of antifungal emulsion revealed the following characteristic elements (Tab. 5).

Boumerdes emulsion 0.5, 1.5 and 2 g of lentiscus oil (B1, B2 and B3), Tizi Ouzou emulsion 0.5, 1.5 and 2 g of lentiscus oil (T1, T2 and T3) and Phanazol reference (FR)

Emulsiony texture, Odor of lentisk "*Pistacia lentiscus* L" and color varies from pale yellow to paler yellow (yellowish) to dark yellow. The color of the formulated emulsions varies depending on the quantity of lentisk (*Pistacia lentiscus* L.) vegetable oil in the emulsion.

7.2 Hydrogen potential determination

According to the data in Table 5, the reference emulsion "Phanazol" is too acidic. In contrast, antifungal emulsions formulated with *Pistacia lentiscus* L. vegetable oil (VO) are characterized by an acid pH profile close to skin pH, with no skin irritation. This profile is considered satisfactory for use on the face, where the pH should be between 4.5 and 8 (S.N. 1996).

Apriliana *et al.* (2018), stated that an over alkaline pH can cause scaly skin, while an over acidic pH can cause irritation. The pH values obtained for emulsion formulations were always considered to meet the requirements for use on the face, with pH values ranging from 4.5 to 8. A pH that is too acidic pH can cause irritation, while one that is too alkaline pH can lead to skin peeling.

7.3 Emulsion stability

The stability study was carried out at centrifugation, to assess the degree of phases separation. In this study, the stability of the antifungal emulsion formulated with Pistacia lentiscus L. vegetable oil was analyzed according to its storage time (approximately two months). The results of this test found are mentioned in Table 5 and Figure 5.

The results obtained (Tab. 5) show that all six antifungal emulsion formulations have perfect stability without any separation of the two phases (oily and aqueous) after centrifugation. The quality of the formulated emulsion was assessed using different evaluation methods. No change in physical properties were observed in formulation. The formulated emulsion showed good consistency and spreadability, a pH near to skin pH and there is no phase separation during the formulation study period. In addition, stability parameters like visual appearance, nature and viscosity of the formulated emulsion showed no variation during the study period.

Several research have shown that when phase separation occurs, all the characteristics of emulsions are affected (Casteli *et al.,* 2008).

7.4 Emulsion direction

7.4.1 Methylene blue staining method

The result of the methylene blue emulsion staining experiment is shown in Figure 6.

Table 6. Electrical conductivity measurements of antifungal emulsions formulated with Pistacia lentiscus L. vegetable oil.

Emulsions formulas	B1	B2	B3	T1	T2	T3
Electrical conductivity (µs/cm)	15.49	18.545	17.07	17.18	16.13	19.85

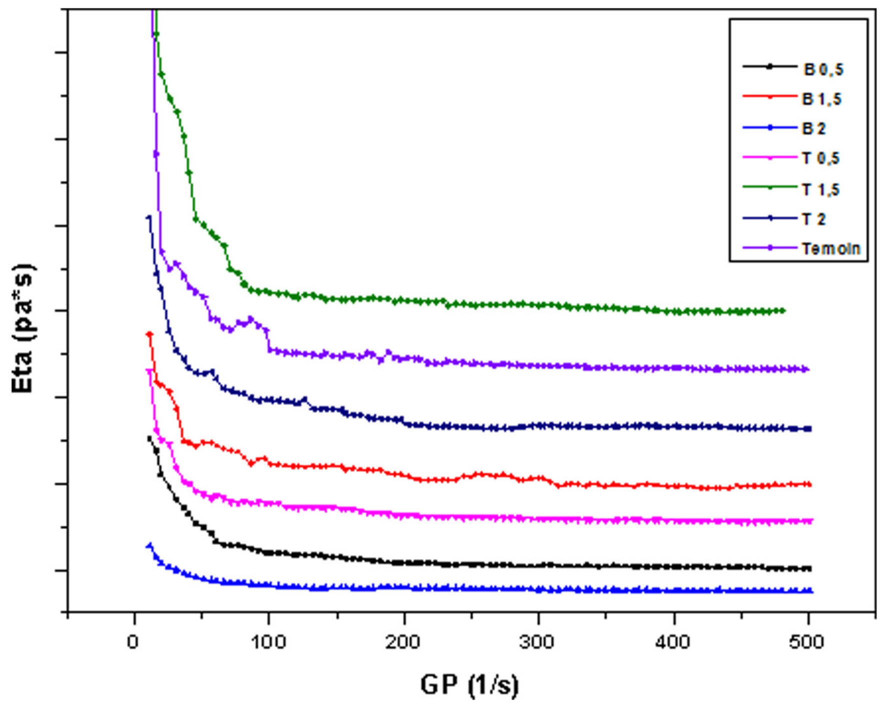

Fig. 7. Rheological curve of antifungal emulsions formulated with Pistacia lentiscus L vegetable oil from the two regions of Algeria and Phanazol reference.

This test revealed that methylene blue did not diffuse into all the emulsions prepared (Fig. 6). This could be justified by the fact that the emulsion/dye mixture is heterogeneous, so the antifungal emulsion formulated in this study does not absorb the dye. It can therefore be said that the emulsions used are of the water/oil type.

7.5 Electrical conductivity determination

In general, the electrical conductivity is the ability of a solution or all types of materials to pass an electric current. In solution, anions and cations are responsible for carrying current. The results are presented in Table 6.

According to the results obtained for this test (Tab. 6), we note that all the emulsions formulated with different percentages of lentisk "*Pistacia lentiscus L.*" vegetable oil have similar electrical conductivity values. These results agree with those of stability since these formulas are all stable.

On the other hand, the electrical conductivity values depend on the composition of the antifungal emulsion formulated.

7.6 Viscosity determination

This test makes it possible to study the variation in the viscosity of antifungal emulsions formulated with *Pistacia lentiscus L* vegetable oil as a function of the shear speed at room temperature. The results obtained are illustrated in Figure 7.

The rheological essays showed that these emulsions are non-Newtonian or pseudo-plastic (*i.e.*, as shear rate increases, viscosity decreases) corresponding to Farah *et al.* (2005) and Lavaseli *et al.* (2012).

From the results obtained, we note that the appearance of the viscosity of the Phanazol® (reference) is proportional to the curve of the emulsions formulated with *Pistacia lentiscus* L. vegetable oil from the two regions of Algeria.

7.7 Infrared analysis of formulated emulsion

The results of Infrared analysis of antifungal emulsion formulations of *Pistacia lentiscus* L. are shown in Figure 8.

7.8 Sensory analysis

After collecting the evaluation sheets, a statistical study was carried out to obtain the evaluation levels for each criterion examined. The results obtained are represented on the histogram (Fig. 9).

Reading the data on the histogram shows that most people appreciated the quality of the emulsion with percentages of appreciation between 80-100%. Therefore, we can say that the

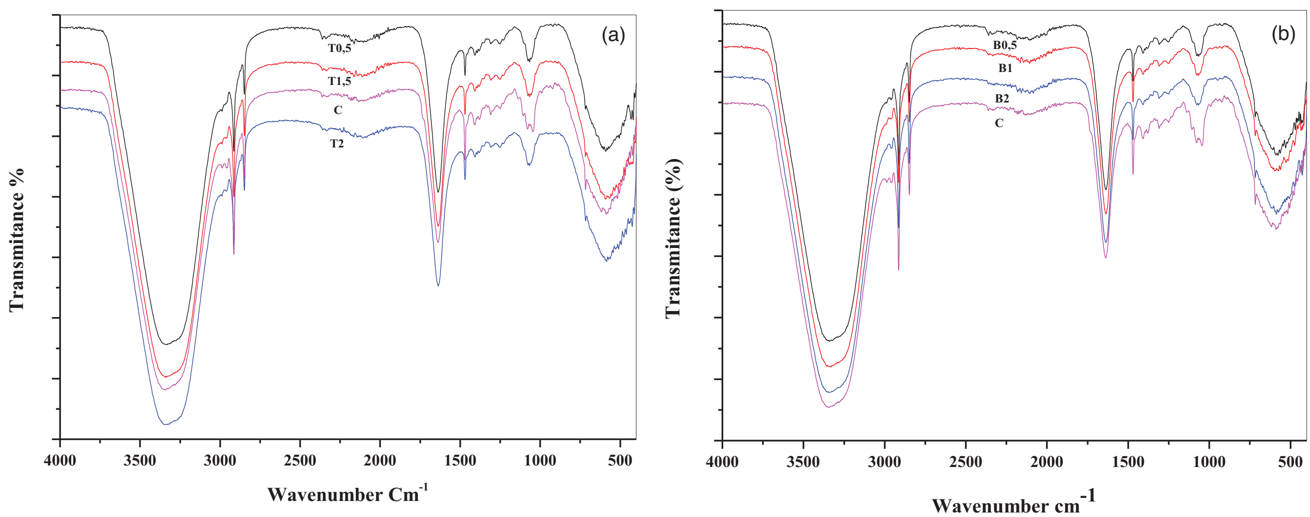

Fig. 8. IRTF spectra of control antifungal emulsions and these prepared with *Pistacia lentiscus* vegetable oil from two Algerian regions : (a) IRTF spectra of Tizi Ouzou lentisk oil (b) Boumerdes lentisk oil. Boumerdes emulsion 0.5, 1.5 and 2 g of lentiscus oil (B1, B2 and B3), Tizi Ouzou emulsion 0.5, 1.5 and 2 g of lentiscus oil (T1, T2 and T3) and Phanazol reference (c).

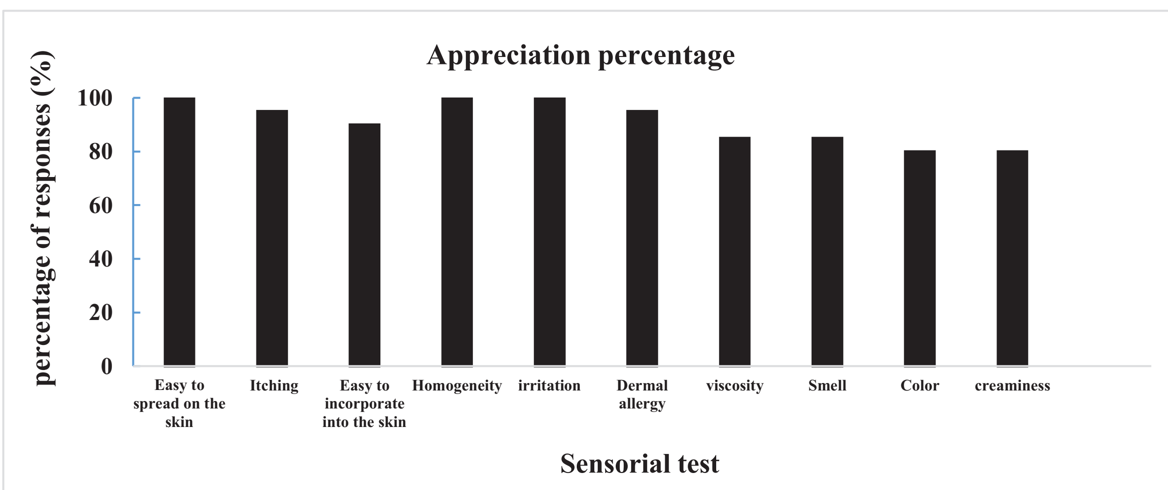

Fig. 9. Results of the sensory study, expressed as percentage of appreciation for each criterion of the emulsion formulated with Pistacia lentiscus vegetable oil from two Algerian regions.

incorporation of lentisk oil in the formulation of an antifungal emulsion does not affect the quality of the latter.

7.9 Antifungal and antibacterial activities of the lentisk emulsion

Several preliminary tests of formulation of antifungal emulsions with three percentages (0.5-1.5–2 g) of vegetable oil from two Algerian lentisk "*Pistacia lentiscus* L" regions (Boumerdes, Tizi Ouzou) were carried out. The results of the analysis of these emulsions, were compared to Phanazol Reference (FR) manufactured by the company Saidal and the quality of the antifungal emulsion formulated in this study was analyzed in terms of Macroscopic analysis (Texture, color, Ordor), physical appearance (stability...) and chemical characteristic of emulsion (pH, ...).

7.9.1 Antibacterial activity of emulsion

The antimicrobial action of antifungal emulsion formulated was checked against microorganisms tested (bacteria; gram positive and gram negative, yeast) by using the modified agar well diffusion method.

Our results show that these emulsions have a moderate antimicrobial effect against all the microorganisms tested. There were differences in their activities depending on the microorganism tested (Tab. 7).

The data in Table 7 showed that the antifungal emulsion formulated with 2% of the vegetable oil was able to inhibit the growth of microorganisms tested. The results of antibacterial tests of emulsions formulated with *Pistacia lentiscus* vegetable oil from the two Algerian regions showed moderate inhibition of bacterial growth (11 mm).

Table 7. Diameters of inhibition zones produced by emulsions formulated with vegetable oil from Pistacia lentiscus L. fruit from the two Algerian regions on the microbial strains tested.

Emulsion formula	Bacterial strains			Yeast strains
	(G+) Staphyloccocus aureus	(G-) Pseudomonas aeruginosa	(G-) Escherichia coli	Condidas albicans
CT-C (100%)	11 mm(S+)	–	–	9 mm(S+)
CT-CD (50%)	–	–	–	10 mm(S+)
CB-C (100%)	–	11 mm(S+)	11 mm(S+)	12 mm(S+)
CB-CD (50%)	11 mm(S+)	11 mm(S+)	–	10 mm(S+)
FR-C (100%)	–	–	7 mm(R+)	11 mm(S+)
CT-C (100%)	6 mm(R+)	7 mm(R+)	7 mm(R+)	12 mm(S+)

Gram-negative bacterial strains "*Pseudomonas aeruginosa*" and "*Escherichia coli*", showed resistance to emulsions formulated with *Pistacia lentiscus* vegetable oil from Tizi Ouzou. Emulsions formulated with *Pistacia lentiscus* vegetable oil from Boumerdes showed moderate antibacterial activity (11 mm). Whereas "*Escherichia coli*", showed sensitivity to the pure formulated emulsion (100%) with inhibition zones of 11 mm, and zero for the diluted emulsion (50%). While the diluted emulsion (50%) based on *Pistacia lentiscus* vegetable oil from the Boumerdes region, revealed average inhibition of the growth of the Gram-positive bacterial strain of *Staphylococcus aureus* (11 mm).

Regarding the results of the reference emulsion "Phanazole" showed a negative antibacterial effect of the pure emulsion (100%) compared to the diluted emulsion (50%) with small diameters of inhibition zones (7 mm). The three bacterial strains tested showed resistance against the reference emulsion "Phanazole". The emulsion formulated in this study was found to have varying degrees of antibacterial activity. What remains to be done is to determine the optimal dose of vegetable oil, or to incorporate an essential oil into the emulsion formula. Indeed, several scientific studies show the antibacterial activity of essential oils is greater than that of vegetable oils.

Ours results of the bio-chemical study, revel the richness of vegetable oil of *Pistacia lentiscus* fruit on polyphenolic and flavonoids contents. According to Supomo *et al.* (2015) in Apriliana *et al.* (2018), the phenolic compounds have antibacterial activities such as flavonoids, saponins and tannins. In addition, Belabbas *et al*. (2023) determined the antibacterial effect of the phenolic extract from *Pistacia lentiscus* leaves (PLLPE). These extracts rich in the total polyphenols contents and flavonoids contents showed also a significant antioxidant activity (concentration-dependent) and antibacterial. Poeloengan *et al*. (2010) stated the antibacterial effect of saponin as an active substance that can increase membrane permeability resulting cell hemolysis, when saponins interact with germ cells, the bacteria will be lysed. Research of Cushnie and Lamb (2005) indicated the relationship between the flavonoid structure and antibacterial activity.

7.9.2 Antifungal activity of emulsion

The zones of inhibition produced by the *Pistacia lentiscus* L. antifungal emulsion formulations are shown in Table 7. Our results show an antifungal effect of the emulsion formulated against the yeast tested "*Candida albicans"*, with inhibition zones of inhibition between 9 and 12 mm diameters. These results recorded in the emulsions developed are comparable to those found for the reference emulsion Phanazol® (11-12 mm). This emulsion shows the good antimicrobial action against the gram positive and gram-negative bacteria.

According to Hartanto (2013), Tannin can react on cell membranes, inactivate enzyme, and inactivate fungi genetic material. Furthermore, tannin can shrink the cell wall or cell membrane so that interferes the permeability of the cell, which ultimately causes the cell cannot perform life activities so that the growth is hampered or even death (Hartanto, 2013). It is known that *Candida albicans* is yeast that can naturally colonize the skin, (oral, intestinal and vaginal mucosa). It is an opportunistic pathogen fungus, which causes many infections (Moussaid *et al.,* 2019). Since the vegetable oil did show activity against bacteria and fungi, it was therefore considered necessary to include these doses of vegetable oil in antifungal emulsion formula.

This is like the earlier reports from previous studies (Eichie *et al.,* 2011), noted that this low sensitivity of these organisms to the emulsion may be attributed to dilution of the vegetable oil doses by the antifungal emulsion base. Or possible partitioning of the active principles between two phases of the emulsion (aqueous and organic) during formulation. This implies that a higher concentration of the extract will be required in formulating the emulsion to obtain an effective concentration that will render a beneficial therapeutic effect. The results showed that the vegetable oil of *Pistacia lentiscus* can be formulated in antifungal emulsion dosage form. Emulsion formulae were more effective against yeast than bacteria. The antifungal emulsion dosage form formulated in this study meets the best physical properties at 2%.

This present study is carried out for the first time to produce an antifungal emulsion using vegetable oil of *Pistacia lentiscus* from two Algerian regions (Boumerdes and Tizi Ouzou).

8 Conclusion

The aim of this work is to substitute the synthetic active ingredient with a natural antioxidant, and thus to contribute to the development of vegetable oil extracted from the Pistacia lentiscus.

In this study, we first undertook the physical and chemical characterization of vegetable oil extracted from the lentisk fruit. The results show that this oil has a significant antioxidant activity due to its polyphenol content (843.55 and 499.26 meq g gallic acid /ml oil from the Boumerdes and Tizi Ouzou regions, respectively) and flavonoids (39.15 and 17.85 mg Eq / mg oil extract from the Boumerdes and Tizi-Ouzou regions, respectively), recognized as secondary metabolites with antioxidant properties.

Next, we formulated an antifungal emulsion using this oil in the first time, then characterized it physically and chemically, and evaluated its biological activity.

The formulated emulsion showed good consistency and spreadability, a pH close to that of the skin (between 4,178 and 4,626), and there was no phase separation during the formulation study period. In addition, stability parameters such as visual appearance, nature and viscosity of the formulated emulsion showed no variation over the study period. The results of the physicochemical analyses obtained showed that the quality indices are in accordance with standards and are consistent with those found in previous studies. Also, emulsions formulated with lentisk vegetable fruit oil have remarkable antioxidant activity, due to their high polyphenols (843.5583 meq g of gallic acid/ml for Boumerdes oil and 499.2683 meq g of gallic acid/ml for Tizi Ouzou oil) and flavonoids content (from 17,854 mg Eq/mg extract to 39,150 mg Eq/mg oil extract). The latter are secondary metabolites with strong antioxidant power.

The formulated emulsion has shown encouraging antifungal effects but still needs to optimize the quantities of oil to be incorporated alone or in synergy with other active ingredients before it can be marketed.

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Cite this article as: Bouhadi N, Rouane A, Aklil D, Riabi R, Alileche K, Boudriche L. 2024. Valorization of *Pistacia Lentiscus* L. oil: antifungal activity in an emulsion formula. *OCL* 31: 26.