

Study of the Antibacterial and Antifungal Activities of Oils of *Pistacia lentiscus l.*

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Abstract

This work is considered as a prospect of a qualitative evaluation of the antibacterial and antifungal activities of the ' extracts of *Pistacia lentiscus* in relation to the microbic stocks linked to several human pathologies.

We began our work with the recovery of our samples from five places in Algeria: «ANNABA, GUELMA, EL MILIA, SKIKDA and EL TARG».

These tests of antibacterial and antifungal biological extracting of respectful operated on the pathogenic stocks according to two Protocols (CMI, CMB), results showed that two of our extracts manifested an antibacterial and antifungal activity.

The curative plants constitute a source of new molecules with activity antibiotic economically accessible which ommit the effects of the resistance phenomena of germs.

Keywords: vegetable oil, *Pistacia lentiscus*, bacterium, *Staphylococcus aureus*, *Bacille klebsiella*, *Candida albicans*, antibacterial activity, antifungal acitivity

INTRODUCTION

For thousands of years, the use of medecinal plants is considered as a principal resort to cure humans. It is generally adapted to simple pathologies targeting a symptomatic treatment.

Approximately, there are 500 000 plants on Earth, among which about 100 000 possess medicinal characteristics contributing to its active ingredients that act straight on the organism.

In addition, these can also be used in classical medicine in phytotherapy ; in fact they provide benefits that conventional medications often lack, as cited ^{1, 2} Also, the usage of medicinal plants can immediately bring answers to some health problems. However, before recommending the use of a particular species for a disease, it is important to validate the traditional usage that is made from. In other words, it is necessary and obligatory to scientifically evaluate the pharmacological activity of the selected medicinal plant, to assess whether it confirms its reputation and also to check the absence from toxicity from it .Moreover, the use of local medicinal plants that can solve health problems ,may be perceived as an alternative to conventional medicines.

Pistacia lentiscus is a shrub or dioecious tree that belongs to the Anacardiaceae's family. From its edible fruit an oil is extracted which was commonly utilized for food, lighting and also in making soap. In Algeria, this oil is traditionally produced in the east of the country, particularly in the coastal areas such as (El Milia, Skikda), where the species is abundant.

Traditional medicines practiced on both sides of the Mediterranean, attribute to the *lentiscus*'s efficacy in treating ; ulcers, sores and light burns. Traditional Algerian medicine mainly uses the obtained oil of the *lentiscus* fruits to treat small wounds, light burns and erythema. Furthermore, the oil is also used orally against respiratory problems of allergic origin and stomach ulcer. It widely employed in Tunisia for the same indications.

Despite its wide utilization, no toxicological studies of any kind have been carried out on *lentiscus* oil. Even its chemistry and pharmacology remains rarely studied.

This work is devoted mainly to the study of the safety of vegetable oil, traditionally used in the treatment of wounds and superficial burns. This oil is extracted from the fruit of *Pistachia lentiscus*, a shrub of the family Anacardiaceae, common to all the countries of the Mediterranean basin³.

The *lentiscus* fruit oil is employed for its medicinal benefits, advised for diabetics, for the treatment of stomach pains and in circumcision ^{4,5}. In addition, it is used as an external topical remedy in the form of an ointment to treat burns ⁶ or back pain⁷.

MATERIAL AND METHODS

Vegetal Material

In Algeria, the genus *Pistacia* is represented by four species, namely *Pistacia lentiscus*, *Pistacia terebinthus*, *Pistacia vera* and *Pistacia atlantica* ,as cited in⁸

Among the species of the genus of *Pistacia*, *Pistacia lentiscus* L. is a very common shrub in our country as cited.^{9,10}

Method of Extraction

The method of extracting fatty oil from lentiscus is very old. The harvest of the berry is done between the months November and December. After harvesting sufficient quantity of mature berries, as in the case of the olive, we apply the same principle. After drying for 7 days, all the berries whose pulp has been sufficiently disintegrated is bubbled in water. We collect the boiled, to fill two bags of long and narrow canvas and then practice the pressing with the help of two wooden tails to extract a kind of siccative oil⁵. This method is very similar to the method of extraction in the iles of Sardinia¹¹. In Algeria, the recovery of lentiscus oil passes through maceration in hot water of the fruit, followed by a decantation (eastern region of Algeria, El-harouch)

Methodes

The harvest of the lentiscus's vegetable oil (*Pistacia lentiscus*) samples come from the regions of: SKIKDA, EL TARF "EL MATROUHA", GULMA, EL MILIA and ANNABA.

Date of extraction :

- SKIKDA : November 2015
- EL TARF : 10th November 2015
- GUELMA : 01st December 2015
- ELMILIA : November 2012
- ANNABA : 20th November 2015

Microbiological Analysis procedure Antibacterial and antifungal activities were evaluated on different micro-organism.

Staphylococcus Species:

Staphylococcus is one of the species *Staphylococcus* that is found in the air at the level of the O.R.L sphere and the skin.

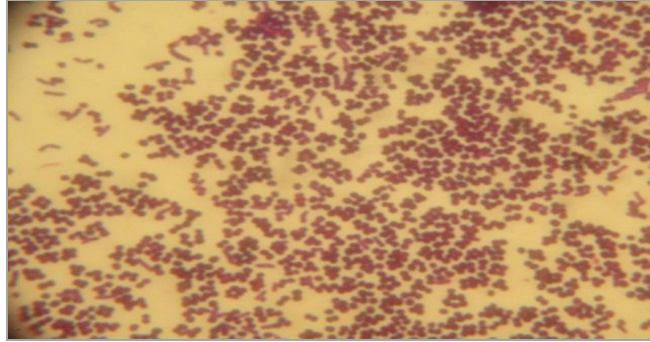


Figure 01

Staphylocoque aureus Gram(+)

There are three species that are considered noxious because :

1. *Staphylococcus aureus* the most pathogenic.
2. *Staphylococcus epidermidis* more or less toxic .
3. *Staphylococcus saprophyticus*.

General characteristics: These are Gram (+) bacteria in the form of Cocci arranged in clusters, in diplococcus, and grapes.

Culture: These are very demanding germs that can be grown on ordinary media such as nutrient agar or M.H (Muller Hinton), selective media for staphylococci "CHAPMAN"

CHAPMAN: it is a hyper salted medium that contains mannitol sugar, a color indicator, phenol red.

Only the staphylococcus can survive in the medium by fermenting the mannitol sugar which gives a yellowish color on the medium thus acidification of the medium which increases the PH (in acid medium) which turns the color red into yellow.

Biochemical Characteristics of Staphylococcus Aureus:

- Mannitol (+)
- Catalase (+): it is the release of oxygen air bubbles by putting a few drops on the seeding.
- Staphylococculase (+): using the plasma of a lyophilized rabbit already prepared (1 ml of rabbit plasma with 1 ml of staphylococcal microbial

suspension) after homogenization and incubation 24 hours at 37 ° C. if the test is positive we have Coagulation.

BACILLE Species:

The species of klebsiella which belongs to the family of enterobacteriaceae are Gram (-) bacilli, coloured from red to pink .The cultivation is carried out on ordinary nutritive agar medium or a selective one, the lactose agar to bromocresol purple (BCP) which gives a Yellowing of the colonies (Acidification of the medium) proving that the medium contains Lactose.

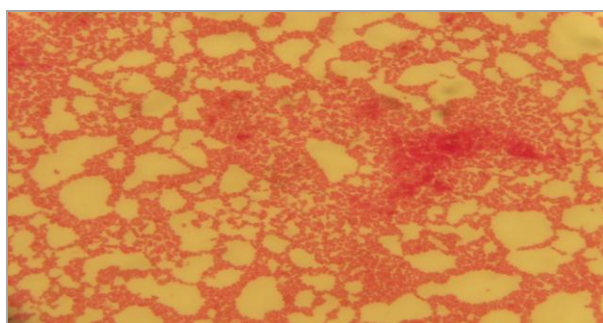


Figure.02

Bacille **Klebsiella** Gram(-)

Candida ALBICANS :

These are Yeasts that can be found in the O.R.L sphere, in the digestive tubes and the urogenital apparatus. They can be grown on ordinary media (nutrient agar) or selective media such as actidione sabouraud + chloramphenicol. (**Figure 03**).

Origin of strains:

In total 3 strains two bacteria and one fungus were isolated from various pathological products from the laboratory of human microbiology belonging to the polyclinic MERZOUG IBRAHIM EPSP TARF during a period of May , 2016. The origin of the samples of the strains is urine or stink.

Verification of the purity of the strains:

From the medium storage, the isolation of the strains was carried out on cetrimide agar in order to check their purity, the incubation was carried out at 30 ° C. for 18 to 24 hours.

The minimum inhibitory concentration (MIC):

It is possible to determine the lowest concentration (expressed in micrograms / ml) capable of inhibiting the growth of the bacterium considered (minimum inhibitory concentration or MIC).

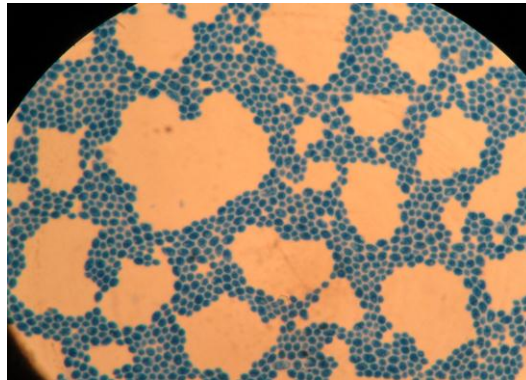


Figure 3. Candida albicans

It Inhibiting minimal concentration CMI : it makes it possible to determine the smallest concentration (expressed in microgrammes/ml) able to inhibit the growth of the bacterium considered (inhibiting minimal concentration or CMI). Based on gradients of concentration, it is the manual method most usually used. It founded on the fact that a disc impregnated of antibiotic and “deposited” on an agar nutritive is beforehand inoculated by the bacterial suspension to test, will diffuse according to a gradient of concentration, and that the bacterium will not develop in the presence of concentrations equal or higher than the inhibiting minimal concentration. The process uses discs 6 millimetres in diameter whose paper was impregnated of an antibiotic with known concentration¹².

The minimum bactericidal concentration (CMB):

The lowest concentration of antibiotic capable of causing the death of at least 99.99% of the bacteria of an inoculum (<0.01% survivors) is the indicating value of the bactericidal power of an antibiotic .

Definition of the word ufc / ml:

UFC: Unit Making Colony. This is a measure of the concentration of mycoplasma in a sample. The concentration is considered to be abnormal from 5 CFU / ml (10,000 CFU / ml).

our work is about the number of colonies appearing in our agar after inoculation of the diluted oil and the microbial suspension is clear it is called bactericidal (kills all the bacteria) the more it is turbid it is bacteriostatic (ash The multiplication of the bacterium).



Figure 04. Dilutions CMI.

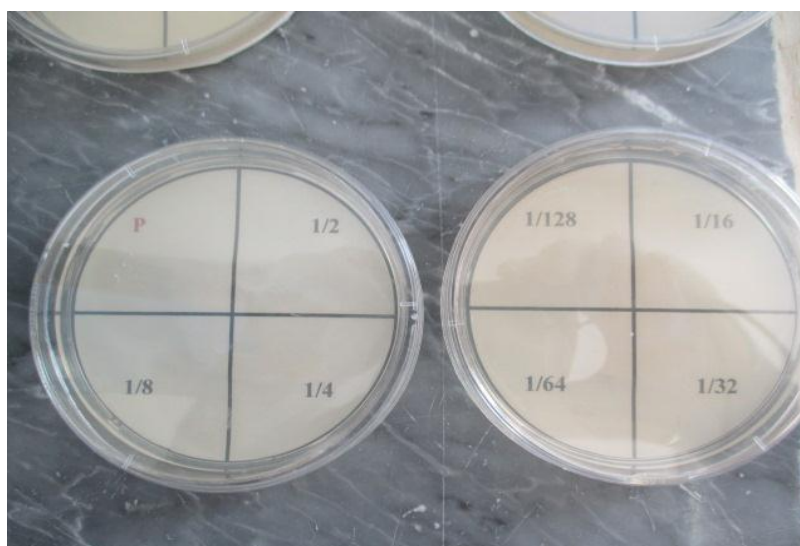


Figure 05 : Dilutions CMB.

Results and Discussion:

The results of the study of antibacterial and antifungal activity of lentiscus oil explained by the CMI and CMB reader. The MIC is done after the incubation by marking a zone of inhibition at the peripheries of the disc. If there is a clear zone at

Table 4 : Determination of the CMI for SKIKDA region.

Dilutions SKIKDA	P	1/2	1/4	1/8	1/16	1/32	1/64	1/128
B (-)	-	-	-	-	-	-	-	-
C (+)	+	+	+	-	-	-	-	-
Candida	+	+	+	+	+	-	-	-

Table 5 : Determination of CMI for d'EL TARF « EL MATROUHA » region

Dilutions EL MATROUHA	P	1/2	¼	1/8	1/16	1/32	1/64	1/128
B (-)	-	-	-	-	-	-	-	-
C (+)	+	+	+	-	-	-	-	-
Candida	+	+	+	+	-	-	-	-

CMB Interpretation:

According to the positive cultures (cfc > 10³ / ml) a number of colonies will be obtained which will decrease from dilution of 1/128 to dilution 1/2 and disappear pure.

The results of the CMB confirm that the comparison of the bacterial counts carried out after incubation made it possible to determine a number greater than 10² cfu / ml in all the boxes, which corresponds to a number greater than 0.01% of the initial number of the bacteria, which means that this Oil has a bacteriostatic and non-bactericidal action.

Table 6 : Determination of the CMB for SKIKDA region.

Dilutions SKIKDA	P	1/2	1/4	1/8	1/16	1/32	1/64	1/128
C (+)	-	-	-	+/-	+	++	++	+++
Candida	-	-	-	-	-	+/-	++	+++

Table 6 : Determination of the CMB for EL TARF « EL MATROUHA »region.

Dilutions	P	1/2	1/4	1/8	1/16	1/32	1/64	1/128
EL MATROUHA								
C (+)	-	-	-	+/-	+	++	++	+++
Candida	-	-	-	-	+/-	+	++	+++

After a positive C.M.I we obtained a C.M.B of which: $ufc > 10^3$ / ml. When we obtain a result at 10^2 ufc / ml it means that this oil has a bacteriostatic and non-bactericidal action (according to the C.M.B result)

Following our results we can take into consideration the following factors:

- The climate of the region
- The date of extraction and storage conditions.

We have noticed that the best climate for oils of better quality was that of: SKIKDA and EL TARF "EL MATROUHA", that of SKIKDA with a warm and temperate climate. Winter is characterized by much more precipitation than in summer the second Climate is Mediterranean with hot summer.

The extraction date also plays a very important role in view that the method of conservation of the oil and could alter its effectiveness. Also the oil must be kept in a dark place (cover with aluminum foil) and Not wet.

CONCLUSION

The usage of Pistacia lentiscus's vegetal oil in five different regions in Algeria for the purpose of evaluating the potentialities related to each oil and to determine its antibacterial and antifungal effects on the strains (bacteria and fungus), where we found an encouraging results for the microbiological tests performed (MIC, CMB). That confirm the use of oil in phytoterapy.

These results are very motivating. They have opened the possibility to look for the molecule or molecules responsible for the inhibitions declared taking into consideration the strains (bacteria and fungus).

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