

Evaluation of Antifungal and Antibacterial Activities of Tunisian Lentisc (*Pistacia Lentiscus L.*) Fruit Oil

Cyrine Dhieb^{1,*}, Hajer Trabelsi², Sadok Boukhchina², Najla Sadfi-Zouaoui¹

¹Laboratoire de Mycologie, Pathologies et Biomarqueurs (LR16ES05),

Faculté des Sciences de Tunis, Université de Tunis El Manar, 2092 Tunis, Tunisie

²Laboratoire de Neurophysiologie, Physiopathologie cellulaire et Valorisation des Biomolécules,

Faculté des Sciences de Tunis Université de Tunis El Manar, 2092, Tunis, Tunisie

*Corresponding author: dhcyrine@gmail.com

Received February 12, 2021; Revised March 18, 2021; Accepted March 26, 2021

Abstract Background: Intensive studies and experiments have proven that the drug resistance of microorganisms is evolving very disturbingly. As a consequence, new avenues of research become strongly essential to expand knowledge on this phenomenon and develop highly effective alternatives targeting it. Therefore, there is increasing interest in the use of medicinal plants for treatment of many infections. **Objective:** In accordance with these findings, this study aims to evaluate the *in vitro* activities of fruit oil extracted from Tunisian varieties of *Pistacia lentiscus*, growing in Rimel forest (Bizerte) in north of Tunisia, against some medically important and emerging species of bacteria and yeasts. **Methods:** The antimicrobial activities were performed using the disc diffusion method. We evaluate antimicrobial activities of this oil against many species like *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *Escherichia coli* and *C. albicans* and to our knowledge, in our current study, we demonstrated for the first time his activities against the following species: *Listeria innocua*; *Salmonella enterica*; *Enterococcus faecalis*; *Shigella flexneri*; *Candida parapsilosis*; *Candida tropicalis* and *Candida glabrata*. **Results:** The results showed positive activities of fruit oil against all tested bacteria and yeasts with some differences depending on the microorganism tested. The maximum antibacterial activities were obtained against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. For yeasts, the oil exhibited high activity against *C. parapsilosis*. **Conclusion:** This study elaborately confirmed that Tunisian *P. lentiscus* fruit oil contains compounds that can be used to treat many infections.

Keywords: *P. lentiscus*, fruit oil, yeasts, bacteria, disc diffusion

Cite This Article: Cyrine Dhieb, Hajer Trabelsi, Sadok Boukhchina, and Najla Sadfi-Zouaoui, "Evaluation of Antifungal and Antibacterial Activities of Tunisian Lentisc (*Pistacia Lentiscus L.*) Fruit Oil." *Journal of Food and Nutrition Research*, vol. 9, no. 4 (2021): 177-181. doi: 10.12691/jfnr-9-4-1.

1. Introduction

During the last decades, microorganisms have emerged as new life-threatening agents causing infections in humans and animals. The alarming increase of the resistance to antimicrobial drugs caused several problems and drove scientists to search for new and efficient antimicrobial substances from different sources and especially medicinal plants known for their harmless character. Antimicrobial resistance is hindering the effective prevention and treatment of an increasing number of infections caused by bacteria, parasites, viruses and fungi [1]. These infections are highly frequent and their impact on human and animal health is not negligible. As a matter of fact, this resistance causes a growing threat to public health worldwide and requires major actions in all sectors as well as in society as a whole. To face on this increasingly dangerous phenomenon, crucial measures need to be necessarily and urgently taken. Among these

actions, is the search for new treatment alternatives, such as the promotion of the use of natural products as antimicrobials [2,3]. In fact, plant compounds are often used to treat fungal and bacterial diseases. This constitutes an old practice that has always been carried out in most countries.

For example, *Pistacia* genus that is a flowering plants belonging to the Anacardiaceae family containing about twenty species. This plant native to the Mediterranean basin is known on a traditional scale for its multiple therapeutic virtues. More importantly, it is glorious for its anti-ulcer, anti-diarrheal and antiseptic effects. The five most popular species of *Pistacia* include *P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk* and *P. lentiscus* that were used for a wide range of purposes. To illustrate this, we can state Lentisc "*P. Lentiscus L.*", an evergreen shrub commonly called "Dharw" in the Tunisian Arabic dialect. *P. lentiscus* has been known since antiquity for its highly effective medicinal properties, traditional medicine attributes many virtues to it. Several studies have demonstrated that the essential oil extracted from

the aerial parts exhibits antifungal, antioxidant, anti-inflammatory, antimicrobial, and antiatherogenic activities [4,5,6]. The leaves are used for the treatment of eczema, diarrhea, and as a potent antiulcer agent [7].

Furthermore, *in vitro*, the ethanolic extract of Chios mastic gum of *P. lentiscus* inhibited the proliferation of cancer cells in the human colon and induced apoptosis [8].

The vast use of this plant in traditional medicine is justified by its richness in chemical components having an aromatic odor such as essential oils, flavonoids, tannins [9] fatty acids, sterols and aliphatic alcohols [10,11,12]. In addition, Benhammou *et al.* reported that the oil extracted from *Lentisc* fruit has good nutritional qualities thanks to its richness in monounsaturated fatty acids [6].

Traditionally, it is often used as an external topical for back pain. It is also employed orally against respiratory problems of allergic origin and ulcers of the stomach. As demonstrated in previous work, this oil showed bactericidal power against *Clostridium perfringens*, one of the most common causes of foodborne illnesses and against *C. albicans* and *Aspergillus flavus* [13].

Therefore, this investigation aims to evaluate the activities of Tunisian *P. lentiscus* fruit oil; through the determination of its antifungal and antibacterial properties. In our study we evaluate antimicrobial activities of this oil against many species like *S. aureus*; *P. aeruginosa*; *E. coli* and *C. albicans* and to our knowledge, in our current study, we demonstrated for the first time his activities against the following species: *L. innocua*; *S. enterica*; *E. faecalis*; *S. flexneri*; *C. parapsilosis*; *C. tropicalis* and *C. glabrata*.

2. Material and Methods

2.1. Fruit Oil Extraction

P. Lentiscus fruits were collected from plants growing in Rimel forest (Bizerte) in north of Tunisia. The extraction of the fatty oil is carried out using the traditional method practiced by rural women in forest areas as described by Mezni *et al.*, [14].

Fruits are ground using grindstones; the paste is mixed by the hands well and then left to rest overnight. The next day, the mixing is repeated, and the paste is first of all heated to boiling and then, placed in a tissue in order to be pressed by hands, separating the oil from the oil meal. The liquid phase is heated until total evaporation of the water. The oil is thus recovered, filtered and then stored at 4°C.

2.2. Tested Microorganisms

To determine the antimicrobial activities of the oil extract described in this paper; Gram-positive bacteria (*E. faecalis*, *S. aureus*, *L. innocua*), Gram-negative bacteria (*S. flexneri*, *E. coli*, *S. enterica*, *P. aeruginosa*) and yeasts (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*) were taken from the collection of the Laboratory of Mycology, Pathologies and Biomarkers at the Faculty of Sciences of Tunis. Each bacterial species was cultivated in Brain Heart Infusion (BHI) agar medium (Biorad, France). *Candida* species, isolated from clinical samples and identified using molecular tools [15,16] were

cultivated in Sabouraud dextrose agar (SA) medium (Biorad, France).

2.3. Antibacterial and Antifungal Activities Assay

The agar disk diffusion method was used for antibacterial and antifungal susceptibility tests. Actually, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts testing [17].

The oil extracted was diluted in Ethanol absolute (C₂H₅OH) (Panreac Quimica SA. Barcelona, Spain) so that to have a final concentration of 80%. Then the oil was sterilized using 0.22 µm filter (Sartorius Stedim Biotech, Tunisia) before assessment for antimicrobial activities. The selected bacteria and yeasts (24 h culture) were mixed with physiological saline solution and the turbidity was adjusted to a Mac Farland turbidity standard of 0.5. After that, the Petri dishes containing Muller Hinton Agar (Biolife, Italy) were inoculated with the standardized inoculum of microorganisms. Sterile filter paper discs (Whatmans paper No. 1; 5 mm diameter) were impregnated with 20 µl of this oil, allowed to dry at room temperature and placed on the agar surface. The Petri dishes were then incubated at 38°C for 48h. The test was performed in triplicate and the inhibition zone was measured in millimeters after a period of 48h. The diameters of all zones of inhibition are measured and compared to bacteria with Gentamicin as well as to yeasts with fluconazole drugs and interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS) [18]. The negative control was done with ethanol only.

3. Results

The evaluation of the *in vitro* antimicrobial activities of Tunisian *P. lentiscus* fruit oil were performed using the agar disk diffusion method. Our results showed that this oil have antimicrobial effects against all of the tested microorganisms. There were significant differences in their activities depending on the microorganism tested.

Antibacterial tests showed the diameters of inhibition zones ranged between 6 and 15mm.

For Gram-positive bacteria the results are: *Staphylococcus aureus* (15 mm); *Listeria innocua* (12.3 mm) and *Enterococcus faecalis* (12 mm).

For Gram-negative bacteria we have found the following diameters: *Pseudomonas aeruginosa* (15mm); *Escherichia coli* (12.3 mm); *Salmonella enterica* (10 mm) and *Shigella flexneri* (6 mm). Then we have found that *S. aureus* and *P. aeruginosa* were the most susceptible to Tunisian *P. lentiscus* fruit oil with maximal inhibition zone of 15 mm. Nevertheless moderate activities were showed with *E. faecalis*; *L. innocua* and *E. coli* in order of 12 mm of diameter and the worst ones are against *S. enterica* (10 mm) and *S. flexneri* (6 mm) (Figure 1).

In the second time, Tunisian *Pistacia lentiscus* fruit oil has been tested against the most medically important and emerging species of *Candida*. Our results showed the oil has antifungal activities. Our assay on *Candida* showed

little different results in the level of species, in fact the following diameters of inhibition zones were obtained:

C. parapsilosis (13mm); *C. albicans* (12mm);
C. glabrata (11mm) and *C. tropicalis* (11 mm).

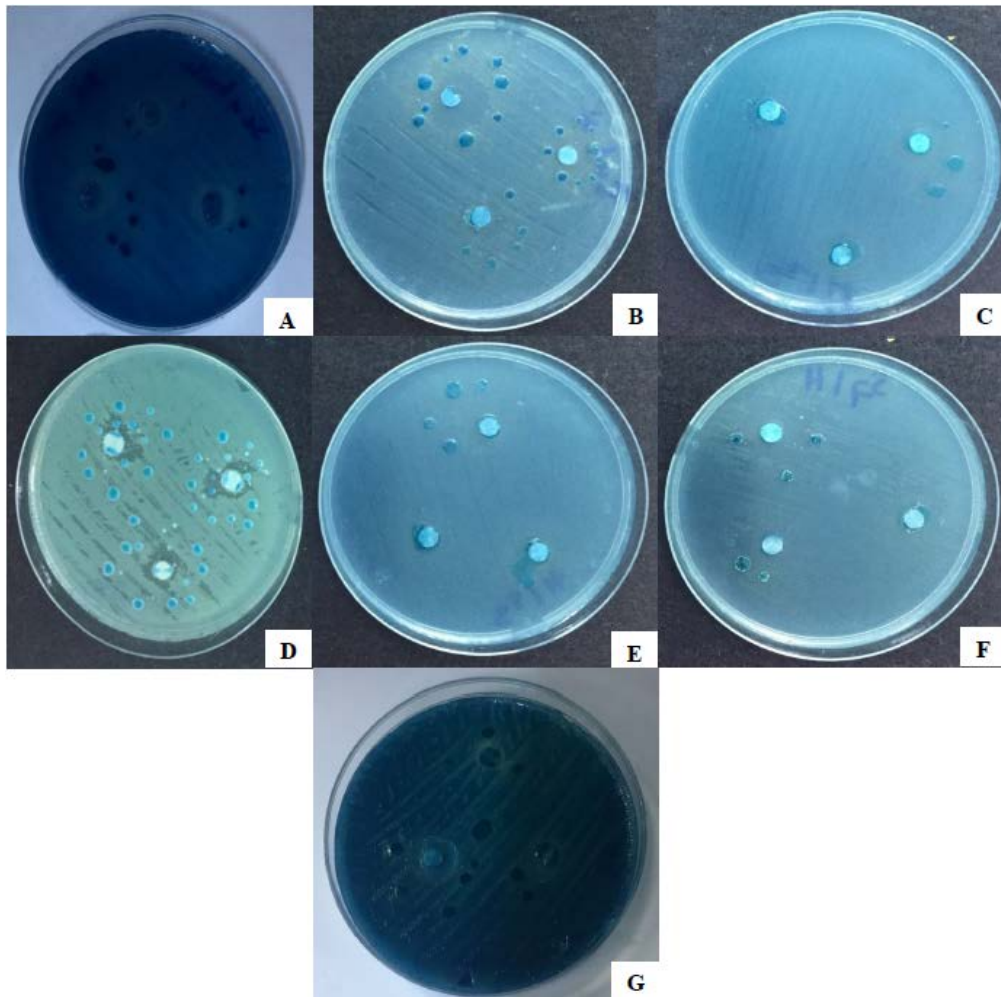


Figure 1. *In vitro* antibacterial activities of Tunisian *Pistacia lentiscus* fruit oil against some Gram-positive and Gram-negative bacteria. **A:** *Enterococcus faecalis*; **B:** *Staphylococcus aureus*; **C:** *Listeria innocua*; **D:** *Pseudomonas aeruginosa*; **E:** *Salmonella enterica*; **F:** *Shigella flexneri*; **G:** *Escherichia coli*.

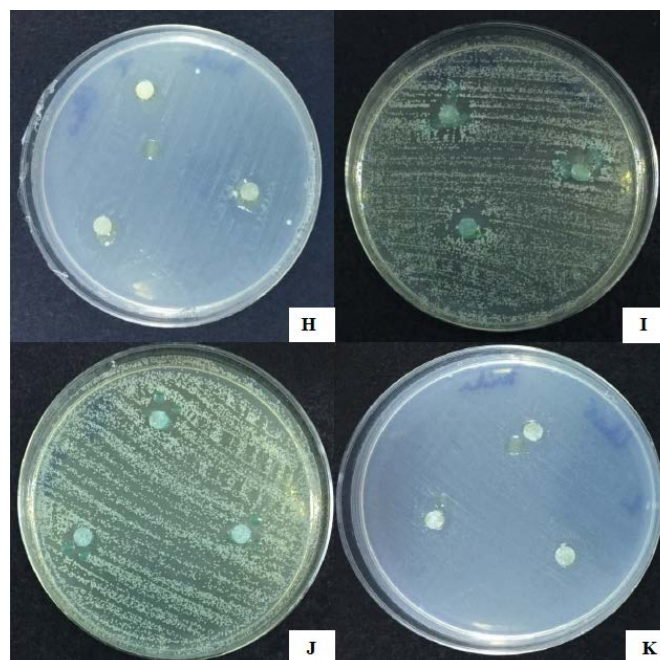


Figure 2. *In vitro* antifungal activities of Tunisian *Pistacia lentiscus* fruit oil against *Candida* species. **H:** *Candida parapsilosis*; **I:** *Candida albicans*; **J:** *Candida tropicalis*; **K:** *Candida glabrata*.

Therefore, the maximum inhibition zone obtained for yeasts was in the order of 13 mm for *C. parapsilosis* while the low activities were against *C. glabrata* and *C. tropicalis* with 11 mm of diameter (Figure 2).

4. Discussion

A review on current researches about the genus *Pistacia* L. highlighting pharmacological studies on crude plant parts, extracts, and some pure metabolites has provided scientific evidence for traditional uses and has revealed this genus to be a valuable source for medicinally important molecules. The results of this study indicate that fruit oil extracted from Tunisian *P. lentiscus* plants showed *in vitro* antimicrobial activities against some medically important bacteria and yeasts that were tested by disc diffusion method. In our current study, we demonstrated antibacterial activities of this oil against many species like *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *Escherichia coli* and in our knowledge for the first time against: *Listeria innocua*; *Salmonella enterica*; *Enterococcus faecalis*; *Shigella flexneri*; most of the bacteria studied are found to be harmful both to humans and/or animals. As a matter of fact, some virulent genetic variants may cause many diseases such as gastroenteritis and urinary tract infection. Our results showed positive activities obtained against all of the bacteria tested. Then we have found that *S. aureus* and *P. aeruginosa* were the most susceptible to Tunisian *P. lentiscus* fruit oil with maximal inhibition zone; moderate activities were showed with *E. faecalis*; *L. innocua* and *E. coli* and the worst ones are against *S. enterica* and *S. flexneri*.

Many studies demonstrate the pharmacological activities, such as antimicrobial, antioxidant and antiproliferative activities, from various parts of this plant (leaf, resin, fruit, seeds, stem, bark, root...) [19].

Indeed Mezni et al., have tested the fixed oil of *P. lentiscus* and its phenolic extract Oil extracted from fruits harvested from six provenances located in Tunisia, he concluded that the antimicrobial activity of the tested extract and oil varied significantly depending on the microbial strain and the geographic origin of the tested substances. Indeed they found activities against *S. aureus* with an inhibition zone of 9.33 mm and no activity against *E. coli* [13].

Nevertheless; in our results we have obtained activities against these two species. Here, we can explain these differences by some factors that can influence the composition of the extract such as soil composition and plant nutrition.

Furthermore, in Morocco, Bammou et al., have compared different extracted oil essential oil, crude extracts total and different fractional compounds and their derived fractions (aqueous; methanol; ethyl acetate) against some pathogenic bacteria they have concluded that total extracted are the most powerful however other fraction were found to be active to a different degree so the plant have many actives antimicrobial substances.

They also concluded that the Gram positive bacteria *Staphylococcus aureus* is the most susceptible bacteria compared with other Gram-negative tested [20].

This is not consistent with our results because we have found higher activities against *S. aureus* (Gram-positive) and *P. aeruginosa* (Gram-negative). We think that they need to test more than one Gram+ bacteria to justify this assumption.

On the other hand, studies evaluated antimicrobial activities of ethanolic (80%) extracts from leaf and stem of *P. lentiscus* and their fractions showed that fractionation increased mainly the antibacterial activity of *P. lentiscus* leaf; the ethyl acetate fraction showed higher activity against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* than that obtained from the crude ethanolic extract and the aqueous fraction displayed highest activity against *S. aureus* [21].

For other bacteria (*Listeria innocua*, *Salmonella enterica*, *Enterococcus faecalis*, *Shigella flexneri*) we don't find publication evaluated fruit oil activities of *P. lentiscus* against him.

The antifungal activities have also performed against the most medically important *Candida* species such as: *C. albicans* *C. parapsilosis*; *C. tropicalis* and *C. glabrata*. Positive activities have been demonstrated for all tested yeasts. Therefore, the maximal inhibition zone obtained for yeasts that are sensitive to the *P. lentiscus* fruit oil was for *C. parapsilosis* while the low activities were against *C. glabrata* and *C. tropicalis*.

Some other studies have shown the activity of oil extracted from different medicinal plants against *Candida* species [21,22,23]; for example the essential oils extracted from the aerial parts of cultivated *Salvia officinalis* L. and the berries of *Schinus molle* L. both showed significant antifungal activity against *C. albicans* [24].

Mezni et al., have obtained activity against *C. albicans* with an inhibition zone of 9 mm in diameter with the fixed oil of *P. lentiscus* fruits [13]. However, little work has been done on the antifungal effects of their fixed oils.

In our study, *P. Lentiscus* fruits were collected from plants growing in Rimel forest (Bizerte) in the north of Tunisia. Trabelsi et al., have studied triacylglycerol (TAG) molecular species and fatty acid compositions of the oil of our used plant and their found that our *P. lentiscus* oil is rich in di- and tri-unsaturated TAGs and less rich in monounsaturated TAGs [12] and that the Oleic acid was the major fatty acid followed by the palmitic and linoleic acids [10]. This composition can depend on the habitat and the ecological source of the derived plant material. According to several studies, oleic and linoleic acids have potential antibacterial properties that are attributable to their unsaturated chain lengths [22,25].

5. Conclusion

In recent years high resistance to antibiotics and antifungal agents has emerged worldwide. According to our study, we can consider that the fruit oil of Tunisian *P. lentiscus* plant is with important antibacterial and antifungal capacity; in fact, we showed that this oil possesses antifungal and antibacterial activities against all tested bacteria and yeasts. The fatty acid components of vegetable oils are assumed to play a major role in the wound-healing process; this could be due to its richness in unsaturated fatty acids. However, in-depth

studies are needed to gain knowledge about *in vivo* oils' effects.

Acknowledgments

We thank the Ministry of Higher Education and Scientific Research of Tunisia for financially supporting this investigation.

Statement of Competing Interests

We affirm that the manuscript has not been previously published, is not currently submitted for review to any other journal and will not be submitted elsewhere before a decision is made.

The authors declare that they have no conflict of interest.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

References

- [1] Marston, H.D., et al., *Antimicrobial Resistance*. JAMA, 2016. 316(11): p. 1193-1204.
- [2] Nikitina, V.S., et al., *Antibacterial activity of polyphenolic compounds isolated from plants of Geraniaceae and Rosaceae families*. Applied Biochemistry and Microbiology, 2007. 43(6): p. 629-634.
- [3] Bobis, O., et al., *Influence of phytochemical profile on antibacterial activity of different medicinal plants against gram-positive and gram-negative bacteria*. Applied Biochemistry and Microbiology, 2014. 51(1): p. 113-118.
- [4] Duru, M.E., et al., *Chemical composition and antifungal properties of essential oils of three Pistacia species*. Fitoterapia, 2003. 74(1-2): p. 170-176.
- [5] Dedoussis, G.V., et al., *Antiatherogenic effect of Pistacia lentiscus via GSH restoration and downregulation of CD36 mRNA expression*. Atherosclerosis, 2004. 174(2): p. 293-303.
- [6] Benhammou, N., F. Atik Bekkara, and T. Kadifkova Panovska, *Antioxidant and antimicrobial activities of the Pistacia lentiscus and Pistacia atlantica extracts*. African Journal of Pharmacy and Pharmacology., 2008. 2: p. 022-028.
- [7] Ali-Shtayeh, M.S., et al., *Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area*. J Ethnopharmacol, 1998. 60(3): p. 265-71.
- [8] Balan, K.V., et al., *Antiproliferative activity and induction of apoptosis in human colon cancer cells treated in vitro with constituents of a product derived from Pistacia lentiscus L. var. chia*. Phytomedicine, 2007. 14(4): p. 263-72.
- [9] Romani, A., et al., *Identification and quantification of galloyl derivatives, flavonoid glycosides and anthocyanins in leaves of Pistacia lentiscus L.* Phytochem Anal, 2002. 13(2): p. 79-86.
- [10] Trabelsi, H., et al., *Total lipid content, fatty acids and 4-desmethylsterols accumulation in developing fruit of Pistacia lentiscus L. growing wild in Tunisia*. Food Chemistry, 2012. 131(2): p. 434-440.
- [11] Trabelsi, H., et al., *LC-ESI-QTOF-MS, MS/MS Analysis of Glycerophospholipid Species in Three Tunisian Pistacia lentiscus Fruit Populations*. Journal of the American Oil Chemists' Society, 2013. 90(5): p. 611-618.
- [12] Trabelsi, H., et al., *Triacylglycerols and aliphatic alcohols from fruits of three Tunisian Pistacia lentiscus populations*. J Sci Food Agric, 2015. 95(10): p. 2028-32.
- [13] Mezni, F., et al., *In vitro antimicrobial activity of Pistacia lentiscus L. edible oil and phenolic extract*. Nat Prod Res, 2015. 29(6): p. 565-70.
- [14] Mezni, F., et al., *Effect of growing area on tocopherols, carotenoids and fatty acid composition of Pistacia lentiscus edible oil*. Nat Prod Res, 2014. 28(16): p. 1225-30.
- [15] Dhieb, C., et al., *Comparison of MALDI-TOF mass spectra with microsatellite length polymorphisms in Candida albicans*. J Mass Spectrom, 2015. 50(2): p. 371-7.
- [16] Dhieb, C., et al., *MALDI-TOF typing highlights geographical and fluconazole resistance clusters in Candida glabrata*. Med Mycol, 2015. 53(5): p. 462-9.
- [17] Balouiri, M., M. Sadiki, and S.K. Ibsouda, *Methods for in vitro evaluating antimicrobial activity: A review*. J Pharm Anal, 2016. 6(2): p. 71-79.
- [18] Canton, E., et al., *Epidemiological cutoff values for fluconazole, itraconazole, posaconazole, and voriconazole for six Candida species as determined by the colorimetric Sensititre YeastOne method*. J Clin Microbiol, 2013. 51(8): p. 2691-5.
- [19] Bozorgi, M., et al., *Five Pistacia species (P. vera, P. atlantica, P. terebinthus, P. khinjuk, and P. lentiscus): a review of their traditional uses, phytochemistry, and pharmacology*. ScientificWorldJournal, 2013. 2013: p. 219815.
- [20] Bammou, M., et al., *Valorisation du lentisque «<i>Pistacia lentiscus L.</i>»: Étude ethnobotanique, Screening phytochimique et pouvoir antibactérien*. Journal of Applied Biosciences, 2015. 86(1): p. 7966.
- [21] Emtinan, A.A., et al., *In vitro antioxidant and antimicrobial activities of Pistacia lentiscus, Phyllanthus anderssonii and Cinnamomum verum crude extracts and fractions*. Journal of Medicinal Plants Research, 2018. 12(16): p. 186-193.
- [22] Manohar, V., et al., *Antifungal activities of origanum oil against Candida albicans*. Mol Cell Biochem, 2001. 228(1-2): p. 111-7.
- [23] Agoramoorthy, G., et al., *ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF FATTY ACID METHYL ESTERS OF THE BLIND-YOUR-EYE MANGROVE FROM INDIA*. Brazilian Journal of Microbiology, 2007. 38(ISSN 1517-8382): p. 739-742.
- [24] Hayouni el, A., et al., *Tunisian Salvia officinalis L. and Schinus molle L. essential oils: their chemical compositions and their preservative effects against Salmonella inoculated in minced beef meat*. Int J Food Microbiol, 2008. 125(3): p. 242-51.
- [25] Zheng, C.J., et al., *Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids*. FEBS Lett, 2005. 579(23): p. 5157-62.

