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Antimicrobial activity of Olive (*Olea europaea* L.) from BeniMellal in Morocco

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Abstract

The aim of this work was to investigate the antimicrobial activity of essential oil and some extracts and bacteriocins of olive (*Olea europaea L.*) collected from BeniMellal in Morocco against bacteria and yeast. The Powder of leaves *O. europaea* L., was extracted by Soxhlet extraction. The antimicrobial activity was determined by agar diffusion method and the determination of minimum inhibitory concentration (MIC) was done by microtitration technique. Result showed that the essential oil was active against Gram-positive more than Gram-negative bacteria. The essential oil had strong antibacterial activity against *Streptococcus pneumonaie* (32 mm) with MIC of 125 µg/ml and *Klebsiella oxytoca* (32 mm) with MIC of 125µg/µl and the olive mille wastewater was active against Gram positive bacteria more than Gram-negative bacteria but not active against yeast strains; the maximum zone of inhibition was noted against *Staphylococcus aureus* (22 mm) and against *Staphylococcus epidermis* (19 mm) and *Acinetobacter baumannii* (18 mm). Bacteriocins inhibited 87.5% of bacteria and yeast. The extracts obtained with Ethanol inhibited some bacteria [the *Klebsiella oxytoca* (14 mm) and *Proteus mirabilis* (14 mm)]. The results indicate that olive composedof phenolic compounds, especially hydroxytyrosol and oleuropein can be used for the development of new antimicrobial agents.

Keywords: Antimicrobial, Olive, Essential oil, Bacteriocins, *Olea europaea* L., Beni Mellal.

Introduction

Globally, there are 800 million olive trees divided into 400 species (Firestone, 2005). Research has been publicshed on the impact of some olive extracts on microorganisms, such as olive leaf extracts (water extract and methanol extract) by researcher (Lahdibi Sahraoui *et al*., 2017) showing its impact on the prevention of harmful, urinary, dermatological , vaginal and also bacteria responsible for food poisonings and nosocomial infection (Lahdibi Sahraoui *et al*., 2017).

In another research by Abeer Ismail on the impact of olive waste water samples from Fez, Esmail *et al.* (2015) showed his strength in the fight against harmful germs, causing urinary, skin, vaginal or food poisoning.

There is a consensus of scientific studies on its role in preventing the growth of harmful germs, which encourages its use in food preservation. It also plays an active role in stopping enzyme activity.

In olives, there are elements that have the characteristic of changing color with oxidation and light, such as polyphénols, tannins, and Antocyans. A Research (Reaven, 1996) has shown that fatty acids, monounsaturated or multiple, prevent a range of heart diseases (de Lorgeril *et al*., 1994).

Polyinsaturated fatty acids n-3, or Omega-3 has an important role to play in platelet functioning and protects the body from atherosclerosis.

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In research of Garcia *et al*. (2000), phenol elements found in olive tree leaves have a paramount importance, for example, flavonoids prevent oxidation and the appearance of cancer-causing free radicals, and the antioxidant hydroxytyrosol (Soni *et al*., 2006) is the primary compound of olive phenol.

Al-Azzawie & Alhamdani (2006) showed that oleuropein controls blood sugar and prevents oxidative stress for diabetics. Olive residue (solid olive residues) was also valued after the addition of a starter containing lactic acid bacteria (Mennane *et al.*, 2010), and a product rich in essential food compounds (proteins, fats, minerals, antioxidants, etc.) was obtained, which could be used to feed livestock.

Bacteriocins can be used in the pharmaceutical industry and in food preservation. Some lactic acid bacteria are isolated from fermented products such as olive and dairy products currently used to treat diarrhea, reduce the risk of infection, rebalance intestinal bacteria, stimulate the immune system, improve lactose digestion, reduce blood cholesterol levels, reduce genetic toxicity, reduce carcinogenic molecules. Odamaki *et al.* (2011) showed that Bifidobacterium contributes signify-cantly to the production of individual vitamins such as vitamin D, and confirmed its importance for the health of consumers of fermented products.

The aim of this work was to investigate the antimicrobial activity of essential oil and some extracts (ethanolic extrat and the olive mille waste water) and bacteriocins of olive (*Olea europaea* L.) collected from BeniMellal in Morocco against Gram-positive, Gram-negative bacteria and yeast.

Materials and Methods

Plants collection

Olive leaves and olive mille waste water were taken from the Beni Mellal region of Morocco. The leaves were cleaned, washed, dried at a mesophilic temperature, crushed into powder and well stored to preserve their biological power.

Preparation of the olive leaves extract

Essentials oils

Soxhlet extraction: 100 g of prepared powder is mixed with half a liter of acetone for a day, and dried by a rotary evaporator. After the essential oils were recovered for testing biological activity (Harborne, 1998).

Organic extracts

100 g of prepared powder is mixed with half a liter of ethanol for a day, and dried by a rotary evaporator. After ethanolic extracts were recovered for testing biological activity (Dub & Dugani, 2013).

Selection and identification of lactic bacteria

Lactic strains were isolated from the fermented table olives on the MRS. culture medium and we identified the different morphological and biochemical characteristics: catalase, growth temperature, production of carbon dioxide, fermentation of different sugars.

Preparation of the bacteriocin of lactic bacteria

After placing the lactic bacteria in the liquid medium, 50 millimeters were centrifuged in the 10,000 rpm cell for 10 minutes. We add a basic solution to modify supernatant acidity and add catalase to two hours before testing.

Antimicrobial assay

Microorganisms used

The test organisms used included 9 bacteria strains and 2 yeasts: *Streptococcus pneumoniae, Staphylococcus epidermidis, Staphylococcus aureus, Acinetobacter baumannii, Pseudomonas aeruginosa, Escherichia coli, Klebsiella oxytoca, Serratia marcescens, Proteus mirabilis, Candida tropicalis* and *Candida albicans*. These strains were collected from the National Institute of Health (NIH) Rabat-Morocco.

Antimicrobial assay

The antimicrobial activity was performed using the diffusion method on agar media Muller-Hinton Agar (MHA) as recommended by Nongpanga et al. Thus, the organisms were spread on MHA: Sabouraud Dextrose Agar (SDA) plates by cotton swab. Wells of 6 mm diameter were punched into the agar medium and filled with 50 µl of plants extracts. The plates were incubated for 24 h at 37°C. Antimicrobial activity was evaluated by measuring the inhibitory zone against the test organism.

Minimum Inhibitory Concentration (MIC)

The determination of the minimum inhibitory concentration of essential oils and fixed extracts of plants, against microbial strains was made using, the technique of microtitration described by Eloff (1998).

Results and Discussion

Firstly, we identified the lactic acid bacteria. The results showed that the majority of the species belong to the genus lactobacillus and the species *L. yamanashiensis, L. acidophilus* and *L. amylovorus.*

The bacteriocin results showed activity for the majority of the strains (Figure 1) tested with the difference between the strains of bacteria studied (36 mm for *Staphylococcus aureus*), it can be ranked second after the essential oils.

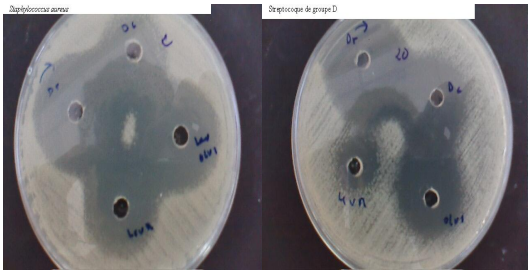

Figure 1. Activity of bacteriocins on bacteria.

The results show that the essential oil of the leaves and the olive trees has the power to inhibit the proliferation of almost all the bacteria and yeasts studied, in particular the enterobacteria such as *Klebsiella*, *E coli* ATCC and *Serratia maresens* with an inhibition diameter ranging from 14 to 32 mm (Table 1). Concerning Gram-negative, oxidase-positive bacteria the plant inhibited *Pseudeumonas aeruginosa* and *Acenitobacter baumanii* with an average inhibition diameter of about 26 mm and a minimum inhibitory concentration (MIC) of 250 mg/ ml, the inhibition also covers Gram-positive bacteria, notably *Staphylococcus aureus* and *Streptococcus pneumoniae* with a diameter of up to 32 mm and a MIC of 125 mg/ml, and pathogenic yeasts such as candida tropicalis.

The inhibition power of essential oil of olive leaves was compared with ethanolic extract, olive mille wastewater from the same region and bacteriocin, and it was found that the inhibition rate is 100% for HE, 87.5% for bacteriocin, while it is 18% for EE and exceeds the others especially for *Klebsiella oxytoca*, *E. coli* ATCC, *Acenitobacter boumanii*, *Pseudomonas aeruginasa* and *Streptococcus pneumoniae.*

Also other works as Yakhlefa *et al.* (2018) for Algerian olive mille waste water, Markin *et al.* (2003), Pereira et al. (2007) and Keskin *et al.* (2012) for aqueous extracts of leaves, and Sousa *et al.* (2006) for aqueous extracts of olive fruits found that they are effective against *Staphylococcus aureus* and *E. coli*.

Sudjana *et al.* (2009) found that olive leaf extracts also inhibited the following germs as our results: *Staphylococcus epidermidis, Staphylococcus aureus, Acinetobacter baumannii, Pseudomonas aeruginosa*, *Escherichia coli, Serratia marcescens* and *Candida albicans.*

Table 2. Screening of antimicrobial activity of essential oil and some extracts of olive (Diameter mm).

Microbial Group	Microbes	The olive mille wastewater	Essential oil	Ethano-licextrat	Bacterio-cins
Enterobacteriae (Gram-negative)	**Serratiamarcescens*	10	14	0	0
	**Proteus mirabilis*	11	-	14	-
	**Klebsiellaoxytoca*	10	32	14	
Not Enterobacteriae (Gram-negative)	**E.coli ATCC 25922*	14	24	0	24
	**Acenitobacterboumanii*	18	26	0	25
	**Pseudomonas aeruginasa*	16	26	0	24
Gram+ Cocci	**Staphylococcus aureus*	22	22	0	36
	**Staphylococcus epidermidis*	19	26	0	25
	Streptococcus pneumonaie	13	32	0	-
Yeasts	*C. albicans*	-	34	0	24
	Candida tropicalis	-	14	0	12

Conclusions

This study emphasizes the antimicrobial activity of essential oil and some extracts (ethanolicextrat and the olive mille waste water) and bacteriocins of olive (*Olea europaea* L.) collected from Beni Mellal in Morocco against human pathogenic bacteria. The maximum inhibitory zone was noted against *Streptococcus pneumonaie* and *Klebsiella oxytoca*. The strongest activity observed is that of the essential oil, followed by the bacteriocins, the olive waste water and lastly the ethanolic extract.

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