Antimicrobial Effects of Hydroxytyrosol Extracted From Olive Leaves, on Propionibacterium Acnes

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Abstract

Introduction: The olive tree consists of twenty species that belong to the olive genus (olea). These trees are always green and have small, seamless leaves that are facing each other. Acne vulgaris is one of the most common skin diseases and the most common cause for referral of patients to dermatologists. Propionibacterium acnes is a gram-positive bacteria, anaerobic rod that is found on the human skin surface as a natural flora, causing acne. It can also cause chronic blepharitis and endophthalmitis in the eye.

Materials and Methods:
Standard well assay method in Mueller-Hinton agar based on CLSI protocol was used to evaluate the zone of inhibition of different hydroxytyrosol concentrations. Then the result of this assay was compared with standard antibiogram test by antibiotics choice for acne treatment such as Clindamycin, Tetracycline and Erythromycin.

To determine MIC and MBC, a sterile microplate was first prepared from hydroxytyrosol 20 dilutions consecutively from 10 fold serial dilution in sterilized Mueller-Hinton Broth from 200mg/ml until 0.00038mg/ml. Standard suspension of Propionibacterium acnes opposite 5×105 cfu/ml was prepared and equally added to the tubes containing different dilutions of hydroxytyrosol.

Findings: Until the fifth dilution, growth was observed; the concentration of 12.5 mg / ml of the tested compound had the minimum bactericidal concentration. MBC converted unit to 12500 µg / ml.

In the 6th dilution (6.25 mg/ml) growth was observed, therefore this concentration was introduced as minimum inhibitory concentration (MIC) and the converted unit to 6250 µg / ml.

Conclusion: In this study, it was found that hydroxytyrosol has an antibacterial effect against Propionibacterium acnes. But it is weaker than existing standard antibiotics.

Key words: Olives, Hydroxytyrosol, Acne vulgaris, Propionibacterium acnes

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Introduction and Importance of Research

(Olive) and scientific name Oleaeuropaea contains approximately twenty species of small trees of the olive family and are found in the ancient world of the Mediterranean Sea, North Africa, Southeast Asia, North to South China, Scotland and Eastern Australia so has a wide dispersion. They are always green and have small, seamless leaves that are facing each other. In Syria, where olive oil has always been important for the economy of its inhabitants, it can be concluded that Syria has been the birthplace of olive. Olive contains a large amount of unsaturated oil, that contains plant chemicals that reduce cholesterol and the risk of cancer, and it contains a lot of antioxidants. Its durability and shelf life are higher than other oils, and it can even be frozen. (1)

Hydroxytyrosol, the scientific name of 2- (3,4-Di-hydroxyphenyl) -ethanol, is a phenylethanoid that is made in a laboratory environment. In nature, there is olive oil and olive seed. It has been shown in laboratory studies that hydroxytyrosol prevents platelet aggregation. It also prevents the formation of thromboxane b2 (Thromboxane B2) (a non-active Thromboxane A2 metabolite) (2).

In the early studies, the antioxidant and anti-bacterial effects have been proven (3).

In rat studies, low hydroxytrylosol has been shown to reduce the effects of cigarette oxidative stress (4).

In some studies, the effect of anti-UV hydroxytyrosol on the skin has been proven (5).

Propionibacterium acnes is a gram-positive bacteria that is an anaerobic form found on the human skin surface as a natural flora, and causes acne. It can also cause chronic blepharitis and endophthalmitis in the eye (6).

Acne vulgaris is one of the most common skin diseases and is the most common cause of referral of patients to dermatologists. It usually begins at the same time as puberty. In the division of skin diseases, acne is one of the diseases of the skin's appendages, namely, hair. The root of the hair is located and grown in the localized canal space called the hair follicle. On the other hand, fat glands, as one of the other skin components, discharge their secretions into the hair follicle, which move upwards and spread on the skin surface. Acne treatment costs a lot at a high cost per day, causing many side effects to the skin, causing scars, wrinkles, and bad skin, which reduces self-confidence, and can lead to depression and even suicide. In some cases, acne can be found in the folic acid known as acne fulminans and can be life threatening (7).

An important part of anti-Propionibacterium acnes antibiotic therapy is clindamycin, tetracyclines, erythromycin, metronidazole, nadifluxin and dapsone. In some cases, Propionibacterium acnes have been shown to be resistant to some of the drugs used, and some drugs are associated with complications that are painful for the patient (8).

Antibiotic resistance, the pathogenic microbes used to fight antibiotics, resist gene mutations in relation to these drugs and create new generations that cannot be combated. One of the most important factors in this type of drug resistance is the use of antibiotics arbitrarily or excessively. This phenomenon looks at the whole of the human society, which has put it at the risk of terrorism. The resistance of bacteria to antibiotics is one of the greatest challenges that threaten the health of the modern age (9).

Based on the above, finding new antibiotic treatments for acne is essential. It is also necessary to find out more about antibacterial hydroxytyrosol effects and its effect, by way of doing basic research, which is the basis for research on humans. Also, using the information obtained from this study, it opens the way for the creation of herbs and ointments of plant origin that have antibacterial and sun protective effects.

The purpose of this study was to determine the effect of hydroxytyrosol on bacteria Propionibacterium acnes. Using the results of this study, a way to build new antibiotics may be realised.

Materials and Methods

This research was a descriptive cross-sectional study in which the studied population of Propionibacterium acnes was conducted in the Department of Microbiology of Yasuj University of Medical Sciences. The data gathering tool was a data registration form, direct observation, and culture results, and the research variables (MIC-MBC) were the no-growth field.

Determination of zone of inhibition

CLSI protocol was used to determine ZOI by different concentrations of hydroxytyozol. Six 10 fold serial dilutions from hydroxytyrosol were prepared. The concentrations prepared included 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml =. With the well method antibiogram test was performed on different concentrations of hydroxytyrosol on the Mueller-Hinton agar and results were compared with standard antibiograms. Standard antibiotics were included: Clindamycin, Tetracyclin and Erythromycin. The test was repeated three times.

Determination of MIC and MBC

To determine MIC and MBC, a sterile flask tube was first prepared from hydroxytyrosol 20 dilution consecutively 1: 10 in sterilized Mueller-Hinton broth. Microplate dilution was then prepared from Propiunibacterium acnes bacteria to the 5×105 cfu/ml and equally added to the wells containing different dilutions of hydroxytyrosol. The wells were then incubated in a 37 ° C for 24 hours. After 24 hours, each well containing hydroxytyrosol and a bacterium was considered as ELISA reader at wavelength 620 nm wells that were not turbidity cultured on blood agar and kept in 37° C for 24 hours. After incubation time, bacterial growth was checked. At least the concentration of hydroxytyrosol that inhibits growth did not produce opacity, but had grown on the associated agar base, as the MIC and the least concentration causing the loss of 99.9% of the bacteria, or more in the well didnot increase turbidity and did not develop on related agar. MBC was considered.

Findings

Microplate dilution method was used to determine MIC and MBC with10 fold 20 serial dilutions from 200 mg/ml until 0.00038mg/ml concentrations. The result showed that hydroxytyrosol in 6.25 mg/ml concentration icontained MIC and at a concentration of 12.5 mg/ml showed MBC.

Results of diameter of the zone of inhibition in the well assay antibiogram at concentrations of 100mg/ml , 50 mg/ml, 25mg/ml, 12.5mg/ml , 6.25mg/ml and 3.125mg/ml were 25mm,21mm,19mm,16mm,13mm and 10mm respectively.

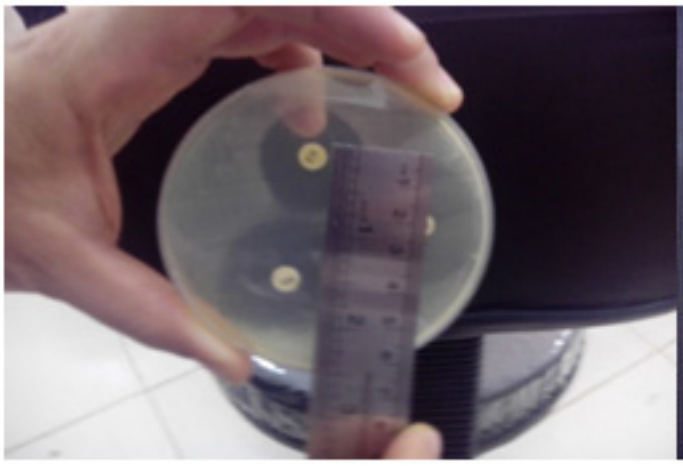
Table 1: Diameter of zone of inhibition for different concentration of hydroxytyrosol (mm).

Concentrations of hydroxytyrosol (mg/ml)	Zone of Inhibition (mm)
100	25
50	21
25	19
12.5	16
6.25	13
3.125	10

Table 2: Diameter of zone of inhibition for standard antibiotics (mm).

Antibiotics	The diameter of ZOI (mm)
Clindamycin (CC)	37
Erythromycin (E)	32
Tetracycline (TE)	29

Photo – 1



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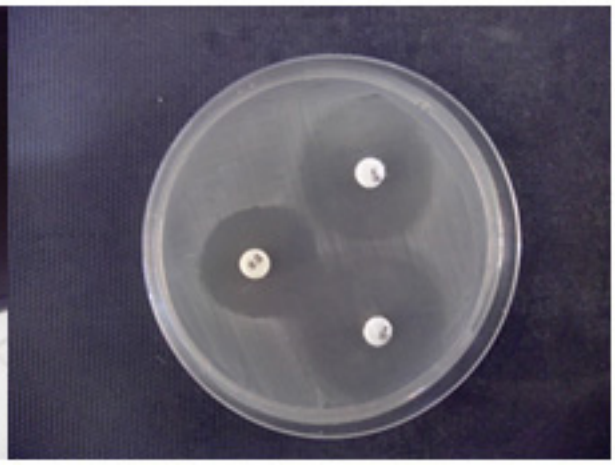

Discussion and Conclusion

In this study, it was found that hydroxytyrosol has an antibacterial effect against Propionibacterium acnes.
Until the fifth dilution of growth was observed, the concentration of 12.5 mg / ml of the tested compound had minimum bactericidal concentration. The MBC was calculated at 12500 µg / ml at unit conversion.

From dilution 6, growth was observed, i.e. a concentration of 6.25 mg / ml was minimum inhibitory concentration and converted unit to 6250 µg / ml.

Pio Maria Furneri, et al in a study titled Antimycoplasmal Activity of Hydroxytyrosol said: “Hydroxytyrosol has an antibacterial effect on twelve species of Mycoplasma hominis and two species of Mycoplasma fermentans and one species of Mycoplasma pneumoniae.” The MIC for these three species was 0.03, 0.25 and 0.5 µg / ml, respectively. In this study, the drug was safe and sufficient for use as an antibiotic (13).

According to this result and in comparison with the results of the present study, the effect of hydroxytyrosol on Propionibacterium acnes is less than Mycoplasma hominis, Mycoplasma fermentans and Mycoplasma pneumoniae.

Table 3

Antibiotic	MIC µg/ml
erythromycin	0.01 to 0.015
clindamycin	0.12 to 0.015
tetracyclin	0.12 to 0.015
Hydroxytyrosol	6250

In Wang WL et al's study entitled "*Susceptibility of Propionibacterium acnes to Seventeen Antibiotics"*: the MIC was calculated for Propionibacterium for Clindamycin 0.015 to 0.12, for erythromycin 0.01 to 0.015, and 0.015 to 0.01 for tetracycline (14).

According to the results of this study, hydroxytyrosol has a weaker anti-microbial effect than standard antibiotics against Propionibacterium acnes.

Bisignano et al, in a study titled on the in vitro antimicrobial activity of Oleuropein (OL) and hydroxytyrosol (HT) OL and HT showed good antibacterial activity in the laboratory environment. But there is not enough information on human bacteria. The bacteria in this study included Haemophilus influenzae, Moraxella catarrhalis, Salmonella typhi, Vibrio parahaemolyticus, and Staphylococcus aureus. In this study, the MIC for hydroxytyrosol was measured for standard strains between 0.24 µg / ml and 7.85 µg / ml, and for clinical strains between 0.97 µg / ml and 31.25 µg / ml. This value was much lower for Oleuropin, for standard strains between 31.25 µg / ml and 250 µg / ml, and for clinical strains between 62.5 µg / ml and 500 µg / ml (3).

In the above study, it was found that hydroxytyrosol has much more antibacterial activity than Oleuropein. Clinical strains also had more bacterial resistance than standard strains.

Comparing the above research with the present study, the antibacterial effect of hydroxytyrosol on Propionibacterium acnes is much less than that of Haemophilus influenzae, Moraxella catarrhalis, Salmonella typhi, Vibrio parahaemolyticus and Staphylococcus aureus.

Table 4:

Bacteria	MIC µg/ml
Mycoplasma hominis	0.03
Mycoplasma fermentans	0.25
Mycoplasma pneumoniae	0.5
Propionibacterium acnes	6250

Table 5

Bacteria	MIC µg/ml
Standard bacteria	7.85 _ 0.24
Clinical bacteria	31.25 _ 0.97
Propionibacterium acnes	6250

Richard Kimura et al in A Novel Borinic Acid Ester with Antibacterial Activity Against Propionibacterium acnes stated that: ANO 128 is a new bionic acid that has a large antibacterial effect on Gram positive bacteria. In our study, it was found that this substance has an antibacterial effect against Propionibacterium acnes. MIC = 2 µg / mL. (15) Compared to the current study, hydroxytyrosol with MIC = 6250 µg / mL is much weaker than ANO 128 with MIC = 2 µg / mL.

Hardy et al. in *Susceptibility of Contemporary Propionibacterium acnes to Fusidic Acid* stated: Fusic acid is a new substance tested for bone and joint infections. In this study, MIC was measured for 51 µg / mL Propionibacterium species between 0.5 and 1 µg / mL. As a result, we conclude that fusicide acid is effective against Propionibacterium acnes as it affects Staphylococcus aureus infection. (16)

Compared to the current study, hydroxytyrosol with MIC = 6250 µg / mL is much weaker than fusidic acid with a MIC of between 0.5 and 1 µg / mL.

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