



STUDY OF THE SYNERGISM EFFECT OF GARLIC AND EXTRA VIRGIN OLIVE OIL AGAINST SOME OTITIS EXTERNA AND OTITIS MEDIA BACTERIA *IN VITRO*

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Abstract

The result of synergism effect of Garlic - Extra Virgin Olive Oil (G-EVOO) against three otitis externa isolates (*Staphylococcus aureus*, *Staphylococcus capitis* and *Enterococcus faecalis*) and *Pseudomonas aeruginosa* (otitis externa isolate), was clearly shown that EVOO had no antibacterial effect only against *Enterococcus faecalis* with maximum inhibition 12 mm in diameter, while Garlic alone inhibited all isolates, with maximum antibacterial effect 34 mm in diameter against *Enterococcus faecalis* (gram positive) and 21 mm in diameter against *Pseudomonas aeruginosa*. While the antibacterial effect of G-EVOO was less than the garlic, the maximum effect was 27 mm in diameter against *Enterococcus faecalis* and 20 mm in diameter against *Pseudomonas aeruginosa*. When we compared the result of G-EVOO antibacterial effect and antibiotics sensitivity test, we can clearly still say that using G-EVOO considered as a good alternative treatment of ear infection with no side effect compared to antibiotics, which are known for their side effect and developing of resistance bacteria.

Key words: Virgin olive oil, garlic, otitis externa, otitis media, *Staphylococcus* and *Pseudomonas*

Introduction

Ear Infections:

Otitis media is one of the most common reasons that children visit a physician so it is considered as the most frequent diseases of childhood and early infancy. The affected children suffered from either complain of manifest behavior that indicating ear pain or earache. Treatment during the course of acute otitis media (AOM) accelerates the healing process and decreases both the child's discomfort and parental anxiety. There has been a substantial increase worldwide in the diagnosis of otitis media during the past two decades. In the United States, AOM is diagnosed >5 million times per year (Sarrell *et al.*, 2018).

Otitis externa is called swimmer ear and is defined as an inflammatory process of the external auditory canal (Baugh 2017). Otitis externa causative agents may be a bacterial infection, although fungal overgrowth is a principal cause in 10 percent of cases. Noninfectious dermatologic processes can lead also to otitis externa (Baugh 2017; Sander 2001).

Olive Oil:

Is considered as the main component of the

Mediterranean diet with its unique taste and flavor, expressed from ripe olive fruits in different ways (*Olea europea*). Depending on the chlorophyll to carotene ratio, its color appears as a pale yellow and may have greenish tint, (Duran 2010). Olive oil composition includes, micronutrients, represented mainly by vitamins (E, A and β -carotene), a large proportion of unsaturated fatty acids (oleic, linoleic and linolenic acids), and micro constituents (e.g. chemicals or phenolic compounds present in the unsaponifiable fraction). The total phenol content in virgin olive oil has been reported to be varied from 1 g/kg to 100 mg/kg (Dahl *et al.*, 2016; Daïdelen 2016).

Olive oil can be classified to: Virgin olive oil is obtained by several physical or mechanical means under specific conditions. Virgin olive oil is suitable for consumption. Extra virgin olive oil is a free acidity virgin olive oil expressed as oleic acid. Refined olive oil is obtained by refining methods that applied on virgin olive oil, which make it suitable for consumption in the natural state. Refined olive-pomace oil is the oil obtained by extraction by using solvents the olive pomace and made edible by refining processes (Karaosmanoğlu 2009).

Olive oil health benefits: Protective Against Heart Disease, Antioxidants, Anti-Cancer Properties, Reduce

Type 2 Diabetes Risk Osteoporosis (Dahl *et al.*, 2016), Antibacterial activity (Karaosmanoilu 2009), Not Associated With Weight Gain and Obesity (Edraki, *et al.*, 2014), Help Treat Rheumatoid Arthritis (Kemer *et al.*, 1990), Anti-Inflammatory (Amiot 2014) and Fight Alzheimer's Disease (Abuznait 2013).

Garlic:

Garlic (*Allium sativum*) is member of the Lilliceae plant family (onion family) and is one of natural plant that have multipurpose medicinal application. Garlic is also used widely as a flavoring in cooking, but it has also been used as a medicine in the modern and ancient account, it has been taken to treat and prevent a wide range of diseases and ailments (Labu and Rahaman 2019; Sulaiman *et al.*, 2014). Like other spices and herbs, over the years there are obvious increasing of using of *A. sativum* in alternative medicine. Garlic can be prepared in various forms: powder, oil, extracts and raw juice (Labu and Rahaman 2019; Daka 2011). Oil- and water-soluble organosulfur compounds of garlic have therapeutic effect and which are responsible for the typical flavor and odor of garlic. The antibiotic activity of garlic may be as a result to thiosulfates (Labu and Rahaman 2019; Durairaj *et al.*, 2009), When the bulb is crushed or ground, the sulfur compound alliin (S-allyl-L-cysteine sulfoxide) produced allicin (diallyl thiosulfinate) via the enzyme allinase (Labu and Rahaman 2019).

The aim of study was to make scientific evidence for one of the most ancient remedy for antimicrobial and ear pain (Garlic- Olive Oil), 1 clove of fresh minced garlic or cut garlic with 2 tablespoons of high quality olive oil , also to see if there is any synergistic effect between them In vitro.

Materials and methods

Culture medium preparation:

All manufacture media were prepared according to the company preparation, method using D.W. and sterilized by autoclave at 121°C/15poud/inch for 15 min. Blood agar was prepared from nutrient agar ,before pouring the (5%)blood was add and allowed to solidify.

Otitis Externa Sample collection:

Twenty ear samples were collected from patients with otitis externa by swabs (the swab should be gently inserted into the ear and rotated to collect material) ,directly the swab was streaked on MacConkey agar and Blood agar. Incubated at 37°C for 24 hrs. Isolated colony was obtained by sub culturing on Nutrient agar (ABC streaking).

The colonies for each sample were observed and

sub cultured on nutrient agar, then cultured on nutrient slant to preserve bacteria for a long time.

Identification:

Vitek 2 systems was used for identification of 3 otitis externa isolates. Otitis Media isolate....*Pseudomonas aeruginosa* (Vitek identification) was obtained from Biology department/ college of science/Baghdad University ,cultured on Hicrome agar and preserved on Nutrient slant.

Antibiotic sensitivity Test:

The bacteria was enriched in nutrient broth at 37°C for 18 hrs, using sterile cotton swabs, the bacteria was swabbed on Muller Hinton agar plate, allowed to dry. Antibiotic discs were placed over the plates (Using an ethanol dipped and flamed forceps) , plates were incubated at 37°C for 24 hrs. The diameter of the inhibition zones was measured in mm (Durairaj *et al.*, 2009).

Preparation of Garlic- Extra Virgin Olive Oil (G-EVOO) stock:

The cloves of garlic (G) were weighted 10 gm, sterilized the clove surface by ethanol and allowed to evaporate. The clove was mashed (mG) by sterile mortar and pests, and the cut garlic (cG) was cut by sterile knife. Two types of extra virgin olive oil (EVOO) were used, 1st EVOO (made in Italy) and the 2nd EVOO (made in Jordan), both of them were purchased from the supermarkets. EVOO1 and EVOO2 were tested directly, and with solvent methanol 40% .Stocks were prepared as the following:

(1) mG stock and cG stocks : mix 1 clove with 2 table spoons of sterile D.W.

(2) mG-EVOO1 and mG-EVOO2 stocks: mix 1 minced clove with 2 table spoons of EVOO

(3) cG-EVOO1 and cG-EVOO2 stocks: mix 1 cut clove with 2 table spoons of EVOO 1, let rest for 24 hrs. let rest for 24 hrs . Each stock was filter by sterile gauze, filtered mixtures were considered as stocks.

Antibacterial Susceptibility Test:

Antibacterial activity was tested by agar well diffusion method. Mueller-Hinton agar was poured into Petri dishes at 45°C and allowed to solidify. Each bacteria was Enriched in nutrient broth at 37°C for 18 hrs (adjusted to 0.5 McFarland standard), then swabbed onto the agar by using a sterile cotton swab. Four wells (5mm in diameter) were bored into the agar with a sterile cork borer (0.5cm), 50ml of each stock was pipette into each well. Incubation at 37°C for 24 hrs, then the diameter of inhibition zone was measured in mm. Each experiment was duplicated

(Sulaiman *et al.*, 2014).

Results and Discussion

Isolates identification:

All 20 samples had no growth on MacConkey agar, isolates obtained from blood agar were gram positive cocci.

Only 3 isolates were identified by vitek 2 Identification system : I 6(*Staphylococcus capitis*), I 7(*Enterococcus faecalis*) and I 17(*Staphylococcus aureus*).

Sander, R. (2001) and Baugh, R.F. (2017) refer that the skin of ear canal as on skin of other body parts, the external auditory canal has a normal bacterial flora and remains free of infection and without any harms unless its defenses are disrupted. New pathogenic flora develops when disruption occurs, that is dominated by *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Antibiotic Sensitivity Test:

The diameter of inhibition zone was measured in mm after incubation and compared with the standards susceptibility test. The result of antibiotic sensitivity (Table 1) showed that *S.aureus* (Fig. 1) was resist to 6 from 10 antibiotics with intermediate resistance to GEN (suggest multiple drug resistance),The maximum diameter was to IPE 45 mm.

S. capitis was resist to 4 from 10 antibiotics, with intermediate resistance to CEC. The maximum diameter was to GEN 49 mm . *E.faecalis* was resist to 5 from 10 antibiotics .The maximum diameter was to GEN 41 mm. *Paeruginosa* was resist to 2 from 9 antibiotics. The maximum diameter was to MEM 33 mm

Antibacterial susceptibility of G-EVOO:

First EVOO was tested for having any contaminated bacteria by spreading on nutrient agar incubated at 37°C for 1-2 days, both olive oil were free from bacteria. Our

Table 1: Antibiotics sensitivity test.

Antibiotic	<i>S.aureus</i>	<i>S.capitis</i>	<i>E. faecalis</i>	<i>Paeruginosa</i>
CEC	R	I	R	S
KF	R	R	R	
AMC	S	S	S	S
CX	R	R	R	R
TI	R	S	S	S
C	S	S	S	Not test
ATM	R	R	R	Not test
GEN	I	49mm	41mm	S
APX	R	R	R	R
IPE	45mm	S	S	S
PI	Not test	Not test	Not test	S
MEM	Not test	Not test	Not test	33mm

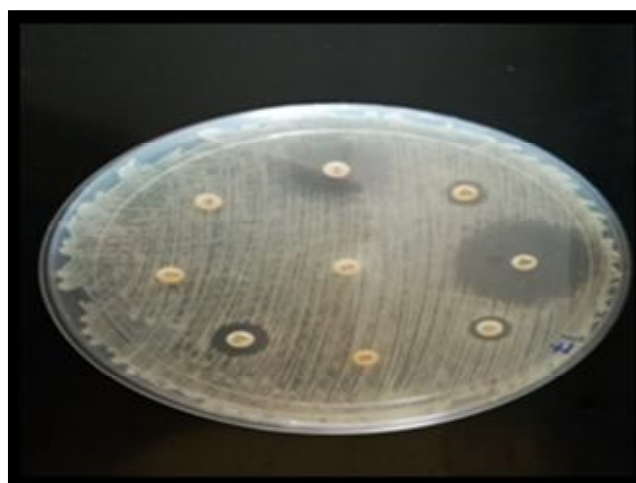


Fig. 1: *S aureus* Antibiotics sensitivity test on Muller Hinton agar.

result showed that both EVOO1 and EVOO2 had no antibacterial activity alone or with solvents (methanol 40% or Tween 20) only against *E.faecalis* with diameter 12 mm for EVOO1 and 10 mm for EVOO2. This result differ from Karaosmanoglu, H. (2009), EVOOs showed bactericidal activity while tested refined olive, hazelnut and canola oils did not cause any significant decrease in microbial population. Daïdelen, A. (2016) test two fungal strains and Fourteen pathogenic bacteria to determine the antimicrobial activity, Phenolic extracts from VOO showed antimicrobial effect against a wide range of microorganisms. The highest antimicrobial activities were determined against *Campylobacter jejuni* ATCC 33291 and *Clostridium perfringens* ATCC 13124 in samples, While Pereira, J.A. *et al.*, (2006) were investigated antimicrobial activity of table olive oils, the samples inhibited the growth of tested organisms : *Bacillus subtilis*, *Bacillus cereus*, *E.coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans* and *Cryptococcus neoformans*.

The difference between the studies maybe related to the fact that EVOO contain high concentration of phenolic compounds and its concentration differ from EVOO to another, but refined oils do no, Also for this

Table 2: Antibacterial of susceptibility of G-EVOO(diameter in cm).

Bacteria	mG-EVOO1	cG-EVOO1	mG-D.W.	cG-D.W.
<i>S.capitis</i>	24mm	-	27mm	18mm
<i>E. faecalis</i>	27mm	14mm	34mm	24mm
<i>S.aureus</i>	24mm	-	30mm	19mm
<i>Paeruginosa</i>	20mm	-	21mm	12mm

mG=minced garlic , cG=cut garlic , - = no effect
mG-EVOO1=minced garlic-extra virgin olive oil, cG-EVOO1=cut garlic-extra virgin olive oil.

study the antibacterial activity was used against only a few number of bacterial strains.

Garlic (Garlic- D.W.) showed a great antibacterial activity against all bacteria (Gram positive and Gram negative), with maximum zone was 34 mm in diameter against *E. faecalis* and the minimum zone was 12 mm in diameter against *P. aeruginosa* (Table 2).

Between the two ways we used for garlic preparation minced garlic (mG) was the best way to obtain the highest antibacterial activity 34 mm against *E. faecalis* compared to cut garlic (cG) the highest antibacterial activity was 24 mm for the same strain. This result may be due to minced garlic contain more amount of allicin compared with cut garlic.

This result similar to Durairaj, S. *et al.*, (2009), The antibacterial effects of aqueous garlic extract (AGE) of different concentrations against 17 multidrug-resistant 15 gram-negative and 2 gram-positive bacterial strains, including: *Proteus* spp., *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* were studied by well-diffusion method. The maximum zone of inhibition was observed in *Bacillus subtilis* that equal to 54 mm and the minimum zone was observed for *Proteus* spp reach to 22 mm. Also Daka, D. (2011) revealed that Garlic (*A. sativum*) has antimicrobial properties against *S. aureus*. It has both a bactericidal and bacteriostatic activity when tested crude preparation of garlic In Vitro. Therefore, garlic may be used successfully for treating *S. aureus* that cause food poisoning.

The exact mechanism remains unknown of action underlying garlic's effects and may vary according to the therapeutic effect and preparation method. The principal of active substance of fresh garlic extract, which diffuse readily through permeable membrane portion (phospholipid membranes) may contribute to its biological activity or allicin can penetrate very rapidly into different compartments of the cells and exert its biological effects (Labu and Rahaman 2019).

When we study the synergistic effect of cG and mG with two type of EVOO, the result we obtained clearly shown that using garlic with D.W. as minced or cut had highest antibacterial activity than using it with EVOO Table 2, with maximum inhibition 27 mm in diameter against *E. faecalis* and 20 mm in diameter against *P. aeruginosa*. Also the result of using mG-EVOO had highest antibacterial activity compared to cG-EVOO, this result depend on the amount of allicin which is increased during mince or crush rather than cut method.

Tympanic membrane is not permeable to

antimicrobial agents in its liquid form, acute otitis media treatment is not advised through the external ear canal. However, the vapors of essential oils may diffuse to the middle ear from the external ear canal in quantities that are sufficient to produce an antimicrobial effect on acute otitis media (Kristinsson *et al.*, 2005), also they found to be enhance local immunologic activity. Finally, herbal extracts are well tolerated owing to their long elimination time), less expensive than the new antibiotics and easy to administer (Sarrell *et al.*, 2018).

Sarrell, E.M. *et al.*, (2018) suggests that in cases of acute otitis media that cause ear pain in children in which active treatment, besides a simple 2- to 3- day waiting period, is needed, an herbal extract solution may be beneficial.

In another study applied on cat Yýpel, F.A. *et al.*, (2016) found that garlic, ozonated olive oil, and marjoram represent easily applicable, cheap and safe alternatives to conventional treatments with no side effects for ear mites (*Otodectes cynotis*) in cats.

EVOO seem to be less antibacterial than garlic which is known for their powerful antibacterial activity, but still EVOO considered as an important in treating ear infection, as soothing agent and its known as the base (healing salves) also depend on its ability to reduce inflammation. Also G-EVOO can help ease the pain of ear infection and reduce healing time.

Further studies are need for antibacterial activity of EVOO phenolic extract rather than crude EVOO. Also there is a need of In Vivo application.

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