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# Antibacterial Activity of Carvacrol against Different Types of Bacteria

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**Abstract**
In the present study, antibacterial efficiency of Carvacrol was studied on nine types of pathogenic bacteria isolated from different clinical samples, *S. aureus*, *S. epidermidis*, *St. pneumonia*, *E. coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeroginosa*, *Enterobacter* spp. and *Serratia* spp. the inhibitory effects of this oil were compared with standard antibiotics, ciprofloxacin. The inhibition effect of Carvacrol in different concentration of bacterial growth were studied, the results showed that there is a great inhibition growth on all studied bacterial isolates except *Pseudomonas aeroginosa*.
**Keywords**: Carvacrol, Antibacterial, Thymus, Vulgaris, Ciprofloxacin.

## Introduction

An alternative strategies or more effective agents exhibiting activity against microorganism are of great interest (Dorman and Deans, 2000). Natural drugs could represent an interesting approach to limit the emergence and the spread of these organisms, which currently are difficult to treat (Lambert *et al*., 2001). The spread of anti-drug resistant strains of microorganisms necessitates the discovery of new classes of antibacterial and compounds that inhibit these resistance mechanisms. Natural products continue to play major role active substances, model molecules for the discovery, and validation of drug targets (Bnyan, *et al*., 2013). On approach may be the essential oils that have been shown to be potential agents in the treatment of infections, and are safe in terms of human and animal health. Oregano oil and it major phenolic components, Carvacrol [2-methyl-5-(methylethyl) phenol] is known for wide spectrum of antimicrobial activity, which has been the subject of several investigations in vitro (Nostro *et al*., 2007). Carvacrol possess multiple biological properties such as anti-inflammatory, anti-leishmanial, antioxidant, hepatoprotective and anti- tumoral activates (Robledo *et al*., 2005).thymus vulgaris essential oil is a mixture of monoterpenes. The main componds of this oil are the natural terpenoid thymol and its phenol isomer Carvacrol (Amiri, 2012), which have antioxidative, antimicrobial, antitussive, antispasmodic and antibacterial effects (Youdim *et al*., 1999). Ciprofloxacin is a second generation flour quinolone antibiotic. Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal and abdominal infections including gram negative and gram positive bacteria (Brunton, 2005).

## Aims of study

The present study aimed to determine the antibacterial potential of Carvacrol against different types of pathogenic bacteria.

## Material and methods

**Bacterial isolation**: both gram positive and gram negative microorganisms were used for the test. All swabs and samples were collected from each patient plated on to blood agar, nutrient agar and MacConkey agar and incubated aerobically at $37^{O}$C overnight. Isolates were identified to the species level based on the standard biochemical and microbiological methods (McFadden, 2000).

**Preparation of different concentration of Carvacrol**

Different concentration of Carvacrol (sigma Alrich chemicals) prepared in Deionized water (DDW) (800, 400, 200, 100 and 50) µg/ml. all these gradient concentration were tested against the bacterial growth to clarifiey the minimum inhibitory concentration after filtrated through 0.2 µm pore size filter.

**Minimal inhibitory concentration (MIC)**

A minimum inhibitory concentration test was carried out to determine the lowest concentration of Carvacrol needed to inhibit visible (99%) bacterial growth of fixed concentration of experimental microorganisms after an overnight incubation. The MIC value was confirmed based on the inhibition and growth observed on the agar plate which had been spot inoculated. The test was carried out in triplicate and the mean value of MIC was calculated (AL-Bayaty *et al*., 2011).

**McFarland tube standard (0.5)**

A barium sulfate turbidity standard solution equivalent to 0.5 McFarland standards was prepared as described by (CLSI, 2010).

**Detection of bacterial growth by optical density**

The optical density of each tube was measured at a wavelength of 750nm against the standard medium, and the measurement being performed every 1 to 2 hrs. During the logarithmic phase to growth, The OD results were

collected as the means of three measurements.

**Screening of Carvacrol effect in bacterial growth**

Carvacrol effect in different concentration was analyzed for inhibition activates against indicator bacteria (*S. aureus*, *S. epidermidis*, *St. Pneumonia*, *E. coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeroginosa*, *Enterobacter* spp. and *Serratia* spp.) by agar-well diffusion assay (Barefoot and Kalanhammer, 1989). Muller Hinton agar seeded with bacterial isolates. The inoculums to be used in this test were prepared by adding 5 isolated colonies grown on blood agar plate to 5ml nutrient broth and incubated at 37$^{O}$C for 18 hrs. and compared with (0.5) McFarland standard tube. A serial swabs was used to obtain an inoculums was streaked on a Muller-Hinton agar plate and left to dry. Wells 5mm were hollowed out in agar using a sterile cork borer, volume of 50µl of tested Carvacrol were droppend separately in each well, and incubated at 37$^{O}$C for 24 hrs. and inhibition zone around in millimeter after subtraction 5 mm (well diameter).

**Results**

In the present study investigation antimicrobial effects of Carvacrol against nine microbial species were recorded. Table (1) summarizes the microbial growth inhibitory by different concentration of Carvacrol. It was found that this oil have the ability to inhibit the growth of bacteria isolated from different sites of infection. And the minimum inhibitory concentration was determined as (400 µg/ml) and the inhibition zone increased in the last concentration (800µg/ml) Carvacrol showed maximum inhibition against *Serratia* spp. (28mm), followed by *E. coli* (26 mm) and *Enterobacter* spp. (25 mm), *Klebsiella pneumonia (*23 mm*), Proteus mirabilis* (22 mm), *S. aureus* (20 mm), *S. epidermidis* (22 mm) and *St. pneumonia* (16 mm), there are no effect on *Pseudomonas aeroginosa.*

Table (2) and Figure (1) summarized the antibacterial effect of ciprofloxacin against different types of tested pathogenic bacteria. Results shown that bacterial isolates from different site of infection were sensitive to antibacterial ciprofloxacin, and the effect of this antibacterial was less than the effect of Carvacrol. The mean optical density for the *S. aureus* and *Proteus mirabilis* at the beginning of the culture where Carvacrol was used in 800µg/ml (0.63) and (0.64) respectively, while the mean OD after 24hrs for the same bacteria reach to (0.11) and (0.12), these results as showed in Figure (2).

**Discussion**

Although the antimicrobial properties of essential oils and their components of action have been reviewed in the past, the mechanism of action has not been studied in great detail (Lambert *et al*., 2001). Considering the large number of different groups of chemical compounds present in essential oils, it is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell (Skandamis et al., 2001; Carson et al., 2002). The microbial activity of selected concentration of Carvacrol oil agenist the experimental organisms are shown in table (1). It is illustrates the bacterial growth seen on agar plates after overnight incubation in the confirmation step. The lowest Carvacrol concentration that inhibits the growth of these organisms is regarded as the minimum inhibitory concentration (MIC) value. This study showed minimum activity with a MIC value 400µg/ml which is the concentration is recommended for inhibition of all clinical bacterial growth except *Pseudomonas aeroginosa*. The results obtained by revealed the fact that Carvacrol has the ability to cause marked inhibition of both gram positive and gram negative bacteria. The other suggested that the mode of action of Carvacrol, one of the major components of oregano and thyme oils, appear to dis-integrate that outer membrane of bacterial cells (Lambert *et al*., 2001). Carvacrol is able to disintegrate the cell membrane of gram negative bacteria releasing lipopolysaccharides (LPS) and increasing the permeability of the cytoplasmic membrane to ATP (Helander *et al*., 1998). Heipieper *et al*., (1994) described the adaption of *Pseudomonas* to ethanol and Carvacrol attribute to changes in the fatty acid composition were observed as an adaption mechanism the compounds. The essential oils possessing the strongest antibacterial properties against pathogens contain a high percentage of phenolic compounds such as Carvacrol (Juliano *et al*., 2000). It seems responsible that the mechanism of action would therefore be similar to other phenolics; this is generally considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force (PMF), electron flow, active transport and coagulation of cell contents (Davidson, 1997).

**Conclusions**

This study showed the possibility to use the Carvacrol as antibacterial. The underlying mechanism of Carvacrol induced inhibition of growth both gram positive and gram negative bacteria by similar to that of other phenolic compounds and occurs via membrane damage resulting in an increase in membrane permeability and disruption of cell wall.

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Table (1) antibacterial activity of different concentration of Carvacrol on bacterial growth

| Microorganism | Carvacrol | | | | |
|---|---|---|---|---|---|
| | 50 | 100 | 200 | 400 | 800 |
| *S. aureus* | No effect | No effect | No effect | 16 mm | 20 mm |
| *S. epidermidis* | No effect | No effect | No effect | 16 mm | 22 mm |
| *St. pneumonia* | No effect | No effect | No effect | 20 mm | 26 mm |
| *E. coli* | No effect | No effect | No effect | 22 mm | 26 mm |
| *Klebsiella pneumonia* | No effect | No effect | No effect | 20 mm | 23 mm |
| *Proteus mirabilis* | No effect | No effect | No effect | 18 mm | 22 mm |
| *Pseudomonas aeroginosa* | No effect | No effect | No effect | No effect | No effect |
| *Enterobacter* spp. | No effect | No effect | No effect | 20 mm | 25 mm |
| *Serratia* spp. | No effect | No effect | No effect | 24 mm | 28 mm |

 

Table (2): antibacterial activity of ciprofloxacin against the pathogenic test isolates

| Microorganism | ciprofloxacin |
|---|---|
| *S. aureus* | 16 mm |
| *S. epidermidis* | 16 mm |
| *St. pneumonia* | 16 mm |
| *E. coli* | 20 mm |
| *Klebsiella pneumonia* | 20 mm |
| *Proteus mirabilis* | 16 mm |
| *Pseudomonas aeroginosa* | Resistance |
| *Enterobacter* spp. | 22 mm |
| *Serratia* spp. | 24 mm |

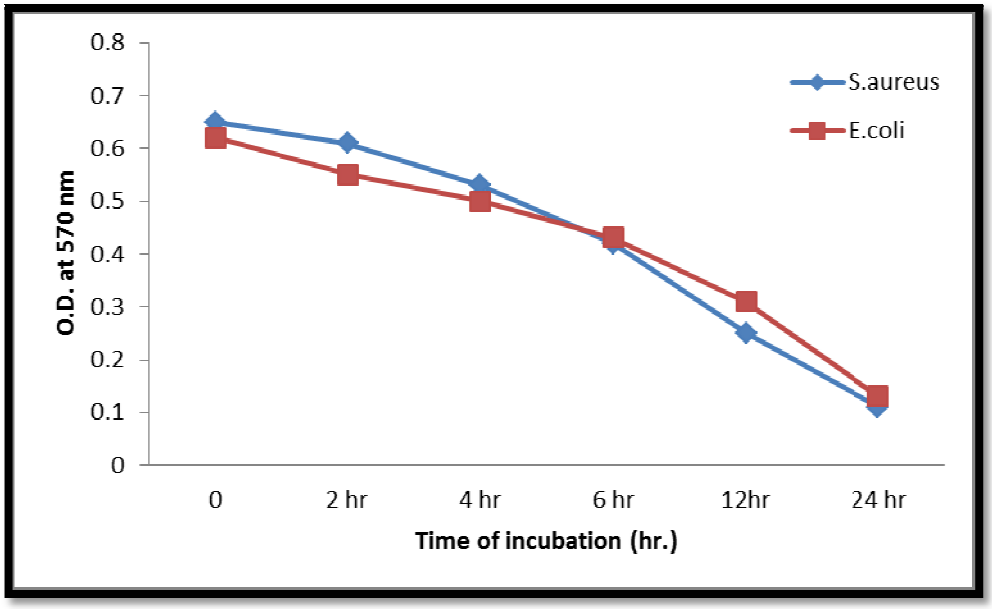


**Figure (1): effect of Carvacrol (800 µg/ml) on bacterial growth in different times**

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