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# Modifying effects of boswellia carteri on clarithromycine action: In vitro antibacterial study against common sensitive bacterial strains

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## Abstract

**Background:**Plant-derived compounds have action alongside Gram-positive and Gram-negative bacteria and numerous compounds, inhibit efflux pumps and hence have become known as efflux pump inhibitors. Clarithromycin is a macrolide antibiotic used to treat pharyngitis, tonsillitis, acute maxillary sinusitis and acute bacterial exacerbation of chronic bronchitis the antibacterial range is the similar as erythromycin but it is active against *Mycobacterium avium complex, M.leprae* and atypical mycobacteria.
The in vitro antibacterial activity results of different boswellic acid compounds discovered alpha keto-boswellic acid (AKBA) to be the preponderance potent antibacterial compound alongside Gram-positive pathogens, but it showed no significant antibacterial activity (MIC >128 µg/ml) against the Gram negative bacteria .
**Aim:** The aim of present study, is to illustrate the effectiveness of Boswellia carteri against Gram positive and negative bacteria alone and in combination with clarithromycine to elucidate the synergestic antibacterial effects and how Boswellia carteri modifying the antibacterial activity of clarithromycine.

**Material and methods:** The bacteria strains used in this study included five Gram-positive bacteria (*Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus, Staphylococcus epidermidis, Staphylococcus saprophyticus*) and three Gram-negative bacteria (*Klebsiella pneumoniae and Escherichia coli and Pseudomonas aeruginosa*) five for each strains. Antibacterial activities were evaluated by measuring inhibition zone diameters by Agar-well diffusion ,while Broth dilution method determine MIC .Then fractional inhibitory concentration determine the in vitro interaction of clarithromycine and boswellia carteri combination.

**Results** :The result of present study showed that zone of inhibition of clarithromycine ranged from 4mg/ml for Pseudomonas aeruginosa and 19mm toward Klebsiella pneumonia while zone of inhibition of Boswellia carteri ranged from 6mmfor Pseudomonas aeruginosa to 14mm for Klebsiella pneumonia the *p* value was insignificant $p > 0.05$ but the combined mixture showed significant differences from either clarithromycine or boswellia carteri $p < 0.05$. The MIC ranged from 8-32mg/ml,8-64mg/ml and 8-16mg/ml for clarithromycine, boswellia carteri and combined clarithromycineand boswellia carteri respectively and the Fractional inhibitory concentration of the combined mixture is more potent than clarithromycine and boswellia carteri alone.

**Conclusions** Boswellia carteri produced valuable property when combined with clarithromycine for sensitive bacteria and as a result others studies desirable for in vivo synergistic studies.

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## Introduction

Previously there has been a dramatic decrease in the figure of pharmaceutical companies rising new antimicrobial agents [1]. In equivalent, the number of

antibiotic-resistant bacteria has augmented [2]. With the reduction in the numeral of new agent and in antibiotic advance, there has been a renaissance of interest in the search for compounds that will renovate the activity of licensed antimicrobial agents that until recently had brilliant activity against Gram-negative bacteria and Gram positive bacteria. Plant-derived compounds have potent antibacterial activity and numerous compounds, inhibit efflux pumps and hence have become known as efflux pump inhibitors [3,4]. Since that time, frequent phytochemicals have been shown to have activity against bacteria, or to act as potential efflux pump inhibitors (EPIs) with antimicrobials for pathogenic bacteria but, the majority of the plant-derived compound has little or no activity with antibiotics against Gram-negative bacteria and it was suggested that, as many plant pathogens are Gram-negative bacteria, plants may not produce molecules effective against these organisms [5–11]. Gram-negative bacteria have innate multidrug resistance to many antimicrobial compounds due to the presence of efflux pumps in the enterobacteriaceae, the efflux pump most commonly associated with this innate multidrug resistance is the AcrAB–TolC efflux system,similar of this pump are found in other Gram-negative bacteria, including *Pseudomonas aeruginosa, Acinetobacter* spp. and *Campylobacter jejuni* , the attendance of these pumps and their broad substrate profile is the cause of the innate resistance to many of the agents that have good antimicrobial activity against Gram positive bacteria. These efflux pumps confer clinically applicable resistance to many antimicrobial agents, including ciprofloxacin and tigecycline, in Enterobacteriaceae species [12, 13,14].

Clarithromycin is a macrolide antibiotic used to treat different bacterial infections, the antibacterial range is the similar as erythromycin but it is more active against Mycobacterium *avium complex, M.leprae* and atypical mycobacteria. Clarithromycin prevent bacteria from growing by interfering with their protein synthesis also Clarithromycin binds to the subunit 50S of the bacterial ribosome and thus inhibits the translation of peptides and has parallel antimicrobial spectrum as erythromycin but is more effective against certain gram-negative bacteria, particularly *Legionella pneumophila*. Besides this bacteriostatic effect, clarithromycin also has bactericidal effect on certain strains such as *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*. Many Gram positive microbes quickly develop resistance to clarithromycin after standard courses of treatment, most frequently via acquisition of resistance gene, which confer high-level resistance to all macrolides [15].

Conventionally, the oleogum resin of Boswellia carteri has been used in many countries for the treatment of rheumatic and other inflammatory diseases such as Crohn's disease and ulcerative colitis [16].

Moreover, olibanum has gained increasing awareness from scientists and pharmaceutical companies to better describe its medical effects and identify the constituents responsible for these effects and the Boswellia carteri created various physiological property like immunomodulatory activity anti-inflammatory activity anti-cancer effects and inhibition of topoisomerases enzyme [17,18, 19, 20].

There is a intensifying awareness in using natural antibacterial compounds such as extracts and herbs for food preservation [21] and these could be an option source of novel therapeutics.The in vitro antibacterial activity of Boswellia carteri due to different boswellic acid compounds like alpha keto-boswellic acid (AKBA) which is the predominance potent antibacterial compound alongside bacterial pathogens, but it showed no significant antibacterial activity (MIC >128 µg/ml) against the Gram negative bacteria also AKBA exerted bacteriostatic antibacterial activity against S. aureus ATCC 29213 and exhibited a good post antibiotic effect (PAE) [22].

In the present study, the effectiveness of Boswellia carteri as plant-derived natural antimicrobials against Gram positive and negative bacteria alone and with combination with clarithromycine to elucidate the synergestic antibacterial effects.

## Material and Methods

This study was voted in Department of Pharmacology, College of Medicine, Al-mustansiriya University and Department Of Biology, College Of Science, Baghdad University, Baghdad – Iraq 2012. It is official by scientific judges of Department of Pharmacology and qualified by board of medical college.

The bacteria used in the study included five Gram-positive bacteria (*Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus, Staphylococcus epidermidis, Staphylococcus saprophyticus*) and three Gram-negative bacteria (*Klebsiella pneumoniae and Escherichia coli and Pseudomonas aeruginosa*) five for each strains. All bacterial cultures were obtained from the Department of Microbiology, college of sciences, Baghdad University.

**Agar-well diffusion**: For a short time, microorganisms from growth on nutrient agar incubated at 37°C for 18 h were suspended in saline solution 0.85% NaCl and adjusted to a turbidity of 0.5 Mac Farland standards (108 cfu/ml). The suspension was used to inoculate in 90 mm diameter Petri plates with a sterile non toxic cotton swab on a wooden applicator. Six millimeters diameter wells were punched in the agar and filled with 50 µl of 2000 µg/ml Boswellia carteri alcoholic solvent. The dissolution of the alcoholic oil solution was aided by 1% (v/v) DMSO. Perez *et al* method [23]. Commercial antibiotics were used as positive reference standard to determine the sensitivity of the bacterial strains. Discs were directly placed onto the bacterial culture and plates were incubated at 37°C for 24 h. Antibacterial activities were evaluated by measuring inhibition zone diameters.

**Broth dilution method**

The minimum inhibitory concentrations (MIC) of the methanolic solution were determined for the sensitive bacteria by broth dilution method. Every test extracts were successively diluted from 200 mg/ml to 20, 40, 60, 80 mg/ml. To 9 ml of sterile Mueller-Hinton broth in test tubes, 1 ml of varying concentrations of the extracts were added and then a loopful (approximately 0.01 ml) of the bacterial suspensions in advance adjusted with sterile saline (0.9% w/v) according to 0.5 McFarland turbidity standard, were introduced to the tubes. Subsequent to incubation the lowest concentration at which no obvious growth was observed was regarded as minimum inhibitory concentration.[24]

In similar manner clarithromycine 500mg diluted to 50,100,150,250 and 250mg/ml and for each bacterial strain, negative controls were maintained where distlled water was used instead of the extract and for positive control, 4 antibiotics, namely Chloramphenicol (30 mcg/disc), Gentamicin (10mcg/disc), Ciprofloxacin (5 mcg/disc) and Imipenem (10 mcg/disc) were used. The experiment was performed two times and the mean values are presented. Drugs Were Obtained From Private Pharmaceutical Company Ltd;luban oil 200mg/ml and clarithromycine (clarcide LTd Syria 500mg) . MIC of the antibiotics was determined in the presence or absence of methanol extract of Boswellia carteri.

**Determination of in vitro interaction**:

Antimicrobial interactions between Boswellia carteri and clarithromycin against 8 clinical bacterial isolates were estimated via fractional inhibitory concentrations (FIC) method. The fractional inhibitory concentrations (FICs) were calculated FIC = (MIC of drug A in combination/MIC of drug A

alone) + (MIC of drug B in combination/MIC of drug B alone). The FIC indices were interpreted as follows: <0.5, synergy; 0.5–1, additive; 1–4.0, indifference; >4, antagonism. [25]

The data analyzed statistically using the unpaired student´s t test, regarding P< 0.05 as significant and expressed as mean ±SD

**Table 1:** The characteristics of study

| | |
|---|---|
| No of isolation | 8 |
| Gram negative | 3 |
| Gram positive | 5 |
| Material used for Negative control | Distilled water |
| Material used for Positve control | Antibiotics |
| Clarithromycine | 500 mg |
| Boswellia carteri | 200mg/m |
| Duration of incubation | 24 hr. |

## Results

The present study characterized and planned in specific manner table (1).

The standard antibiotics susceptibility test of the selected bacterial strains showed in table (2).

**Table 2:** Standard antibiotics susceptibility of selected bacteria.

| | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| **Bacterial types** | **Chloramphenicol (30 mcg/disc)** | **Ciprofloxacin (5 mcg/disc)** | **Gentamicin (10 mcg/disc) a** | **Piperacillin (100 mcg/disc)** |
| Escherichia coli | 12 | 16 | 14 | 4 |
| Klebsiella pneumoniae | 14 | 15 | 12 | 5 |
| Pseudomonas aeruginosa | 8 | 14 | 16 | 2 |
| Staphylococcus aureus | 15 | 17 | 17 | 19 |
| Streptococcus faecalis | 16 | 14 | 13 | 4 |
| Bacillus cereus | 16 | 15 | 12 | 5 |
| Staphylococcus epidermidis | 17 | 14 | 4 | 18 |
| Staphylococcus saprophyticus | 15 | 22 | 12 | 20 |

Zone of inhibition of clarithromycine ranged from 4mm for Pseudomonas aeruginosa and 19mm toward Klebsiella pneumonia while Boswellia carteri ranged from 6mm for Pseudomonas aeruginosa to 14mm for Klebsiella pneumonia the *p* value was insignificant $p > 0.05$ but the combined mixture showed significant differences from either clarithromycine or boswellia carteri $p < 0.05$ table(3).

**Table 3:** Antibacterial activity of Clarithromycine ,Boswellia carteri alone and combination of both.

| Zone of Inhibition (mm) | | | |
|---|---|---|---|
| Bacterial types | Clarithromycine | Boswelliacarteri | Combined |
| Escherichia coli | 17 | 12 | 24^ |
| Klebsiella pneumoniae | 19 | 14 | 23^ |
| Pseudomonas aeruginosa | 4 | 6 | 15^ |
| Staphylococcus aureus | 15 | 12 | 26^ |
| Streptococcus faecalis | 17 | 8 | 22^ |

^ $p < 0.05$(significant)

The MIC ranged from 8-32mg/ml,8-64mg/ml and 8-16mg/ml for clarithromycine, boswellia carteri and combined clarithromycine and boswellia carteri respectively table (4).

**Table 4:** Minimal Inhibitory Concentration of clarithromycine,boswellia carteri and both.

| MIC (mg/ml) | | | |
|---|---|---|---|
| Bacterial types | Clarithromycine | Boswelliacarteri | Combined |
| Escherichia coli | 8 | 16 | 8 |
| Klebsiella pneumoniae | 8 | 8 | 8 |
| Pseudomonas aeruginosa | 32 | 64 | 16 |
| Staphylococcus aureus | 8 | 32 | 8 |
| Streptococcus faecalis | 8 | 64 | 8 |
| Bacillus cereus | 16 | 16 | 8 |
| Staphylococcus epidermidis | 16 | 16 | 8 |
| Staphyloccus saprophyticus | 16 | 64 | 8 |

Fractional inhibitory concentration of the combined mixture is more potent than clarithromycine and boswellia carteri alone table (5).

**Table 5:** Fractional Inhibitory Concentration (FIC) of combined clarithromycine and Boswellia carteri

| Bacterial types | FIC |
|---|---|
| Escherichia coli | 1.5 |
| Klebsiella pneumoniae | 2 |
| Pseudomonas aeruginosa | 0.75 |
| Staphylococcus aureus | 1.25 |
| Streptococcus faecalis | 1.125 |
| Bacillus cereus | 1 |
| Staphylococcus epidermidis | 1 |
| Staphyloccus saprophyticus | 0.625 |

## Discussion

Through the increase in the occurrence of resistance to antibiotics, alternative natural products of plants could be of concern and several plant extracts and phytochemicals are recognized to have antimicrobial actions, which might be of significance in the treatments of infectious diseases and a range of studies have been conducted in different countries, indicative of the efficacy of this type of treatment [26, 27]**.** Numerous herbs have been evaluated not only for direct antimicrobial activity but also as resistance-modifying agents [28]. Diverse chemical compounds, synthetic or natural have direct antibacterial activity alongside many species of bacteria strains, via enhancing the activity of a specific antibiotic, reversing the usual resistance of bacteria to specific antibiotics, causing the elimination of plasmids and inhibiting the active efflux of antibiotics throughout the plasma membrane [29]. The potentiation of antibiotic activity or the reversal of antibiotic resistance permits the classification of these compounds as modifiers of antibiotic activity [30].

This study has showed that methanolic extracts of Boswellia carteri in combination with clarithromycine inhibited the growth of tested bacteria at a lower concentration than when the single drugs were tested separately. This effect was synergistic for the most of the tested strains and the combinations between methanolic extracts of Boswellia carteri and clarithromycine showed a potent synergy. The presence of sub-inhibitory concentrations of the methanolic extracts of Boswellia carteri modulated the activity of clarithromycine by reducing the concentration of antibiotic needed to inhibit the growth of bacteria these findings indicated the potentiality of Boswellia carteri as a source of antibiotic modifying agent and the synergy was observed against all test bacteria,. Horiuchi *et al* 2007 study reported synergistic activity between acetone extract from Boswellia carteri and aminoglycosides against vancomycin-resistant enterococci [31]. The methanolic extracts of Boswellia carteri demonstrated potent antibacterial activity (MIC = 8 mg/mL) against Klebsiella pneumonia and MIC =16-64mg/ml for others tested bacteria, so Boswellia carteri showed an important ability to improve activity of clarithromycine against tested bacteria. This is interesting to note that the synergistic capacity of plant extracts could be investigated independently of their antimicrobial activity. In this study, according to FIC(fractional inhibitory concentration) indices, methanolic extracts of Boswellia carteri showed synergestic effects with clarithromycine this supported by Aqil *et al* 2007 study which showed synergism between ethanolic extract from Boswellia carteri and tetracycline, chloramphenicol and ciprofloxacin, but it was tested by disk-diffusion method and synergistic effect was observed on the basis of enlargement of inhibition zone [32].

Subsequently, numerous semisynthetic derivatives of erythromycin, like clarithromycin and azithromycin were planned to broaden the antimicrobial spectrum, reduce gastrointestinal side effects, and increase acid stability and bioavailability in this class of antibiotics [33].

The boswellic acids of boswellia carteri are organic acids, comprising of a pentacyclic triterpene, Alpha-boswellic acid and beta-boswellic acid, they differ only in their triterpene structure [34] and there

is profuse information reachable on the antibacterial activity of Boswellia carteri, Weckessera et al 2007 study showed significant antibacterial activity of Boswellia alongside aerobic and anaerobic bacteria such as *Streptococcus, Corynebacteria, C. perfringens and P. acnes.* [35]

Keto-ß-boswellic acid from boswellia carteri act through distortion of the cell membrane structure, and disruption of the permeability barrier of microbial membrane structures also the antibacterial activity of Keto-ß-boswellic acid against Gram-negative bacteria was low which may be attributed due to the presence of lipophilic outer membrane, this external layer of the Gram-negative outer membrane is composed primarily of lipopolysaccharide molecules and forms a hydrophilic permeability blockade as long as guard against the effects of highly hydrophobic compounds [36,37] .Moreover; Keto-ß-boswellic acid is known to be a DNA intercalator and an inhibitor of bacterial DNA synthesis through topoisomerase inhibition [38,39].

All the previous studies compatible with results of the present study about the antibacterial activity of boswellia carteri. Therefore; the bactericidal activity of boswellia carteri via cell wall inhibition and DNA distortion ,this synergy the bactericidal effects of clarithromycine since this antibiotic act as dual cidal /static effects.

Moreover the fractional inhibitory concentration (FIC) of the combined form (boswellia carteri and clarithromycin) appear within the synergestic range from 0.75-2(< 5) so boswellia carteri potentiate and synergized clarithromycine effects and act as clarithromycine modifying agent.

The clarithromycin/ boswellia carteri combination verified a synergistic effect against all of the strains and no indifferent or antagonistic effects were observed against any bacterial strains . Therefore, this study demonstrated that boswellia carteri synergistically enhanced the antibacterial activity of clarithromycin against the tested bacterial strains. This propensity is in agreement with a previous report of clarithromycin susceptible bacteria. Koga *et al.*2002 study which showed that as boswellia carteri modify in membrane fluidity and increased membrane permeability, so boswellia carteri extracts might potentiate the antibacteria activity of clarithromycin by increasing the permeability of the membranes of even clarithromycin-resistant bacteria[40,41,42].

Therefore;boswellia carteri produced beneficial effects when combined with clarithromycine for sensitive bacteria and so others studies needed for in vivo synergistic studies.

## Conclusions

Boswellia carteri produced valuable property when combined with clarithromycine for sensitive bacteria and as a result others studies desirable for in vivo synergistic studies.

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