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Synergistic Antibacterial Effects of *Syzygium aromaticum* and Citrus Lemon Combination on Some Local Bacterial Isolates

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ABSTRACT

The current study was conducted to estimate the synergistic anti-bacterial action of a combination of ground cloves and crude lemon juice as natural anti-bacterial. Combination of citrus lemon (lemon juice) and *Syzygium aromaticum* (dried flowers of clove) possessed significant antibacterial effect on hazardous bacteria. The antibacterial activity was estimated by different bacterial species including *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia* and *Enterobacter aerogenes* by means of agar well diffusion method (cork borer method). The results showed a strong anti-bacterial activity of clove-lemon combination in comparison with anti-bacterial activity of each one of them alone. Also, in comparison with clove soaked in boiling water, clove soaked in drinking water, as well as the effect of the lemon juice alone. Among the tested bacterial species, *B. subtilis* exhibited maximum inhibition with 30 mm diameter of zone of inhibition, followed by *S. aureus* (25 mm), *E. aerogenes* (25 mm), *E. coli* (23 mm), *P. aeruginosa* (20 mm) and *K. pneumonia* (18 mm). In conclusion, the use of herbs/fruits combination improved the efficacy of benefits of both phytogenic components by taking advantages of their synergistic and additive effects. It was preferred over other chemical options, mainly being natural in origin. The combination of clove and lemon exhibited antibacterial activity that indicated the synergism action of both the antibacterial activity of clove and lemon combination higher than if they were used separately.

Key words: *Syzygium aromaticum*, citrus limon combination, phytogenic combinations, synergistic action

INTRODUCTION

Exacerbating bacterial resistance to antibiotics is an important medical problem, so there is an urgent need to find new types of antibacterial agents, in particular from natural sources. Compared to manufactured medicines, plants based anti-bacterial is not accompanied by adverse effects, besides excellent curative efficacy in healing many disorders and infections (Poudel *et al.*, 2016; Sharma *et al.*, 2018). At times using one type of antibiotic does not give the potent therapeutic results; so, the combination of two or more drugs often exerts their synergistic effect that exceeds their individual effect (Coates *et al.*, 2020). The synergistic action of combining two or more types of drugs may result in the formation of a new compound or complex that has high therapeutic efficacy against a specific type of pathogens by inhibiting the growth or killing of these pathogens (Wei *et al.*, 2019; Álvarez-Martínez *et al.*, 2020). Synergism is observed in phytogenic components by notable effects; mostly accompanied with the concept "the whole is greater than the sum of the parts" (El-Saber Batiha *et al.*, 2020; Mejia-Argueta *et al.*, 2020). When herbs or plants are used in combination, as herb-herb or herb-fruit combination, the various interactions can occur among the individual components. Additional therapeutic benefits are the expected outcome from herbal combination therapy, such combinations are expected to show synergistic effects as a therapy (Wang *et al.*, 2021a, b).

Clove (*Syzygium aromaticum*) has drawn attention because of its antioxidant and antimicrobial activities which help in relieving toothache, gastrointestinal and inflammation disorders (Shrivastav *et al.*, 2019; Faujdar *et al.*, 2020). Many studies mentioned the high antibacterial efficacy of clove, indicating that clove's phenolic extract has antibacterial activity against the respiratory system bacteria and growth of *E. coli* and *S. aureus* (Hu *et al.*, 2018; Mejía-Argueta *et al.*, 2020).

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According to the high antibacterial activity of lemon juice, it is considered a good source of several pharmaceutical and effective natural compounds like citric acid, ascorbic acid, minerals, flavonoids and essential oils (Basak *et al.*, 2017; Makni *et al.*, 2018). Besides the physiological, medicinal and pharmacological activities, it also includes hypoglycaemic, anticancer, anti-inflammatory and antioxidant actions (Henderson *et al.*, 2018). The method of combination or synergistic therapy of natural extracts of clove and lemon offers a synergy action between benefits of the therapeutic components that were found in natural extracts of clove and lemon, so, the level of antibacterial therapy was undertaken in this study.

MATERIALS AND METHODS

Clinical bacterial isolates subjected in this study; *S. aureus, E. coli, P. aeruginosa, B. subtilis, K.* p*neumonia* and *E. aerogenes* were obtained from Microbiology Research Laboratory, Department of Clinical Laboratory Sciences, College of Pharmacy, University of Basrah. Both clove seeds and lemon fruits were brought up from local markets in Basrah city (south of Iraq).

Ripen fresh fruits of lemon were washed in tap water and cut open with a sterile knife. Lemon juice was pressed out into a sterile container separately. Seeds and other tissues were removed. Dried flower buds of clove were ground to a fine powder to keep in clean container. Ten ml lemon fruits juice was added to 1 g of clove ground powder in clean container and mixed well. One gm of clove ground powder was added to 10 ml of boiling water, the same procedure was repeated with drinking water (clove and drinking water) in ambient conditions, all mixed well. All the mixtures were left at room temperature.

The antibacterial activity of combination was accomplished firstly by disc diffusion method; however, the results were not encouraging. Therefore, antibacterial activity of clove and lemon combination was done by using agar well diffusion method (Nyamath and Karthikeyan, 2018), against different Gram-positive and Gram-negative pathogenic bacterial species. Nutrient agar plates were inoculated with 24 h bacterial growth suspension. Fifty µl inoculums of each selected bacteria were uniformly spread by using glass spreader on plates. By using sterile borer, four wells approximately of 5 mm diameter were bored. Fifty µl of clove-lemon combination was poured into the wells, and then plates were incubated at 37°C for 24 h. The procedure was made with three repetitions.

Under the same conditions and procedures, clove-lemon combination was repeated with mixtures of clove with boiling water, drinking water and lemon juice alone. The procedure was made with three repetitions for each component.

RESULTS AND DISCUSSION

The growing concern about finding alternative ways to control bacterial resistance has recently led to the development of natural antibiotics. This study was conducted in the natural home conditions of eating clove with lemon, based on the principle that natural food substances have their biological effectiveness in the same way that a person deals with in his daily life, then their effectiveness is measured under the same conditions, and this gives a great perception of the way in which the body benefits from these nutrients and how they enhance the body's ability and immune system defenses, as they are honest and realistic. In addition, the use of a combination of herb (clove) and fruit (lemon) gives many benefits (Malongane *et al.*, 2017; Yeddes *et al.*, 2022), due to the synergy that occurs between their active ingredients.

The clove-lemon combination exhibited synergy and positive interaction, as good antibacterial activity was obtained against all tested pathogenic bacteria isolates (Table 1). Amongst them, *B. subtilis* displayed maximum inhibition with 30 mm diameter of zone of inhibition, followed by *S. aureus* with 25 mm diameter zone of inhibition, *E. aerogenes* (25

Table 1. Antibacterial activity of clove-lemon combination against pathogenic bacterial isolates

Bacterial isolates	Zones of inhibition (mm)
B. subtilis	30
S. aureus	25
E. aerogenes	25
E. coli	23
P. aeruginosa	20
K. pneumonia	18

mm), *E. coli* (23 mm), *P. aeruginosa* (20 mm) and *K. pneumonia* (18 mm) (Figs. 1, 2, 3, 4 and 5).

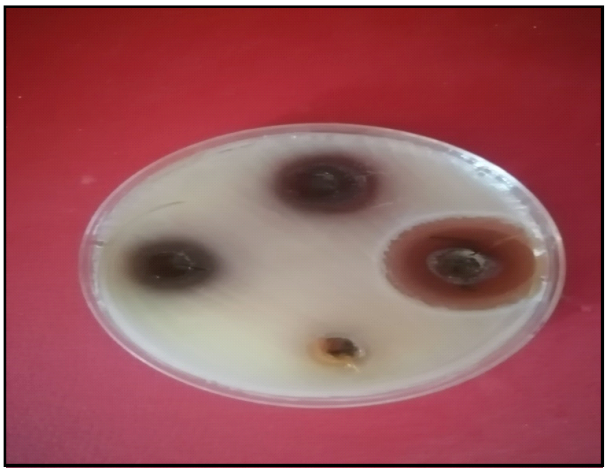

Fig. 1. Antibacterial activity of clove-lemon combination against *Bacillus subtilis*.

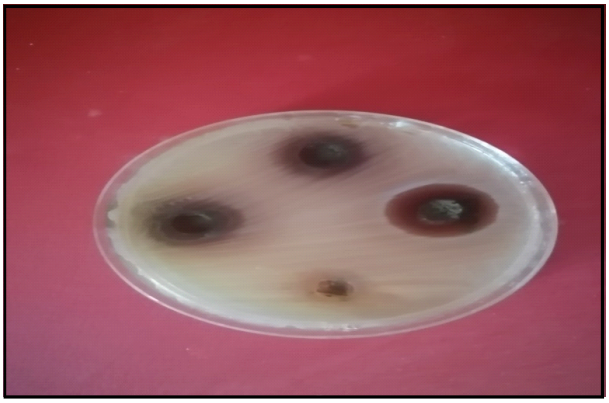

Fig. 2. Antibacterial activity of clove-lemon combination against *S. aureus*.

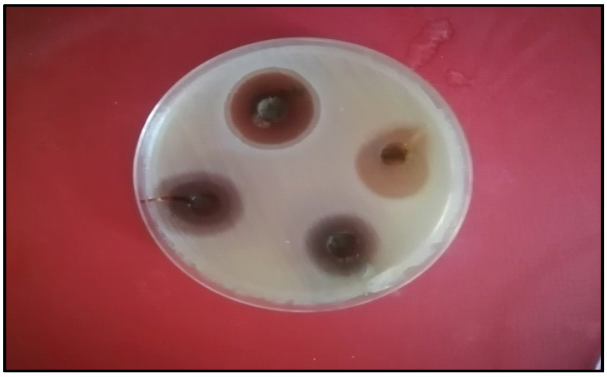

Fig. 3. Antibacterial activity of clove-lemon combination against *E. coli*.

A mixture of clove powder with boiling and drinking water was also made to find out which of these methods was the best to get the most benefits from the medicinal components of cloves. Note that each of these three ways of taking clove powder was one of the methods used by most sectors of Iraqi society. Also, antibacterial activity of lemon juice alone (without any additives) was experimented to compare it with clove-lemon antibacterial activity (Table 2, 3 and 4).

Fig. 4. Antibacterial activity of clove-lemon combination against *P. aeruginosa*.

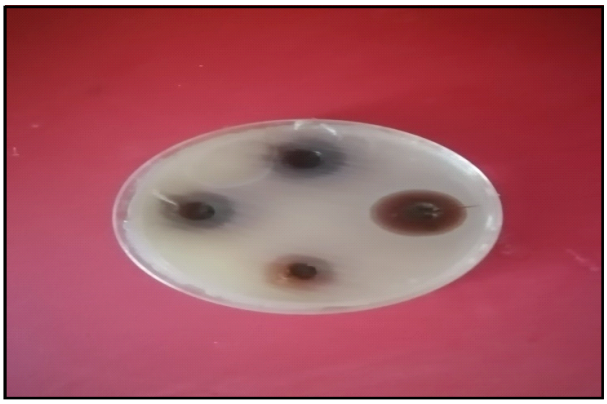

Fig. 5. Antibacterial activity of clove-lemon combination against *K. pneumonia*.

Table 2. Antibacterial activity of clove-boiling water mixture against bacterial isolates

Bacterial isolates	Zones of inhibition (mm)
B. subtilis	10
S. aureus	20
E. aerogenes	No effect
E. coli	No effect
P. aeruginosa	No effect
K. pneumonia	No effect

Table 2 shows the results of the effect of a mixture of clove powder with boiling water on bacterial isolates growth. The mixture gave antibacterial activity against *B. subtilis* and *S. aureus* with 10 and 20 mm diameters of inhibition zones, respectively.

Lemon juice showed good antibacterial effect

Table 3. Antibacterial activity of clove-drinking water mixture against bacterial isolates

Bacterial isolates	Zones of inhibition (mm)
B. subtilis	15
S. aureus	21
E. aerogenes	No effect
E. coli	No effect
P. aeruginosa	No effect
K. pneumonia	No effect

Table 3 shows antibacterial activity of mixture of clove dissolved in drinking water against *B. subtilis* and *S. aureus* with 15 and 21 mm diameters of inhibition zones, respectively.

Table 4. Antibacterial activity of lemon juice against bacterial isolates

Bacterial isolates	Zones of inhibition (mm)
B. subtilis	20
S. aureus	20
Er. aerogenes	15
E. coli	13
P. aeruginosa	11
K. pneumonia	13

against all bacterial isolates, but when compared the antibacterial effect of lemon alone, it was found that it was less than the antibacterial activity of clove-lemon combination in all cases.

According to results of this study, the combination of clove-lemon represented best synergistic antibacterial influence against the experimented pathogenic bacterial isolates (Hartanti *et al.*, 2020; Poudel *et al.*, 2021). While the single-use of clove or lemon generally resulted in the relatively low to moderate antibacterial potencies, and these results were confirmed by a comparison between the effect of clove-lemon combination with the mixture of clove with boiled water, drinking water and lemon juice alone to find out which was the most effective (Hartanti *et al.*, 2020).

Lemon is known to have high antioxidant and antimicrobial effects attributed to limonene. In addition to the anti-carcinogenic, anti-mutagenic, anti-inflammatory activities which are attributed to the presence of eugenol as its major constituent and anti-microbial benefits of cloves, especially its action against respiratory system germs and being a good analgesic and pain reliever (De and De, 2019; Maqbool *et al.*, 2023), therefore, making a mixture of lemon juice and cloves soaked will inevitably led to a synergistic action between the antibacterial substances for each of them, resulting in a wonderful mixture of high-impact antibacterial benefits taking advantages of their synergistic and additives effect. Besides this, the high acidity of lemon provided an unsuitable growth environment for the growth of a wide range of pathogens (Ekawati and Darmanto, 2019).

Thus, this mixture had a strong antibacterial action against bacteria isolated from clinical cases characterized by their high resistance to antibiotics (Guan and Liu, 2020). Gram-positive bacteria were the most sensitive to the mixture of lemon and cloves, and this was a logical and acceptable result because of the complex nature of cell wall structure of these bacteria and its difference in composition of the Gram-positive bacteria cell wall. The Gram-negative bacteria exhibited sensitivity to clove-lemon combination, but to a lesser extent than the Gram-positive bacteria, and this was reflected in the diameters of inhibition. The results of current study are compatible with work of Otang and Afolayan (2016), who proved that eugenol, thymol and limonene were found to be very active molecules and endowed with a considerable antimicrobial activity, and their synergistic effect resulted in the improvement of the antibacterial effectiveness.

Presence of the outer membrane within the composition of the cell wall of Gram-negative bacteria considered underlying reason for the resistance of these bacteria, which impeded passage of many antibiotic types, like quinolones, lactams (Yeddes *et al.*, 2022). To reach their targets, antibiotics must cross the outer membrane, as hydrophobic drugs can pass by a diffusion pathway, while β-lactams considered a hydrophilic antibiotics which can pass through porins, other types of antibiotics with complex structure don't have the ability to cross the outer membrane, like vancomycin (De Araújo *et al.*, 2020). Gram-negative bacteria continued to alter the characteristics of their outer membrane to increase their resistance to antibiotics, like varying the hydrophobic properties or changing the porins structure by mutations. Gram-positive bacteria lacked this important layer, which made them more sensitive to antibiotics in comparison with Gram-negative ones (Breijyeh *et al.*, 2020).

Finally, perhaps one of the things that must

be mentioned here; one of the main reasons that prompted to conduct this research, was the effective influence of clove-lemon combination in reducing the symptoms of infection with COVID- 19 among patients infected with this virus during the corona pandemic period, as the respiratory functions improved well in the infected individuals and the time period for infection was reduced. It also boosts the immunity of people who keep daily drinking of this mixture. Many studies have proven the efficiency of both cloves and lemon in resisting infections of the respiratory system caused by bacteria or viruses, as in the following studies (Lutgring, 2019; El-Shouny *et al.*, 2020).

CONCLUSION

The use of herbs - fruits combination improved the efficacy of benefits of both of them in pharmaceutics by taking advantages of their synergistic and additive effects, it preferred other therapeutic options, mainly of natural origin. The combination of clove and lemon exhibited antibacterial activity that indicated the synergism action of both. The antibacterial activity of clove and lemon combination was higher than if they were used separately, and better than antibacterial activity of clove soaked in boiling water, clove soaked in cold water, as well as the effect of the lemon juice only.

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