RESEARCH ARTICLE

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***Article info:***
*Received:* 11 July 2024
*Accepted:* 25 September 2024

# Synergistic Effect of Meropenem and Vancomycin Antibiotics with *Ecballium elaterium* and *Senna italica* Extracts

**ABSTRACT**

The aim of this study is to determine whether the use of plant extracts like *Ecballium elaterium* and *Senna italica* leaf aqueous extracts helps support the activity of meropenem and vancomycin antibiotics against *Staphylococcus aureus* and *Staphylococcus epidermis* bacteria. The minimum inhibitory concentration (MIC) and the fractional inhibitory concentration (FIC) were used to find out the antibacterial effect of *E. elaterium* and *S. italica* extracts in combination with meropenem and vancomycin antibiotics by employing microbroth dilution assay and checkerboard assay. According to the obtained results, all combinations showed a synergistic effect against *S. aureus* except vancomycin and *S. italica* combinations which exhibited an additive effect. Moreover, all combinations exhibited an additive effect against *S. epidermis* except vancomycin and *E. elatenium*, which showed a synergistic effect. In conclusion, *E. elaterium* and *S. italica* leaf aqueous extracts have great potential as sources of antibacterial compounds against microorganisms and they can be used to treat infectious diseases caused by antibiotic-resistant microorganisms.

**Keywords:** *Ecballium elaterium*; *Senna italica*; Synergy; Meropenem; Vancomycin; Antibiotics

## Introduction

Antimicrobial resistance is a result of antibiotic misuse. This involves either using antibiotics when they are not necessary or using antibiotics that were not received with a prescription (Nisbet, 2021). To overcome this problem, it is essential to find new classes of traditional antibiotics. Therefore, scientists characterized plant natural products to discover and formulate new effective drugs (Salam & Quave, 2018). Medicinal plants contain hundreds to thousands of bioactive molecules, so the combined impact of different molecules with antagonistic, additive, and synergistic effects ultimately contributes to the overall activity of medicinal plants (Enke & Nagels, 2011; Junio et al., 2011). In this regard, *Ecballium elaterium*, also known as squirting cucumber (*Cucurbitaceae*), is a wild medicinal plant that is distributed in the Mediterranean region (Linnaeus, 1753; Latté, 2009). Fever, cancer, sinusitis, jaundice, constipation, hypertension, and rhinosinusitis have all been medicated with this plant (Felhi et al., 2017). Several studies revealed the antibacterial effect of *E. elaterium* plant in different parts (Adwan et al., 2011; Felhi et al., 2017; Hamidi et al., 2020). In addition to *E. elaterium*, *Senna italica* (*Fabaceae*) is a plant species that grows primarily in tropical and subtropical regions. Many varieties of *Senna* are frequently used traditionally to treat a variety of diseases, including digestive disorders, circulatory system issues, and sexually transmitted diseases (Sulieman et al., 2017). Moreover, *S. italica* is used as an antipyretic, antibacterial, antifungal, antiviral, antineoplastic, anti-inflammatory, and analgesic (Amutha et al., 2014). Some studies have been conducted to assess the antibacterial activity of *S. italica* (Masoko et al., 2010; Sulieman et al., 2017). Based on that, research in medicinal plant-derived mixtures tends either to focus on one or two bioactive compounds. The interactive effects of antimicrobial combinations lead to the enhancement of antimicrobial effects of the individual compounds, exclusion of antagonistic interactions, and minimization of toxicity and adverse effects of these compounds (Eliopoulos & Eliopoulos, 1988). As antibiotic resistance is one of the most significant global health problems, there is a need to renew the supply of antimicrobials depending on natural sources such as plants, either alone or in combination with antibiotics. One of the key strategies in the



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field of antimicrobial therapy is the combination of antibiotics and compounds derived from medicinal plants. Some medicinal plants can inactivate mechanisms of antibiotic resistance. This ability resulted from the synergy between the compounds of medicinal plants and antibiotics, whose activity would be low in the absence of these plant-derived compounds. Additionally, some of these plant compounds only exhibit relevant activity when used in combination with antibiotics (Williamson, 2001). Regarding this, some studies in the literature support the synergetic effectiveness of using a combination of *E. elaterium* extracts and antibiotics, including ciprofloxacin, cefuroxime, penicillin and oxacillin (Elbashiti et al., 2017). Besides that, other studies provide evidence of the synergistic efficacy of combining *S. italica* with antibiotics against different isolates of bacteria (Sulieman et al., 2017).

In this study, the combination effect of the aqueous extracts of *E. elaterium* and *S. italica* with the two common antibiotics meropenem and vancomycin was evaluated against *Staphylococcus aureus* and *Staphylococcus epidermis*. The meropenem exhibits high bactericidal action *in vitro* against practically all clinically important anaerobes and aerobes. Its great activity is explained by the ease with which it can enter bacteria and the fact that it has a high affinity for penicillin-binding proteins, particularly those involved in cell lysis (Hurst & Lamb, 2000). Another antibiotic vancomycin, is used in treating patients with antibiotic-induced enterocolitis, and prophylaxis in patients with reduced renal function (Griffith, 1984). Several research findings in the literature pointed to the combination benefits of employing plant extracts with meropenem and vancomycin antibiotics (Vithya et al., 2011; Yang et al., 2018; Kalumbi et al., 2019; Nafis et al., 2020; Su et al., 2020).

## Materials and Methods

### *Studied Plants and Extract Preparation*

The wild plant species (*Ecballium elaterium* and *Senna italica*) were collected from West Bank, Palestine, and identified by Ghadeer Omar, Department of Biology & Biotechnology, Faculty of Science, An-Najah National University; Palestine. The plant materials for the antibacterial study were washed, air-dried, ground into powder, and stored in a dry place at room temperature. Both studied extracts were prepared as follows: five grams of the plant powders from each studied plant species were soaked in 100 mL of warm and sterile distilled water. The aqueous mixtures were subjected to rotary shaking at room temperature for a week. After that, the extract mixtures were centrifuged for 10 minutes at 4500 rpm. Then, the obtained supernatants from the two extracts were filtrated and evaporated by freeze-drying. Finally, 200 mg/mL of each plant extract was prepared by dissolving the weighed samples in distilled water and sterilizing them by microfiltration (Omar et al., 2013).

### *Studied antibiotics*

Meropenem and vancomycin (Laboratorio, Spain) were prepared by dissolving the weighed samples in distilled water and sterilizing them by microfiltration.

### *Studied bacteria*

The two bacterial isolates, *Staphylococcus aureus* (ATCC 6538P) and *Staphylococcus epidermis* (ATCC 12228) were obtained from the American Type Culture Collection (ATCC). The tested bacteria were grown overnight on nutrient agar plates. Broth turbidity was adjusted to 0.5 McFarland ($1.5\times10^8$ CFU/mL). Then it was diluted in saline to obtain $1\times10^7$ CFU/mL.

### *Minimum inhibitory concentration (MIC) test*

The antimicrobial effectiveness of the prepared extracts and antibiotics in this study against the two chosen reference isolates was analyzed through the determination of the minimum inhibitory concentrations (MIC) using microbroth dilution assay (NCCLS, 2000). Twofold serial dilution of each extract and each antibiotic in Muller-Hinton broth medium was prepared as follows, for each 100 µL Muller Hinton (MH) broth, 100 µL of the antibacterial agent was added horizontally in the first column and duplicated, the serial dilution of each compound was (50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195 and 0.098 mg/mL). To the 96-well micro-plates, one µL of the adjusted bacterial inoculum ($1 \times 10^7$ CFU/mL) was applied horizontally to all studied concentrations. and to the growth control wells (wells with MH broth medium and the tested bacteria). While the sterility control wells were not inoculated with bacteria (wells with a sterile Muller-Hinton broth). The plates were then incubated overnight at 37 °C. So, the lowest concentration that inhibited bacterial growth was then considered the MIC. This can be seen by the absence of turbidity.

### *Fractional inhibitory concentration (FIC) test*

A twofold serial dilution of plant extract in MH broth medium was prepared as follows: to each 100 µL of MH broth, 100 µL 4X plant extract was added to the first row and diluted vertically. Then 100 µL of 8X antibiotic was added to the last column. After that, 100 µl of 4X plant extract was applied to the last column and serial dilution was performed horizontally. At the end, 1µL of $1\times10^7$ CFU/ml of the tested bacterium was added to all combinations, and plates were incubated at 37°C for 18 hours. Measurement of bacterial growth was done by using a microplate reader (Labtech, UK) at 625 nm. The fractional inhibitory concentration (FIC) was derived from the lowest concentration of antibiotic and extract combination, permitting no visible growth of the test organisms on the plates



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(Bellio et al., 2021). The FIC value for each agent was calculated using this formula:

FIC (antibiotic) = MIC of antibiotic in combination / MIC of antibiotic alone

FIC (extract) = MIC of extract in combination / MIC of extract alone

Combinations were classified as synergistic, if the FIC indices were less than 0.5, additive if the FIC indices were between 0.5 and 1, indifferent if the FIC indices were between 1 and 4 and antagonistic if the FIC indices were more than 4.

## Results

The first part of the current research was performed to find out the minimum inhibitory concentration (MIC) of the two antibiotics, *E. elaterium* and *S. italica* leaf aqueous extracts (Table 1). Results demonstrated that the two extracts under study were effective bacteriostatic agents with MIC values equal to 6.25 mg/mL against *S. aureus*. Moreover, results showed a stronger inhibitory effect of *E. elaterium* against *S. epidermis* with a MIC value of 3.125 mg/mL.

**Table 1.** *Minimum inhibitory concentration (MIC) for Ecballium elatenium, Senna italica. meropenem and vancomycin against Staphylococcus aureus and Staphylococcus epidermis.*

| | MIC | |
|---|---|---|
| | ***S. aureus*** | ***S. epidermis*** |
| *E. elaterium* | 6.25 mg/mL | 3.125 mg/mL |
| *S. italica* | 6.25 mg/mL | 6.25 mg/mL |
| Meropenem | 2 µg/mL | 1 µg/mL |
| Vancomycin | 4 µg/mL | 8 µg/mL |

The second part of this study is to search for potential natural antimicrobial agents like *E. elaterium* and *S. italica* leaf aqueous extracts that can reinforce the effectiveness of meropenem and vancomycin antibiotics. To fulfill this, the fractional inhibitory concentration index was measured for the combination of meropenem and vancomycin antibiotics with *E. elaterium* and *S. italica* extracts. The indexes of these combinations are summarized in Table 2. The interpretations of the activity of most combinations showed a remarkable synergistic effect against both *S. aureus* and *S. epidermis* isolates. It is noticeable that all combinations showed a synergistic effect against *S. aureus* except the vancomycin and *S. italica* combination which exhibited an additive effect. Moreover, all combinations exhibited an additive effect against *S. epidermis* except vancomycin and *E. elatenium* which showed a synergistic effect.

## Discussion

The extensive use of antibiotics reduces their effect on the fight against pathogens, including bacteria. The common view in society and the medical community is that plant-based products are healthier, safer, and more reliable than synthetic products (Benli et al., 2008). Besides that, most plant products are less expensive and they have a wide spectrum against resistant microbes. In addition, plants continue to be one of the key sources of natural products for new medications, including antibacterial agents, especially in developing nations (Ozolua et al., 2010). In this aspect, the use of plant extracts like *E. elaterium* and *S. italica* helps support the activity of antibiotics against these pathogens. This may lead to a stronger antimicrobial effect on several pathogens with a lower dosage. In the literature, no articles consider the combination activity between the antibiotics meropenem and vancomycin with *E. elaterium* and *S. italica* leaf aqueous extracts against microbes like *S. aureus* and *S. epidermis*. The mechanisms through which antibiotics can achieve synergy are relatively understood. However, the synergistic mechanisms of medicinal plant-derived compounds have not yet been fully understood (Wagner, 2005). Suppression of biochemical pathways; inactivation of enzymes that degrade antimicrobial agents; and interaction with the microbial cell wall, which leads to increased uptake of other antimicrobial agents, are all considered among possible synergistic mechanisms of plant derived compounds and other antimicrobial agents (Pillai et al., 2005). To emphasize the synergistic role of *E. elaterium* and *S. italica* aqueous extracts with meropenem and vancomycin antibiotics, it is necessary to determine the types of phytochemicals in each plant species. According to a literature search, *E. elaterium* chemical analysis showed the presence of hydrodistillated essential oils in the leaves and fruits of this plant species as determined by GC-MS. Octyloctanoate, 3-(6,6-dimethyl-5-oxohept-2-enyl)-cyclohexanone, and hexahydro farnesyl acetone were the main components among the 21 constituents characterized in the leaf oil. In addition to that, twenty-one compounds were also identified in the oil of the *E. elaterium* fruits, with E-anethol

**Table 2.** *Fractional inhibitory concentration (FIC) index for the combination between Ecballium elatenium and Senna italica and the two antibiotics Meropenem and Vancomycin against Staphylococcus aureus and Staphyloccocus epidermis.*

| Combinations | *S. aureus* | | *S. epidermis* | |
|---|---|---|---|---|
| | **FIC index** | **Interpretation** | **FIC index** | **Interpretation** |
| Meropenem & *E. elatenium* | 0.36916 | Synergistic | 0.5625 | Additive |
| Meropenem & *S. italica* | 0.28125 | Synergistic | 0.625 | Additive |
| Vancomycin & *E. elatenium* | 0.375 | Synergistic | 0.3125 | Synergistic |
| Vancomycin & *S. italica* | 0.75 | Additive | 0.5625 | Additive |

and 3-(6,6-dimethyl-5-oxohept-2-enyl)-cyclohexanone as the main constituents (Razavi et al., 2010). Moreover, the GC-MS analysis of *S. italica* leaves revealed the existence of nine phytochemical constituents that could contribute to the medicinal quality of the plant. They are, 1-butanol, 2-methyl-, acetate; Phytol; 3-o-methyl-d-glucose; N-hexadecanoic acid; 9,12,15-octadecatrienoic acid, methyl ester, (z z,z)-; 11,14,17-eicosatrienoic acid, methyl ester; Z-5,17-octadecadien-1-ol acetate; Dodecyl cis-9,10- epoxyoctadecanoate; 1-hentetracontano (Chandran, 2015).

Some of these active compounds, such as ethyl acetate and n-butanol extracts of *S. italica* act on the bacterial cell wall, so they may reinforce the action of meropenem and vancomycin that also target the cell walls of bacteria. A previous study on *S. italica* leaf water extract with the antibiotic ampicillin against several bacterial isolates showed synergy against *S. aureus* (Sulieman et al., 2017). Accordingly, both antibiotics in the ongoing research act on the bacterial cell wall, like ampicillin, so they may facilitate the entrance of plant active ingredients into the bacterial cell wall and cause a synergistic effect.

Considering the outcomes that were obtained, it can be concluded that the *E. elaterium* and *S. italica* leaf aqueous extracts have great potential as sources of antibacterial compounds and they can be used in the treatment of infectious diseases caused by antibiotic-resistant microorganisms (Suleiman et al., 2017). Findings from this study significantly contribute to the development of effective natural medications that support antibiotic action. Although plant extract therapy is useful in the treatment of infection, it may exhibit some side effects. For example, *S. italica* causes stomach cramps, diarrhea, and a serious allergic reaction. So, further investigations on the bioavailability of combined therapy and their effects in the human body should be analyzed.

## Acknowledgments

All thanks to the Biology and Biotechnology Department at An-Najah National University for allowing us to access their laboratories. Also, all thanks to Dr. Ghadeer Omar for her role in plant classification.

## References

Adwan G, Salameh Y, Adwan K. 2011. Effect of ethanolic extract of *Ecballium elaterium* against *Staphylococcus aureus* and *Candida albicans*. Asian Pac J Trop Biomed., 1(6): 456-460.

Amutha PR, Suganya V, Suganthi B, Aishwarya K. 2014. Evaluation of free radical scavenging activity of selected parts of *Cassia senna*. Int J Pharm Sci Rev Res., 27:74-78.

Bellio P, Fagnani L, Nazzicone L, Celenza G. 2021. New and simplified method for drug combination studies by checkerboard assay. MethodsX, 8: 101543.

Benli M, Bingol U, Geven F, Guney K, Yigit N. 2008. An Investigation on the antimicrobial activity of some endemic plant species from Turkey. Afr. J. Biotechnol., 7(1): 001-005.

Chandran M. 2015. Phytocompounds screening in the methanolic leaf extract of plant *Senna italica* by GCMS analysis. J. Emerg. Technol. Innov. Res., 2(6): 389-400.

Elbashiti T, Abou elkhair E, Abu Draz WS. 2017. The antibacterial and synergistic potential of some Palestinian plant extracts against multidrug resistant *Staphylococcus aureus*. J Med Plants Stud., 5: 54-65.

Eliopoulos GM, Eliopoulos CT. 1988. Antibiotic Combinations: Should They Be Tested? Clin. Microbiol. Rev., 1: 139-156.

Enke CG. Nagels LJ. 2011. Undetected Components in Natural Mixtures: How Many? What Concentrations? Do They Account for Chemical Noise? What Is Needed to Detect Them? Anal. Chem., 83: 2539-2546.

Felhi S, Saoudi M, Daoud A, Hajlaoui H, Ncir M, Chaabane R, Kadri, A. 2017. Investigation of phytochemical contents, *in vitro* antioxidant and antibacterial behavior and *in vivo* anti-inflammatory potential of *Ecballium elaterium* methanol fruits extract. Food Sci. Technol., 37: 558-563.

Griffith RS. 1984. Vancomycin use – an historical review. J. Antimicrob. Chemother., 14(suppl_D): 1-5.

Hamidi M, Ghasemi S, Bavafa Bighdilou B, Eghbali Koohi D. 2020. Evaluation of antioxidant, antibacterial and cytotoxic activity of methanol extract from leaves and fruits of Iranian squirting cucumber (*Ecballium elaterium* (L.) A. Rich). Res. J. Pharmacogn., 7(1):23-9.

Hurst M, Lamb HM. 2000. Meropenem: a review of its use in patients in intensive care. Drugs. 59: 653-680.

Junio HA, Sy-Cordero AA, Ettefagh KA, Burns JT, Micko KT, Graf TN, Richter SJ, Cannon RE, Oberlies NH, Cech NB. 2011. Synergy Directed Fractionation of Botanical Medicines: A Case Study with Goldenseal (Hydrastis Canadensis). J. Nat. Prod., 74: 1621-1629.

Kalumbi MH. 2019. Effect of Vancomycin, tetracycline, Persia americana leaf extract and combinations thereof on antibacterial activity against pathogenic organisms. J Pharmacogn Phytochem., 8: 2733-2737.

Latté KP. 2009. *Ecballiun elaterium* (L.) Rich.–die Eselgurke. Zeitschrift für Phytotherapie. 30: 148-154.

Linnaeus C. 1753. Species *Plantarum*. London.

Masoko P, Gololo SS, Mokgotho MP, Eloff JN, Howard RI, Mampuru LJ. 2010. Evaluation of the antioxidant, antibacterial, and antiproliferative activities of the acetone extract of the roots of *Senna italica* (Fabaceae). Afr. J. Tradit. Complement. Altern. Med., 7(2): 138-148.

Nafis A, Kasrati A, Jamali CA, Custódio L, Vitalini S, Iriti M, Hassani L. 2020. A Comparative Study of the in Vitro Antimicrobial and Synergistic Effect of Essential Oils from *Laurus nobilis*, L. and *Prunus armeniaca*, L. from Morocco with Antimicrobial Drugs: New Approach for Health Promoting Products. Antibiotic, 9: E140.

National Committee for Clinical Laboratory Standards NCCLS. 2000. Methods for Dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standards- fifth edition. NCCLS document M7-A5. NCCLS: Wayne, PA, USA.

Nisbet R. 2021. National Antibiotic Awareness Week: Combating the Rise of Antibiotic Resistance. Retrieved from https://blog.amopportunities.org/2021/11/16/national-antibiotic-awareness-week-combating-the-rise-of-antibiotic-resistance/

Omar G, Abdallah LA, Ismail S, Almasri MY. 2013. Screening of selected medicinal wild plant extracts antibacterial effect as natural alternatives. Int J Indig Med Plants, 46: 1299-1304.

Ozolua RI, Idogun SE, Tafamel GE. 2010. Acute and sub-acute toxicological assessment of aqueous leaf extract of *Bryophyllum* *pinnatum* (Lam.) in Sprague-Dawley rats. Am J Pharmacol Toxicol., 5(3):145-151.

Pillai SK, Moellering RC, Eliopoulos GM. 2005. Antimicrobial Combinations. In Antibiotics in Laboratory Medicine, 5th ed.; Lorian, V., Ed.; The Lippincott Williams & Wilkins Co.: Philadelphia, CP, USA. pp. 365-440.

Razavi SM., Nejad-Ebrahimi S. 2010 Phytochemical analysis and allopathic activity of essential oils of *Ecballium elaterium* A. Richard growing in Iran, Nat. Prod. Res., 24(18): 1704-1709.

Salam AM, Quave CL 2018. Opportunities for plant natural products in infection control. Curr Opin Microbiol. 45: 189-194.

Su PA, Li SL, Tang HJ, Chen CC, Lu YC, Cheng KC, Lin YC, Chuang YC, Lai CC. 2020. *In Vitro* Synergy of *Pongamia pinnata* Extract in Combination with Antibiotics for Inhibiting and Killing Methicillin-Resistant *Staphylococcus aureus*. Antibiotics (Basel, Switzerland)., 9(3): 103.

Sulieman AME, Alghamdi AA, Veettil VN, Abdelgadir M, Ibrahim NA. 2017. Evaluation of antimicrobial activity of Senna (*Senna italica* Mill) plant and its synergistic effect with antibiotic drugs. European J. Biotechnol.Biosci., 5(1): 69-73.

Vithya A, Basri D, Huyop F. 2011. Determination of the post-antibiotic effect (PAE) of combinations of extracts from galls of Quercus infectoria with vancomycin against methicillin-resistant Staphylococcus aureus (MRSA). Afr. J. Biotechnol., 10(79): 18274-18278.

Wagner H. 2005. Natural Products Chemistry and Phytomedicine in the 21st Century: New Developments and Challenges. Pure Appl. Chem., 77: 1-6.

Williamson EM. 2001. Synergy and Other Interactions in Phytomedicines. Phytomedicine, 8: 401-409.

Yang SK, Yap PSX, Krishnan T, Yusoff K, Chan KG, Yap WS, Lai KS, Lim SHE. 2018. Mode of Action: Synergistic Interaction of Peppermint (Mentha x Piperita, L. Carl) Essential Oil and Meropenem against Plasmid-Mediated Resistant, *E. Coli*. Rec. Nat. Prod., 12: 582-594.



http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20242173