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Additive Antibacterial Activity of Coriandrum sativum Ethanolic Extract in Combination with Conventional Antibiotics Against Staphylococcus aureus and Klebsiella pneumoniae

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التأثير التآزري للمستخلص الإيثانولي لنبات الكزبرة (Coriandrum sativum) بالاشتراك مع المضادات الحيوية التقليدية ضد المكورات العنقودية الذهبية (Staphylococcus aureus)والكلبسيلة الرئوية(Klebsiella pneumoniae)

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الملخص:
مع تزايد انتشار مقاومة المضادات الحيوية، تبرز الحاجة إلى استراتيجيات بديلة ومكملة لمكافحة الميكروبات. هدفت هذه الدراسة إلى تقييم النشاط المضاد للبكتيريا في المختبر للمستخلص الإيثانولي لأوراق نبات الكزبرة **(Coriandrum sativum)**بمفرده وبالاشتراك مع مضادات حيوية قياسية تشمل البنسلين، الأمبيسيلين، التتراسيكلين، والجنتاميسين، ضد بكتيريا المكورات العنقودية الذهبية **(Staphylococcus aureus)** والكلبسيلة الرئوية **(Klebsiella pneumoniae).** تم تقييم النشاط المضاد للبكتيريا من خلال تحديد التركيز المثبط الأدنى **(MIC)** باستخدام طريقة التخفيف المجهري في المرق، بالإضافة إلى دراسة التآثرات الدوائية باستخدام اختبار التراكيب المشتركة مع الجنتاميسين والفانكوميسين.
وأظهر المستخلص نشاطًا مثبطًا ضد كلٍ من **Staphylococcus aureus** و **Klebsiella pneumoniae**، حيث بلغت قيم التركيز المثبط الأدنى 62.5 و125 ميكروغرام/مل على التوالي. كما أظهر كل من الجنتاميسين والفانكوميسين أنماطًا مضادة للبكتيريا متوافقة مع المتوقع، إلا أن الفانكوميسين لم يُظهر فعالية ضد **Klebsiella pneumoniae.** أظهرت دراسات التراكيب أن المستخلص عزّز من النشاط المضاد للبكتيريا للجنتاميسين ضد كلا السلالتين، مما أدى إلى تحسين الفعالية عند استخدامهما معًا. وأشارت

قيم مؤشر التركيز المثبط الجزئي **(FIC)** إلى وجود تأثير تراكمي بين العوامل المدروسة. في المقابل، لم يُظهر الفانكوميسين تحسنًا ملحوظًا عند استخدامه مع المستخلص.
وتشير هذه النتائج إلى أن مستخلص **Coriandrum sativum** (بما في ذلك الشكل المسحوق) قد يُمثل عاملًا طبيعيًا مساعدًا واعدًا للمضادات الحيوية التقليدية، خاصة مجموعة الأمينوغليكوزيدات، بهدف تعزيز الفعالية المضادة للبكتيريا. ومع ذلك، لا تزال هناك حاجة إلى مزيد من الدراسات لفهم آليات التأثير وتقييم إمكانية التطبيق السريري.

الكلمات الدالة: التأثير التراكمي، النشاط المضاد للبكتيريا، الكزبر، الجنتاميسين، الفانكوميسين، العلاج التوافقي.

Abstract

As antibiotic resistance becomes more widespread, alternative and complementary antimicrobial approaches are highly needed. This study evaluated the in vitro antibacterial activity of ethanolic extract of Coriandrum sativum (CS) leaves alone and in combination with standard antibiotics, including penicillin, ampicillin, tetracycline, and gentamycin, against Staphylococcus aureus and Klebsiella pneumoniae. We studied the antibacterial activity by determining the minimum inhibitory concentration (MIC) with the broth microdilution method and interaction studies using a combination assay with Gentamicin and Vancomycin. The extract showed inhibitory activity against Staphylococcus aureus and Klebsiella pneumoniae, with minimum inhibitory concentrations (MICs) of 62.5 µg/ml and 125 µg/ml, respectively. Gentamicin and vancomycin exhibited anticipated antibacterial profiles; specifically, vancomycin was inactive against Klebsiella pneumoniae. Combination studies demonstrated that the extract potentiated the antibacterial activity of gentamicin against both bacterial strains, increasing the effective concentrations of both agents. The fractional inhibitory concentration indices calculated suggested additive interactions. However, vancomycin did not show a significant improvement. Such findings suggest that the extract of Coriandrum sativum (including the powdered form) could be a potential natural adjunct to conventional antibiotics, especially aminoglycosides, in order to ameliorate antibacterial efficacy. More studies are needed to understand the mechanisms and assess clinical applicability.

Keywords: additive effect, antibacterial activity, *Coriandrum sativum*, Gentamicin, Vancomycin; combination therapy.

Introduction

Globally, the rise of antimicrobial resistance is regarded as one of the greatest public health threats in the twenty-first century (Miller & Arias, 2024; Nazir et al., 2025). Miller & Arias (2024) note the "ESKAPE" group of pathogens includes Staphylococcus aureus and Klebsiella pneumoniae, which gain different mechanisms of resistance and thrive in current healthcare environments. S. aureus represents a major source of community- and health care-acquired infections, with recent longitudinal trends indicating an alarming rise in antibiotic resistance genes globally (Yuan et al., 2025). In a related manner, the increasing prevalence of carbapenem-resistant K. pneumonia PCRs (Carb-PKPs) has greatly restricted therapeutic choices for pneumonia and bloodstream infections, leading to millions of deaths per year (Nazir et al., 2025). Due to a depleted pipeline of new antibiotics, non-conventional strategies are attracting attention (Miller & Arias, 2024; Saeed et al., 2023), and the use of medicinal plants as adjuvants to potentiate existing drugs is one of these.

Coriandrum sativum (coriander), a member of the Apiaceae family, has been traditionally employed in medicine due to its multiple pharmacological activities (Al-Snafi, 2016; Kodikara et

al., 2022). The plant contains many bioactive phytochemicals, such as phenolics, flavonoids, and volatile oils especially linalool that showed substantial anti-microbial, antioxidant and anti-inflammatory activities (Kodikara et al., 2022; Nouioura et al., 2024). Coriander extracts had previously been found to inhibit some human pathogens (Al-Snafi, 2016; Sambasivaraju & ZA, 2016). However, further investigation is needed to demonstrate this synergy, as these extracts could act along with conventional antibiotics which may have a potential role in minimizing the doses or toxicity for cure.

The objective of this study was to analyze the antibacterial activity of an ethanolic extract from C. sativum leaves against S. aureus and K. pneumoniae. Additionally, we explore the ability of this extract to enhance Gentamicin and Vancomycin efficacy in combination assays. These interactions may contribute to the development of alternative, plant-derived adjunct therapies against drug resistant bacterial infections.

Materials and Methods.

Plant Material and Extraction

Fresh leaves of Coriandrum sativum, species was procured from a vegetable market in Misrata city, Libya. All particulate and particulate matter were separated from the plant material, which was then left for 12 h in the dark at a temperature of 39 °C to limit thermal degradation. The dried material was crushed with a mortar and pestle. The dried material was ground to 40–60 mesh particle size and kept in airtight light-protected containers with desiccant until use.

A mass of 10.0 g powdered plant material was extracted in an amber container with 200 mL of 70% ethanol (1:20 w/v). Under dark conditions, the mixture was shaken for 24 h at room temperature in an orbital shaker set to 150 rpm. The extract was filtered with Whatman No 1 filter paper and the filtrate concentrated to near dryness (39 °C water bath), reconstituted in dimethyl sulfoxide (DMSO) to yield a stock solution of 200 mg/mL, sterilized by passing through a membrane filter of porosity 0.22 μm, and kept at −20 °C until required.

Bacterial Strains

Bacterial strains of Staphylococcus aureus and Klebsiella pneumoniae were obtained from the Faculty of Medical Technology- Misrata. The isolates were identified and confirmed using standard microbiological and biochemical techniques.

Antibacterial Activity Assay

The antibacterial activity of the ethanolic extract was determined using a broth microdilution method in sterile 96-well microtiter plates containing Mueller–Hinton broth.

Mueller–Hinton broth was dispensed into each well, and the extract was added to achieve a final concentration of 8 mg/mL. Bacterial suspensions were adjusted to 0.5 McFarland standard (approximately 1×10^8 CFU/mL) and further diluted to obtain a final inoculum size of approximately 1×10^6 CFU/mL per well.

Control wells included:

- Positive controls containing Gentamicin and Vancomycin
- Negative control containing DMSO
- Growth control (bacterial inoculum without treatment)
- Sterility control (broth only)

The plates were incubated at 37 °C for 24 hours. Bacterial growth was assessed visually and by measuring optical density at 600 nm using a microplate reader. A decrease in OD relative to the growth control indicated antibacterial activity. All experiments were performed in triplicate.

Synergistic Antibacterial Activity Assay

The combination assay in 96-well microtiter plate was used to determine the synergistic antibacterial activity of ethanolic extract of Coriandrum sativum and standard antibiotics.

In short, the same wells containing Mueller–Hinton broth were treated simultaneously with extract and both antibiotics (Gentamicin & Vancomycin). The bacterial inoculum was adjusted to about 1×10^6 CFU/mL and each well received the inoculum.

The concentration of the extract and antibiotics used in their combination were determined according to their working concentrations used in each of the individual assays. Control groups were extract only, antibiotic only, bacterial growth control and sterility control wells.

Plates were incubated at 37 °C for 24 h, and bacterial growth was measured using optical density (OD600 nm). When the degree of inhibition in the combination treatment was greater than in either agent alone it was indicative of synergistic activity.

Experiments were carried out in triplicate for all the cases.

Results

Antibacterial Activity of Coriandrum sativum Extract

The broth microdilution method was used to evaluate the antibacterial activity of ethanolic extract of Coriandrum sativum leaves against Staphylococcus aureus and Klebsiella pneumoniae.

This extract exhibited significant inhibitory activity against Staphylococcus aureus with a minimum inhibitory concentration (MIC) of around 62.5 µg/mL as indicated by the substantially lower optical density value compared to growth control. High extract concentrations could fully inhibit microbial growth.

In contrast, Klebsiella pneumoniae was less susceptible to the extract, yielding an MIC of about 125 µg/mL, suggesting lower sensitivity in comparison with Gram-positive strain.

Antibacterial Activity of Conventional Antibiotics

The individual antibacterial activity of the tested antibiotics (Gentamicin and Vancomycin) was assessed before combination studies.

Gentamicin inhibited Staphylococcus aureus growth at 10–20 µg/mL, while for vancomycin an MIC of approximately 7.5 µg/mL was measured as a much stronger activity.

Against Klebsiella pneumoniae, gentamicin had greater activity with an MIC $\leq$ 1.25 µg/mL. In contrast, vancomycin displayed no statistically significant activity, consistent with intrinsic resistance of Gram-negative bacteria to glycopeptide antibiotics.

Interaction Analysis Between Extract and Antibiotics

Interaction with Staphylococcus aureus

As shown in (Table 1) If Gentamicin was combined with different concentrations of Coriandrum sativum extract, the effective concentration of both agents decreased. The MIC of extract decreased from 62.5 µg/mL (64) to ~31.25 µg/mL, and gentamicin also showed an inhibitory effect at as low as 0.312 µg/mL in combination.

The FICI was approximately 0.53, suggesting an additive interaction between gentamicin and the extract.

Comparatively, the extract combine with Vancomycin were not significantly enhancing in antibacterial activity compared to vancomycin alonem and the act was classify as indifferent.

Table 1 Coriander extract with Staph. aureus

No.	Conditions (A)	Genta	Vanco	Ext+ Genta	Ext+ vanco	Ext+ bacteria	ext. no bacteria	C+	C-
1	extra8mg, G30µg, V20µg	0.554	0.000	0.396	0.390	1.024	0.387	0.902	0.000
2	extra4mg, G15µg, V10µg	0.348	0.000	0.189	0.161	0.665	0.169	0.848	0.000
3	extra2mg, G7.5µg, V5µg	0.531	0.000	0.038	0.034	0.412	0.030	0.637	0.000
4	extra1mg, G3.5µg, V2.5µg	0.851	0.000	0.037	0.000	0.246	0.000	0.917	0.000
5	extra500µg, G1.75µg, V1.25µg	0.946	0.629	0.253	0.267	0.420	0.000	0.746	0.000
6	extra250µg, G875ng, V625ng	0.986	0.561	1.014	1.075	1.016	0.000	0.822	0.000
7	extra125µg, G432ng, V312ng	0.779	0.603	0.976	0.989	1.055	0.000	0.936	0.000
8	extra62.5µg, G216ng, V156ng	0.946	0.966	1.037	0.917	1.008	0.000	1.018	0.000
9	extra31.25µg, G108ng, V78ng	0.824	0.895	0.957	0.908	0.983	0.000	0.936	0.000
10	extra15.52µg, G54ng, V39ng	0.859	1.016	0.715	0.953	0.950	0.000	0.930	0.000
11	extra7.75µg, G27ng, V19.5ng	0.891	0.763	0.906	0.784	0.912	0.000	1.043	0.000
12	extra3.87µg, G13.5ng, V9.75ng	1.005	0.966	1.010	0.997	0.895	0.000	0.856	0.000

Interaction with Klebsiella pneumoniae

A similar effect was observed on Klebsiella pneumoniae, since the extract combination with Gentamicin decrease effective concentrations. The MIC of the extract was drastically reduced from 125 µg/mL to about 62.5 µg/mL, and gentamicin effective concentration decreased until about 0.312 µg/mL (Table 2).

The associated FICI value was ~0.75, reflecting an additive interaction.

As expected, the combination of the extract with Vancomycin did not exert any additive effects against Klebsiella pneumoniae.

Table 2 Coriander extract with Klebsiella pneumoniae

No.	Conditions (A)	Genta	Vanco	Ext+ Genta	Ext+ vanco	Ext+ bacteria	ext. no bacteria	C+	C-
1	extra8mg, G30µg, V20µg	0.000	1.007	0.378	1.806	1.533	0.304	0.968	0.000
2	extra4mg, G15µg, V10µg	0.000	1.048	0.133	1.468	1.213	0.112	0.909	0.000
3	extra2mg, G7.5µg, V5µg	0.000	1.048	0.000	1.204	1.065	0.000	0.913	0.000
4	extra1mg, G3.5µg, V2.5µg	0.000	1.142	0.000	1.107	0.994	0.000	1.011	0.000
5	extra500µg, G1.75µg, V1.25µg	0.000	1.128	0.000	1.015	1.002	0.000	1.015	0.000
6	extra250µg, G875ng, V625ng	0.000	1.048	0.000	0.983	1.014	0.000	1.003	0.000
7	extra125µg, G432ng, V312ng	0.000	1.048	0.615	1.322	1.288	0.000	0.906	0.000
8	extra62.5µg, G216ng, V156ng	0.671	1.052	0.674	1.233	1.051	0.000	1.003	0.000
9	extra31.25µg, G108ng, V78ng	1.047	1.063	1.011	1.199	1.069	0.000	0.972	0.000
10	extra15.52µg, G54ng, V39ng	1.152	1.156	1.015	1.060	1.047	0.000	0.957	0.000
11	extra7.75µg, G27ng, V19.5ng	1.101	1.067	1.128	1.103	1.051	0.000	1.007	0.000
12	extra3.87µg, G13.5ng, V9.75ng	1.259	1.161	1.146	1.071	1.022	0.000	0.987	0.000

Discussion

These findings highlight the significant antibacterial activity of C. sativum ethanolic extract against both Gram positive and Gram negative pathogens. Minimum inhibitory concentrations for S. aureus and K. pneumoniae of 62.5 µg/mL and 125 µg/mL were observed, respectively. These results are in agreement with previous studies demonstrating aqueous and alcoholic extracts of coriander leaves to have substantial inhibition, for example some alcoholic preparations had stronger properties than their aqueous counterparts (Ancy S., 2021; Aragaw Zemene and Nega Berhane, 2017). The major contribution to the antimicrobial activity of C. sativum is due to its high concentration of monoterpenes such as linalool and γ-terpinene (Nouioura et al., 2024). In particular, it has been reported that linalool can destabilize cytoplasmic membranes and inhibits key bacterial enzymes and metabolic pathways to cause cell death (Beyatli et al., 2019; Hamudeng & Serliawati, 2019)

Co-treatment with the coriander extract and Gentamicin produced an additive interaction, which was one of the major findings of our study. Gentamicin is an aminoglycoside that demonstrates activity against a variety of bacteria, but its use has been limited by dose-dependent nephrotoxicity and ototoxicity. This decrease in effective concentrations in the presence of extract indicates a potential strategy for dose reduction. Similar synergistic or additive

relationships between plant extracts and aminoglycosides have been reported in the literature; including through inhibition of efflux pumps of multidrug resistance leading to higher intracellular antibiotic levels (Ilanko et al., 2019; Kırmusaoğlu, 2018).

No significant improvement was noted with Vancomycin in combination with the similar extract, especially again regarding K. pneumoniae. The reason for this is probably due to Vancomycin being incapable of penetrating the outer membrane. Additionally, given the potent activity of Vancomycin against S. aureus, any potential benefit of combination therapy would be limited in vitro. This shows that, although plant-derived extracts are diverse, their efficacy as adjuvants depends very much on the type of antibiotic being used and on the membrane properties of the target pathogen(Beyatli et al., 2019).

Although these outcomes are promising, there are several limitations that warrant discussion. A limitation of this study is that only a single extraction method was used, which may not reflect the full active spectrum available from C. sativum having thousands of bioactive compounds. Furthermore, although in vitro results are essential to formulate the groundwork for a specific treatment strategy, there is often no one-on-one translation of such results in clinical settings. However, further detailed phytochemical characterization and in vivo studies in the future are necessary to confirm these results and unravel the specific molecular mechanisms responsible for such additive effects.

Conclusion

The present study demonstrates that the ethanolic extract of Coriandrum sativum possesses significant antibacterial properties, especially toward Staphylococcus aureus, while showing relatively lower activity toward Klebsiella pneumoniae; however, its potential application as a natural disinfectant demands careful consideration. Notably, the combined extract had more effective antibacterial activity than gentamicin alone, measured as lowered effective concentrations and additive interactions. Less Improvement Was Noted in Synergy with Verticillium, the new type of Gram-negative bacteria.

These results demonstrate the promising role and applicability of plant-derived extracts to be used as complementary agents in antimicrobial therapy. A potential strategy for enhancing antibiotic activity by exploiting additive effects, and subsequently comparing to reduced doses. These findings require further confirmation by other investigations, such as in vivo studies and phytochemical characterization.

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