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In vitro Evaluation of Antibacterial Activity of 2-benzoxazolinone and Its Combination Interaction with Ciprofloxacin

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Abstract

In the present study, it was aimed to demonstrate the antibacterial and synergistic activities of 2-benzoxazolinone, a promising allelochemical, against *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 25923 *via* broth microdilution method. 2-benzoxazolinone exhibited promising antibacterial activity against all tested bacteria with 4 mg/mL against *E. coli* and *E. faecalis*, and *K. pneumoniae* and *S. aureus* 2 mg/mL against. When used in combination with ciprofloxacin, no antagonistic activity was observed. However, the combination interaction revealed indifferent activity against all tested strains. Overall, the antibacterial data obtained from the study demonstrated that 2-benzoxazolinone can further be used as a potential antibacterial agent in the treatment of bacterial infections.

Keywords

2-benzoxazolinone, antibacterial, checkerboard, ciprofloxacin.

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INTRODUCTION

As the antimicrobial resistance crisis continues, there is an inevitable need for the development of new antimicrobial options. 2-benzoxazolinone, a secondary metabolite produced by plants, algae, and microorganisms, possess variety of pharmacological activities including antioxidant, anti-inflammatory, and anticancer (Verma and Silakari, 2018). Furthermore, it has been previously demonstrated that 2-benzoxazolinone compounds revealed promising antimicrobial activities against pathogenic microorganisms (Bravo et al., 1997; Koksal et al., 2002; Siugzdaite et al., 2024).

Koksal et al. screened the antimicrobial activity of 2-benzoxazolinone derivatives against various microorganisms. The antimicrobial results revealed promising activity against two Gram negative bacteria: *E. coli* and *Pseudomonas aeruginosa*, one Gram positive bacteria: *S. aureus*, and three fungi: *Candida albicans*, *C. krusei*, *C. parapsilosis* (Koksal et al., 2002). Additionally, novel 2-benzoxazolinone derivatives were shown to possess significant antibacterial activity against various pathogenic bacteria. The most sensitive strains against the compounds were shown to be *E. coli* and *Bacillus subtilis*. However, *S. aureus* and *Salmonella* Enteritidis were shown to be more resistant in comparison to the sensitive strains with higher MIC values (Siugzdaite et al., 2024).

In this study, we aim to evaluate the antibacterial activity of 2-benzoxazolinone, while also evaluating its combination interaction with ciprofloxacin, one of the most commonly prescribed antibiotic, against selected pathogenic bacteria.

MATERIALS AND METHODS

Inoculum preparation

The evaluation of antibacterial activity of 2-benzoxazolinone (Sigma-Aldrich) was conducted against *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 25923. Each inoculum of bacteria were sub-cultured on Mueller Hinton Agar (MHA) at 37 ^{o}C for 24 hours prior to the antibacterial experiments. Each inoculum was adjusted to 0.5 McFarland (1.5 x 10^8 cfu/mL) within Mueller Hinton Broth (MHB).

Evaluation of minimum inhibitory concentration

Broth microdilution method was conducted to evaluate the antibacterial activity of 2-

benzoxazolinone (Wikler, 2006). The final concentrations of 2-benzoxazolinone ranged from 0.06 – 4 mg/mL. The highest concentration of 2-benzoxazolinone in MHB was used as regular sterility control and ciprofloxacin was used as the positive control. Incubation of 96-well plate was done at 37 ^{o}C for 18 hours. The MIC for each bacterium was accepted as the minimum concentration of 2-benzoxazolinone that clearly prevented the growth of each tested bacteria.

Evaluation of minimum bactericidal concentration

10 µL of 2-benzoxazolinone from the wells that clearly prevented the growth of each tested bacteria and was loaded onto MHA. Incubation of media was done at 37 ^{o}C for 18 hours. MBC was accepted for the lowest concentration that has completely eliminated the bacteria on the MHA.

Evaluation of the interaction of 2-benzoxazolinone with ciprofloxacin

The evaluation of the combination interaction of 2-benzoxazolinone with ciprofloxacin was done *via* checkerboard assay as previously described (Bellio et al., 2021). The final concentrations of the 2-benzoxazolinone ranged from 0.03 to 4 mg/mL, whereas ciprofloxacin ranged from 0.001 to 1 mg/L. Incubation of 96-well plate was done at 37 ^{o}C for 18 hours.

The combination interaction of the 2-benzoxazolinone and ciprofloxacin was determined *via* fractional inhibitory concentration (FIC) index calculation (FICI = A / MIC_A + B / MIC_B). 'A' and 'B' in the calculation are the MIC of each antibacterial agent in combination within a single well plate; and MIC_A and MIC_B in the calculation are the MIC of each agent individually. The interaction is accepted as synergistic if the FIC index was < 0.5; additive between 0.5-0.9; indifference between 1-4; and antagonistic if it was > 4, respectively.

Statistical analyses

The antibacterial experiments were conducted three times for each bacteria. The analyses were done *via* Students t-test using Excel.

RESULTS AND DISCUSSION

Antibacterial activity of 2-benzoxazolinone

The antibacterial activities of 2-benzoxazolinone were evaluated using microdilution method against pathogenic bacteria: *K. pneumoniae*, *E. coli*, *E. faecalis*, and *S. aureus*. MIC of each bacteria of 2-Benzoxazolinone is shown in Table 1.

Table 1: Minimum inhibitory concentrations (MICs) of 2-benzoxazolinone against tested bacteria.

Agents		Gram positive bacteria		Gram negative bacteria	
		S. aureus **ATCC 25923**	***E. faecalis*** **ATCC 29212**	***E. coli*** **ATCC 25922**	***K. pneumoniae*** **ATCC 700603**
Sample (mg/mL)	2-benzoxazolinone	2 ± 0	4 ± 0	4 ± 0	2 ± 0
Control (mg/L)	Ciprofloxacin	0. 25 ± 0	1 ± 0.	0.015 ± 0	0.06 ± 0

*Data represented as the standard error of mean (±S.E.M).

2-benzoxazolinone showed promising antibacterial activity against all tested Gram positive and Gram negative bacteria with 2 mg/mL against *S. aureus* and *K. pneumoniae*, whereas, 4 mg/mL against *E. faecalis* and *E. coli*, respectively. Furthermore, MBC assays revealed no bactericidal effect even at the highest tested concentration for each bacterial strain. However, there was a decrease in the colonies of *K. pneumoniae* treated with 4 mg/mL in comparison to 2 mg/mL of 2-benzoxazolinone as shown in Figure 1. Similarly, 2-benzoxazolinone derivatives were investigated where their antimicrobial properties demonstrated notable activity against two Gram negative bacteria: *E. coli* and *P. aeruginosa*, as well as one Gram positive bacterium: *S. aureus* (Koksal et al., 2002). In a more recent study, it was reported that novel 2-benzoxazolinone derivatives exhibited substantial antibacterial activity against various pathogenic bacterial strains. Among these, *E. coli* and *B. subtilis* were identified as the most susceptible, whereas *S. aureus* and *S. Enteritidis* displayed comparatively higher MICs (Siugzdaite et al., 2024). Furthermore, Ozalp et al. screened the antimicrobial activity of several 2-benzoxazolinone derivatives against various pathogenic microorganisms and found promising results, particularly against *E. faecalis* (Ozalp et al., 2001). These findings collectively suggest that 2-benzoxazolinone hold promise as potential leads for the development of new antibacterial agents.

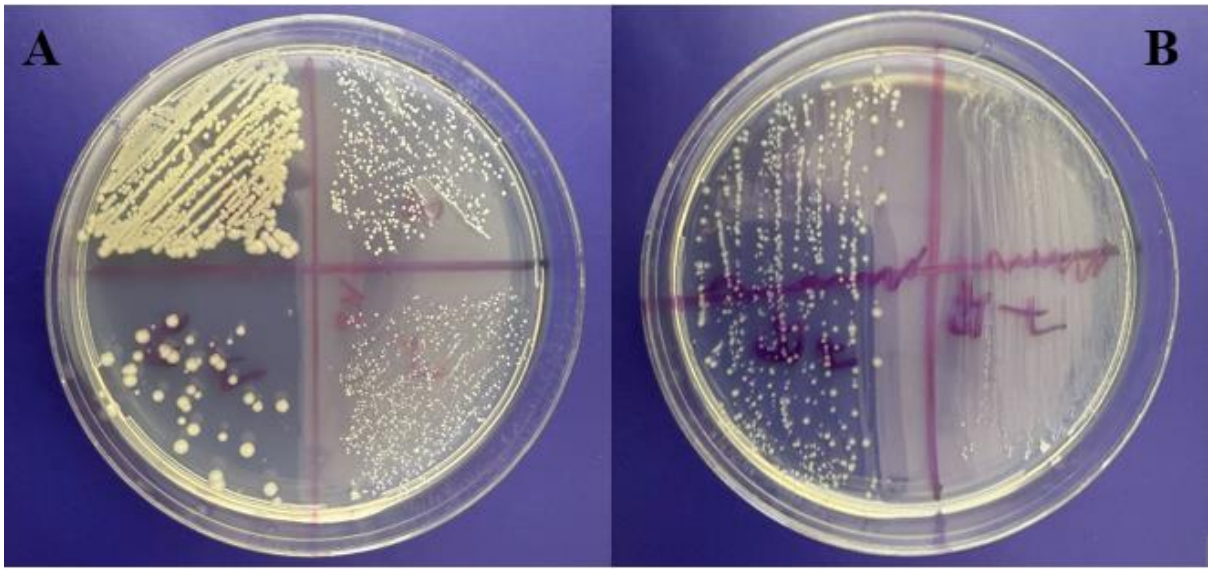

Figure 1: Minimum bactericidal concentration (MBC) results of 2-benzoxazolinone from the wells that clearly prevented the growth of each tested bacteria. **A-** 4 mg/mL (*bottom left*) and 2 mg/mL (*top left*) of 2-benzoxazolinone against *K. pneumoniae*; 4 mg/mL (*bottom right*) and 2 mg/mL (*top right*) against *S. aureus*. **B-** 4 mg/mL (*left*) of 2-benzoxazolinone against *E. coli*; 4 mg/mL (*right*) against *E. faecalis*.

Furthermore, when binary combinations of 2-benzoxazolinone and ciprofloxacin were tested against selected bacteria, no antagonistic activity was observed as shown in Table 2. Furthermore, all of the results revealed indifference effect against tested bacterial strains.

Table 2: Fractional inhibitory concentration index (FICI) of 2-benzoxazolinone and ciprofloxacin combinations against tested bacteria.

Samples	Best Combination		FICI Index	
	Ciprofloxacin (mg/L)	2-benzoxazolinone (mg/mL)	< 0.5	> 0.5
S. aureus	0.001	2		1.004 (I)
E. faecalis	0.001	4		1.001 (I)
E. coli	0.001	4		1.067 (I)
K. pneumoniae	0.001	2		1.017 (I)

I: Indifference.

CONCLUSION

2-benzoxazolinone, a promising allelochemical, is known for its antioxidant, anti-inflammatory, and anticancer properties. In addition, antimicrobial screenings with the compound and its derivatives revealed promising antimicrobial activities against various pathogenic microorganisms. In our study, the MIC values were determined were promising against all tested Gram positive and Gram negative bacteria. Furthermore, the combination of the compound with ciprofloxacin revealed no antagonistic activity against all tested strains.

REFERENCES

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

Bravo HR, Copaja SV, Lazo W (1997). Antimicrobial activity of natural 2-benzoxazolinones and related derivatives. *J Agric Food Chem* **45**: 3255-3257.

Koksal M, Gokhan N, Erdogan H, Ozalp M, Ekizoglu M (2002). Synthesis of 3-(4-substituted benzoylmethyl)-2-benzoxazolinones and screening antimicrobial activities. *Il Farmaco* **57**(7): 535-538.

Ozalp M, Calis U, Gokhan N, Erdogan H (2001). Evaluation of antimicrobial activities of some 2-benzoxazolinone derivatives. *FABAD J Pharm Sci* **26**: 169-172.

Siugzdaite J, Lelesius R, Grybaite B, Vaickelioniene R, Mickevicius V (2024). Synthesis and biological studies of new 2-benzoxazolinone derivatives as antibacterial agents. *Appl Sci* **14**: 4783.

Verma H, Silakari O (2018). Chapter 10 - Benzoxazolinone: A scaffold with diverse pharmacological significance. Key Heterocycle Cores for Designing Multitargeting Molecules p. 343-367. Punjabi University, Patiala, India.

Wikler MA (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard, vol. 26, p M7–A7. CLSI, Wayne, Pennsylvania.