

Alkaloid-Rich Extracts from *Ocimum gratissimum* and *Zanthoxylum zanthoxyloides* as Potential Antibiotic Adjuvants Against Resistant Bacterial Pathogens

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Research Article

Keywords: Antimicrobial resistance, Antibiotic modulation, *Zanthoxylum zanthoxyloides*, *Ocimum gratissimum*, Alkaloidal extract

Posted Date: August 26th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9997414/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background

Ocimum gratissimum and *Zanthoxylum zanthoxyloides* are medicinal plants widely used in African traditional medicine for the treatment of infectious diseases. The increasing prevalence of antimicrobial resistance (AMR) has renewed interest in plant-derived bioactive compounds as potential antibacterial agents and antibiotic adjuvants capable of restoring or enhancing the efficacy of existing antibiotics.

Aim of the study

This study investigated the antibacterial activity of total alkaloid extracts from *O. gratissimum* (OG-TAE) and *Z. zanthoxyloides* (ZZ-TAE), and evaluated their ability to modulate the efficacy of tetracycline against selected bacterial pathogens.

Materials and methods

Total alkaloid extracts from *O. gratissimum* (OG-TAE, 1.18% w/w) and *Z. zanthoxyloides* (ZZ-TAE, 1.40% w/w) were evaluated against Gram-positive and Gram-negative bacteria using checkerboard and time–kill kinetic assays. The chemical composition of the extracts was characterized by LC–ESI–MS analysis to identify putative bioactive alkaloids associated with antibacterial activity.

Results

Both extracts exhibited strain-dependent bacteriostatic activity with greater potency against Gram-positive bacteria. OG-TAE enhanced tetracycline activity against *Salmonella poona*, producing additive interactions and reducing the antibiotic concentrations required for antibacterial activity. Time–kill studies confirmed bactericidal effects for the combinations, with 5–10-fold improvements in antibacterial activity comparable to ciprofloxacin after 24 h. LC–ESI–MS analysis identified the following bioactive alkaloids; Zanthoamide I, nor-chelerythrine, 8-acetonyldihydro-chelerythrine, skimmianine, and benzimidazol-5-amine.

Conclusions

These findings demonstrate the potential of alkaloid-rich extracts from *O. gratissimum* and *Z. zanthoxyloides* as effective antibiotic adjuvants that enhance tetracycline efficacy, enable antibiotic dose reduction and offer a plant-based combination strategy to combat AMR.

Introduction

Antimicrobial resistance (AMR) is responsible for an estimated 1.27 million deaths annually and is projected to cause up to 10 million deaths per year by 2050 if effective interventions are not implemented (1). It has emerged as a critical global health challenge, threatening to reverse decades of progress in the management of infectious diseases. The alarming pace at which resistance emerges has

severely compromised the effectiveness of existing antibiotics, leading to increased mortality, prolonged hospital stays, and substantial economic burden worldwide (2). In response to this escalating crisis, the World Health Organization (WHO) has published a priority list of antibiotic-resistant bacteria to guide research and antibiotic development efforts. This list identifies pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* as critical or high-priority threats, owing to their extensive resistance profiles, limited treatment options, and significant clinical impact (2–4). The designation of these organisms highlights the urgent need for novel antibacterial agents and alternative therapeutic strategies capable of circumventing established resistance mechanisms (5).

Against this backdrop, increasing attention has been directed toward combination-based therapeutic strategies, including the use of medicinal plant-derived natural products as partners to conventional antibiotics (6). Medicinal plants synthesize a wide range of secondary metabolites as part of their innate defense against microbial attack, many of which exhibit antibacterial activity or the capacity to modulate microbial susceptibility to antibiotics. Growing evidence demonstrates that such natural products can enhance the efficacy of standard antimicrobial agents, mitigate AMR and restore or enhance antibiotic activity when used in combination, either by potentiating antibacterial activity or by interfering with bacterial resistance mechanisms (Obiri et al., 2025; Hussain et al., 2025; Vaou et al., 2022; Yap et al., 2021; Maurya et al., 2013). This approach has gained prominence as traditional single-agent therapies increasingly fail against multidrug-resistant (MDR) pathogens. In clinical practice, antibiotic combination therapy has long been employed to improve therapeutic outcomes by broadening antimicrobial spectra, reducing the emergence of resistant subpopulations, and achieving enhanced bactericidal effects compared to monotherapy (7). Classical examples include the co-administration of β -lactam antibiotics with β -lactamase inhibitors to neutralize enzymatic resistance and enhance intracellular drug uptake ((8, 9). Another well-documented strategy is the pairing of vancomycin with aminoglycosides, particularly in the treatment of severe enterococcal and streptococcal infections, where vancomycin-induced disruption of cell wall synthesis enhances aminoglycoside penetration and bactericidal activity, while reducing the likelihood of therapeutic failure associated with monotherapy (10, 11). Together, these principles highlight the potential of multi-agent and adjuvant-based strategies as a rational and effective means of managing resistant bacterial infections in the era of AMR.

Ocimum gratissimum L., commonly known as African basil or scent leaf, is widely distributed across tropical regions and holds a prominent place in traditional medicine systems, including in West Africa, where it is used to treat infections, diarrhea, inflammation, and other ailments (12, 13). Phytochemical investigations of *O. gratissimum* have consistently reported the presence of alkaloids, flavonoids, phenolic acids, and essential oils such as eugenol, thymol, and β -caryophyllene, which are thought to contribute to its antimicrobial activities (12–15). Empirical antibacterial studies have shown that leaf extracts of *O. gratissimum* exert inhibitory effects against clinically relevant bacteria, including *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*, suggesting broad antimicrobial potential (12, 16). *Zanthoxylum zanthoxyloides* Lam. (Rutaceae), an African tree species, also holds a rich ethnomedicinal history. Various parts of the plant, including roots, bark, and leaves, have been

traditionally used in West Africa to manage conditions such as malaria, rheumatism, urinary tract infections, and oral diseases, and the roots are frequently employed as chewing sticks for dental health (17, 18). Phytochemical screenings of *Z. zanthoxyloides* have detected alkaloids as one of the major phytoconstituent in the plant and associated with antimicrobial, anti-inflammatory, and antifungal activities (17, 19, 20).

Although *Ocimum gratissimum* and *Zanthoxylum zanthoxyloides* have documented antibacterial activities, most previous studies have focused primarily on crude extracts or essential oils using simple inhibition assays such as zone of inhibition and minimum inhibition concentrations (21–28). The antibacterial potential of alkaloid-enriched fractions remains unexplored. Alkaloids, a structurally diverse class of plant-derived nitrogenous compounds, have gained considerable attention as antibacterial agents due to their efficacy against a broad spectrum of pathogens, including multidrug-resistant strains (29–32). Unlike total crude extracts, which contain complex mixtures of phytochemicals with variable bioactivity, alkaloids-enriched fractions concentrate compounds that directly target bacteria. Specifically, alkaloids can disrupt bacterial cell membranes, leading to loss of membrane integrity and leakage of cellular contents (30, 33); inhibit nucleic acid and protein synthesis, thereby halting replication and transcription (30); and interfere with bacterial efflux pump systems, reducing bacterial resistance to antibiotics (31, 34, 35). Recent reviews of plant alkaloids highlight their broad-spectrum antibacterial potential and their relevance in overcoming antimicrobial resistance (7, 29, 30). Therefore, we hypothesized that alkaloids-enriched fractions from *Ocimum gratissimum* and *Zanthoxylum zanthoxyloides* could potentially serve as effective adjuvant for improving efficacy of existing antibiotic agents.

This study systematically examines the antibacterial activity of alkaloid-enriched extracts from *Ocimum gratissimum* and *Zanthoxylum zanthoxyloides* against pathogens identified as high-priority by WHO. Using high-throughput spot culture growth inhibition (HT-SPOTi) assays, time-kill kinetics, and modulation experiments with tetracycline, the research evaluates both standalone and combinatorial effects with these extracts. Additionally, LC-ESI-MS/MS analysis was conducted to identify the alkaloid constituents potentially responsible for the observed antibacterial properties, providing a pharmacological basis for their efficacy.

Materials and Methods

Plant Material Selection and Authentication

Fresh plant materials of *Ocimum gratissimum* and *Zanthoxylum zanthoxyloides* were collected from Nkanfua (5.14012° N, 1.24316° W) and Elmina (5.08419° N, 1.35380° W) respectively in the Central Region of Ghana. The identities of the collected specimens were authenticated by Mr. Felix Fynn a taxonomist at the Department of Botany, University of Cape Coast, Ghana. Voucher specimens were deposited in the university herbarium under the following numbers: Labiate CC 960a (*O. gratissimum*) and Fagara CC 4226 (*Z. zanthoxyloides*). *O. gratissimum* and *Z. zanthoxyloides* are common, non-protected plant species that grow widely along roadsides and in unenclosed areas in Ghana. Both plant

species were collected from private land, and verbal permission was obtained from the respective landowners prior to sample collection.

Crude Extraction of Plant Materials

The plant samples were washed to remove soil and other debris and air-dried. Powdered leaves of *Ocimum gratissimum* (730 g) and *Zanthoxylum zanthoxyloides* (820 g) were separately macerated in 1.5 L methanol-dichloromethane (1:1, v/v) mixture for 72 h with regular shaking. The mixtures were filtered, and the marc remacerated in 1.5 L methanol for another 72 h. Combined filtrates were concentrated under reduced pressure at 45°C to yield crude extracts: *O. gratissimum* – 42.70 g (5.85% w/w) and *Z. zanthoxyloides* – 53.19 g (6.49% w/w). Extracts were stored in a desiccator until use.

Phytochemical Screening

Preliminary qualitative phytochemical screening was conducted on the crude extracts and total alkaloid fractions to detect the presence of major secondary metabolite classes, specifically alkaloids, triterpenoids, steroids, flavonoids, tannins, saponins, and glycosides. The analyses were performed using standard qualitative phytochemical procedures as described in literature (36–39)

Total Crude Alkaloidal Extraction

The total alkaloids were selectively extracted from the total crude extracts (TCE) following the procedure described by Acheampong et al. (2021). Briefly, *Zanthoxylum zanthoxyloides* (40.11 g) and *Ocimum gratissimum* (22.63 g) crude extracts were separately dissolved in 30 mL 10% (v/v) acetic acid and 30 mL 15% (v/v) acetic acid respectively. The resulting acidic solutions were defatted by repeated extractions with hexane (7 × 50 mL) until the organic phases were nearly colourless. The combined aqueous layers were made alkaline with aq. NH₃ (70 mL) to a pH 8–9 and extracted with chloroform (5 × 30 mL). The combined chloroform extracts were concentrated under reduced pressure at 40°C using a rotary evaporator to obtain the crude alkaloidal fractions. The dried alkaloidal extracts of *O. gratissimum* (0.2672 g, 1.18% w/w) and *Z. zanthoxyloides* (0.5627 g, 1.40% w/w) were labelled as OG-TAE and ZZ-TAE respectively and stored in a desiccator at room temperature until use. The presence of alkaloids was confirmed by standard tests and the absence of other secondary metabolites was also confirmed using standard procedures (36–39).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MICs) of the total crude extracts and alkaloids-enriched fractions were determined using the high-throughput spot culture growth inhibition assay (HT-SPOTi), as

described by Danquah et al. (2016). Bacterial growth inhibition was assessed by visual inspection after 18–24 h of incubation. Tetracycline and ciprofloxacin were used as positive control antibiotics.

Antibiotic Resistance Modulation (Checkerboard Assay)

The ability of the alkaloidal extracts to modulate the antibacterial activity of tetracycline was evaluated using the checkerboard assay, as described by Trabelsi et al. (2020), with minor modifications. Bacterial strains exhibiting inhibition by the alkaloidal extracts at MIC values $\leq 250 \mu\text{g/mL}$ and reduced susceptibility to tetracycline ($\text{MIC} \geq 15.6 \mu\text{g/mL}$) were selected for the study. The test organisms included *Escherichia coli* ATCC 43888, *Staphylococcus aureus*, *Salmonella poona*, *Shigella* spp., and *Staphylococcus lentus*.

For each bacterial strain, stock solutions of tetracycline and the total alkaloid extract were prepared at concentrations equivalent to 8 times their respective MICs. Two-fold serial dilutions were performed to obtain concentration ranges from $8 \times \text{MIC}$ to $1/16 \times \text{MIC}$. Equal volumes ($10 \mu\text{L}$) of tetracycline and alkaloid extract dilutions were combined in a 96-well microtiter plate. Aliquots ($2 \mu\text{L}$) of each combination were transferred into a second 96-well plate containing molten nutrient agar ($196 \mu\text{L}$ per well), mixed gently, and allowed to solidify. Standardized bacterial inocula ($2 \mu\text{L}$) were then added, and plates were incubated at 37°C for 24 h. Bacterial growth inhibition was assessed by visual inspection.

Fractional inhibitory concentration (FIC) indices were calculated according to Kohanski et al. (2010) using the equation:

$$\frac{A}{\text{MIC} (A)} + \frac{B}{\text{MIC} (B)} = \text{FIC} (A) + \text{FIC} (B) = \text{FIC Index}$$

FIC indices were interpreted as follows: synergy ($\text{FIC} < 0.5$), additive interaction ($\text{FIC} 0.5\text{--}4.0$), and antagonism ($\text{FIC} > 4.0$).

Time-Kill Kinetics Assay

Time-kill kinetic studies of the alkaloidal extracts and alkaloid–tetracycline combinations were performed over 24 h according to the method described by Mojsoska et al. (2015), with minor modifications. Assays were conducted in duplicate at concentrations corresponding to the MICs ($\leq 250 \mu\text{g/mL}$) of the alkaloidal extracts against the selected test strains. Aliquots of the alkaloidal extracts ($2 \mu\text{L}$) were dispensed into appropriate wells of a 96-well microtiter plate, followed by Mueller–Hinton broth ($196 \mu\text{L}$). Standardized bacterial suspensions adjusted to 0.5 McFarland turbidity ($2 \mu\text{L}$) were then added. The optical density (OD) at 620 nm was measured at time zero ($t = 0$) using a microplate reader. Plates were sealed, protected from light, and incubated at 37°C . OD measurements were recorded at 30-min intervals during the first 6 h and subsequently at 1 h intervals between 18 and 24 h. Blank and negative control experiments were run concurrently. Changes in bacterial growth over time were used to generate time-kill curves for the alkaloidal extracts alone and in combination with tetracycline.

Chromatographic Isolation and Purification

The total alkaloidal extracts (TAE) of *Ocimum gratissimum* (OG-TAE) and *Zanthoxylum zanthoxyloides* (ZZ-TAE) were subjected to flash column chromatography for preliminary compounds separation. OG-TAE (0.40 g) was dissolved in dichloromethane (5 mL), adsorbed onto silica gel and dried under reduced pressure at 40°C. The dried sample was loaded onto a silica gel flash column and eluted with solvents of increasing polarity: hexane (100%), hexane–ethyl acetate (50:10 v/v), and hexane–ethyl acetate (50:30 v/v) and methanol (100%). A total of 63 fractions were collected and pooled into nine bulk fractions (F1–F9) based on TLC profiling and spraying with Dragendorff's reagent. The flash column chromatography procedure was repeated using ZZ-TAE (0.50 g) to afford seventy-one (71) fractions. The fractions were pooled based on TLC profiles into twelve bulk fractions (F1–F12). All combined fractions were concentrated to < 1 mL, transferred into 1.5 mL HPLC vials, further dried in a desiccator, and stored until LC–MS analysis.

LC–ESI–MS Analysis

Alkaloids profiling of the chromatographic fractions was performed using reverse-phase liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS). Analyses were carried out on a triple quadrupole mass spectrometer (AB Sciex, USA) interfaced with an Agilent 1200 HPLC system (Agilent Technologies, USA) equipped with a binary gradient pump, degasser, autosampler, and column oven. Chromatographic separation was achieved on a Zorbax SB-C18 column (2.1 × 50 mm, 1.8 µm; Agilent Technologies, USA) maintained at 25°C. The mobile phase consisted of 0.1% formic acid in water (solvent A) and methanol–acetonitrile (1:1, v/v; solvent B). A gradient elution program adapted from Nowacka et al. (2014) was employed over a total run time of 16 min at a flow rate of 100 µL min⁻¹, with an injection volume of 2 µL. Mass spectrometric detection was conducted in the positive ion mode under the following conditions: capillary temperature, 300°C; curtain gas pressure, 30 psi; nebulizer gas pressure, 60 psi. Nitrogen was used as both curtain and collision gas.

Results

Extraction and Crude Alkaloidal Extract

Extraction with methanol-dichloromethane (1:1, v/v) followed by methanol afforded the phytoconstituents from the leaves of *Zanthoxylum zanthoxyloides* (53.19 g, 6.49% w/w) and *Ocimum gratissimum* (42.70 g, 5.85% w/w). Selective partitioning of alkaloids by exploiting their acid-base properties is a cost-effective method for separating alkaloids from other secondary metabolites (Obiri et al., 2025). We added acetic acid to convert alkaloids into their water-soluble salts to enable their selective partitioning into the aqueous phase. The salts were further converted into their neutral form with aqueous ammonia and extracted with chloroform to afford the total alkaloidal extracts of *O. gratissimum* (OG-TAE 1.18% w/w) and *Z. zanthoxyloides* (ZZ-TAE 1.40% w/w). The yields are consistent with generally low levels of alkaloids reported in plants. Phytochemical screening of the total crude

extract (TCE) and crude alkaloidal extracts (OG-TAE and ZZ-TAE) is shown in Table 1. The TCE contained multiple classes of secondary metabolites including alkaloids, triterpenoids, steroids, tannins, and glycosides. The presence of alkaloids in the TCE, OG-TAE and ZZ-TAE were confirmed using standard methods. Addition of Dragendorff's reagent gave orange red precipitate. Addition of Meyer's reagent resulted in yellowish precipitate, and the use of Wagner's reagent formed a large brown amorphous and flocculent precipitate. It was gratifying to observe that the alkaloidal extracts contained no other secondary metabolites when we repeated the phytochemical screening assays, indicative of successful acid-base extraction method for selective alkaloidal extraction.

Table 1
Profile of Phytoconstituents of the crude and alkaloidal extracts of *O. gratissimum* and *Z. zanthoxyloide*

Phytoconstituent	<i>O. gratissimum</i> ^{a (b)}	<i>Z. zanthoxyloide</i> ^{a (b)}
Alkaloid	+ (+)	+ (+)
Triterpenoid	+ (-)	+ (-)
Steroids	+ (-)	+ (-)
Flavonoid	+ (-)	- (-)
Tannin	+ (-)	+ (-)
Saponins	- (-)	- (-)
Glycosides	+ (-)	+ (-)
Key: a = total crude extract; b= total alkaloid extract; + = detected; - = not detected		

Table 2

MICs for Total Crude Extracts and Total Alkaloidal Extracts of *O. gratissimum* and *Z. zanthoxyloides* Against Ten Different Bacteria

Drug/ Extract	Minimum Inhibition Concentration (MIC) (µg/ml)									
	<i>E. coli</i> (10)	<i>E. coli</i> (43)	<i>S. aureus</i>	<i>MRSA</i>	<i>Kleb</i>	<i>Shigella</i>	<i>S. typhi</i>	<i>S. poona</i>	<i>S. lentus</i>	<i>S. pyogenes</i>
OG-TCE	N/A	> 500	> 500	N/A	> 500	N/A	N/A	> 500	> 500	> 500
ZZ-TCE	N/A	500	> 500	N/A	> 500	N/A	N/A	500	500	> 500
OG-TAE	> 500	250	250	500	500	125	500	125	250	> 500
ZZ-TAE	> 500	250	< 3.9	< 3.9	500	< 3.9	< 3.9	< 3.9	< 3.9	< 3.9
CIPX	< 3.9	3.9	15.6	125	< 3.9	125	7.8	15.6	15.6	< 3.9
TCLN	< 3.9	15.6			15.6					
Key: OG-TCE = <i>O. gratissimum</i> Total Crude Extract; ZZ-CE = <i>Z. zanthoxyloides</i> Total Crude Extract; OG-TAE = <i>O. gratissimum</i> Total Alkaloidal										
Extract; ZZ-TAE = <i>Z. zanthoxyloides</i> Total Alkaloidal Extract; CIPX = Ciprofloxacin; TCLN = Tetracycline; <i>E. coli</i> (10) = <i>Escherichia coli</i>										
ATCC 10455; <i>E. Coli</i> (43) = <i>Escherichia coli</i> ATCC 43888										

In-vitro Antibacterial Activity

The antibacterial activity of the total crude extracts and total alkaloid extracts of *Ocimum gratissimum* (OG) and *Zanthoxylum zanthoxyloides* (ZZ) was evaluated against ten clinically relevant bacterial strains using the HT-SPOTi assay. The MIC values for each extract are summarized in Table 2.

Against all bacterial strains, the total alkaloid fractions (OG-TAE and ZZ-TAE) generally exhibited enhanced antibacterial activity compared to the corresponding crude extracts (OG-TCE and ZZ-TCE). For example, OG-TAE inhibited *S. poona* at 125 µg/mL and both *E. coli* (43) and *S. aureus* at 250 µg/mL, whereas OG-TCE showed no activity (MIC > 500 µg/mL) against the same strains. Similarly, ZZ-TAE exhibited inhibitory effects against *E. coli* ATCC 43888, *S. aureus*, *S. lentus* all at 250 µg/mL and both *Shigella* and *S. poona* at 250 µg/mL, while its crude extract (ZZ-TCE) showed negligible activity.

The reference antibiotic ciprofloxacin consistently displayed the highest potency across all tested strains (MIC < 3.9 µg/mL), whereas tetracycline was less active against several strains, including *MRSA*, *Shigella*, *S. aureus*, *S. lentus* and *S. poona*, reflecting reduced susceptibility of these bacteria. These observations highlight the antibacterial effects of the alkaloids-enriched fractions, particularly against strains exhibiting reduced susceptibility to tetracycline.

Time-Kill Kinetics Study

The negative control growth curves showed uninterrupted bacterial proliferation over the 24 h incubation period, confirming that the assay conditions adequately supported bacterial growth. Optical density (OD) increased steadily during the first 6 h, reflecting successful transition from the lag to exponential growth phase across all strains.

A marked rise in OD values was observed between 18 and 24 h, indicating continued biomass accumulation with no evidence of growth inhibition or nutrient limitation. Average optical densities for the final 6 h ranged from 0.68 (*Shigella*) to 1.14 (*Klebsiella*), with *S. aureus* and *Klebsiella* exhibiting the highest growth levels, consistent with their known growth characteristics (40, 41). The consistent growth patterns validated the viability of the inocula and established a reliable baseline for interpreting antibacterial effects in the treated time-kill experiments.

Time-Kinetics of Total Alkaloidal Extracts from *O. gratissimum* (OG-TAE) and *Z. zanthoxyloides* (ZZ-TAE) Leaves

The time-kill kinetics of OG-TAE demonstrated notable strain-dependent bacteriostatic activity, often surpassing tetracycline in limiting bacterial growth. For *E. coli* (43), OG-TAE maintained lower OD values (~ 0.51 – 0.53) throughout the 24-h period, whereas tetracycline-treated cultures rose above 0.64 after 19 h, indicating that the extract provided more sustained suppression of proliferation. Against *S. aureus*, OG-TAE consistently kept OD values low (~ 0.01 – 0.18) during the first 6 h and maintained a lower OD_{ave} at 24 h (~ 0.14) compared to tetracycline (~ 0.19 – 0.21), reflecting superior inhibition of this Gram-positive pathogen. Similarly, for *S. poona*, OG-TAE exhibited pronounced late-phase suppression (OD_{ave} ~ 0.035 at 5.5–6 h) relative to tetracycline (~ 0.093 – 0.095), demonstrating effective bacteriostatic activity even in strains with moderate susceptibility. Against *S. lentus*, OG-TAE maintained OD values between 0.024 and 0.171 over 24 h, showing prolonged inhibition comparable to or slightly better than tetracycline in the later stages. For Gram-negative strains like *Klebsiella* and *Shigella*, OG-TAE partially inhibited growth; initial OD values remained low (< 0.1), but growth resumed gradually, with final OD values still lower than the negative control. Ciprofloxacin fully inhibited growth across all strains, while tetracycline showed moderate suppression. These patterns mirror the MIC data, confirming that the total alkaloidal extract delays bacterial proliferation rather than causing immediate killing. The results support known bacteriostatic mechanisms of plant alkaloids, including disruption of nucleic acid synthesis, interference with membrane integrity, and possible efflux modulation, with Gram-positive bacteria showing greater sensitivity than Gram-negative species (30, 34, 42–44).

The time-kill kinetics of ZZ-TAE showed clear and sustained growth suppression across all tested strains, frequently exceeding the inhibitory performance of tetracycline. Against *E. coli*, ZZ-TAE maintained low OD values (0.028–0.054) during the first 6 h, compared to 0.033–0.200 for tetracycline, with a marked difference at 6 h (0.048 vs. 0.200). This advantage persisted at 24 h, where ZZ-TAE limited growth to an

OD of 0.386 compared with 0.655 for tetracycline. A similar trend was observed for *Shigella*, where early OD values under ZZ-TAE (0.036–0.055) were substantially lower than tetracycline (0.122–0.266), and growth at 24 h remained more effectively suppressed (0.295 vs. 0.346). Against *Salmonella poona*, ZZ-TAE showed particularly strong inhibition, maintaining very low OD values (0.014–0.021) within the first 6 h and sustaining suppression at 24 h (OD 0.026), markedly outperforming tetracycline (OD = 0.150). Against the Gram-positive *Staphylococcus lentus*, ZZ-TAE produced stable inhibition comparable to tetracycline in early phases and slightly lower OD at 24 h (0.093 vs. 0.103), indicating prolonged bacteriostatic activity.

Against MRSA, OD values remained low (0.011–0.030) and were lower than tetracycline at key points (2 h: 0.0135 vs 0.0225), although regrowth occurred by 24 h (OD 0.443 vs 0.082). In *Klebsiella*, ZZ-TAE produced moderate early suppression (OD 0.100–0.149 up to 6 h), remaining below the negative control but less effective than tetracycline at 24 h (0.555 vs 0.246). For *S. Typhi*, ZZ-TAE showed clearer early superiority, maintaining lower OD values up to 6 h (6 h: 0.046 vs 0.081), though recovery was observed at 24 h (0.464 vs 0.127). Overall, ZZ-TAE exhibits pronounced early bacteriostatic activity, occasionally outperforming tetracycline, but with limited persistence at later time points.

Overall, both alkaloidal extracts demonstrated notable bacteriostatic effects, often matching or exceeding tetracycline in the early suppression of bacterial growth, particularly for Gram-positive strains. This suggests that these total alkaloidal extracts interfere effectively with bacterial proliferation, likely through mechanisms affecting nucleic acid synthesis and cell membrane integrity, similar to conventional antibiotics but with a distinct mode of action that may complement or even outperform tetracycline in certain cases supporting the conclusion that alkaloids are key drivers of the antibacterial activity and can provide sustained control of bacterial proliferation.

In-vitro Modulation Study

Table 3: Fractional Inhibitory Concentration (FIC) Indices for the Combination of OG-TAE and Tetracycline Against Selected Bacterial Strains

MIC Combinations (µg/mL:µg/mL)	OG-TAE:Tetracycline (TCLN)		MIC Combinations (µg/mL:µg/mL)	OG-TAE:Tetracycline (TCLN)	
	<i>S. poona</i>			<i>E. coli</i> (43)	
	FIC	Activity		FIC	Activity
1000/15.6	9	A	1000/3.9	8.25	A
500/7.8	4.5	A	500/3.9	4.25	A
250/7.8	2.5	I	31.25/62.5	4.25	A
125/15.6	2	I	125/125	9	A
62.5/62.5	4.5	A			

Table 4: Fractional Inhibitory Concentration (FIC) Indices for the Combination of ZZ-TAE and Tetracycline Against Selected Bacterial Strains

MIC Combinations (µg/mL:µg/mL)	ZZ-TAE:Tetracycline (TCLN)		MIC Combinations (µg/mL:µg/mL)	ZZ-TAE:Tetracycline (TCLN)	
	<i>S. poona</i>			<i>E. coli</i> (43)	
	FIC	Activity		FIC	Activity
1000/1000	16	A	1000/125	9	A
500/500	8	A	500/62.5	4.5	A
250/250	10	A			

The checkerboard modulation assay was performed to assess whether total alkaloid extracts from *O. gratissimum* and *Z. zanthoxyloides* leaves could enhance or support tetracycline activity, particularly given the incomplete growth suppression observed for this antibiotic in the time–kill assays. For *OG-TAE*, the interaction with tetracycline was clearly strain- and ratio-dependent. In *Salmonella poona*, additive interactions were observed at both 1MIC:1MIC combination (125µg/mL *OG-TAE*:15.6µg/mL tetracycline; FIC = 2) and 2MIC:½MIC combination (250µg/mL *OG-TAE*:7.8µg/mL tetracycline; FIC = 2.5). These findings are notable because they demonstrate that antibacterial activity can be retained even when the tetracycline dose is reduced, while at higher proportion of the alkaloidal extract. This indicates a dose-sparing effect, in which the natural product compensates for reduced antibiotic exposure, a feature that may help limit toxicity and selective pressure associated with conventional antibiotics. In contrast, all *OG-TAE*–tetracycline combinations against *E. coli* (43) were antagonistic (FIC = 4.25–9), suggesting strain-specific interference, potentially arising from competing uptake pathways or overlapping cellular targets.

For *ZZ-TAE*, all combinations with tetracycline were antagonistic across the tested strains. In *Shigella*, high FIC values (8–16) indicated marked reduction of tetracycline efficacy, while similar antagonistic effects were observed in *S. poona* (FIC = 4.5–9). These results indicate that, despite its intrinsic antibacterial activity, *ZZ-TAE* does not favourably modulate tetracycline under the conditions tested.

Overall, although synergistic interactions were not detected, the additive effects observed for *OG-TAE* against *S. poona* provide clear evidence that plant alkaloidal extracts can support reduced doses of conventional antibiotics without loss of antibacterial efficacy. This positions *OG-TAE* as a promising candidate for further optimization in adjunct antibacterial strategies aimed at antibiotic dose reduction rather than replacement, a concept of growing relevance in the context of antimicrobial resistance.

Time-Kill Kinetics for Modulated Combination with Additive Effect

Following the additive interactions observed in the checkerboard modulation assay, time–kill kinetic studies were performed to determine whether the OG-TAE–tetracycline combinations exert bacteriostatic or bactericidal effects. The optical density data clearly demonstrate that combining OG-TAE with tetracycline resulted in markedly enhanced bacterial killing compared with either agent alone.

Across the 24-h period, the results demonstrated that OG-TAE–tetracycline combinations elicited markedly superior bactericidal effects compared to either compound in isolation. OG-TAE monotherapy exhibited a predominantly bacteriostatic profile, with OD₆₂₀ values rising modestly from 0.06–0.08 within the initial 4 h to 0.10–0.11 by 24 h, reflecting limited killing and gradual bacterial persistence. Tetracycline alone fared even worse, displaying progressive bacterial recovery as evidenced by a steady OD₆₂₀ escalation from ~ 0.06 at time zero to ~ 0.15 at 24 h, indicating incomplete inhibition and suboptimal efficacy.

In stark contrast, both tested ratios (1MIC OG-TAE:1MIC tetracycline and 2MIC OG-TAE:1/2MIC tetracycline), sustained OD₆₂₀ values near baseline levels (approx. 0.01–0.03) throughout the incubation period, with no observable regrowth, indicative of rapid and persistent bacterial eradication. The OG-TAE–tetracycline combinations not only surpassed tetracycline monotherapy but also rivalled or exceeded the performance of ciprofloxacin, a potent fluoroquinolone benchmark. Ciprofloxacin maintained OD₆₂₀ at 0.02–0.03 over 24 h, yet the combinations achieved equivalent or lower values during early- and mid-lag phases, signifying accelerated onset and sustained bactericidal action. This synergy persisted even at sub-MIC tetracycline concentrations (0.5× MIC), substantiating a pronounced dose-sparing effect that could mitigate antibiotic exposure while preserving therapeutic potency.

Quantitative analysis further reinforced these observations. In the 1MIC:1MIC (OG-TAE: Tetracycline) combination, the average optical density (OD_{avg}) of tetracycline alone, previously 0.1806 (0–6 h) and 0.1995 (19–24 h), dropped drastically to 0.0189 and 0.0333, respectively. This represents an approximately 10- and 6-fold improvement in bactericidal effect during the first and last 6 h respectively. Similarly, in the 2MIC:½MIC (OG-TAE: Tetracycline) ratio, tetracycline's performance improved markedly (approximately 5- to 6-fold) upon combination with total alkaloids from *Ocimum gratissimum* (OG-TAE). During the first 6 h, OD_{avg} decreased from 0.0932 (Tetracycline alone) to 0.0156 (combination). From 19–24 h, it fell from 0.1405 to 0.0285, again reflecting about a 5-fold enhancement. In fact, from the 19–24 h, this extract-drug combination recorded smaller optical densities indicating a marginally better action than standard ciprofloxacin. The OD_{ave} from 19–24 h for ciprofloxacin was 0.0327 and 0.0283 for the modulated standard tetracycline. These kinetic data corroborate the additive modulation observed in static checkerboard assay, revealing a mechanistic shift from bacteriostatic (OG-TAE or Tetracycline alone) to robust bactericidal activity upon combination at reduced tetracycline concentrations, with efficacy comparable to ciprofloxacin, highlighting the potential of this alkaloidal extract as an adjunct agent for antibiotic dose reduction while maintaining robust antibacterial activity.

LC-MS/MS (ESI+) Analysis of Crude Alkaloid Extracts

Following chromatographic purification, LC-MS/MS analysis in positive electrospray ionization mode (ESI+) was conducted to identify and characterize the bioactive constituents of the total alkaloid extracts from *Zanthoxylum zanthoxyloides* (ZZ-TAE) and *Ocimum gratissimum* (OG-TAE) (Supplementary Document, S1). This analysis provided a chemical basis for the possible alkaloid types contributing to the antibacterial activities observed *in-vitro*. The structures of the identified alkaloids are shown in Fig. 5.

For ZZ-TAE, four compounds were identified. Compound A displayed a molecular ion peak $[M + H]^+$ at m/z 322.4, a strong doubly charged pseudo-molecular ion $[M + 2H]^{2+}$ at m/z 161.7, a minor $[M + H_2O + H]^+$ adduct at m/z 340.4, and an additional peak at m/z 261.1. This compound was characterized as Zanthoamide I (45), consistent with a molecular weight of 321.42 amu. Compound B showed the molecular ion $[M + H]^+$ at m/z 334.3 and the most intense doubly charged peak $[M + 2H]^{2+}$ at m/z 167.6, with additional ions at m/z 176.2, 298.3, and 117.1. This compound was identified as Nor-chelerythrine (46), consistent with a molecular weight of 333.34 amu. Compound C presented a protonated molecule $[M + H]^+$ at m/z 406.3, along with doubly charged ions at m/z 203.8 and 222.7, and minor peaks at m/z 136.5 and 319.4. This compound was assigned as 8-acetyldihydro-chelerythrine (47, 48), consistent with a molecular weight of 405.4 amu. Compound D exhibited a molecular ion $[M + H]^+$ at m/z 260.1, a weak $[M - H_2O + H]^+$ adduct at m/z 242.1, an intense doubly charged pseudomolecular ion $[M + 2H]^{2+}$ at m/z 130.6, and minor peaks at m/z 78.1, 185.1, and 111.1. This compound was assigned as Skimmianine (49), consistent with a molecular weight of 259.08 amu. For OG-TAE, Compound E was observed with a molecular ion $[M + H]^+$ at m/z 134.3, a doubly charged ion $[M + 2H]^{2+}$ at m/z 67.8, and a minor $[M + H_2O + H]^+$ adduct at m/z 152.2. This compound was characterized as Benzimidazol-5-amine (50), consistent with a molecular weight of 133.15 amu.

Discussion

Alkaloids represent a class of structurally diverse compounds with documented antibacterial activity in both pure and fractionated forms (51, 52). This study examined the antibacterial activity of total alkaloid extracts from *Ocimum gratissimum* (OG-TAE) and *Zanthoxylum zanthoxyloides* (ZZ-TAE) and their capacity to modulate the efficacy of tetracycline against selected bacterial pathogens. Collectively, the results demonstrate that alkaloid-enriched fractions from these two West African medicinal plants possess meaningful antibacterial activity and, in the case of OG-TAE, can enhance the antibacterial performance of an existing antibiotic at reduced doses. These findings contribute to the growing body of evidence supporting the development of plant-derived alkaloids not only as direct antibacterial agents but also as adjuvants that may help extend the utility of conventional antibiotics in the face of escalating antimicrobial resistance.

The markedly superior antibacterial activity of OG-TAE and ZZ-TAE relative to their corresponding total crude extracts (OG-TCE and ZZ-TCE) highlights the value of targeted fractionation in the discovery of bioactive phytochemicals. Crude plant extracts contain complex mixtures of secondary metabolites, many of which are pharmacologically inert or may interact antagonistically, thereby diluting or masking

the activity of genuinely bioactive constituents (51, 53, 54). Selective acid-base extraction of alkaloids concentrates the bioactive fraction and removes interfering non-alkaloid compounds, as confirmed by the phytochemical screening data in this study. This approach is consistent with findings from comparable investigations, where alkaloid-enriched fractions from plants such as *Phyllanthus fraternus*, *Piper guineense*, and *Enantia chlorantha* demonstrated significantly improved antibacterial activity over their unfractionated crude counterparts (7, 33, 53, 55, 56). Alkaloids exert antibacterial effects through well-characterized mechanisms including inhibition of nucleic acid synthesis, disruption of bacterial membrane integrity, and interference with protein synthesis, all of which are more effectively expressed when non-active phytochemicals are removed (30, 33, 57, 58). The MIC data further revealed that Gram-positive organisms (*S. aureus*, *S. lentus*) were consistently more susceptible to both extracts than Gram-negative species (*E. coli*, *Klebsiella*, *Shigella*, *S. typhi*). This differential susceptibility reflects the well-established role of the lipopolysaccharide-rich outer membrane of Gram-negative bacteria as a selective permeability barrier that restricts the intracellular access of both hydrophilic and hydrophobic phytochemicals, necessitating higher concentrations for equivalent antibacterial effects (59, 60).

Time-kill kinetic analyses provided further understanding into the mode of action of each extract, demonstrating that both OG-TAE and ZZ-TAE primarily exerted bacteriostatic rather than bactericidal effects at their respective MIC concentrations. Growth suppression was generally more pronounced against Gram-positive strains, consistent with the MIC data. ZZ-TAE was particularly notable for its strong early-phase inhibitory activity, outperforming tetracycline during the initial six hours of incubation for several strains, though variable bacterial regrowth at 24 hours indicated that sustained sterilizing activity was not consistently achieved. The progressive recovery of bacteria at later time points, particularly for certain Gram-negative species, likely reflects the inability of bacteriostatic agents at MIC-level concentrations to maintain complete growth arrest over extended periods, a recognized limitation of this class of antimicrobial action. Nonetheless, the capacity of these extracts to suppress bacterial proliferation during the critical early growth phase has potential clinical relevance, as early bacterial load reduction is an important determinant of favourable infection outcomes (34, 42, 43, 61).

Importantly, the most clinically significant finding of this study was the additive interaction between OG-TAE and tetracycline against *Salmonella poona*. Checkerboard modulation assays demonstrated additive effects at both 1MIC:1MIC and 2MIC:½MIC (OG-TAE:tetracycline) ratios, yielding FIC indices of 2.0 and 2.5 respectively. Crucially, these combinations preserved antibacterial activity even when the tetracycline concentration was reduced by half, establishing a clear dose-sparing effect. The therapeutic implications of this are considerable: reducing antibiotic dosage without compromising efficacy can limit dose-dependent adverse effects, reduce selective pressure that drives the emergence of resistance, and potentially prolong the clinical utility of existing antibiotics. This concept aligns with established combination strategies in clinical practice, such as the co-administration of β -lactam antibiotics with β -lactamase inhibitors and the pairing of vancomycin with aminoglycosides, where complementary agents jointly achieve enhanced bacterial killing at individually sub-therapeutic concentrations (8–11). The observation that additive interactions were specific to *S. poona* and not reproduced against *E. coli* (43), where antagonism was instead detected, highlights the strain-dependent nature of plant-antibiotic

interactions and reinforces the importance of testing such combinations across a broad panel of clinically relevant pathogens before drawing generalized conclusions about adjuvant efficacy.

Subsequent time-kill kinetic studies of the OG-TAE–tetracycline combinations confirmed a unique transition from bacteriostatic to bactericidal activity, a shift that neither agent achieved independently. At both combination ratios, bacterial OD₆₂₀ values were maintained near baseline throughout the 24-hour period, with no detectable regrowth, in stark contrast to the progressive bacterial recovery observed with either agent used alone. The observed 5- to 10-fold reductions in average optical density relative to tetracycline monotherapy, together with efficacy that matched that of ciprofloxacin, reflect the additive killing dynamics achievable through combination. This bacteriostatic-to-bactericidal transition may be attributable to complementary mechanisms of action: OG-TAE alkaloids may permeabilize bacterial membranes or inhibit efflux pump activity, thereby increasing intracellular tetracycline concentrations and enabling the antibiotic to reach its ribosomal target at otherwise sub-therapeutic doses. Specific alkaloids such as berberine, chelerythrine, and magnoflorine have demonstrated inhibitory effects against bacterial pathogens, acting through mechanisms such as DNA intercalation, disruption of cell membranes, and interference with protein synthesis (29, 47–50, 62, 63). These findings position OG-TAE as a candidate antibiotic adjuvant warranting further mechanistic investigation.

The antibacterial activities observed in this study are further supported by the chemical profiles established through LC–ESI–MS/MS analysis. Four alkaloids were putatively identified in ZZ-TAE: zanthoamide I, nor-chelerythrine, 8-acetyldihydro-chelerythrine, and skimmianine. Nor-chelerythrine and 8-acetyldihydro-chelerythrine are members of the benzophenanthridine alkaloid class, structurally related to chelerythrine, which has been extensively documented to inhibit bacterial growth through DNA intercalation, disruption of cell membrane integrity, and inhibition of cell wall biosynthetic enzymes (29, 43, 62, 64). The presence of these derivatives in ZZ-TAE provides a credible mechanistic basis for the antibacterial activity observed against both Gram-positive and Gram-negative organisms. Skimmianine, a furoquinoline alkaloid frequently reported in *Zanthoxylum* species, has also demonstrated antimicrobial activity in prior investigations and is likely a contributing bioactive constituent in ZZ-TAE (49). While the biological role of zanthoamide I in antibacterial activity is less well characterized, the co-occurrence of these structurally diverse alkaloids suggests that the overall activity of ZZ-TAE is likely the result of multiple compounds acting through complementary mechanisms rather than a single dominant constituent. In OG-TAE, benzimidazol-5-amine was the sole alkaloid identified. Benzimidazole-based scaffolds are recognized pharmacophores in medicinal chemistry with documented antibacterial properties attributed to their capacity to interact with microbial nucleic acids and inhibit key bacterial enzymes (50). This mechanistic profile may partly account for the ability of OG-TAE to modulate tetracycline activity, potentially through effects on bacterial membrane permeability or intracellular target access.

In the broader context of antimicrobial resistance management, the results of this study support a strategy centred on antibiotic dose reduction through natural adjuvants rather than complete antibiotic replacement. The antagonistic interactions observed for ZZ-TAE in combination with tetracycline against

S. poona and *Shigella* serve as a reminder that not all plant-antibiotic pairings produce favourable outcomes, and that systematic evaluation across multiple strains and concentration ratios is essential. Nonetheless, the additive effects demonstrated by OG-TAE represent a meaningful advance toward the identification of natural products with unique adjuvant potential. To move this work toward clinical applicability, future investigations should focus on the isolation and structural characterization of individual bioactive alkaloids, detailed mechanistic studies of their interaction with tetracycline and bacterial targets, and assessment of in vivo efficacy in appropriate infection models. Cytotoxicity profiling against mammalian cell lines will also be an essential step in establishing the safety margin of these extracts. Collectively, this study reinforces the value of African medicinal plants, and alkaloid-enriched fractions in particular, as an underexplored yet promising resource for developing adjunct antibacterial agents to combat the growing challenge of antimicrobial resistance.

Conclusion

This study demonstrates that total alkaloidal extracts from *Zanthoxylum zanthoxyloides* (ZZ-TAE, 1.40% w/w) and *Ocimum gratissimum* (OG-TAE, 1.18% w/w), despite representing a relatively low composition of the plant material, are potent antibacterial agents, exhibiting strain-dependent bacteriostatic activity with pronounced effects against Gram-positive bacterial pathogens. Checkerboard modulation studies demonstrated that OG-TAE could enhance tetracycline activity against *Salmonella poona*, achieving additive effects at reduced antibiotic concentrations, suggesting potential dose-sparing benefits. Subsequent time-kill assays confirmed that these additive combinations exhibited bactericidal activity, outperforming the crude alkaloids and tetracycline when used alone. The combination of OG-TAE with Tetracycline achieved 5–10 folds increase in activity, comparable to the efficacy of ciprofloxacin even at half-MIC tetracycline doses, in 24 h. LC-MS characterization identified several bioactive alkaloids, namely Zanthoamide I, Nor-chelerythrine, 8-acetonyldihydro-chelerythrine, Skimmianine, and Benzimidazol-5-amine, providing a chemical rationale for the observed antibacterial activities of the alkaloidal extracts. Collectively, these findings highlight the therapeutic potential of plant-derived alkaloidal extracts as modulators of conventional antibiotics, supporting their further exploration in combination strategies particularly significant in the context of antimicrobial resistance, as it supports a strategy focused on antibiotic dose reduction while preserving or enhancing therapeutic efficacy rather than complete antibiotic replacement.

Abbreviations

AMR

Antimicrobial Resistance

CIPX

Ciprofloxacin

HT-SPOTi

High-throughput Spot Culture Growth Inhibition

MIC

Minimum Inhibitory Concentration
OD
Optical Density
OD_{ave}
Average Optical Density
OG-TAE
Occimum gratissimum Total Alkaloid Extract
TLCN
Tetracycline
WHO
World Health Organization
ZZ-TAE
Zanthoxylum zanthoxyloides Total Alkaloid Extract

Declarations

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the author(s) used ChatGPT in order to evaluate the initial draft of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Funding declaration: This study received no financial support.

Ethics and Consent to Participate declarations: not applicable

Clinical trial number: not applicable

Author Contribution

SAO: Experimental methods design and implementation, methods validation, data curation, results analysis and interpretation and drafting original manuscript. **DOK:** Data curation, formal analysis, investigation, methodology **FAA** Conceptualization, research design, results analysis and interpretation, supervision, manuscript review and editing. **YOB:** Conceptualization, research design, results analysis and interpretation, supervision, manuscript review and editing **IA:** Conceptualization, research design and supervision, project administration, data analysis, manuscript writing, review and editing. All authors contributed to this study, read and approved the final manuscript

Acknowledgement

The authors wish to thank our colleagues in the Medicinal Chemistry and Drug Discovery Research Group at the Department of Chemistry, Universities of Cape Coast (UCC), for their constructive

feedback during group presentation. We are also thankful to the Department of Biomedical Science, UCC, for providing access to their microbiology laboratory.

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Figures

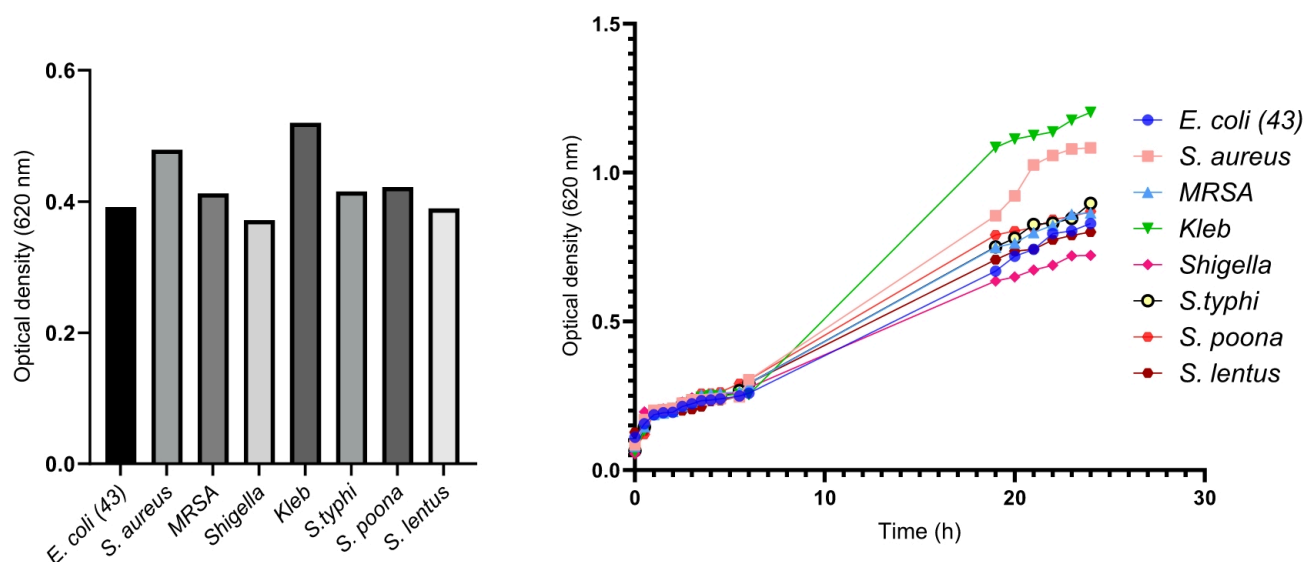


Figure 1

Uninhibited Bacterial Growth Patterns Used as Reference for Time-Kill Kinetic Analysis. Normal bacterial proliferation in the absence of antimicrobial agents was monitored *via* optical density at 620

nm (OD_{620}) measurement over 24 h. Measurements were recorded at frequent early intervals (0–6 h; every 0.5 h) and subsequently at hourly intervals from 19 to 24 h to capture lag and exponential phases. These untreated growth profiles provided the reference framework for interpreting time–kill kinetics and assessing the inhibitory effects of the total alkaloid extracts and standard antibiotics.

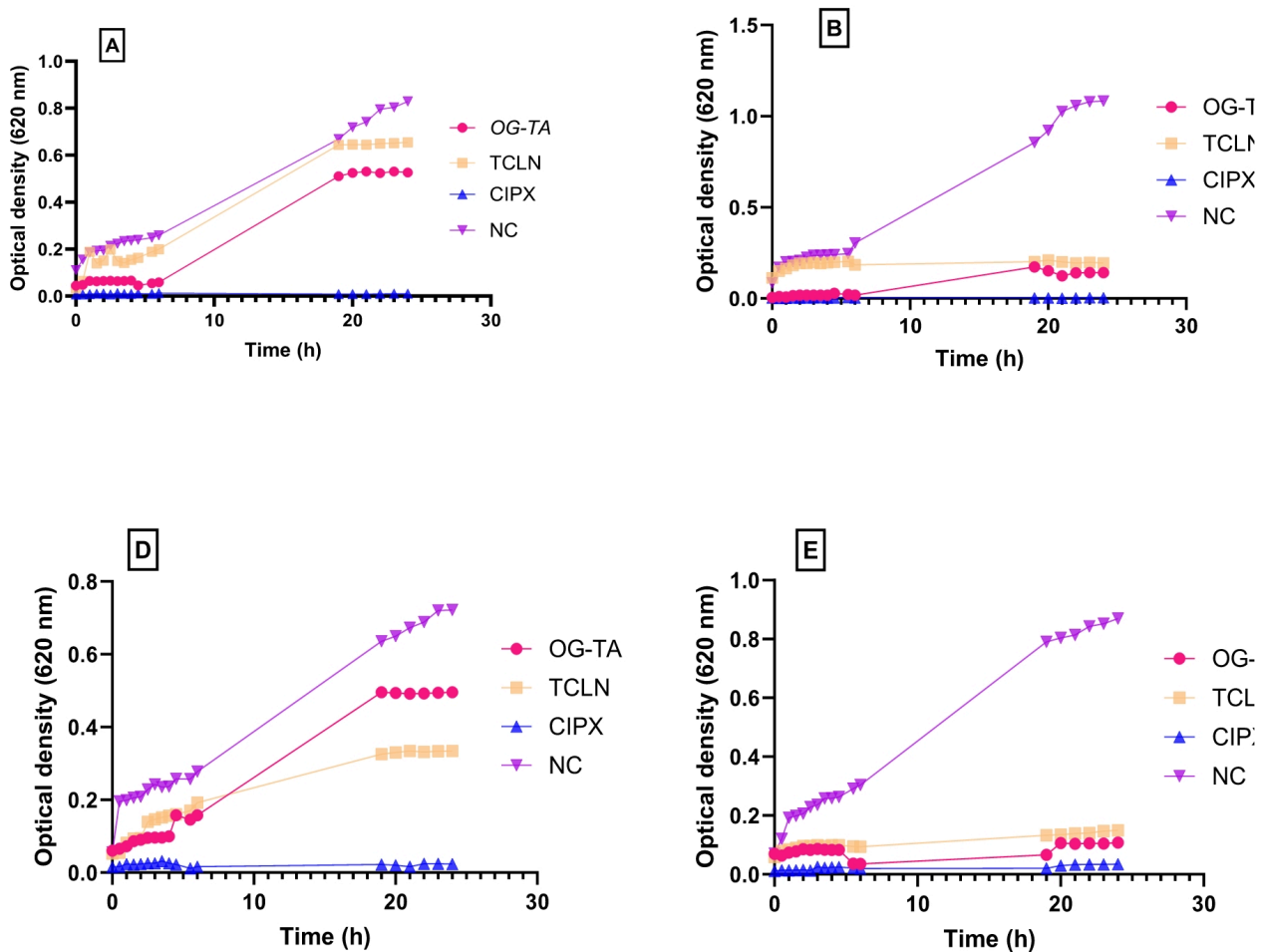


Figure 2

Time-kill kinetics curve of the total alkaloid extract from *Ocimum gratissimum* (OG-TAE) against *E. coli* (43)(A), *S. aureus* (B), *Klebsiella pneumoniae* (C), *Shigella spp.* (D), *S. poona* (E), *S. lentus* (F) growth over 24 h, compared with tetracycline (TCLN), ciprofloxacin (CIPX), and the negative control (NC). OG-TAE produced sustained bacteriostatic activity across a number of strains, maintaining lower optical density (OD_{620}) values than tetracycline at several time points, particularly during the late growth phase (18 – 24 h). Ciprofloxacin exhibited near-complete growth inhibition throughout the assay, demonstrating complete bactericidal activity. Similar trends were however not observed for tetracycline. The assay was performed at the minimum inhibitory concentrations (MICs) with viable cells monitored by measuring optical density at 620 nm (OD_{620}).

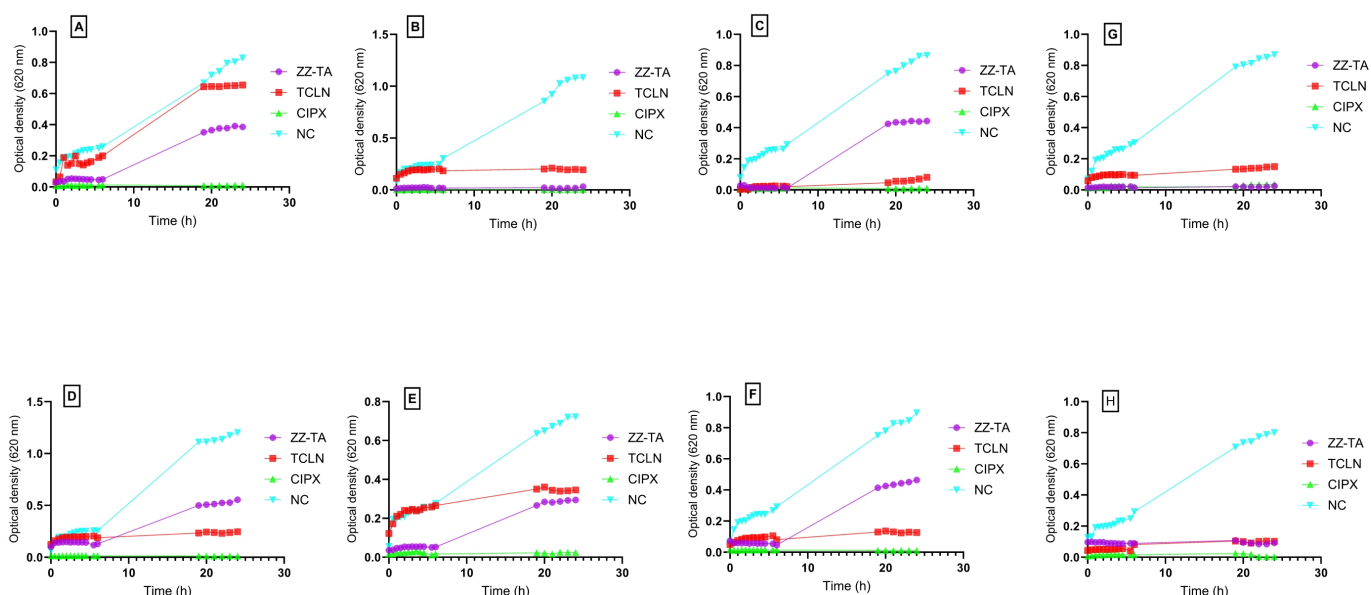


Figure 3

Time-kill kinetics curve of the total alkaloid extract from *Zanthoxylum zanthoxyloides* (ZZ-TAE) against *E. coli* (43)(A), *S. aureus* (B), *MRSA* (C), *Klebsiella pneumoniae* (D), *Shigella spp.* (E), *S. typhi* (F) *S. poona* (G), *S. lentus* (H) growth over 24 h, compared with tetracycline (TCLN), ciprofloxacin (CIPX), and the negative control (NC). ZZ-TAE showed pronounced early-phase growth suppression, in some cases outperforming tetracycline during the first 6 h, followed by variable regrowth at later time points, indicating strain-dependent and time-dependent bacteriostatic activity. Ciprofloxacin exhibited near-complete growth inhibition throughout the assay, demonstrating complete bactericidal activity, whereas comparable trends were not consistently observed for tetracycline. All assays were conducted at the respective minimum inhibitory concentrations (MICs), with bacterial growth monitored by measuring optical density at 620 nm (OD₆₂₀).

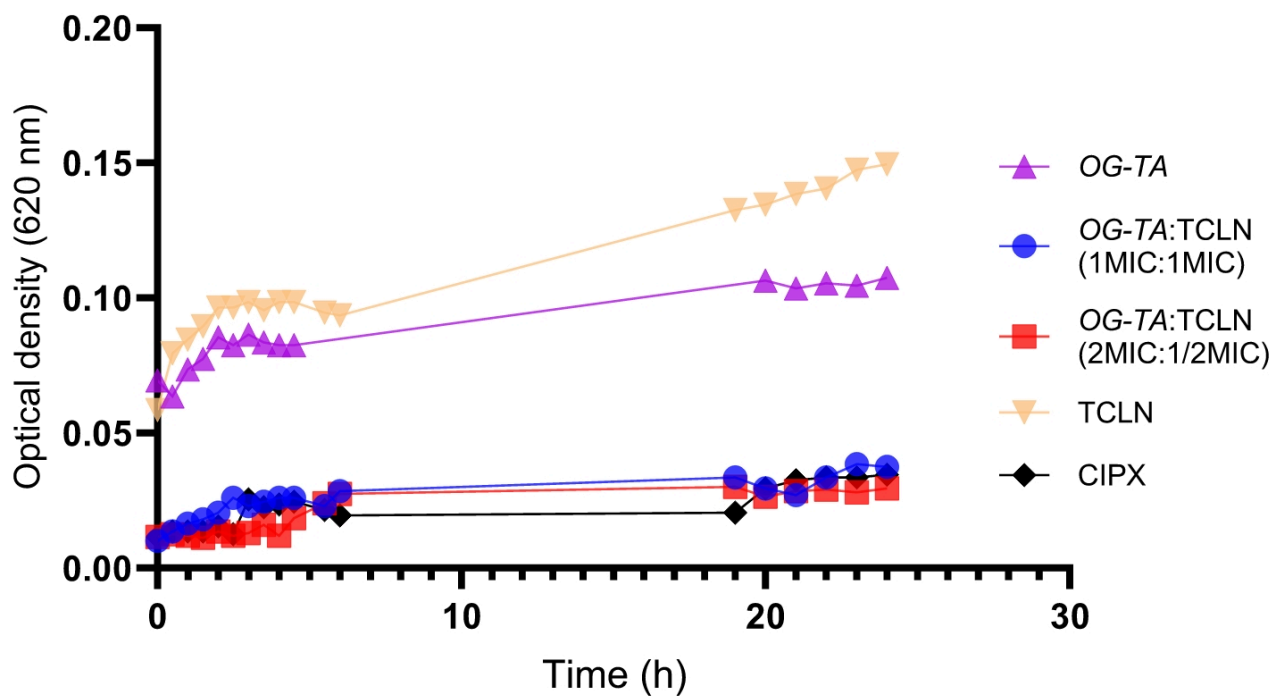


Figure 4

Time-kill kinetic curves showing the antibacterial effects of OG-TAE–tetracycline combinations compared with single agents (OG-TAE, Tetracycline and Ciprofloxacin) against *Salmonella poona*. Bacterial growth was monitored by optical density at 620 nm (OD_{620}) over 24 h. The OG-TAE:tetracycline combinations at 1 MIC:1 MIC (125 $\mu\text{g/mL}$:15.6 $\mu\text{g/mL}$) and 2 MIC: $\frac{1}{2}$ MIC (250 $\mu\text{g/mL}$:7.8 $\mu\text{g/mL}$) revealed sustained bactericidal activity comparable to ciprofloxacin.

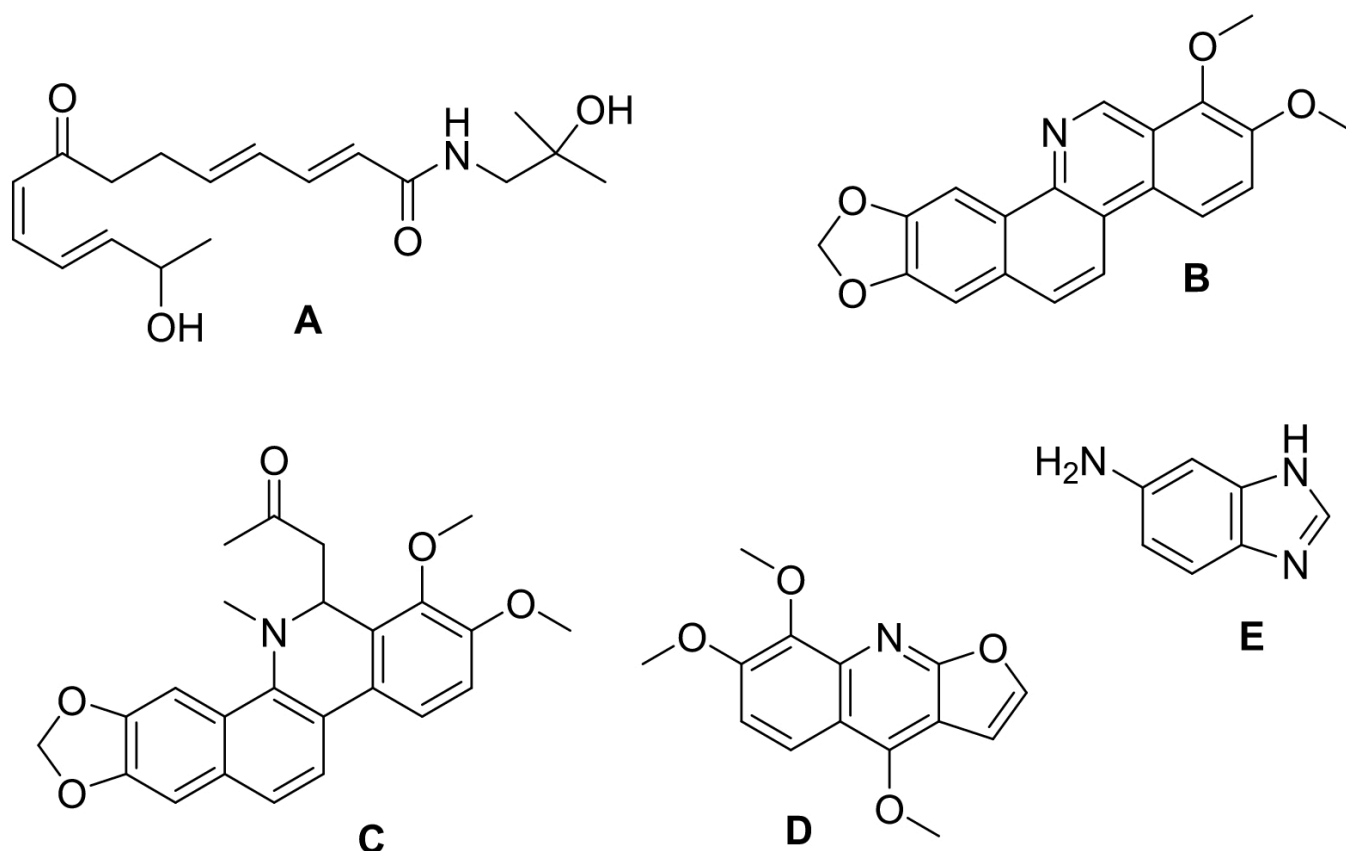


Figure 5

Chemical structures of bioactive alkaloids identified from the total alkaloid extracts of *Zanthoxylum zanthoxyloides* (ZZ-TAE) and *Ocimum gratissimum* (OG-TAE) by LC-MS/MS analysis. Compounds A–D correspond to Zanthoamide I, Nor-chelerythrine, 8-acetyldihydro-chelerythrine, and Skimmianine, respectively, while Compound E represents Benzimidazol-5-amine.

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