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Research Article

Enhancing Antibiotic Efficacy: Exploring Synergistic Interactions between Plant Extracts and Conventional Antibiotics

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Abstract: Medicinal herbs including *Senna alata*, *Ricinus communis*, and *Lannea barteri* have been utilized for centuries to cure a variety of illnesses caused by microbial infections. This study looked at the synergistic effects of these drugs with traditional antibiotics on clinical isolates of *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The test bacteria were chosen based on their minimal potential for monotherapy and susceptibility to at least one antibiotic with a known genetic basis. The interactions of plant extracts with antibiotics against the chosen pathogenic microorganisms were investigated using the agar well diffusion, broth microdilution, and checkerboard methods. Calculated fractional inhibitory concentration index (FICI) values were used to describe how the extracts and antibiotics interacted. All the extracts from the three plants combined with fluconazole exhibited a synergistic interaction against *C. albicans* (FICI < 0.5). The minimum inhibitory concentration (MIC) of ampicillin against *E. coli* was demonstrated to be reduced by the combination of the ethanol extract of *S. alata* with ampicillin, with a FICI value of 0.4 indicating a synergistic effect. With a synergistic action (FICI < 0.5) against *P. aeruginosa*, the ethanol extract of *S. alata* and amoxicillin were successful in reducing the MIC of amoxicillin from 0.32 to 0.17 mg/mL. Aqueous *L. barteri* extract combined with amoxicillin exhibited synergism (FICI < 0.2) against *S. aureus* with a reduction of MIC from 0.20 to 0.03 mg/mL. The current study is the first to investigate the aforementioned plants in combination with conventional antibiotics for their antimicrobial activities. The findings of this study could be used to create a useful, applicable, feasible, and alternative source of novel antimicrobial agents.

***Keywords*:** antimicrobial, fractional inhibitory concentration index, *Senna alata*, *Ricinus communis*, *Lannea barteri*, synergy, combination

1. Introduction

Devastating consequences are linked to infections brought on by bacteria that are extensively drug-resistant (XDR).[1-3] XDR has been discovered to represent a serious hazard to human health.[1,2] For instance, *Klebsiella pneumoniae* is linked with the outbreak of many infections with high mortality and morbidity rates in hospitalized and

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immunocompromised patients, including outbreaks in neonatal intensive care units (NICUs).[4] Treatment failures of urinary tract infections and sepsis in pediatric patients are attributed to the emergence of XDR *K. pneumoniae* strains.[4,5] Given the lack of available treatments, the World Health Organization (WHO) has stated the necessity for research and development of novel therapeutic medicines against XDR.[2] Even though there have been many novel drugs discovered in the last ten years to treat XDRs, further research is still necessary because of the emergence of drug resistance.

Ampicillin, amoxicillin, and fluconazole are used as monotherapeutic drugs, although there are questions about their safety and effectiveness.[6,7] Treatment failures of antimicrobials have been ascribed to factors such as superinfections, untreatable infectious diseases, inadequate spectrum coverage, inadequate antimicrobial blood levels, and antimicrobial tissue penetration problems.[8] However, the most frequent reason is attributed to the emergence of resistant microorganisms. Although antimicrobial resistance is a natural phenomenon that occurs over time through genetic changes in pathogens, the principal driving forces behind the development and dissemination are the excessive and inappropriate usage of antimicrobial agents in the domains of humans, animals, and plants.[9] According to Ayukekbong and collogues,[10] the presence of antimicrobial resistance in developing nations can be linked to factors such as improper prescription methodologies, insufficient patient instruction, restricted diagnostic capabilities, illicit dissemination of antimicrobials, and the absence of effective drug regulatory mechanisms.

It is for this reason that combination therapy is recommended as a viable strategy for the treatment of multidrug-resistant and complex infections such as human immunodeficiency virus (HIV), tuberculosis (TB) and malaria; and complicated diseases like cancer, cardiovascular diseases and diabetes.[11] The main concept of combination therapy is to use drugs that work by different mechanisms, thereby decreasing the likelihood of resistance developing, improving therapeutic efficacy through the additive or synergistic activity, and reducing dose resulting in reduced adverse effects.[12]

Currently, one of the most frequently used antimicrobials in emergency departments and primary health care facilities globally is amoxicillin-clavulanic acid. The clavulanic acid, a beta-lactamase inhibitor, in conjunction with amoxicillin, which is a bacterial cell wall biosynthesis inhibitor, broadens the spectrum of the latter and combats resistance.[13] Other successful combination chemotherapies are artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated *P. falciparum* malaria; streptomycin in combination with isoniazid and rifampicin for the treatment of TB;[11] and food and drug administration (FDA) approved drug combinations used in breast cancer including Adriamycin and Cyclophosphamide (AC), Adriamycin and Cyclophosphamide, followed by Taxol (AC-T), Cyclophosphamide, Adriamycin and 5-Fluorouracil (CAF), and 5-Fluorouracil, Epirubicin and Cyclophosphamide (FEC).[14]

It has been established that the minimum inhibitory concentrations of existing conventional antibiotics against bacterial strains could be significantly reduced when plant extracts are used in combination with the antibiotics.[15] For example, the literature reports that although carbapenems alone have special pharmacological properties against complicated bacterial infections, *in vitro* studies revealed that they are very effective when combined with colistin. This has led to many experts to recommend using this combination to treat infections brought on by Gram-negative *bacilli* that are resistant to carbapenems.[16]

Plant-derived chemicals specifically exercise their potential as antibacterial agents via synergism, a favorable interaction that occurs when two drugs are combined, resulting in an inhibitory impact higher than the sum of their separate effects.[15,17,18] These crude extracts contain complex mixtures of compounds that inhibit the growth of bacteria by interfering with various mechanisms, such as disrupting membrane functionality and structure, interrupting DNA/RNA synthesis and operation, interfering with intermediate metabolism, and causing coagulation of cytoplasmic constituents.[19] It is also established that plant extracts may also exhibit a modulation effect on bacterial virulence by inhibiting biofilm formation and quorum sensing.[20] Moreover, there are reports indicating that the combination of plant extracts and conventional antibiotics leads to synergistic interactions through various mechanisms. These mechanisms include the inhibition of protective enzymes, the combination of membrane active agents, the sequential inhibition of shared biochemical pathways, and the use of membranotropic agents to enhance the diffusion of other antibiotics.[21]

According to earlier research by our colleagues,[22] the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was successfully inhibited by several extracts from the leaves of *Senna alata*, *Ricinus communis*, and the stem bark of *Lannea barteri*. Additionally, it was established that binary combinations of the aqueous and ethanol extracts of these three plants exhibited either a synergistic or an additive effect against some microorganisms associated with wound infection.[12] The aim of this research was to assess the effects of conventional antibiotics in combination

with the crude extracts of these plants on *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*.

2. Materials and methods

2.1 *Chemicals and solvents*

All chemicals and solvents used were analytical grades, unless otherwise stated. They include ethanol (VWR Chemicals BDH, France), dimethyl sulphoxide (DMSO) (VWR Chemicals BDH, France), Tween-80 (Avishkar Lab Tech Chemicals, India), amoxicillin trihydrate (Tobinco Pharmaceutical Limited, Ghana), fluconazole powder (Tobinco Pharmaceutical Limited), ampicillin sodium salt (AdvaCare Pharma, USA). Distilled water was prepared in the laboratory using a water distiller (WS-100-4-A Stuart Water Still, Cole-Parmer, UK).

2.2 *Collection of plant material*

Plant material from the leaves of *S. alata* and *R. communis*, as well as the stem bark of *L. barteri*, was collected between August and September, 2021, from several neighborhoods in Navrongo (10° 53′ 5.00″ N, 1° 05′ 25.00″ W), Upper East Region of Ghana. A reasonably small quantity of the same plant species was collected from a single plant and from multiple locations. There were no noticeable differences in each plant species during collection. All the plant materials were collected after they had been authenticated by a plant taxonomist at the University for Development Studies herbarium of Ghana Herbaria, Northern Savanna Biodiversity; Savanna Herbarium, Nyankpala, Northern Region. The voucher specimens with numbers of SH 710 (*S. alata*), SH 720 (*R. communis*) and SH 790 (*L. barteri*) were deposited in the herbarium.

2.3 *Preparation of plant crude extracts*

For two weeks, plant components from *S. alata*, *R. communis*, and *L. barteri* were air dried at room temperature. The stem bark was pulverized using a pestle and mortar, and the individual leaf samples were blended into a homogeneous powder using a blender. Five hundred grams (500 g) of the powdered plant material from each plant was soaked in 2 L of solvent at room temperature for 48 h to produce the ethanol and aqueous extracts. Each extract was filtered using Whatman filter paper No. 42, and the filtrate was then concentrated using a rotary evaporator (Laborota 4001 ENcient, Heidolph Instruments GMBH, Germany), followed by warming on a water bath at 70 °C for the aqueous extract and 50 °C for the ethanol extract to obtain a solid or semi-solid product of constant mass. All traces of solvent were removed after placing the samples in a desiccator for one week. Samples were then put in sterile containers and placed in the refrigerator at 2-4 °C until further use. The yields of *L. barteri* extracted in aqueous and ethanol were 12.40 and 12.10% respectively; *S. alata* extracted in aqueous and ethanol were 7.40 and 7.20% respectively; *R. communis* extracted in aqueous and ethanol were 6.01 and 5.20% respectively.

2.4 *Test microorganisms*

Clinical isolates of four bacteria, *E. coli*, *S. aureus*, *P. aeruginosa*, and *K. pneumoniae*; and a fungus, *C. albicans*, were used for the study. All the isolates were obtained from the Public Health and Reference Laboratory, Tamale Teaching Hospital (TTH), Tamale, Ghana. While the fungal isolates were maintained at 4 °C on potato dextrose agar, the bacterial isolates were maintained in nutrient broth at 2-8 °C.

2.5 *Agar well diffusion assay*

For the agar well diffusion experiment, our colleagues' approach from earlier research was applied.[23] Briefly, 6 mm-thick sterile petri dishes were filled aseptically with molten Mueller Hinton agar (OXOID, Basingstoke, England) at 40 °C and allowed to set. On the solidified agar, a bacterial suspension in a solution of normal saline (100 µL) was administered, followed by the creation of 6 mm-diameter wells that were subsequently filled with 100 µL of each of

the varied concentrations (200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 mg/mL) of plant extracts. To assess the antibacterial activity, a positive control of Amoxicillin at a concentration of 250 mg/mL and a negative control of dimethyl sulfoxide (DMSO) (1%, v/v) were employed. Duplicate tests were conducted, allowing the plates to remain at room temperature for a duration of 30 min, after which they were incubated at a temperature of 37 °C for a period of 24 h. Through the measurement of the diameter of the zones of inhibition in millimeters (mm), the antibacterial activity was evaluated. Quality control set up comprised of the extracted solvents on inoculated agar plates, inoculated agar plates without any extract/standard antibiotic, and extract on non-inoculated agar plates.

2.6 *Test for antifungal activity*

The microdilution method was used to examine the antifungal activity of the extracts.[23] The fungus spores were removed from the agar plates' surface using sterile 0.85% saline that contained 0.1% Tween 80 (v/v). The spore suspension was diluted with sterile saline to a concentration of around 1.0×10^7 cfu/mL, or 0.5 McFarland scale. The inocula were stored at 4 °C in preparation for future use. Dilutions of the inocula were grown on solid potato dextrose agar to check for contamination and to determine the effectiveness of the inoculum.

2.7 *Inoculum preparation for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)*

Suurbaar et al.[24] described a method for producing the inoculum for the MIC and MBC tests. From an agar plate culture, at least three to five distinct colonies with the same morphology were collected. Each colony's top was touched with a sterile loop before being placed in a tube with 5 mL of ordinary saline and vortexed. The broth culture was kept under observation for about 4 h at 37 °C until it reached the turbidity of the 0.5 McFarland standard (1.5×10^8 cfu/mL).

2.8 *Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)*

The method described by Donkor et al.[25] was employed for the determination of MBCs and MICs.

2.9 *Determination of minimum fungicidal concentration (MFC)*

By subculturing 2 µL from each of the wells that showed no growth into microtiter plates with 100 µL of broth per well and subsequently incubating for 72 h at 28 °C. The minimum fungicidal concentrations (MFCs), which represent 99.5% killing of the original inoculum, were determined as previously described.[24,26] Fluconazole, a commercial standard, was utilized as a positive control (1.00-30.00 mg/mL), and DMSO (1%, v/v) was used as a negative control. In order to reproduce results, each experiment was run twice and then repeated three times.

2.10 *Estimation of the interactions between plant extracts and antibiotics*

The utilization of the National Committee for Clinical Laboratory Standards (NCCLS) recommended checkerboard assay, as discussed by Vaou et al.[27] and Britton et al.[28], with slight modification in volume and concentration, was employed to evaluate the interactions between the conventional antibiotics and the plant extracts. In order to achieve a MIC increase of at least 2-fold, the stock solutions and subsequent 2-fold dilutions of each antibiotic and extract were prepared. A quantity of 50 µL of Mueller-Hinton broth was introduced into each well of the microtitre plates. The extract was diluted progressively along the x-axis, while the antibiotic of the combination was diluted along the y-axis. Every bacterial isolate was utilized to generate an inoculum that aligned with the 0.5 McFarland turbidity scale in Mueller-Hinton broth. The plates were incubated at 37 °C for 48 h under aerobic conditions after each microtiter well had been infected with 100 µL of a bacterial inoculum containing 5×10^5 cfu/mL. Each extract and antibiotic combination was then arranged in a checkerboard pattern, with the tubes holding the highest concentrations of each antibiotic in the opposite corners. The NCCLS guidelines for broth microdilution defined the MIC as the lowest medication concentration that did not cause the organism's development to be visible to the naked eye. When the ratio

of the concentration of each antibiotic to its MIC was the same for all components of the mixture, synergy was more likely to be exhibited.[12,29]

The plant extracts (drug A) and antibiotics (drug B) were examined for synergistic interactions. The agents were serially diluted five times, starting at a concentration that was twice their MIC value. The effects of the combinations were evaluated using the Fractional Inhibitory Concentration Index (FICI) expressed by Equation (1).[12,29,30]

$$FICI = FIC_A + FIC_B \quad (1)$$

where, FIC_A = MIC of drug A in combination/MIC of drug A alone;

FIC_B = MIC of drug B in combination/MIC of drug B alone.

An FIC index ≤ 0.5 was used to determine synergy; FICI values between 1.0 and 0.5 were regarded as an additive interaction; FICI values > 1.0 to ≤ 4 indicated indifferent interaction; and FICI values > 4 indicated antagonistic interaction between the two agents.[12]

3. Results

3.1 *Minimum inhibitory concentration (MIC)*

In this study, the antibacterial potential of plant extracts from the leaves of *S. alata*, *R. communis*, and *L. barteri* stem bark was evaluated. The effectiveness of the extract against the pathogens *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, and *C. albicans* was investigated. Our research team has previously shown that the plant extracts alone examined had antibacterial activity.[22] The MICs of the extracts alone are shown in Table 1.

Table 1. Minimum inhibitory concentrations (MIC) (mg/mL) of plant extracts and antibiotics alone

	E. coli	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*
Extracts					
Aqueous *S. alata*	3.13	12.50	6.25	6.25	12.50
Aqueous *L. bateri*	6.25	12.50	6.25	12.50	12.50
Aqueous *R. communis*	6.25	3.13	3.13	12.50	12.50
Ethanol *S. alata*	6.25	12.50	6.25	12.50	25.00
Ethanol *L. bateri*	6.25	25.00	6.25	6.25	12.50
Ethanol *R. communis*	6.25	25.00	6.25	6.25	25.00
Antibiotics					
Ampicillin	0.39	0.05	0.02	0.20	-
Amoxicillin	0.39	0.20	0.39	0.20	-
Fluconazole	-	-	-	-	4.69

- = not determined

The lowest MICs (3.13 mg/mL) were seen in the aqueous extracts of *S. alata* and *R. communis*, whereas the highest MICs (25.00 mg/mL) were seen in the ethanol extracts of *L. barteri* and *R. communis*.

Besides the antimicrobial potential of the plant extracts alone, we also looked at how the extracts from the various

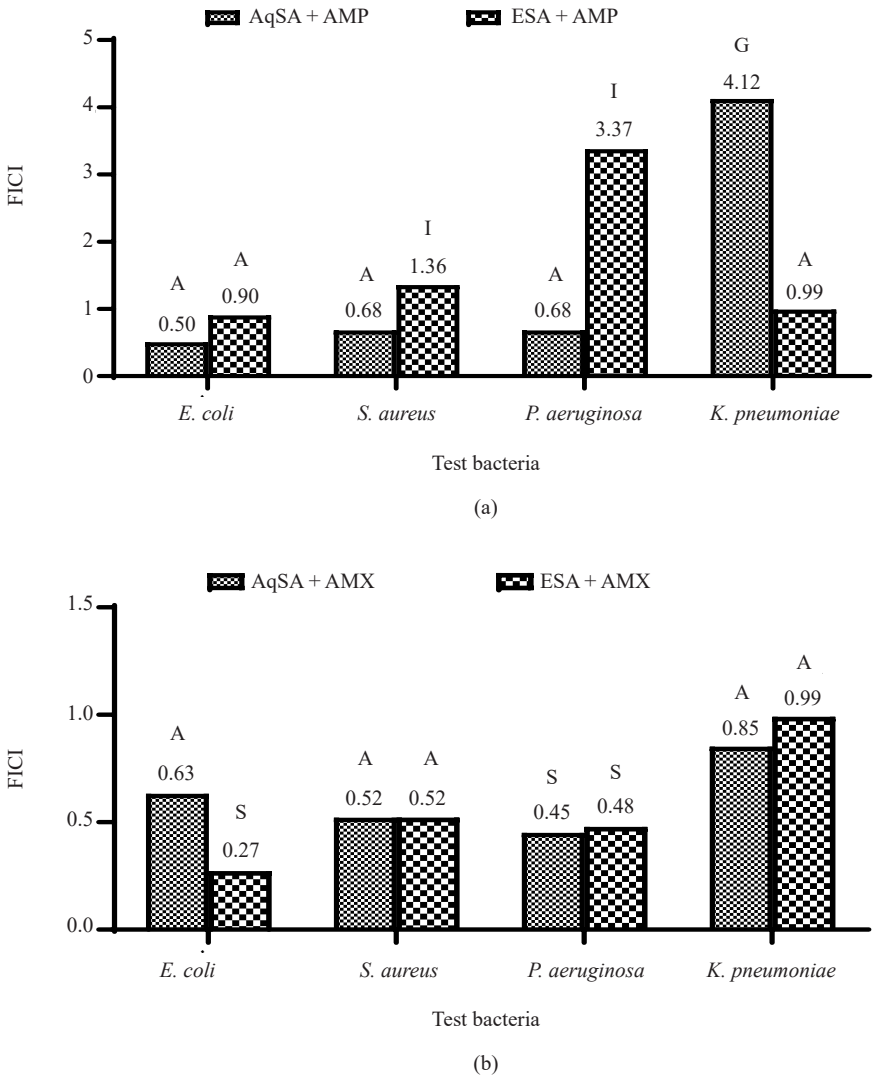
extractants interacted with antibiotics that are frequently used to treat wounds and other serious infections but are less effective when used as monotherapies. The MICs of two beta-lactamase antibiotics, ampicillin and amoxicillin, as well as an antifungal, fluconazole, are shown in Table 1.

Table 2. Minimum inhibitory concentrations (MIC) (mg/mL) of extracts in combination with antibiotics

Extract + antibiotic	Microorganisms				
	E. coli	*S. aureus*	*P. aeruginosa*	*K. pneumonia*	*C. albicans*
	S. alata leaf extracts				
AqSA +AMX	0.22	0.10	0.17	0.16	-
ESA + AMX	0.10	0.10	0.18	0.19	-
AqSA + AMP	0.15	0.32	0.16	0.78	-
ESA + AMP	0.33	0.63	0.78	0.19	-
AqSA + FLZ	-	-	-	-	0.93
ESA + FLZ	-	-	-	-	0.13
	L. barteri stem bark extracts				
AqLB + AMX	0.09	0.03	0.17	0.39	-
ELB + AMX	0.17	0.32	0.83	0.81	-
AqLB + AMP	0.34	0.31	0.13	0.40	-
ELB + AMP	1.33	1.25	0.19	0.81	-
AqLB +FLZ	-	-	-	-	0.13
ELB+FLZ	-	-	-	-	1.07
	R. communis leaf extracts				
AqRC + AMX	0.33	0.01	0.44	0.39	-
ERC + AMX	0.43	0.20	0.22	0.10	-
AqRC + AMP	0.31	0.32	0.63	0.39	-
ERC + AMP	1.33	0.78	0.39	0.97	-
AqRC +FLZ	-	-	-	-	0.46
ERC + FLZ	-	-	-	-	0.23

AqSA = aqueous leaf extract of *S. alata*; ESA = ethanol leaf extract of *S. alata*; AqLB = aqueous stem bark extract of *L. barteri*; ELB = ethanol stem bark extract of *L. barteri*; AqRC = *R. communis*; ERC = ethanol leaf extract of *R. communis*; AMX = amoxicillin; AMP = ampicillin; FLZ = fluconazole; - = not determined

The checkerboard method was used to ascertain how the antibiotics/antifungal and the extracts interacted with one another. When combined, the MICs ranged from 0.01 mg/mL for aqueous leaf extract of *R. communis* in combination with amoxicillin against *S. aureus*, to 1.33 mg/mL for ethanol stem bark extract of *L. barteri* in combination with ampicillin against *E. coli*. The result is depicted in Table 2.



AqSA = aqueous leaf extract of *S. alata*; ESA = ethanol leaf extract of *S. alata*; AMP = ampicillin; AMX = amoxicillin; I = Indifferent; S = Synergism; A = Additive; G = Antagonism; FICI = Fractional Inhibitory Concentration Index

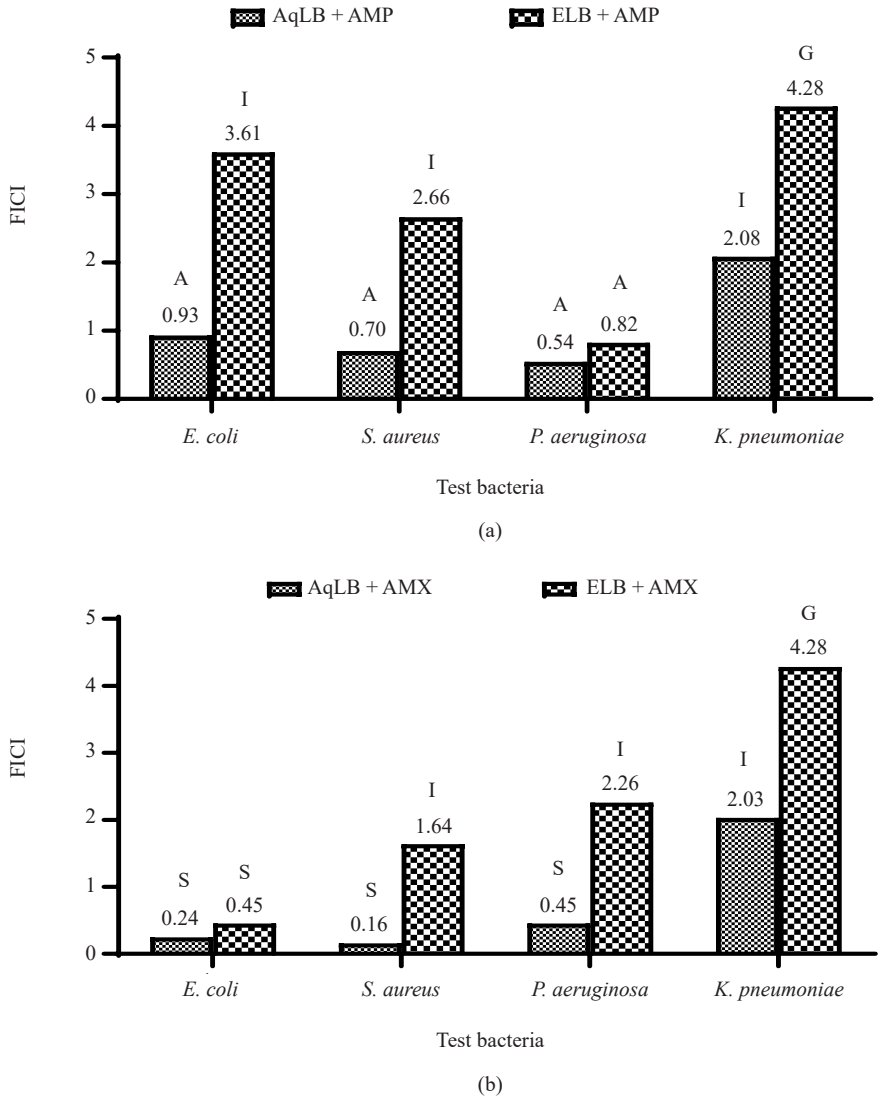
Figure 1. Effects of (a) ampicillin (b) amoxicillin in combination with *S. alata* extracts against bacterial isolates

3.2 *Fractional inhibitory concentration index (FICI) and the extent of reduction fold of MICs*

The fractional inhibitory concentration index (FICI) was computed taking into account the lower combined concentrations where bacterial growth inhibition was felt, and the type of interaction was established (Figures 1-4).

Additive, synergistic, or indifferent interactions were found in virtually all studied combinations of antibiotics and extracts. Only the interactions between *S. alata* aqueous extract and ampicillin (Figure 1a), *L. barteri* ethanol extract and ampicillin (Figure 2a), *L. barteri* ethanol extract and amoxicillin (Figure 2b), and *R. communis* ethanol extract and ampicillin (Figure 3a) were antagonistic. Nearly all of the extracts showed significant MIC reduction (Table 2) and synergistic effects with the antifungal fluconazole (Figure 4). Fluconazole and all of the extracts worked together synergistically, resulting in a 5-fold decrease in the MIC for the aqueous extract and a 36-fold reduction for the ethanol extract of *S. alata*. According to the checkerboard test, the aqueous extract of *L. barteri* in conjunction with fluconazole produced a 36-fold decrease in MIC, but the ethanol extract resulted in a 4-fold reduction. A 10-fold reduction in MIC was shown when fluconazole was mixed with the aqueous *R. communis* extract, but a 20-fold reduction was seen when fluconazole was combined with the ethanol *R. communis* extract. A 2-fold drop in MIC was seen when amoxicillin and

the aqueous extract of *S. alata* were used to treat *P. aeruginosa*. But when used in combination with amoxicillin, the ethanol extract of *S. alata* showed roughly a 4-fold decrease in MIC on treatment against *E. coli* as well as a 2-fold reduction on treatment against *P. aeruginosa*. Additionally, the MIC was reduced by 7-fold when amoxicillin and aqueous *L. bateri* combination was used to treat *S. aureus*, a 4-fold drop when used to treat *E. coli*, and a 2-fold drop when used to treat *P. aeruginosa*.



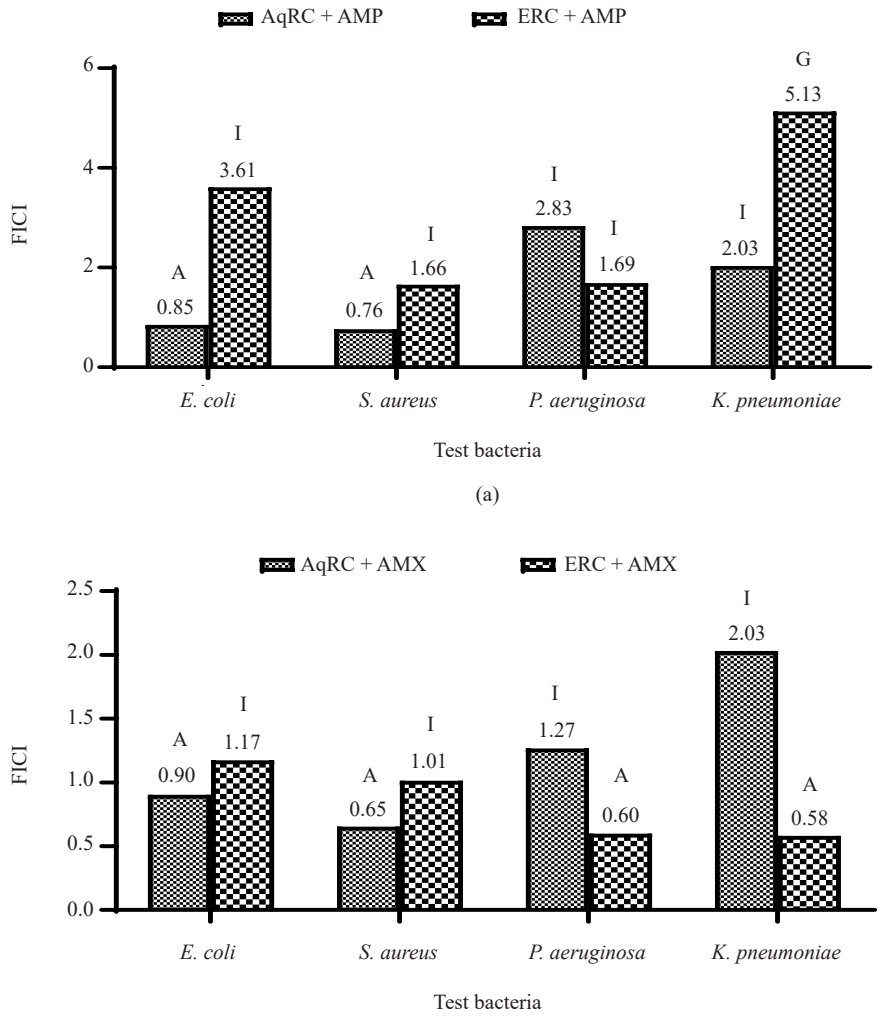
AqLB = aqueous stem bark extract of *L. barteri*; ELB = ethanol stem bark extract of *L. barteri*; AMP = ampicillin; AMX = amoxicillin; I = Indifferent; S = Synergism; A = Additive; G = Antagonism; FICI = Fractional Inhibitory Concentration Index

Figure 2. Effects of (a) ampicillin (b) amoxicillin in combination with *L. bateri* extracts against bacterial isolates

4. Discussion

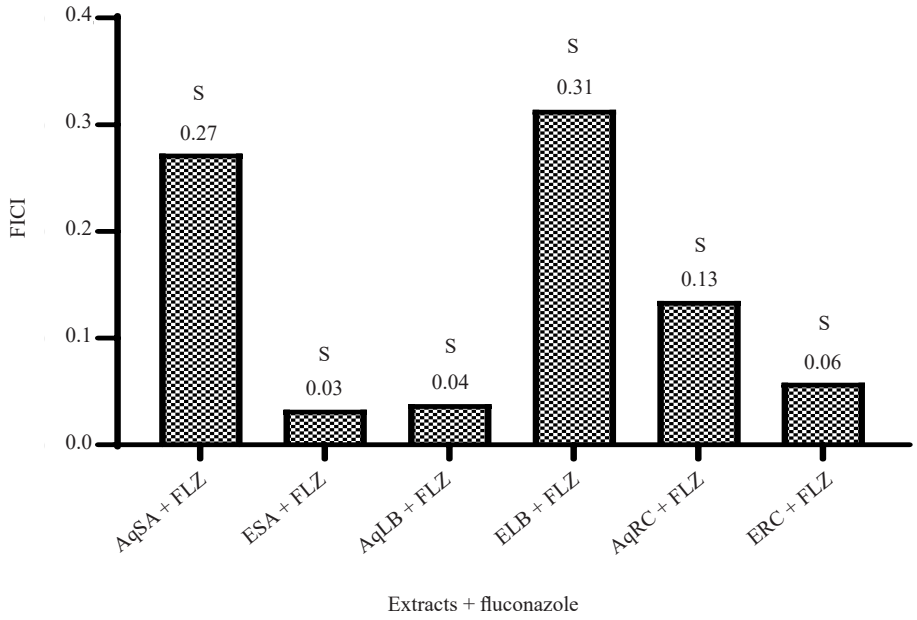
Most nosocomial or hospital acquired bacteremia are caused by *S. aureus*, of which some strains have been shown to be resistant to a variety of medications, including methicillin. *S. aureus* is a crucial pathogen that infects humans as well as an important pathogen that afflicts animals. It has been reported that, this bacterium infects dairy cattle and causes mastitis, an udder infection that results in significant worldwide economic losses.[15] According to reports,

veterinarians often use antibiotics to treat infections, which raises the likelihood of antimicrobial resistance.[31] Saini et al.[32] proposed a relationship between increasing antimicrobial resistance, namely to ampicillin, and the intramammillary injection of penicillin-novobiocin combination in the treatment of mastitis. Bovine *S. aureus* strains may exhibit ampicillin resistance that ranges from 5.2% to 77.3%, according to studies done in several countries throughout the world.[33,34] These statistics highlight the need for creative approaches to reduce antibiotic resistance. *E. coli* often lives as a harmless pathogen in the lower gastrointestinal tract microbiota of animals, including humans. However, there are several pathogenic strains of *E. coli* that may infect both people and animals and cause a wide range of diarrheal and other illnesses.[35] One of the most concerning traits of *P. aeruginosa* is its low antibiotic susceptibility, which is caused by a coordinated action of the multidrug efflux pump with chromosomally encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes.[36] *K. pneumoniae* is developing new strains that are resistant to antibiotics.[37] Currently available information suggests that plasmids are the main source of resistance genes in the Gram-negative rods of the genus *Klebsiella*, which are often resistant to a number of antibiotics.[38] Extended-spectrum beta-lactamase (ESBL)-producing pathogens are resistant to a number of antibiotics. Penicillins, fluoroquinolones, tetracyclines, aminoglycosides, chloramphenicol, and sulfamethoxazole are the antibiotics that are most often resisted.[39]



AqRC = *R. communis*; ERC = ethanol leaf extract of *R. communis*; AMP = ampicillin; AMX = amoxicillin; I = Indifferent; S = Synergism; A = Additive; G = Antagonism; FICI = Fractional Inhibitory Concentration Index

Figure 3. Effects of (a) ampicillin (b) amoxicillin in combination with *R. communis* extracts against bacterial isolates



AqSA = aqueous leaf extract of *S. alata*; ESA = ethanol leaf extract of *S. alata*;
AqLB = aqueous stem bark extract of *L. barteri*; ELB = ethanol stem bark extract of *L. barteri*;
AqRC = aqueous leaf extract of *R. communis*; ERC = ethanol leaf extract of *R. communis*;
FLZ = Fluconazole; S = Synergism; FICI = Fractional Inhibitory Concentration Index

Figure 4. Effect of extracts in combination with fluconazole against *C. albicans*

Several *Candida* species are often not harmful commensals that are found in the genitourinary and gastrointestinal tracts. However, they can also cause a variety of diseases in healthy people, including painful cutaneous infections of the skin and nails, oral thrush (oral candidiasis), and vaginal thrush (vaginal candidiasis), as well as in immunocompromised (HIV) patients, patients receiving chemotherapy, or cancer radiotherapy. Immunocompromised patients in critical care may potentially develop life-threatening systemic infections, particularly those receiving chemotherapy for cancer or immunosuppressive medication after organ or bone marrow transplant operations.[40-42]

Numerous phytochemicals with potential antibacterial action are produced by plant secondary metabolism. It is important to note that several of these compounds have been reported to have less antibacterial action than conventional medications.[15] However, these substances may work in concert with conventional antimicrobials to strengthen their impact and aid the body in fighting off infection.[15] In this work, pathogenic strains of *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, and *C. albicans* were used to assess the antibacterial activity of six extracts from three distinct plant species using two different extractants in combination with the antibiotics. This is the first time the antibacterial activity of the extracts of the three plants has been evaluated in combination with antibiotics against the specified pathogens, despite the fact that these extracts had previously been characterized for use against other bacterial species. It has been reported that, there is disagreement about what constitutes a good degree of inhibition when comparing naturally occurring products to antibiotic criteria.[43] Furthermore, natural medications may only be considered efficacious when their levels of inhibition are at par with those of synthetic antibiotics.

However, according to some studies, natural substances are only efficient if their levels of inhibition are much lower than those often seen with conventional antimicrobials.[43] According to a suggested categorization of plant materials based on MIC data, extracts with strong inhibition have a MIC of 0.5 mg/mL; those with moderate inhibition have a MIC of 0.6 to 1.5 mg/mL; and those with poor inhibition have a MIC of over 1.6 mg/mL.[44] By adopting these criteria, it could be said that the aqueous extracts of *S. alata* and *R. communis* with MICs of 3.13 mg/mL showed weak inhibition. All the other extracts demonstrated comparable inhibitory capacity with MIC values higher than 5 mg/mL. Tatsimo and colleagues[45] revealed that the methanol residue extracts of *S. alata* leaves were subjected to antibacterial assays against *S. aureus*, and multi-drug-resistant *Vibrio cholerae* strains and *Shigella flexneri*, with MICs ranging from 0.512 to 2.048 mg/mL. A previous work by Sabo et al.[46] reported that the crude methanol extract, butanol and ethyl acetate fractions of

L. barteri showed activity against *S. aureus*, *B. subtilis*, *E. coli* and *S. typhi* with MICs of 12.5 mg/mL. Naz and Bano[47] investigated the *in vitro* antimicrobial activities of the leaf extract in different solvents including, methanol, ethanol and water extracts of *R. communis*. The authors revealed that the methanol leaf extract had a significant potential to inhibit the growth of pathogenic bacterial and fungal strains than ethanol and aqueous leaf extracts.

Alkaloids, flavonoids, terpenes, limonene, α-selinene, caryophyllene, germacrene D, cinnamic acid, pyrazol-5-ol, methaqualone, isoquinoline, quinones, reducing sugars, steroids, and volatile oils have all been listed as the main ingredients of *S. alata* extract.[48] The stem bark and root of the *L. barteri* plant contain a variety of phytochemicals, including saponins, coumarins, polyphenols, alkaloids, tannins, quinones, steroids, terpenoids, and flavonoids.[49,50] Saponins, flavonoids, alkaloids, steroids, and glucosides are the main phytochemical components that have been identified in *R. communis*. The leaves of the plant have been shown to contain significant amounts of phenolic compounds such as gallic acid, quercetin, gentilic acid, rutin, epicatechin, and ellagic acid, as well as monoterpenoids (1, 8-cineole), camphor, and α-sesquiterpenoids (β-caryophyllene). The study of plant roots, however, identified indole-3-acetic acid and a number of esters, including palmitic, stearic, arachidic-hexadecenoic, oleic, linoleic, ricinoleic, and dihydroxy stearic acids.[51]

The antibacterial activity of the extracts could be attributed to the presence of phytochemicals like tannins, flavonoids, and terpenoids.[24] Tannins and flavonoids exhibit a similar mechanism by acting as a source of stable free radicals and also forming complexes with nucleophilic amino acids in proteins, leading to protein inactivation and loss of function. The antimicrobial potential of these compounds is significant, as they likely target microbial cell surface adhesions, cell wall polypeptides, and membrane-bound enzymes.[52] Terpenoids are known to impact the dissolution of the cell wall of microorganisms by weakening the membranous tissue.[53] Saponins have also been implicated in their ability to induce protein and enzyme leakage from cells.[54] Alkaloids, which were among the first bioactive compounds isolated from plants, possess the ability to intercalate with DNA, disrupt the activity of enzymes (such as esterase, DNA polymerase, and RNA polymerase), or interfere with cell respiration.[20]

The synergistic interaction exhibited by the plant extracts in combination with the antibiotics could be attributed to the ability of the bioactive compounds in the plants to modify or hinder acquired resistance mechanisms so that the bacterium becomes sensitive to the antibiotic, or the antibiotic acts in lower concentrations.[20,55,56] The combination of these chemicals is thought to help lower the minimal dosage required for effective antibacterial action. This is encouraging since lower dosages can reduce the possibility of negative side effects,[57-59] and cut down on treatment expenditures. However, given the significance of medicinal applications, it is important to explore how the plant extracts work in order to have a complete understanding of the molecular processes behind their interactions.

The rationale behind the synergistic phenomenon is indorsed to the alteration of key binding sites on the bacterial cell surface, inhibition of enzymes responsible for the degradation or modification of antibiotics, enhancement of membrane permeability, and the inhibition of efflux pumps. β-lactam antibiotics hinder the metabolic processes of peptidoglycan by attaching themselves to penicillin-binding proteins (PBPs), which are responsible for facilitating the cross-linking of peptidoglycan in a mesh-like structure. The bacterial cell is comprised of diverse enzymatic systems that render antibiotics ineffective. This phenomenon occurs through mechanisms such as hydrolysis, substitution of active groups (such as acetylation, phosphorylation, glycosylation, and adenylation), and oxidation-reduction processes.[60] The cell wall functions as an initial barrier that antibiotics and other substances must overcome in order to reach their desired targets and exert their inhibitory effects. In Gram-positive bacteria, the cell wall is comprised of multiple layers of peptidoglycan, which generally allows the passage of various substances. However, in Gram-negative bacteria, the cell wall is more complex, consisting of a single layer of peptidoglycan and an outer membrane composed of lipoproteins and lipopolysaccharides. It has been suggested that antagonism refers to a reduction in the activity of one component in the presence of the other. Contrarily to synergism, drug antagonistic interactions are frequently unwanted but may be helpful in preventing drug-resistant mutations.[61]

5. Conclusion

Both aqueous and ethanol extracts of *S. alata*, *L. barteri*, and *R. communis* exhibit synergism against *C. albicans*. Ampicillin, amoxicillin, and the other plant extracts interact in a variety of ways that are both additive and indifferent.

The new findings are promising and may increase the use of natural products in place of conventional antibiotics. Additionally, these items can be used in combination with active extracts to reduce the resistance brought on by the use of modern antimicrobial drugs. Finding the smallest dose necessary for successful antimicrobial effects will come from examining various combinations, which is exciting since it may lessen both the danger of side effects and the financial burden associated with treating infectious diseases. Further investigations are currently being carried out to validate the exact mechanism of action and impact of our plant extracts in combination with the antibiotics by employing scanning electron microscopy for morphological analysis of the selected pathogens.

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Ethics statement

All the test microorganisms used in this study were clinical isolates that were gifted from the Public Health and Reference Laboratory, Tamale Teaching Hospital (TTH), Tamale, Ghana. The authors were granted permission to use the isolates solely for research purposes. The authors were not involved in the collection and isolation of the microorganisms. In addition, the authors did not have contact with any patient or patient data. Therefore, the authors did not submit the current study to ethical approval, approval by an IRB, or informed consent.

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Authors' contribution

AMD, BA, AY and MND conceived and designed the study; AY collected the plant samples and all authors participated in the experimental work; AMD and MND wrote the initial draft. AMD, BA, AY and MND contributed in editing the paper and all authors read and approved the final version of the manuscript.

Conflicts of interest

The authors declare no competing financial interest.

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