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Research Article

Combinative phytochemical compositions and antimicrobial activities of *Xylopia aethiopica* and *Sida acuta extracts*

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Abstract

Xylopia aethiopica (Dunal) A. Rich. and *Sida acuta* Burm.f. are widely recognized for their traditional medicinal properties in treating various ailments. This study seeks to uncover the phytochemical composition and antimicrobial potential of combined extracts from these two valuable plant species. We prepared solvent extracts using aqueous, 70% ethanol, absolute ethanol, and methanol from the fruits and leaves of *Xylopia aethiopica*, along with the leaves of *Sida acuta*. These extracts were then combined into four new formulations: combined aqueous (CoA), combined absolute ethanol (CoE), combined 70% ethanol (CoE_{70}), and combined methanol (CoM). We meticulously analyzed the phytochemical compositions of these formulations. To gauge their antimicrobial activity, we examined the susceptibility of various control strains to different concentrations of the extracts through the agar well diffusion method. The minimum inhibitory concentration (MIC) was determined for the most effective combined extracts. Notably, flavonoids were discovered solely in CoA, whereas cyanogenic glycosides were present only in CoM. At a concentration of 200 mg/ml, the extract from the leaves of *Xylopia aethiopica* (AXL) exhibited the strongest inhibitory effect on *Staphylococcus aureus* ATCC 25923 (17.67 ± 0.47 mm). In comparison, CoE_{70} yielded significant results against *Staphylococcus saprophyticus* ATCC 15305 (14.00 ± 0.82 mm). AXL also showed impressive inhibition of *Salmonella typhi* ATCC 19430 (18.67 ± 0.47 mm) and *Escherichia coli* ATCC 25922 (15.00 ± 0.52 mm). The observed MICs ranged from 25 mg/ml to 3.13 mg/ml. Except for the aqueous extract of *Xylopia aethiopica* leaves, the combined extracts from *Xylopia aethiopica* and *Sida acuta*, produced through various extraction methods, exhibited distinct phytochemical profiles and demonstrated significantly greater antimicrobial activity than their individual counterparts. These compelling findings underscore the potential of these plants in developing more effective antimicrobial treatments.

Keywords: solvent extracts; concentration; zones; inhibitory; bacteria

Introduction

The frequent use of antimicrobials for diverse purposes has driven the development of antimicrobial-resistant pathogens [1]. Bacterial antimicrobial resistance is a major global public health problem because it causes an estimated 1.27 million global deaths [2]. It has been projected that due to antimicrobial resistance, the cost of healthcare will increase to US$ 1 trillion by 2050 [3]. Due to the increasing numbers of antimicrobial-resistant bacteria, attention has been turned to antimicrobial agents from natural products such as plants [4]. Plants possess phytochemicals that have similar antimicrobial mechanisms to synthetic drugs, such as inhibition of bacterial cell wall peptidoglycan formation and disruption of bacterial cell membrane [5].

The study of plants used in folk medicine requires, among other factors, knowledge from ethnobotanical surveys [5]. From this, it is known that combinations of different medicinal plants, that is, herbal mixtures, are often used for treatment. Also, evidence suggests that combinations of extracts from some native Ghanaian plants and Australian plants [6] exhibit antimicrobial synergism. Furthermore, the potential application of adding combined plant extracts to commercial antimicrobial drugs to produce synergy against pathogens is practical [7]. The efficacy of plant combination therapy used by traditional healers has been previously reported [8]. This provides evidence to explore the potential benefits of combining various plants such as *Xylopia aethiopica* (Dunal) A. Rich. and *Sida acuta* Burm.f. used in folk medicine for the treatment of infections. To understand how medicinal plants used in folk medicine can be explored as an alternative to mainstream antimicrobial drugs, it is necessary to study how these interact due to variations in their phytochemical components. *Xylopia aethiopica* and *Sida acuta* are important medicinal plants that have been exploited in various combinations in folk medicine [9,10].

The *Xylopia aethiopica* tree belongs to the family Annonaceae [10]. Traditionally, most parts of the plant have been used in different therapeutic preparations to treat conditions such as fever, bronchitis, cough, skin infections, wounds, and dysentery [11,12]. It has proven antimicrobial activity against a spectrum of Gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus pyogenes*, as well as Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa, Enterobacter aerogenes, Klebsiella pneumoniae, and Serratia marcescens* [12]. *Sida acuta* belongs to the family Malvaceae [13]. Aqueous and ethanolic plant extracts contain tannins, saponins, polyuronides, reducing sugars, terpenoids, flavonoids, and alkaloids [14]. Although all parts of the plant have found their way into folk medicine, the leaves are the most frequently used parts [15]. Traditionally, it has been applied to treat fever, skin diseases, headache, diarrhoea, and dysentery [9]. Its antimicrobial activity has been proven against bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis, Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* during in vitro testing of various extracts [16,17]. However, the in vitro interaction between the combination of these medicinal plants has not been assessed regarding antimicrobial efficacy. This study aimed to determine the phytochemical composition and antimicrobial activity of combinations of plant material from the solvent extracts of *Xylopia aethiopica* and *Sida acuta.*

Materials and methods

Collection of plant material

Fruits and leaves of *Xylopia aethiopica* (Dunal) A. Rich. were bought from the central market of Kpando Municipal in the Volta region of Ghana. Whole plants of *Sida acuta* Burm.f. were collected from Tsakpe, a suburb of the Kpando municipality (7.001819534, 0.292956556). The plant materials were identified morphologically by an expert botanist at the Centre for Plant Medicine Research in Mampong-Akuapem of the Eastern region of Ghana. Voucher specimen numbers were obtained for *Xylopia aethiopica* (CPMR 5236) and *Sida acuta* (CPMR 5237). The name of each plant was verified from http://www.theplantlist.org on May 3, 2024.

Processing of plant material

In total, 200 g of *Sida acuta*, 100 g of *Xylopia aethiopica* leaves and 145 g of *Xylopia aethiopica* fruits were prepared for processing. The plant materials were sorted, washed with water and air dried on plain linen concrete slabs in a ventilated room at room temperature for 25 days. With the aid of an industrial blender, each plant material was blended into powder at ambient temperature for approximately 1 minute and stored in three separate labelled sterile air-tight containers.

Preparation of plant extracts

For solvent extraction, the powdered *Xylopia aethiopica* fruit (XF), powdered *Xylopia aethiopica* leaves (XL), and powdered *Sida acuta* leaves (SL) were divided into four portions. Aqueous extracts of *Xylopia aethiopica* fruit (AXF), *Xylopia aethiopica* leaves (AXL), and *Sida acuta* leaves (ASL) were obtained by

decoction. This involved boiling 1:20 (w/v) of the plant material in distilled water for 5 min. The filtrates were stored in a refrigerator at 4 °C. Absolute ethanolic extracts, 70% ethanolic extracts, and absolute methanolic extracts of each plant material were produced by maceration using a 1:20 ratio (w/v) of the plant material to the respective solvent. After 72 h, each sample was filtered to obtain nine plant material extracts. For absolute ethanol, these were solvent extracts of *Xylopia aethiopica* fruit (EXF), the leaves of *Xylopia aethiopica* (EXL), and the leaves of *Sida acuta* (ESL). For 70% ethanol, these were solvent extracts of *Xylopia aethiopica* fruit (E70XF), leaves of *Xylopia aethiopica* (E70XL), and leaves of *Sida acuta* (E70SL). For absolute methanol, these were solvent extracts of *Xylopia aethiopica* fruit (MXF), *Xylopia aethiopica* leaves (MXL), and *Sida acuta* leaves (MSL). Rotary evaporation was performed to derive pure extracts of each plant material.

The solvent-specific derivatives of the fruits of *Xylopia aethiopica*, the leaves of *Xylopia aethiopica*, and the leaves of *Sida acuta* were homogenized in a ratio of 1:1:1 to obtain combined aqueous extracts (CoA) from the single extracts, combined methanolic extracts (CoM) from the single extracts, combined 70% ethanolic extracts (CoE_{70}) from the single extracts, and combined absolute ethanol extracts (CoE) from the single extracts of each of the three plant materials.

Phytochemical screening

Phytochemical tests were performed on each combined extract (CoA, CoM, and CoE_{70}) to determine their qualitative differences according to the solvent used for extraction. Reducing sugars were detected using Fehling's test [18]. Saponins were detected using the foaming test [19]. Alkaloids were detected using Mayer's test [20]. Flavonoids were detected using a dilute ammonia solution and concentrated sulfuric acid [20]. Phenolic compounds were detected using a 5% ferric chloride solution [20]. Triterpenes and phytosterols were detected using the Libermann-Burchard test [20]. Cyanogenic glycosides were detected using the Borntrager test [20]. Polyuronides were detected using acetone [18]. Anthracenosides were detected using ethyl ether and 1 ml of 25% ammonia [18].

Antimicrobial activity tests

For every single extract and combined extract, 200 mg/ml solutions were prepared in 5% dimethylsulfoxide (DMSO) and filtered through 0.2 microfilter paper. Two-fold dilutions of the stock solution were prepared to obtain 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. The antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *Staphylococcus saprophyticus* ATCC 15305, *Salmonella typhi* ATCC 19430, and *Escherichia coli* ATCC 25922 was determined using the agar diffusion method [21]. Each Mueller-Hinton agar plate was inoculated with 0.5 McFarland standard inoculum of the selected bacteria. Wells were created in each plate using an 8 mm diameter sterile cork-borer. Each well was labeled and loaded with 80 µl of its corresponding single or combined extract solution. All tests were performed in triplicate. Positive controls using a 5 µg ciprofloxacin disk [22] and negative controls with 5% DMSO were established for each control strain. Cultures were incubated at 35-37 °C, and the inhibition zones were read at 24 h.

Minimum inhibitory concentration

The MIC of each extract was determined by employing a modification of the method described by the Clinical Laboratory Standards Institute [22] to enable testing of the plant extract. Each well of a 96-microtitre plate was filled with 0.1 ml of Cation-adjusted Mueller-Hinton broth. Subsequently, 0.1 ml of 200 mg/ml of a combined extract was added to its corresponding well. A 1:2 serial dilution of this concentration was performed in successive wells. A 1:100 0.5 McFarland bacteria suspension was prepared in Mueller-Hinton broth, and 0.1 ml of the prepared inoculum was introduced into each corresponding well to obtain final concentrations of 50 mg/ml to 0.02 mg/ml. Positive and negative growth controls were included. The set-up was incubated at 35-37 °C, and the results were determined at 24 h.

Data analysis

Data were analyzed using Stata Statistical Software: Release 14 (StataMP 14). College Station, TX: StataCorp LLC. The mean ± standard deviation for all zones of inhibition was generated. The relative activity of each single extract was determined as the ratio of the single extract's antimicrobial inhibition halo (AIH) to its corresponding combined solvent extract. A relative activity >1 indicated that the single extract was more potent than its corresponding combined solvent extract.

Results

Phytochemical constituents of combined extracts

The phytochemical identification revealed that CoE_{70} had more phytochemicals present (6/10) than CoA (5/10), CoM (5/10), and CoE (3/10) (Table 1). All combined extracts contained reducing sugars, while none contained triterpenes and anthracenosides. Saponins were present in CoA, CoE_{70}, and CoM. Alkaloids were present in CoE_{70} and CoE. Flavonoids were only found in CoA. Phenolic compounds and polyuronides were found only in CoA and CoE_{70}. Cyanogenic glycosides were present in CoM. Phytosterols were not found in CoA. Polyuronides were found in CoA and CoE_{70}.

Table 1. Screening for phytochemical constituents of combined plant extracts.

Phytochemicals	CoA	CoE_{70}	CoE	CoM
Reducing sugar	+	+	+	+
Saponins	+	+	-	+
Alkaloids	-	+	+	-
Flavonoids	+	-	-	-
Phenolic Compounds	+	+	-	+
Triterpenes	-	-	-	-
Phytosterols	-	+	+	+
Cyanogenic glycosides	-	-	-	+
Polyuronides	+	+	-	-
Anthracenosides	-	-	-	-

Present (+), Absent (-), Combined aqueous extracts (CoA), Combined 70% ethanolic extracts (CoE), Combined absolute ethanol extracts, and Combined methanolic extracts (CoM).

Inhibition of Staphylococcus aureus ATCC 25923

In total, 16 extract preparations of varying composition and concentration were tested against *Staphylococcus aureus* ATCC 25923 (Figure 1). At 200 mg/ml, AXL (17.67 ± 0.47 mm), AXF (16.33 ± 0.47 mm), ASL (13.00 ± 1.41 mm), ESL (13.00 ± 1.41 mm), CoE_{70} (12.66 ± 1.25 mm), and CoE (12.33 ± 0.47 mm) respectively produced the most significant inhibitory effect on *Staphylococcus aureus* ATCC 25923. Furthermore, these were the only extracts to produce an inhibitory effect at 100 mg/ml. All others produced zone sizes less than 11 mm at 200 mg/ml except CoM, EXL, and MXL, which did not produce an inhibitory effect for all concentrations tested. Only AXL (8.67 ± 0.47 mm) and AXF (9.33 ± 0.47mm) produced an inhibitory effect at 50 mg/ml. In addition, none of the extracts inhibited *Staphylococcus aureus* ATCC 25923 at concentrations of 25 mg/ml and 12.5 mg/ml.

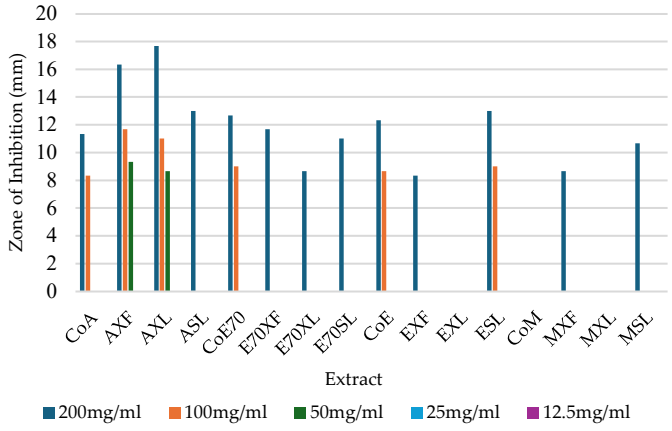


Figure 1. Antimicrobial activity of extracts against *Staphylococcus aureus* ATCC 25923.

Aqueous extract of *Xylopia aethiopica* fruits (AXF), Aqueous extract of *Xylopia aethiopica* leaves (AXL), Aqueous extract of Sida acuta leaves (ASL), Combined aqueous extracts (CoA), 70% ethanolic extract of *Xylopia aethiopica* fruit (E70XF), 70% ethanolic extract of *Xylopia aethiopica* leaves (E70XL), 70% ethanolic extract of *Sida acuta* leaves (E70SL), Combined 70% ethanolic extracts (CoE70), Absolute ethanol extracts of *Xylopia aethiopica* fruit (EXF), Absolute ethanol extract of *Xylopia aethiopica* leaves (EXL), Absolute

ethanol extract of *Sida acuta* leaves (ESL), Combined absolute ethanol extracts (CoE), Methanolic extracts of *Xylopia aethiopica* fruit (MXF), Methanolic extract of *Xylopia aethiopica* leaves (MXL), Methanolic extract of *Sida acuta* leaves (MSL).

Inhibition of Staphylococcus saprophyticus ATCC 15305

In total, 16 extract preparations of different compositions and concentrations were tested against *Staphylococcus saprophyticus* ATCC 15305 (Figure 2). At 200 mg/ml, CoE_{70} (14.00 ± 0.82 mm), AXL (13.67 mm), CoE (13 mm), and CoA (13.00 ± 0.82 mm) respectively produced the most significant inhibitory effect on *Staphylococcus saprophyticus* ATCC 15305. These were the only extracts to produce an inhibitory effect at 100 mg/ml. All others produced zone sizes smaller than 11 mm at 200 mg/ml except AXL, ASL, and ESL, which did not produce any inhibitory effect at all concentrations tested. None of the extracts inhibited *Staphylococcus saprophyticus* ATCC 15305 at concentrations of 50 mg/ml, 25 mg/ml, and 12.5 mg/ml.

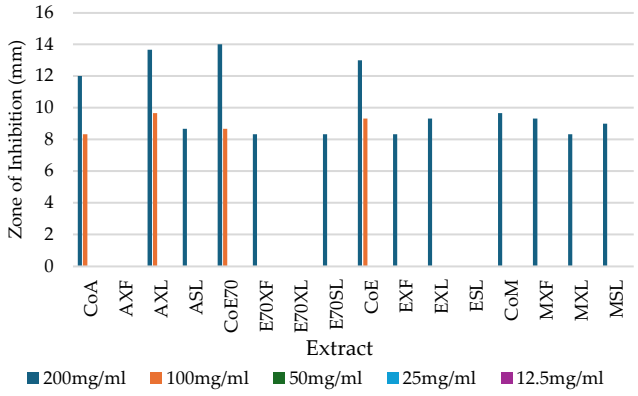


Figure 2. Antimicrobial activity of extracts against *Staphylococcus saprophyticus* ATCC 15305.

Inhibition of Salmonella typhi ATCC 19430

In total, 16 extract preparations of varying composition and concentration were tested against *Salmonella typhi* ATCC 19430 (Figure 3). At 200 mg/ml, AXL (18.67 ± 0.47 mm), AXF (17.33 ± 0.47 mm), CoA (15.67 ± 0.47 mm), CoE (14.00 ± 0.82 mm), MXL (13.67±0.47mm), EXL (13.67 ± 0.82 mm), CoE_{70} (12.33 ± 0.47 mm), and CoM (11.67 ± 0.47 mm), respectively, produced the most significant inhibitory effect on *Salmonella typhi* ATCC 19430. All others produced zones of inhibition measuring less than or equal to 10 mm at 200 mg/ml except EXF, MXF, E70XL, and E70SL, which did not produce any inhibitory effect at all the tested concentrations. At 100 mg/ml, only AXF, AXL, CoA, CoE, EXL, and CoM produced inhibitory effects corresponding to less than 11 mm inhibition zones. Only AXL (8.67 ± 0.94 mm) produced an inhibitory effect at 50 mg/ml. Furthermore, none of the extracts inhibited *Salmonella typhi* ATCC 19430 at concentrations of 25 mg/ml and 12.5 mg/ml.

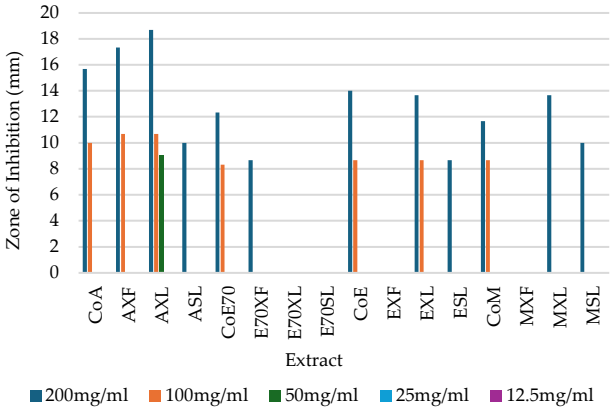
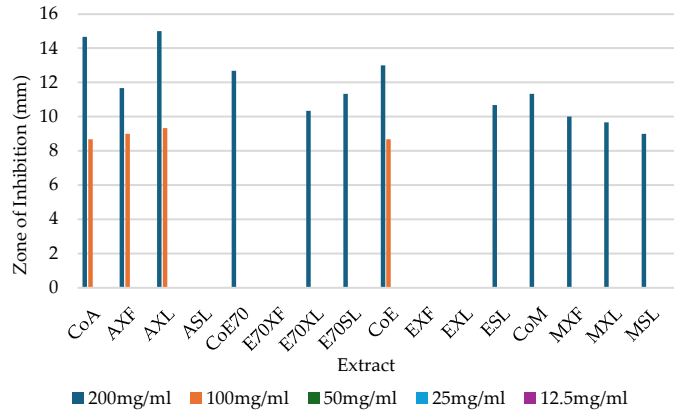


Figure 3. Antimicrobial activity of extracts against *Salmonella typhi* ATCC 19430.

Inhibition of Escherichia coli ATCC 25922

In total, 16 extract preparations of varying composition and concentration were tested against *Escherichia coli* ATCC 25922 (Figure 4). At 200 mg/ml AXL (15.00 ± 0.52 mm), CoE (13.00 ± 0.82 mm), CoE_{70} (12.67 ± 0.47 mm), AXF (11.67 ± 0.47 mm), E70SL (11.33 ±0.94 mm) and CoM (11.33 ± 0.47 mm) respectively produced the most significant inhibitory effect on *Escherichia coli* ATCC 25922. All others produced inhibition zones of less than 11 mm at 200 mg/ml, except ASL, EXF, E70XF, and EXL, which did not produce any inhibitory effect at all the concentrations tested. At 100 mg/ml, only CoA, CoE, AXF, and AXL produced inhibitory effects corresponding to inhibition zones measuring less than 10



mm. None of the extracts inhibited *Escherichia coli* ATCC 25922 at concentrations of 50 mg/ml, 25 mg/ml, and 12.5 mg/ml.

Figure 4. Antimicrobial activity of extracts against Escherichia coli ATCC 25922.

Relative activity

The combined extracts (CoA, CoM, CoE_{70}, and CoE) were frequently (45/48) more potent against *Staphylococcus saprophyticus* ATCC 15305 than their corresponding single extract components (Table II). These were also frequently (42/48) more potent (Relative activity < 1) against *Escherichia coli* ATCC 25922 than their corresponding single extract components, frequently (36/48) more potent (Relative activity < 1) against *Salmonella typhi* ATCC 19430 than their corresponding single extract components, and frequently (35/48) more potent (Relative activity < 1) against *Staphylococcus aureus* ATCC 25923 than their corresponding single extract components.

Table 2. Relative activity of single extracts to combined extracts.

Single	***S. aureus* ATCC 25923**				***S. saprophyticus* ATCC 15305**				***S. typhi* ATCC 19430**				***E. coli* ATCC 25922**			
Extract	CoA	CoM	CoE_{70}	CoE	CoA	CoM	CoE_{70}	CoE	CoA	CoM	CoE_{70}	CoE	CoA	CoM	CoE_{70}	CoE
AX_F	1.44	0.00	1.29	1.32	0.00	0.00	0.00	0.00	1.11	1.48	1.41	1.23	0.79	1.03	0.92	0.90
AX_L	1.56	0.00	1.40	1.43	1.14	1.41	0.98	1.05	1.19	1.60	1.51	1.33	1.02	1.03	1.18	1.15
AS_L	1.15	0.00	1.02	1.05	0.72	0.90	0.62	0.67	0.64	0.86	0.81	0.71	0.00	1.32	0.00	0.00
EX_F	0.73	0.00	0.66	0.68	0.69	0.86	0.60	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EX_L	0.00	0.00	0.00	0.00	0.78	0.96	0.67	0.72	0.87	1.17	1.11	0.98	0.00	0.00	0.00	0.00
ES_L	1.15	0.00	1.02	1.05	0.00	0.00	0.00	0.00	0.55	0.74	0.70	0.62	0.72	0.94	0.84	0.82
$E_{70}X_F$	1.03	0.00	0.92	0.95	0.69	0.86	0.60	0.64	0.55	0.74	0.70	0.62	0.00	0.00	0.00	0.00
$E_{70}X_L$	0.76	0.00	0.68	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.70	0.91	0.82	0.80
$E_{70}S_L$	0.97	0.00	0.89	0.89	0.69	0.86	0.60	0.64	0.00	0.00	0.00	0.00	0.72	0.94	0.84	0.82
MX_F	0.76	0.00	0.68	0.70	0.78	0.96	0.67	0.72	0.00	0.00	0.00	0.00	0.68	0.88	0.79	0.77
MX_L	0.00	0.00	0.00	0.00	0.69	0.86	0.60	0.64	0.87	1.17	1.11	0.98	0.66	0.85	0.76	0.74
MS_L	0.93	0.00	0.84	0.87	0.75	0.93	0.64	0.69	0.64	0.86	0.81	0.71	0.61	0.79	0.71	0.69

MIC of selected combined extracts

The CoA MICs ranged from 25 mg/ml to 3.13 mg/ml for *Staphylococcus aureus* ATCC 25923 (25 mg/ml), *Staphylococcus saprophyticus* ATCC 15305 (25 mg/ml), *Salmonella typhi* ATCC 19430 (6.25 mg/ml), and *Escherichia coli* ATCC 25922 (3.13 mg/ml) (Table 3). Furthermore, the CoE_{70} MICs ranged from 12.5 mg/ml to 6.25 mg/ml for *Staphylococcus aureus* ATCC 25923 (12.5 mg/ml), *Staphylococcus saprophyticus* ATCC 15305 (12.5 mg/ml), *Salmonella typhi* ATCC 19430 (6.25 mg/ml), and *Escherichia coli* ATCC 25922 (6.25 mg/ml). Furthermore, the CoE MICs ranged from 12.5 mg/ml to 6.25 mg/ml for *Staphylococcus aureus* ATCC 25923 (12.5 mg/ml), *Staphylococcus saprophyticus* ATCC 15305 (12.5 mg/ml), *Salmonella typhi* ATCC 19430 (6.25 mg/ml), and *Escherichia coli* ATCC 25922 (6.25 mg/ml). Finally, the CoM MICs ranged from 25 mg/ml to 12.5 mg/ml for *Staphylococcus aureus* ATCC 25923 (25 mg/ml), *Staphylococcus saprophyticus* ATCC 15305 (25 mg/ml), *Salmonella typhi* ATCC 19430 (25 mg/ml), and *Escherichia coli* ATCC 25922 (12.5 mg/ml).

Table 3. Validation of tests and MIC of combined extracts.

Control Strain	**Ciprofloxacin AIH (CLSI Interpretive Category)**	**MIC of Extracts (mg/ml)**			
		CoA	**CoE_{70}**	**CoE**	**CoM**
S. aureus ATCC 25923	23 (S)	25	12.5	12.5	25
S. saprophyticus ATCC 15305	25 (S)	25	12.5	12.5	25
S. typhi ATCC 19430	25 (I)	6.25	6.25	6.25	25
E. coli ATCC 25922	19 (R)	3.13	6.25	6.25	12.5

Antimicrobial Inhibition Halo (AIH), Combined 70% ethanolic extracts (CoE70), Combined absolute ethanol extracts (CoE), Combined methanolic extracts (CoM), Staphylococcus aureus (S. aureus), Staphylococcus saprophyticus (S. saprophyticus), Salmonella typhi (S. typhi), and Escherichia coli (E. coli), Clinical Laboratory Standards Institute (CLSI), Susceptible (S), Intermediate (I), Resistant (R).

Discussion

Xylopia aethiopica (Dunal) A. Rich. and *Sida acuta* Burm.f. are known for their traditional use in treating various diseases, either as single aqueous extracts or in various combinations. This study aimed to determine the potential efficacy of these plant materials used to treat infections. Combinations of specific solvent extracts of medicinal plant material can increase the levels of phytochemical constituents and, as such, were expected to enhance the antimicrobial activity. Apart from AXL, AXF, and ASL, this was frequently the case observed in this study. It has been previously reported that the nature of the interactions between the phytochemical constituents may either enhance or dampen the efficacy of the combination of single-extract plant materials [23].

The phytochemical constituents of the combined extracts may provide a basis for predicting their antimicrobial potential. The antimicrobial activity of plant extracts is a product of phytochemicals that work synergistically against bacteria [24]. Most of the tested phytochemicals were found in CoE_{70}, the most potent extract. It is known that the nonpolar ethyl group of ethanol acts as an adjunct in water to form a less polar medium, which can facilitate the elution of nonpolar constituents [25]. The phytochemical composition of CoE_{70} was uniquely different from that of the other solvent extracts. Alkaloid was the unique constituent that was absent in the other combinations. Although alkaloids were not found in CoM, it has been previously reported that there are more alkaloids than other phytochemicals in MSL [26]. Alkaloids are known components of conventional antimicrobial drugs like quinolones and metronidazole [27], and they are highly effective against bacteria.

CoE_{70} produced a more significant inhibitory effect than the corresponding single extracts, inhibited a broad spectrum of the bacterial species tested, and produced lower MICs. The MIC for CoE_{70} was less than what was previously reported for ASL (9 mg/ml) and equivalent to that reported for ESL (6.3 mg/ml) when evaluated using *E. coli.* [14]. However, it was more significant than what was previously reported for ASL (6.3 mg/ml) and ESL (7.2 mg/ml) when evaluated using *S. aureus* [14]. Also, it was more significant than what was previously reported for EXL (0.1 mg/ml) and AXL (0.015 mg/ml) when evaluated using *E. coli* [28] and more significant than what was previously reported for EXL (0.1 mg/ml) and AXL (0.03 mg/ml) when evaluated using *S. aureus* [28]. Such variations occur because factors such as the method and solvent of extraction, ecology of the plants, and parts of the plant used determine the phytochemical composition, which in turn influences the medicinal properties [29,30].

Different species of bacteria have different structures and, hence, differ in intrinsic resistance [31]. CoE_{70} produced more antimicrobial activity against *Staphylococcus aureus* ATCC 25923 than the other single extract combinations. However, this combination had a lower inhibitory effect than the single and combined aqueous extracts. In the case of *Staphylococcus saprophyticus* ATCC 15305, CoE_{70} was more effective than its single extract components, CoE and CoM. All combined extracts produced more excellent antimicrobial activity against *Staphylococcus saprophyticus* ATCC 15305 than the combined aqueous extract. The difference in the observed antimicrobial activity of the combined extracts against *Staphylococcus saprophyticus* ATCC 15305 and *Staphylococcus aureus* ATCC 25923 supports a species-specific effect of the phytochemical constituents. This is because different species may exhibit differences in resistance mechanisms. To further support this, although, for *Salmonella typhi* ATCC 19430 and *Escherichia coli* ATCC 25922, the most effective combined versus single extract antimicrobial activity was produced by 70% ethanolic extracts, this was limited in antimicrobial activity because the relative inhibitory effect was less than that observed for CoA and CoE.

It has previously been shown that ethanol can elute phytochemicals with potent antimicrobial activity against carbapenem-resistant Enterobacteriaceae [32]. The solvent used to extract plant materials may influence the phytochemical composition and antimicrobial activity. Phytochemical constituents like phytosterols and polyuronides were found in CoE_{70}, which produced a more significant inhibitory effect than the other single extract combinations. Phytosterols and polyuronides were also found in CoA and CoE, respectively. These combinations performed better than CoM, which

performed poorly. Therefore, the factors that influence the inhibition of bacterial growth in the case of the combined extracts may be the species of bacteria against which these were evaluated and the phytochemicals eluted by the extraction solvent.

The aqueous extracts of the plant materials, especially *Xylopia aethiopica*, produced a more potent antimicrobial activity against the control strains. For example, AXL, AXF, and ASL were the only single extracts that were more potent than all the combined extracts evaluated using *Staphylococcus aureus* ATCC 25923. AXL was the only single extract that was more potent than all the combined extracts, except CoE_{70}, that were evaluated using *Staphylococcus saprophyticus* ATCC 15305. AXL and AXF were the only single extracts more potent than the four combined extracts evaluated using *Salmonella typhi* ATCC 19430. AXL was the only single extract more potent than the four combined extracts evaluated against *Escherichia coli* ATCC 25922. Aqueous extracts of plants have previously been reported to produce broad-spectrum antibacterial properties compared to methanolic extracts that showed less antimicrobial activity [33]. The potency of the aqueous extracts suggests the importance of eluting these phytochemicals in water for therapeutic purposes as practiced in traditional healing. The antimicrobial activity described above for the aqueous extracts was observed more frequently in the leaves of *Xylopia aethiopica* than in the fruit. It occurred less frequently in the leaves of *Sida acuta*. This suggests that the phytochemicals with antimicrobial activity were more prominent in the leaves of *Xylopia aethiopica*.

Despite the high concentrations of the single and combined extracts, which are in mg/ml compared to ug/ml for synthetic drugs, which were required to inhibit the test strains as revealed by the MICs, these may still be therapeutically beneficial, primarily because the extracts used were not fractionated. Fractions of plant extracts may result in the separation of the various phytochemical constituents. Pure fractions may demonstrate antimicrobial activity at concentrations lower than whole extracts. Furthermore, despite the high concentrations evaluated, extracts can find application in topical drugs for the potential treatment of wounds and skin infections, as topical drugs are known to have a lower risk of systemic adverse effects [34] that can potentially arise from higher concentrations. Additionally, plant extracts such as *Zephyranthes cetrina* administered as systemic drugs up to 2000 mg/kg of body weight are safe [35], which means that the high concentrations of the extracts produced from these plant materials may not pose a problem, as phytomedicines are generally known to be safe at such doses [36] as those used in this study. The antimicrobial inhibition halos observed demonstrated that the activity of the extracts did not exceed 18.67 ± 0.47 mm. Comparatively, these zones appear smaller than those produced by in vitro tests of synthetic drugs of much lower concentrations. However, the isolation of phytochemicals can result in more potent constituents than whole extracts and potentially lead to the observation of wider zones.

However, the study is limited because the phytochemical composition was determined only for the combined extracts. Quantitative variations in the phytochemical composition of plant materials from various sources may also occur, resulting in differences in reproducibility since pure fractions were not evaluated. Furthermore, high concentrations of the extracts were required to inhibit the test strains; therefore, the toxicity must be assessed. However, fractionation of the phytochemical constituents of the extracts may result in pure fractions, which can demonstrate antimicrobial activity at lower concentrations. This will also allow for testing specific combinations of fractions for their phytochemical constituents and to determine the fractions responsible for the antimicrobial activities against the test strains. Furthermore, there is a need to evaluate these extracts against known hard-core resistant clinical isolates to determine the potential therapeutic benefits when applied in clinical settings. Finally, the study did not employ a chequerboard system that would have confirmed synergism or antagonism in the combined extracts.

Conclusion

This study has revealed that different solvent extracts of the combined plant materials of *Xylopia aethiopica* and *Sida acuta* possess different phytochemicals that may exhibit variations in their antimicrobial activity against different species of bacteria. The potency varied regarding the spectrum of species these inhibit and the minimum inhibitory concentration. The combined extracts enhanced the

antimicrobial activity, which can be exploited for therapeutic purposes. The aqueous extracts were in the lead regarding the antimicrobial potency of the single and combined extracts. Hence, as traditionally applied, water was the best solvent for eluting the active phytochemicals. Additionally, this study has shown that the leaves of *Xylopia aethiopica* possess potent antimicrobial compounds that are active against various bacteria and thus may have high medicinal value. Further studies are required to isolate and characterize the exact phytochemical constituents responsible for the inhibitory effects that occurred.

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Authors contribution

All the authors have contributed equally.

Declaration of interest

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