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In-vitro assessment of antibacterial effects of combined crude extracts of *S. glaucescens* and *C. swynnertonii* with antibiotics

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Currently, there is an upsurge of bacterial resistance in single-drug treatment regimens. This has stimulated a growing interest in research and development of new antibacterial agents containing several ingredients as one of the means to combat bacterial resistance. Herb-antibiotic combination therapy is one of the reported effective treatment regimens to combat antimicrobial resistance. This study was aimed to assess antibacterial effects of combined crude extracts of *Synadenium glaucescens* and *Commiphora swynnertonii* with antibiotics. In this study, three standard antibiotic drugs namely, ciprofloxacin, ampicillin and erythromycin in combination with crude extracts from *S. glaucescens* and *C. swynnertonii* were screened for antibacterial effects against two Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis* and three Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. Broth microdilution technique was used to determine the Minimum Inhibitory Concentration (MIC) while Fractional Inhibitory Concentration (FIC) indices were calculated from MIC values of combined extracts to determine the combination effects. Synergism was observed when ciprofloxacin was combined with all tested crude extracts against *E. coli* (ΣFIC of 0.02), combination of ciprofloxacin with extract from root barks of *C. swynnertonii* (ΣFIC of 0.5) against *S. aureus*, root barks of *Synadenium glaucescens* (ΣFIC of 0.1) against *S. aureus* and combination of ampicillin with all tested crude extracts (ΣFIC of 0.03-0.1) against *E. faecalis*. Moreover, antagonism was observed between the combinations of ampicillin and erythromycin with all tested crude extracts against Gram-negative bacteria (ΣFIC of 4-8). Therefore, the combinations which demonstrated synergism may be promising alternatives for the treatment of infectious diseases caused by *E. coli*, *S. aureus* and *E. faecalis*. However, in the future, toxicity studies for combinations which demonstrated synergism are recommended.

Key words: Antibacterial activity, antibiotic, crude extracts, herbs, synergism.

INTRODUCTION

The “one drug, one target, one disease” approach has for some time remained the conventional pharmaceutical approach to the development of medicines and treatment strategies (Zhou et al., 2016). However, due to rapid development of microbial resistance, this mono-substance therapy model of either herbs or commercially

available antibiotics has gradually shifted toward the adoption of combination therapies in which multiple active components are employed (Sheard et al., 2019). Over the last decade, there have been screening of the mono-essential effective, safe, cheap and available therapeutics from various medicinal plants like herbs for their potential antimicrobial effect (Atef et al., 2019). Despite the fact that plant products proved as more promising antimicrobials therapy their activity is milder than commercially available antibiotics (Bhardwaj et al., 2016). The extracts from S. glaucescens which belong to the family Euphorbiaceae (Mwine and Damme., 2011), (known as “Mvunjakongwa” in Swahili language) have been reported to have potential activity against infections caused by bacteria, fungi, viruses and pests in human and livestock (Mabiki et al., 2013; Max et al., 2014). The tropical tree, C. swynnertonii (known as “Oltemwai” in Masai language), from the family Burs eraceae, has been reported to have antimicrobial activity (Mkangara et al., 2014). These plants contain secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, anthraquinones, steroids and essential oils (Mkangara et al., 2014), responsible for various bioactivities. Herbal drugs alternatively can be used in combination with antibiotics to enhanced activity against bacterial infections. Drug combinations can substantially lower the risk of the development of resistance and they useful as their effects on cells may be amplified or weakened than either drug alone a phenomenon known as synergistic or antagonistic interactions (Bobrowski et al., 2021). Usually the amplified or synergistic effects are the most desired outcome. The present study reports the antibacterial effects between combinations of crude extracts of *S. glaucescens* and *C. Swynnertonii* and ciprofloxacin, ampicillin and erythromycin from three different classes of antibiotics (fluoroquinolones, ß-lactams and macrolides), with different modes of action and all having broad-spectrum of antibacterial activity (Christina and Adelaide, 2013). The effects were tested on strains of the bacterial species (*Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa)* that are major causes of nosocomial infections and nominated by WHO as critical in relation to human health risks due to antimicrobial resistance (Khan et al., 2015).

MATERIALS AND METHODS

Study design and study area

This study was an experimental one whereby the antibacterial combinations of the herb-herb were assessed based on their effects and efficacies against selected bacteria. The study was conducted in the chemistry laboratory, Department of Chemistry and Physics, and microbiology laboratory, Department of Biosciences, of the College of Natural and Applied Sciences of the Sokoine University of Agriculture (SUA).

Plant collection and preparation

The fresh plant samples of *S. glaucescens* were collected from Mtulingala village in Njombe region in the Southern Highland of Tanzania located at 08°34' to 08°49' S and 08°34' to 03°55' E m above the sea level on 30th December 2018. Samples of *C. swynnertonii* were collected from Mirerani area in Simanjiro district of Manyara region in the Northern Highland of Tanzania located at 03°36' to 03°14.73' S and 36°50' to 36°18.05' E m above the sea level on 19th December, 2018. Authentication of plant species was done by a botanist, Mr. Haji O. Seleman and voucher specimen number HOS/FM 3672 for *S. glaucescens* and FMM 3897 for *C. swynnertonii* were stored in the herbarium at the Department of Botany, College of Natural and Applied Sciences of the University of Dar es Salaam, Tanzania. Plants names were verified at http://www.theplantlist.org. The leaves stem and roots of the targeted were collected. Plants parts were washed with clean water then peeled to separate the barks and wood. They were dried in a dark room at 20°C at the Tanzania Tree Seed Agency Laboratory, Morogoro, Tanzania.

Dry samples were grounded separately using a laboratory mill machine (Christy Hunt Engineering Ltd, Manchester-England), approximately 2 mm particle size. The selection of these plant parts was based on a previous report that demonstrated antimicrobial activity against selected bacteria (Max et al., 2014).

Reagents and drugs

Solvents used for extractions were methanol (Finer Chemical, Gujarat, India), dichloromethane and dimethyl sulphoxide (Loba-Chemie, Mumbai, India). The standard antibiotics used as positive control included ciprofloxacin, ampicillin and erythromycin (Sigma-Aldrich, Berlin-Germany).

Crude extracts

Extraction was carried out using the method described by Max et al. (2014). Briefly, 1000 g of dry ground plant materials were extracted by 98% dichloromethane using hot continuous extraction method at 50°C for 4 h whereby the 33 g dry ground samples were injected into each thimble (33 mm diameter, 80 mm length) and extracted using Soxhlet apparatus. The samples were filtered and the obtained solid residues were soaked in 99% methanol at room temperature (25-30°C) for 72 h. All samples were filtered using Whatman No. 1 filter paper (Maidstone-Kent, UK). The filtrates were concentrated in a rotary evaporator (Buchi Labor tetechnik, Flawil, Swtzerland) with a water bath maintained at 40°C. The obtainedcrude extracts were air-dried to remove any remains of solvents. The dried extracts were stored at -20°C until the day of use.

Test bacterial strains

Gram-positive bacteria used were *S. aureus* American Type Culture

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Test bacterial strains

Gram-positive bacteria used were *S. aureus* American Type Culture Collection (ATCC 29213) and *E. faecalis* (ATCC 51559). Gram-negative bacteria used were *E. coli* (ATCC 25922), *K. pneumonia* (ATCC 1145), *and P. aeruginosa* (ATCC 27853). The four (4) strains which are *S. aureus*, *E. faecalis* and *E. coli* and *K. pneumonia* were obtained from Microbiology laboratory in the Department of Biosciences, (SUA), and *P. aeruginosa* was bought from the Muhimbili University of Health and Allied Sciences (MUHAS).

Preparation of individual and combined herb-antibiotic solutions

Stock concentrations of 3 mg/mL from each crude extract and 0.1 mg/mL standard antibiotic were prepared and individually-tested for their antibacterial effects. Depending on the individual MIC of the crude extracts and standard antibiotics, the concentration resulting from combination was calculated thereafter, sample solution of extracts and standard antibiotics were combined in ratio of 1:1v/v to make working bench solutions. All sample solutions were mixed well by overtaxing.

This study was an experimental one whereby the antibacterial combinations of the herb-herb were assessed based on their effects and efficacies against selected bacteria. The study was conducted in the chemistry laboratory, Department of Chemistry and Physics, and microbiology laboratory, Department of Biosciences, of the College of Natural and Applied Sciences of the Sokoine University of Agriculture (SUA).

Minimum inhibitory concentration by broth dilution method

The minimum inhibitory concentration (MIC) was determined by a two-fold microdilution method to assess the antibacterial effects of herb-herb (Kudumela et al., 2018). In brief, sterile, 96-well polystyrene microtitre plate was first preloaded with 50 µL of Mueller-Hinton broth in each well, followed by the addition of 50 µL of extract in combination with standard drug solutions into the first wells of each row to make a total volume of 100 µL. Each of the test sample materials was tested in duplicate. To the first well, the samples were mixed and 50 µL was drown from each well and transferred to the subsequent rows until the last well. Then 50 µL of the mixture from the last well was discarded. Thereafter, 50 µL of the bacterial suspension equivalent to 0.5 MacFarland standard turbidity (1.5×10^6 CFU mL^{-1}) was added to each well. An additional row containing 0.1 mg/mL of standard antibiotic (50 µL of ciprofloxacin, ampicillin or erythromycin) was used as a positive control. Wells containing (50 µL) Mueller-Hinton broth and DMSO and bacteria only were used as negative controls. The plates were incubated at 37°C overnight. MIC was determined visually; whereby the lowest concentration without growth was considered as MIC.

Fractional inhibitory concentration

Among the techniques employed in the evolution of two combinations of antimicrobials activities is the fractional inhibitory concentration (FIC) (Jain et al., 2011). The fractional inhibitory concentration (FIC) technique employs a methodology similar to that used for the determination of minimum inhibitory concentration (MIC) which also appears useful to test the antibacterial activities of combinations of herbs or herbs with antibiotics (Meletiadis et al., 2010). The combined effect was analyzed by using measurements of MIC to calculate the FIC indices, where the combination was defined to have a synergistic effect if the FIC index ≤0.5, additive effect if 0.5 > FIC Index < 4 or antagonistic effect if FIC Index ≥ 4. This interpretation followed the conventional model (Odds, 2003; EUCAST, 2000). The fractional inhibitory concentration index (ΣFIC) is then calculated for each test sample independently as specified in the following algebraic formula **(**Kudumela et al., 2018):

FIC index = FIC *Cs* + FIC Antibiotic or FIC index = FIC *Sg* + FIC Antibiotic

Where:

$$\text{FIC } Cs = \frac{\text{MIC value of } Cs \text{ in combination with antibiotic}}{\text{MIC value of } Cs \text{ independently}}$$

$$\text{FIC } Sg = \frac{\text{MIC value of } Sg \text{ in combination with antibiotic}}{\text{MIC value of the } Sg \text{ independently}}$$

$$\text{FIC Antibiotic} = \frac{\text{MIC value of antibiotic in combination with crude extract}}{\text{MIC value of the antibiotic independently}}$$

RESULTS

Antibacterial Activity of individual extracts and antibiotics

The MICs of the antibacterial activities of individual extracts and antibiotics investigated are indicated in Tables 1 and 2. The MIC values were interpreted based on classification criteria as follows: 0.05-0.5 $mgmL^{-1}$ as strong activity; 0.6-1.5 $mgmL^{-1}$ as moderate activity, and above 1.5 mg/mL as weak activity (Sartoratto et al., 2004). Among the crude extracts tested, methanol extracts of leaves, stem barks and root barks of *S. glaucescens* and *C. swynnertonii* inhibited the growth of Gram-positive bacteria *S. aureus* and *E. faecalis* with the lowest MIC values ranging between 0.01 and 0.37 $mgmL^{-1}$. Dichloromethane extracts of *S. glaucescens* and *C. swynnertonii* showed moderate antibacterial activity against Gram-positive bacteria tested with MIC values ranging from 0.75 to 1.5 $mgmL^{-1}$. Furthermore, all extracts showed weak activity against Gram-negative bacteria (Tables 1 and 2). However, the antibiotics (ciprofloxacin, ampicillin and erythromycin) tested showed strong antibacterial activity individually (Tables 1 and 2).

Minimum inhibitory concentration of crude extracts combined with ciprofloxacin

The study of antibacterial activity was conducted and the MIC of combined extracts with ciprofloxacin was obtained as indicated in Table 3. The MIC values were interpreted based on classification criteria as described by Sartoratto et al. (2004). The crude extracts combined with ciprofloxacin showed strong activity against Gram-negative bacteria (*E. coli*, *K. pneumoniae*, and *P. aeruginosa)* with MIC value ≤ 0.5 mg/mL (Table 3). Additionally, the combinations of crude extracts with the antibiotic showed strong activity against *S. aureus* (MIC value ≤ 0.5 mg/mL) except for dichloromethane root bark

Table 1. Minimum Inhibitory Concentration (mg/mL) of individual crude extracts of *C. swynnertonii* tested against bacteria.

Extracts/antibiotics	MIC (mg/mL)				
	S. aureus	***E. faecalis***	***E. coli***	***K. pneumoniae***	***P. aeruginosa***
*Cs*7D	0.38	0.75	3	3	3
*Cs*7M	0.09	0.38	3	3	3
*Cs*5D	0.75	1.5	3	3	3
Cs5M	0.18	0.75	1.5	3	3
*Cs*2D	1.5	0.38	3	3	3
*Cs*2M	0.04	0.38	3	3	3
Ciprofloxacin	0.0007	0.0003	0.0001	0.0031	0.0012
Ampicillin	0.02	0.025	0.3	0.3	0.3
Erythromycin	0.0015	0.0003	0.2	0.2	0.2

D= Dichloromethane extract, M= Methanol extract, *Cs*= *Commiphora swynnertonii, Cs*7= leaf extract, *Cs*5= stem bark extracts, *Cs*2= root bark extract.
Source: Authors 2022

Table 2. Minimum inhibitory concentration (mg/mL) of individual crude extracts of *S. glaucescens* tested against bacteria.

Extract/antibiotics	MIC mg/mL				
	S. aureus	***E. faecalis***	***E. coli***	***K. pneumoniae***	***P. aeruginosa***
*Sg*7D	0.75	0.75	3	3	1.5
*Sg*7M	0.38	0.38	3	3	0.75
*Sg*5D	0.38	0.75	3	3	3
*Sg*5M	0.02	1.5	3	3	3
*Sg*2D	0.02	0.38	3	3	1.5
*Sg*2M	0.01	0.02	1.5	3	1.5
Ciprofloxacin	0.0007	0.0003	0.0001	0.0031	0.0012
Ampicillin	0.02	0.025	0.3	0.3	0.3
Erythromycin	0.0015	0.0003	0.2	0.2	0.2

D= Dichloromethane extract, M= Methanol extract, Sg= *Synadenium glaucescens, Sg*7= leaf extract, Sg5= stem bark extract, Sg2= root bark extract.
Source: Authors 2022

extract of *C. swynnertonii* and combination of all crude extracts with ciprofloxacin against *E. faecalis*, which showed moderate activity to weak activity (MIC value 0.6-1.5 mg/mL).

Minimum inhibitory concentration of crude extracts combined with ampicillin

The combinations of crude extracts with ampicillin showed weak activity with MIC of 1.6 mg/mL (Table 4) against Gram-negative bacteria (*E. coli*, *K. pneumoniae* and *P. aeruginosa)*. Additionally, methanol leaves and stem barks extracts of *C. swynnertonii* combined with ampicillin showed moderate activity against *S. aureus* (MIC value 0.7 mg/mL) while combination of crude extracts with ampicillin had strong activity against *E. faecalis* (MIC values ≤ 0.5 mg/mL).

Minimum inhibitory concentration of crude extracts combined with erythromycin

Methanol leaves extract of *S. glaucescens* and *C. swynnertonii* and dichloromethane leaves and stem barks extract of *S. glaucescens* combined with erythromycin showed moderate activity (MIC value = 0.7 mg/mL) against *S. aureus* (Table 5). Additionally, combinations of all crude extracts with erythromycin except methanol leaves extract from *S. glaucescens* revealed strong activity against *E. faecalis* (MIC values ≤ 0.5 mg/mL) while combinations of erythromycin with dichloromethane extract of stem barks and root barks of *C. swynnertonii* had strong activity against *P. aeruginosa* (MIC value 0.4 mg/mL). Also, dichloromethane and methanol leaves extract of *C. swynnertonii* combined with erythromycin showed strong activity against *K. pneumoniae* (MIC value 0.4 mg/mL) while methanol root

Table 3. Minimum Inhibitory Concentration (mg/mL) of crude extracts of *C. swynnertonii* and *S. glaucescens* combined with ciprofloxacin against tested selected bacteria.

Extract/ciprofloxacin	MIC mg/mL				
	S. aureus	***E. faecalis***	***E. coli***	***K. pneumoniae***	***P. aeruginosa***
*Cs*7M+CIPRO	0.02	1.50	0.002	0.09	0.02
*Cs*5M+CIPRO	0.09	1.50	0.001	0.04	0.02
*Cs*2M+CIPRO	0.02	1.50	0.002	0.38	0.02
*Cs*7D+CIPRO	0.18	1.50	0.001	0.04	0.02
*Cs*5D+CIPRO	0.37	1.50	0.001	0.04	0.02
*Cs*2D+CIPRO	0.75	1.50	0.001	0.04	0.02
*Sg*7M+CIPRO	0.09	0.75	0.002	0.09	0.19
*Sg*5M+CIPRO	0.01	0.75	0.001	0.09	0.04
*Sg*2M+CIPRO	0.001	0.77	0.001	0.09	0.02
*Sg*7DC+IPRO	0.37	0.75	0.001	0.04	0.02
*Sg*5D+CIPRO	0.18	0.75	0.001	0.04	0.02
*Sg*2D+CIPRO	0.01	0.75	0.001	0.04	0.02

+ = Combination, CIPRO=Ciprofloxacin, D= Dichloromethane crude extract, M= Methanol crude extract, Cs7= *Commiphora swynnertonii* leaf extract, Cs5= *Commiphora swynnertonii* stem bark extracts, Cs2= *Commiphora swynnertonii r*oot bark extract, Sg7= *Synadenium glaucescens* leaf extract, Sg5= *Synadenium glaucescens* stem bark extract, Sg2= *Synadenium glaucescens* root bark extract.
Source: Authors 2022

barks extract of *C. swynnertonii* showed moderate activity (MIC 0.8 mg/mL) against all tested bacteria. Also, combination of erythromycin with all crude extracts showed weak activities against *E. coli* (MIC value1.6 mg/mL) (Table 5).

Fractional inhibitory concentration index (∑FIC) of crude extracts combined with antibiotics selected

Ciprofloxacin combined with methanol root barks extract of *C. swynnertonii* and *S. glaucescens* exhibited synergistic effects against *S. aureus* at ∑FIC 0.5 and 0.1, respectively (Table 6). The combinations of ciprofloxacin with crude extracts against *E. coli, K. pneumoniae* and *E. faecalis* revealed synergistic, additive and antagonistic effects at ∑FIC values 0.03, 0.9-1.9 and 8-481, respectively (Table 6). Additionally, crude extracts of *C. swynnertonii* combined with ciprofloxacin displayed synergistic effects (∑FIC 0.5) against *P. aeruginosa* while combination of ciprofloxacin with methanol leaves extract and dichloromethane leaves extract, stem barks and root barks extracts of *S. glaucescens* showed additive effects (∑FIC 0.9-3.9).

Synergistic effects (∑FIC 0.5) were observed against *S. aureus* in the combinations of ampicillin with methanol leaves, root barks and dichloromethane stem barks and root barks of *C. swynnertonii (*Table 7). Additionally, ampicillin combined with methanol stem barks and root barks extracts of *S. glaucescens* showed synergistic effects (∑FIC 0.03). Moreover, when ampicillin combined with methanol crude extracts of leaves, stem barks and root barks of *C. swynnertonii* then showed synergistic effects of 0.06 were observed against *E. faecalis.* Also (∑FIC), dichloromethane stem barks and root barks of *S. glaucescens* (∑FIC 0.03) and dichloromethane leaves of *S. glaucescens* (∑FIC 0.5). Antagonistic effects were observed against *E. coli*, *K. pneumoniae*, and *P. aeruginosa* when subjected to combinations of all crude extracts with ampicillin (Table 7).

Furthermore, when erythromycin combined with methanol root barks extract of *C. swynnertonii* and *S. glaucescens* was tested against *S. aureus;* it showed two synergistic effects (∑FIC 0.5 and 0.1 respectively) (Table 8). In addition, combinations of erythromycin with root barks extracts of *C. swynnertonii* and *S. glaucescens* against *S. aureus* showed two synergistic effects; however the most of combinations of erythromycin with crude extracts against *E. faecalis, E. coli, K. pneumoniae,* and *P. aeruginosa* exhibited antagonistic effects (∑FIC ≥ 4).

DISCUSSION

Herbal medicines derived directly or indirectly from plants have been successfully used for treatment of different human diseases including infectious diseases for thousand years worldwide (Shakya, 2016). In this study, individual crude extract and antibiotics were screened for antibacterial activity. The extracts showed activity against Gram-positive bacteria while weak activity were observed against Gram-negative bacteria the results of this study concurred with previous studies reporting on antibacterial

Table 4. Minimum Inhibitory Concentration (mg/mL) of crude extracts of *C. swynnertonii* and *S. glaucescens* combined with ampicillin tested against selected bacteria.

Extract/ampicillin	MIC mg/mL				
	S. aureus	***E. faecalis***	***E. coli***	***K. pneumonia***	***P. aeruginosa***
*Cs*7M+AMPI	0.74	0.006	1.55	1.55	1.55
*Cs*5M+AMPI	0.77	0.006	1.55	1.55	1.55
*Cs*2M+AMPI	0.02	0.006	1.55	1.55	0.38
*Cs*7D+AMPI	0.09	0.003	1.55	1.55	0.77
*Cs*5D+AMPI	0.04	0.003	1.55	1.55	1.55
*Cs*2D+AMPI	0.04	0.001	1.55	1.55	1.55
*Sg*7M+AMPI	0.19	0.09	1.55	1.55	1.55
*Sg*5M+AMPI	0.09	0.003	1.55	1.55	1.55
Sg2M+AMPI	0.09	0.006	1.55	1.55	1.55
*Sg*7D+AMPI	0.19	0.04	1.55	1.55	1.55
*Sg*5D+AMPI	0.09	0.04	1.55	1.55	1.55
*Sg*2D+AMPI	0.09	0.003	1.55	1.55	1.55

+ = Combination, AMPI= Ampicillin, D= Dichloromethane crude extract, M= Methanol crude extract, Cs7= *Commiphora swynnertonii* leaf extract, Cs5= *Commiphora swynnertonii* stem bark extracts, Cs2= *Commiphora swynnertonii r*oot bark extract, Sg7= *Synadenium glaucescens* leaf extract, Sg5=*Synadenium glaucescens* stem bark extract, Sg2=*Synadenium glaucescens* root bark extract.
Source: Authors 2022

Table 5. Minimum Inhibitory Concentration (mg/mL) of crude extracts of *C. swynnertonii and S. glaucescens c*ombined with erythromycin tested against selected bacteria.

Extracts/erythromycin	MIC mg/mL				
	S. aureus	***E. faecalis***	***E. coli***	***K. pneumoniae***	***P. aeruginosa***
*Cs*7M + ERYT	0.76	0.375	1.55	0.38	1.55
*Cs 5*M + ERYT	0.38	0.75	1.55	1.55	1.55
*Cs 2*M + ERYT	0.02	0.01	1.55	0.76	1.55
*Cs*7D + ERYT	0.04	0.18	1.55	0.38	1.55
*Cs 5*D + ERYT	0.09	0.38	1.55	1.55	0.38
*Cs 2*D + ERYT	0.04	0.18	1.55	1.55	0.38
Sg 7M + ERYT	0.75	0.75	1.55	1.55	1.55
Sg 5M + ERYT	0.1	0.38	1.55	1.55	1.55
Sg 2M + ERYT	0.006	0.38	1.55	1.55	1.55
Sg 7D + ERYT	0.75	0.38	1.55	1.55	1.55
Sg 5D + ERYT	0.75	0.38	1.55	1.55	1.55
Sg 2D + ERYT	0.1	0.18	1.55	1.55	1.55

+ = Combination, ERYT= Erythromycin, D= Dichloromethane crude extract, M= Methanol crude extract, Cs7= *Commiphora swynnertonii* leaf extract, Cs5= *Commiphora swynnertonii* stem bark extracts, Cs2= *Commiphora swynnertonii r*oot bark extract, Sg7= *Synadenium glaucescens* leaf extract, Sg5= *Synadenium glaucescens* stem bark extract, Sg2= *Synadenium glaucescens* root bark extract.
Source: Authors 2022

activity from similar parts of the selected plants (Max et al., 2014; Mkangara et al., 2014). Antibiotics used in this study also showed wide range of antibacterial activity of inhibiting growth of both Gram-negative and Gram-positive bacteria. Ciprofloxacin exhibited strong antibacterial activity compared to ampicillin and erythromycin, the strong antibacterial activity also reported in previous studies (Grădinaru et al., 2014; Rapper et al., 2016). There are several studies, in the combinations between plant extracts and antibiotics which indicated synergistic effects (Kuok et al., 2017; Sheard et al., 2019).

In this study, standard drugs in combination with methanol and dichloromethane extracts from leaves stem barks, and root barks of *C. swynnertonii* and *S. glaucescens* plants that are used traditionally for

Table 6. Fractional inhibitory concentration of crude extracts of *C. swynnertonii* and *S. glaucescens* combined with ciprofloxacin tested against selected bacteria.

Combination	FIC Index				
	S. aureus	***E. faecalis***	***E. coli***	***K. pneumoniae***	***P. aeuriginosa***
*Cs*7M+CIPRO	15.50	12.12	0.05	1.93	0.48
*Cs*5M+CIPRO	15.32	481.26	0.02	0.96	0.48
Cs2M+CIPRO	0.50	3.78	0.05	7.75	0.48
*Cs*7D+CIPRO	7.84	12.12	0.02	0.96	0.48
*Cs*5D+CIPRO	31.31	15.50	0.02	0.96	0.48
*Cs*2D+CIPRO	60.12	2.13	0.02	0.96	0.48
*Sg*7M+CIPRO	127.47	60.49	0.05	1.93	3.98
*Sg5*M+CIPRO	1.95	250.5	0.05	1.93	0.96
*Sg2*M+CIPRO	0.15	8.01	0.02	1.93	0.48
*Sg*7DC+IPRO	240.49	125.5	0.02	0.96	0.96
*Sg*5D+CIPRO	120.49	125.5	0.029	0.96	0.96
*Sg*2D+CIPRO	2.13	60.49	0.029	0.96	0.96

Source: Authors 2022

Table 7. Fractional inhibitory concentration of crude extracts of *C. swynnertonii* and *S. glaucescens* combined with ampicillin tested against selected bacteria.

Combination	FIC Index				
	S. aureus	***E. faecalis***	***E. coli***	***K. pneumoniae***	***P. aeuriginosa***
*Cs*7M+AMPI	0.5	0.06	4.00	8.01	8.01
*Cs*5M+AMPI	7.80	0.06	8.01	8.01	8.01
*Cs*2M+AMPI	0.25	0.06	4.00	4.13	5.25
*Cs*7D+AMPI	1.01	0.03	4.00	8.01	8.01
*Cs*5D+AMPI	0.5	0.03	8.01	8.01	8.01
*Cs*2D+AMPI	0.5	0.15	8.01	8.01	8.01
*Sg*7M+AMPI	1.01	1	8.01	8.01	8.01
*Sg*5M+AMPI	0.03	0.03	4.00	8.01	8.01
Sg2M+AMPI	0.03	0.61	4.00	8.01	8.01
*Sg*7D+AMPI	2.00	0.5	8.01	8.01	8.01
*Sg*5D+AMPI	1.01	0.5	8.01	8.01	8.01
*Sg*2D+AMPI	1.01	0.03	8.01	8.01	8.01

Source: Authors 2022

treatment of various ailments including bacterial infections in Tanzania, were tested against selected bacteria both individually and in combinations with some selected antibiotics to determine their antibacterial and combination effects. Some synergistic effects were observed in combinations of ciprofloxacin and crude extracts. When methanol extract from stem barks of *C. swynnertonii* and dichloromethane extract of root barks of *S. glaucescens* in combination with ciprofloxacin tested against *S. aureus*, additive effects were observed. Previous studies have also demonstrated synergistic and additive effects when ciprofloxacin is in combination with bioactive compounds such as essential oil tested against some pathogen bacteria (Grădinaru et al., 2014; Rapper et al., 2016). Some combinations of crude extracts with ciprofloxacin in this study displayed antagonistic effects against *S*. *aureus* and *E. faecalis.* In some previously studies, antagonistic effects occurred when a combination of ciprofloxacin with plant materials were tested against some pathogenic bacteria; for instance, this was observed when *Moringa oleifera* in combination with penicillin or tetracycline tested against *P. vulgaris* (Ilanko et al., 2019*).* In the combination of ampicillin with crude extracts tested, the best outcome that is synergism was obtained against Gram-positive bacteria in particular, *S. aureus* and *E. faecalis*. Antagonistic effects were

Table 8. Fractional Inhibitory Concentration of crude extracts of *C. swynnertonii* and *S. glaucescens* combined with erythromycin against tested selected bacteria.

Combination	FIC Index				
	S. aureus	***E. faecalis***	***E. coli***	***K. pneumoniae***	***P. euriginosa***
*Cs*7M +ERYT	15.50	12.12	8.01	4.00	8.01
*Cs 5*M + ERYT	15.32	481.26	8.01	8.01	8.01
*Cs 2*M + ERYT	0.50	3.78	8.01	8.01	8.01
*Cs*7D + ERYT	7.84	120.19	8.01	4.00	8.01
*Cs 5*D + ERYT	31.31	484.13	8.01	4.00	4.00
*Cs 2*D + ERYT	60.12	60.12	8.01	8.01	4.00
Sg 7M + ERYT	121.47	240.49	8.01	8.01	8.01
Sg 5M + ERYT	1.95	481.01	8.01	8.01	8.01
Sg 2M + ERYT	0.15	7.75	8.01	8.01	4.00
Sg 7D + ERYT	240.49	240.63	8.01	8.01	8.01
Sg 5D + ERYT	120.49	240.63	8.01	8.01	8.01
Sg 2D + ERYT	2.13	60.12	8.01	8.01	8.01

Source: Authors 2022

observed against Gram-negative bacteria (*E. coli*, *K. pneumonia* and *P. aeruginosa)*. The results of the combination generated by the model of the study are different from other previously published works (Torres et al., 2017). This may be attributed to different plant samples, different concentrations of bacterial used and strain-dependent factors. In recent research work, two synergistic effects have been observed against *S. aureus* in the combination root barks extracts of *C. swynnertonii* and *S. glaucescens* with erythromycin. In previous study, synergistic effects were reported as a key in phytomedicine research from organic extracts of *Indigofera suffruticosa* leaves with erythromycin against *S. aureus* (Santos et al., 2015). Other synergistic effects have been reported on the combination of penicillin and plant extract against methicillin-resistant *Staphylococcus aureus* (Kuok et al., 2017).

CONCLUSION AND RECOMMENDATIONS

The combinations of plant extracts and antibiotics which demonstrated synergism in this work may be considered as alternatives for treatment of infectious diseases caused by *E. coli*, *S. aureus* and *E. faecalis*. The present *in-vitro* model provides an easy and simple technique for the assessment of herbal-antibiotic combinations specifically when a single antibiotic is combined with the crude extract. The method can be a useful tool to help traditional users in the selections of appropriate combination therapy and consequently, avoid delay in starting treatment of severe infections. Further work needs to be performed involving a large number of clinical Gram-negative and Gram-positive bacteria with a wider section of antibiotics to verify the results obtained by the presented model and eventually, come up with more conclusive recommendations. Furthermore, toxicity studies for the combinations which demonstrated synergism are recommended in order to understand the safety of such combinations.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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