ANTIBACTERIAL EFFECTS OF SINGLE AND COMBINED CRUDE EXTRACTS OF *SYNADENIUM GLAUCESCENS* AND *COMMIPHORA SWYNNERTONII*

OCHOLLAH G. Mary¹*, MSENGWA S. Zaituni¹, MABIKI P. Faith¹, KUSILUKA J.M. Lughano², MDEGELA H. Robinson³ and OLSEN E. John⁴

¹Department of Chemistry and Physics, College of Natural and Applied Sciences, Sokoine University of Agriculture, P.O. Box 3038, Morogoro, Tanzania. ²Mzumbe University, P.O. Box 1, Morogoro, Tanzania. ³Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, P.O. Box 3015, Morogoro, Tanzania. ⁴Department of Veterinary and Animal Sciences, University of Copenhagen, Stigbøljen 4, Frederiksberg C. Denmark.

*Corresponding Author’s E-Mail: ochollahmary50@gmail.com*

**Abstract**

**Background:** *Synadenium glaucescens* and *Commiphora swynnertonii* are among the reported plants used traditionally for treatment of bacterial infections. This study reports antibacterial effects of single and combined extracts from leaves, stem and root barks of *Commiphora swynnertonii* and *Synadenium glaucescens*.

**Materials and Methods:** Plants were collected from Manyara and Njombe regions in Tanzania. Extraction was done using dichloromethane and methanol. The extracts were assessed for antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*). Minimum Inhibitory Concentrations (MIC) was determined by broth microdilution, while Fractional Inhibitory Concentration (FIC) indices were calculated from MIC values of combined extracts to determine combination effects.

**Results:** Strong antibacterial activities were demonstrated by all extracts of *S. glaucescens* (MIC 0.011-0.375mg/mL) against Gram-positive bacteria and methanol extracts of *C. swynnertonii* (MIC 0.047-0.375mg/mL). Synergistic effect was observed when combining methanol extracts of *C. swynnertonii* stem bark with *S. glaucescens* leaves against *S. aureus* (∑FIC 0.5). Other synergistic effects were observed against *E. faecalis* with dichloromethane extracts of *C. swynnertonii* stem bark and *S. glaucescens* stem bark (∑FIC 0.5), and *C. swynnertonii* root bark and *S. glaucescens* root bark (FIC index 0.3). For the remaining combinations, mainly additive effects were observed.

**Conclusion:** Synergistic effects on bacteria were observed by combining different plant parts of *S. glaucescens* and *C. swynnertonii* suggesting that it could be beneficial to combine such extracts when used for antibacterial purposes.

**Keywords:** *Synadenium glaucescens*, *Commiphora swynnertonii*, Antibacterial activity, Synergism, Antagonism, Additive and Crude extracts

**Abbreviations:** MIC= Minimum Inhibitory Concentration, FIC=Fractional Inhibitory Concentration, ATCC=AmericanType Culture Collection, DMSO=Dimethyl sulfoxide, CFU mL⁻¹=Colon Forming Unit per milliliter, mg/mL=Milligram per milliliter, *S. aureus=Staphylococcus aureus*, *E. faecalis= Enterococcus faecalis*, *E. coli=Escherichia coli*, *K pneumoniae=Klebsiella pneumonia*, *P. aeruginosa=Pseudomonas aeruginosa* + = combination, DCM/D= Dichloromethane crude extracts, MeOH/M= Methanol crude extracts, *Cs7=Commiphora swynnertonii* leaves extracts, *Cs5=Commiphora swynnertonii* stem bark extracts, *Cs2=Commiphora swynnertonii* root bark extracts, *Sg7= Synadenium glaucescens* leaves extracts, *Sg5=Synadenium glaucescens* stem bark extracts, *Sg2= Synadenium glaucescens* root bark extracts.
Introduction

Herbal products have been used as medicines since the commencement of human life (Masimba et al., 2014). The recipes for medicinal plant preparation for the treatment of several ailments are evidenced from the earliest Sumerian, Indian, Egyptian, and Chinese publications (Karunamoorthy et al., 2013). Unlike pharmaceuticals, where the ingredients are well defined and characterized, herbal products contain multiple bioactive compounds with little or no understanding of how these compounds function, likewise the effect of herbal combinations is usually poorly characterized (Gupta et al., 2017). When herbal combinations are administered together there is a possibility of causing chemical or pharmacological effects that may increase or decrease the effectiveness or severity of adverse effects via synergistic, additive, or antagonistic effects (Shi and Klotz, 2012; Sheng et al., 2018). In Tanzania, people access a variety of medicines to meet their healthcare needs. At least 70% of the population is estimated to use traditional medicines (Stanifer et al., 2015). Synadenium glaucescens (Munjakongwa in Swahili) and a tropical tree Commiphora Swynnertonii (Oltemwai in Masai) which belong to the families Euphorbiaceae and Burseraceae respectively are among the medicinal plants used by Tanzanians to treat various diseases in humans (Bakari et al., 2012; Mabiki et al., 2013; Mkangara et al., 2014). These plants contain secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, anthraquinones, steroids, and essential oils (Mabiki et al., 2013; Kalala et al., 2014). Such compounds are reported to have activity against infections caused by bacteria, fungi, viruses, and pests in humans and livestock (Bakari et al., 2012; Mabiki et al., 2013; Mkangara et al., 2014). Despite the exhibited potentials of some individual herbal drugs in the treatment of some infectious diseases, there are reported failures of most single drugs or medicines in the treatment of many pathogenic infectious diseases (Wang et al., 2021). The root causes of these hindrances are reported to be the development of anti-microbial resistance, a narrow antimicrobial spectrum, and limited activity of antimicrobials agents (Rubaka et al., 2014; Ayukkebong et al., 2017). As a result, these failures may cause an increase in the number of morbidities, mortality, disability, and socioeconomic costs (Stanifer et al., 2015). Therefore, there is a need for the search for novel antibacterial drugs from natural resources like herbs to combat the reported hindrances for antimicrobial activities (Bhardwaj et al., 2016). Due to synergistic effects resulting between the combination of more than one drugs in the treatment of microbial infections, it has been reported to be the best techniques to fight against hindrances for antimicrobial effects (Vuuren and Viljoen, 2011). Hence, this study focused on evaluation of antibacterial activities of combined extracts from leaves, stem barks, and root barks of S. glaucescens and C. swynnertonii. The results from this study, especially for the combinations which demonstrated synergistic effects, may be adopted for the treatment of bacterial infections. However, further study on safety for these combinations is highly recommended.

Materials and Methods
Study design and study Area

This study was an experimental one where the antibacterial effects of combinations of herbal medicines 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 leaves, stem, and root barks of Synadenium glaucescens were collected from Mtulingala village in Njombe region coordinates 08°34’ to 08°49’S and 08°34’ to 03°55’ E meters above sea level. The root barks, leaves, and stem barks of Commiphora swynnertonii were collected from Mirerani-Simanjiro District in Manyara region coordinates 03°36’ to 03°14.73’ S and 36°50’ to 36°18.05’ E meters above the sea level. Plant parts were washed with clean water then peeled to separate the barks and wood. Plant materials were dried in a dark room at 20°C at the Tanzania Tree Seed Agency Laboratory, Morogoro. Dry samples were grounded separately using a lab mill machine (Christy Hunt Engineering Ltd, England) to obtain approximately 2mm particle size. The selection of these plant parts was based on the previously conducted studies on antimicrobial activity against selected bacteria (Max et al., 2014; Mkangara et al., 2014).

Reagents

Solvents used for extraction and dissolving sample in this study were methanol (Finer Chemical, Gujarat-India), dichloromethane, and dimethyl Sulphoxide (Loba Chemie, Mumbai-India). The standard antibiotic used as positive control was gentamicin (Sigma-Aldrich, Germany).
Extraction and Concentration

Extraction of extracts were carried out using the method used by Bakari et al. (2012) and Max et al. (2014). Briefly, 1000g of dry ground plant materials were extracted by dichloromethane using hot continuous extraction method at 50°C for 4 hours whereby 33g of dry ground samples were injected into each thimble (33mm diameter, 80mm length) and extracted using Soxhlet apparatus. The samples were filtered and the obtained solid residues were soaked in methanol at room temperature (25-30°C) for 72 hours. All samples were filtered using Whatman No.1 filter paper (Maidstone-Kent, UK). The filtrates were concentrated in a rotary evaporator (Buchi Labortertechnik, Flawil, Switzerland) with a bath maintained at 40°C. The obtained crude extracts were air-dried to remove remains of solvents. The Dried extracts were stored in a refrigerator at 6°C until further use.

Test bacterial strain

Gram-positive bacteria used were Staphylococcus aureus American Type Culture Collection (ATCC 29213) and Enterococcus faecalis (ATCC 51559). Gram-negative bacteria used were Escherichia coli (ATCC 25922), Klebsiella pneumonia (ATCC 1145), Pseudomonas aeruginosa (ATCC 27853). These belong to species that are major causes of nosocomial infections, and where antimicrobial resistance is a high treat to human health (WHO, 2002).

Preparation of individual and combined crude extracts solutions

A stock concentration of 3 mg/ml crude extract from leaves, stem barks and root barks of S. glaucescens and C. Swynnertonii was made. Depending on the MIC value of each crude extract, the different concentrations were made to make working bench solutions. The extracts were combined in ratio 1:1v/v, 1:1v/v and 1:1:1v/v.

Minimum inhibitory concentrations (MIC) by broth dilution method

MIC values were determined by a two-fold microdilution method to assess the antibacterial effects of herb-herb combinations according to Kudumela et al. (2018). In brief, sterile, 96-well polystyrene microtiter plates was first preloaded with 50µL of Mueller Hinton broth in each well followed by the addition of 50µL of extract solutions into the first well of each row to make a total volume of 100µL. Each of the test sample materials was tested in duplicate. To the first well, the samples were mixed and 50µL was drawn from each well and transferred to the subsequent wells until the last wells. Then 50µL of the mixture from the last well was discarded. Thereafter, 50µL 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 0.1mg/ml of gentamicin (50µL) was used as a positive control. Wells containing (50µL) solvent 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 of bacteria was considered as the MIC.

Fractional inhibitory concentration (FIC)

Checkerboard assay was employed to determine the Fraction Inhibitory Concentration (FIC) as described (Jain et al., 2011). FIC is determined by a methodology similar to that utilized for the determination of MIC, however modified so that it is useful to test the antibacterial activities of combinations of extracts (Meletiadis et al., 2010). The summation of fractional inhibitory concentration (ΣFIC) was calculated for each tested sample independently as specified in the following algebraic formula (Kudumela et al., 2018).

FIC index = FIC Cs + FIC Sg

Where:

FIC Cs = \( \frac{\text{MIC value of } Cs \text{ in combination with } Sg \text{ crude extract}}{\text{MIC value of } Cs \text{ independently}} \)

FIC Sg = \( \frac{\text{MIC value of } Sg \text{ in combination with } Cs \text{ crude extract}}{\text{MIC value of the } Sg \text{ independently}} \)

Where the combined effect, was interpreted as synergistic if the FIC index ≤0.5, additive if 0.5 > FIC Index < 4, or antagonistic if FIC Index ≥ 4. This interpretation follows the conventional model suggested by (Odds, 2003) and Kassim et al., (2016).
Results

Antibacterial activity of individual extracts

The evaluations of antibacterial activities of individual extracts were conducted and the MIC of each extract was obtained as indicated in Table 1 and 2. The MIC values were interpreted based on classification criteria as follows: 0.05–0.5mg/mL strong activity, 0.6–1.5mg/mL moderate activity and above 1.5mg/mL 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 considerable with the lowest MIC values range 0.011 – 0.375mg/mL as shown in Table 1 and 2. Dichloromethane extracts of S. glaucescens and C. swynnertonii showed moderate antibacterial activity against Gram-positive bacteria tested with MIC values range 0.75mg/mL – 1.5mg/mL. Furthermore, all extracts showed weak activity against Gram-negative bacteria (Tables1 and 2). However, gentamicin showed stronger antibacterial activity than the extracts tested (Tables1 and 2).

Table 1: Minimum inhibitory concentration (mg/mL) of individual crude extracts of Commiphora swynnertonii tested against selected bacteria

| Extracts/Gentamicin | Gram-positive bacteria | Minimum inhibitory concentration (mg/mL) |
|---------------------|------------------------|----------------------------------------|
|                     | Gram-negative          |                                        |
|                     | bacteria               |                                        |
|                     | S. aureus ATCC 29213   | S. aureus ATCC 29213                    |
| Cs7D                | 0.37                   | 0.75                                   |
| Cs7M                | 0.09                   | 0.37                                   |
| Cs5D                | 0.75                   | 1.5                                    |
| Cs5M                | 0.18                   | 0.75                                   |
| Cs2D                | 1.5                    | 0.37                                   |
| Cs2M                | 0.04                   | 0.37                                   |
| Gentamicin          | 0.002                  | 0.002                                  |

Key: D= Dichloromethane extract, M= Methanol extract, Cs= Commiphora swynnertonii, Cs7= leaves extracts, Cs5= stem bark extracts, Cs2= root bark extracts.

Table 2: Minimum inhibitory concentration (mg/mL) of individual crude extracts of Synadenium glaucescens tested against selected bacteria

| Extract/Gentamicin | Gram-positive bacteria | Minimum inhibitory concentration (mg/mL) |
|---------------------|------------------------|----------------------------------------|
|                     | Gram-negative          |                                        |
|                     | bacteria               |                                        |
|                     | S. aureus ATCC 29213   | S. aureus ATCC 29213                    |
| Sg7D                | 0.75                   | 0.75                                   |
| Sg7M                | 0.37                   | 0.37                                   |
| Sg5D                | 0.37                   | 0.75                                   |
| Sg5M                | 0.02                   | 1.5                                    |
| Sg2D                | 0.02                   | 0.37                                   |
| Sg2M                | 0.01                   | 0.02                                   |
| Gentamicin          | 0.002                  | 0.002                                  |

Key: D= Dichloromethane extract, M= Methanol extract, Sg=Synadenium glaucescens, Sg7=leaves extracts, Sg5=stem bark extracts, Sg2= root bark extracts

Antibacterial activity of combined crude extracts and fractional inhibitory concentrations

The combination effects were evaluated with respect to MIC value of each crude extract against bacteria. In the combination of 1:1v/v, the extracts exhibited strong activity against Gram-positive bacteria S. aureus and E. faecalis with MIC values ≤0.5 (Table 3). These combinations include methanol extracts of C. swynnertonii leaves and stem barks of S. glaucescens, C. swynnertonii leaves and root barks of S. glaucescens, stem barks of C. swynnertonii and S. glaucescens leaves, stem barks of C. swynnertonii stem barks of S. glaucescens, stem barks of C. swynnertonii and root barks of S. glaucescens, root barks of C. swynnertonii and S. glaucescens leaves, root barks of C. swynnertonii and stem barks of S. glaucescens, and root barks of C. swynnertonii and root barks S. glaucescens.
However, crude extracts combined in ratios 1:1:1 and 1:1:1:1v/v revealed moderate activity against *S. aureus* with MIC values range 0.6-1.5mg/mL (Table 3). Additionally, these combinations exhibited weak antimicrobial activity with MIC values above 1.5 mg/mL (Table 3) against the tested gram-negative bacteria *E. coli, K. pneumoniae* and *P. aeruginosa*. The FIC values were calculated and antibacterial effects were outlined in Table 4. In 1:1v/v combinations, One (1) synergistic effect observed in combination of methanol extracts of *C. swynnertonii* stem barks and *S. glaucescens* leaves against *S. aureus* (∑FIC 0.5) (Table 4). Other two synergistic effects were observed against *E. faecalis* in dichloromethane extracts of *C. swynnertonii* stem barks and *S. glaucescens* stem barks (∑FIC 0.5), and *C. Swynnertonii* root barks and *S. glaucescens* root barks with FIC index 0.3 (Table 4). Furthermore, three (3) antagonistic effects were observed in the combinations of dichloromethane leaves extract of *C. swynnertonii* and root barks of *S. glaucescens*, stem barks of *C. swynnertonii* and root barks of *S. glaucescens*, and root barks of *C. swynnertonii* and root barks of *S. glaucescens* against *S. aureus* with MIC values range 0.6 (Table 4). In addition, other antagonistic effects were observed against *E. faecalis* in combinations of methanol leaves extract of *C. swynnertonii* and *S. glaucescens* leaves, stem barks of *C. swynnertonii*, and root barks of *S. glaucescens*, and leaves of *C. swynnertonii* and root barks of *S. glaucescens* with FIC Index values 10 and 19 (Table 4). The 1:1:1v/v and 1:1:1:1v/v combination ratios revealed antagonistic effects against *S. aureus* and additive effects against *E. faecalis* (Table 4). Moreover, the extracts in the combination ratio of 1:1v/v and 1:1:1v/v tested against Gram-negative bacteria revealed additive effects with FIC Index value 2 (Table 4), whereby the extracts in the combination ratio of 1:1:1:1v/v showed different antagonistic effects against Gram-negative bacteria with FIC Index values 4, 5, 6 and 8 (Table 4).

| Combinations | Minimum inhibitory concentration (mg/mL) |
|--------------|----------------------------------------|
|              | Gram-positive bacteria                  | Gram-negative bacteria |
|              | *S. aureus* ATCC 29213                  | *E. faecalis* ATCC 1559 |
|              | *E. coli* ATCC 25922                    | *K. pneumoniae* ATCC 1145 |
|              | *P. aeruginosa* ATCC 27853              |
| Cs7D+Sg7D    | 1.5 0.75 3 3 3                         |                           |
| Cs7M+Sg7M    | 0.07 0.75 3 3 3                       |                           |
| Cs7D+Sg5D    | 0.37 0.37 3 3 3                       |                           |
| Cs7M+Sg5M    | 0.02 0.07 3 3 3                       |                           |
| Cs7D+Sg2D    | 0.75 0.28 3 3 3                       |                           |
| Cs7M+Sg2M    | 0.01 0.21 3 3 3                       |                           |
| Cs7M+Sg7M+Sg5M | 0.75 0.37 3 3 3           |                           |
| Cs7M+Sg7M+Sg5M+Sg2M | 0.09 0.37 3 3 3 |                           |
| Cs5D+Sg7D    | 0.75 0.75 3 3 3                       |                           |
| Cs5M+Sg7M    | 0.07 0.14 3 3 3                       |                           |
| Cs5D+Sg5D    | 0.37 0.28 3 3 3                       |                           |
| Cs5M+Sg5M    | 0.11 0.14 3 3 3                       |                           |
| Cs5D+Sg2D    | 0.75 0.11 3 3 3                       |                           |
| Cs5M+Sg2M    | 0.19 0.39 1.5 3 1.5                   |                           |
| Cs5M+Sg7M+Sg5M | 0.18 0.75 3 3 3           |                           |
| Cs5M+Sg7M+Sg5M+Sg2M | 0.18 0.37 3 3 3 |                           |
| Cs2D+Sg7D    | 0.75 0.56 3 3 3                       |                           |
| Cs2M+Sg7M    | 0.05 0.37 1.5 3 3                   |                           |
| Cs2D+Sg5D    | 0.37 0.56 3 3 3                       |                           |
| Cs2M+Sg5M    | 0.008 0.14 1.5 3 3                   |                           |
| Cs2D+Sg2D    | 0.75 0.18 3 3 3                       |                           |
| Cs2M+Sg2M    | 0.01 0.05 0.75 3 3                   |                           |
| Cs2M+Sg7M+Sg5M | 0.09 0.18 3 3 3           |                           |
| Cs2M+Sg7M+Sg5M+Sg2M | 0.09 0.04 3 3 3 |                           |

Key: + = combination, D= Dichloromethane crude extracts, M= Methanol crude extracts, Cs7 Commiphora swynnertonii leaves extracts, Cs5 Commiphora swynnertonii stem bark extracts, Cs2 Commiphora swynnertonii root bark extracts, Sg7 Synadenium glaucescens leaves extracts, Sg5=Synadenium glaucescens stem bark extracts, Sg2 Synadenium glaucescens root bark extracts.
Table 4: Fractional inhibitory concentration Index (FIC Index) of combined crude extracts from Commiphora swynnertonii and Synadenium glaucescens tested against selected bacteria.

| Combinations                        | Gram-positive bacteria | Gram-negative bacteria |
|-------------------------------------|------------------------|------------------------|
|                                     | S. aureus ATCC 29213   | E. faecalis ATCC 1559  |
|                                     |                        | E. coli ATCC 25922     |
|                                     |                        | K. pneumoniae ATCC 1145|
|                                     |                        | P. aeruginosa ATCC 27853|

|                          | Sg7                        | Cs5                        | Cs2                      | Cs7                        |
|--------------------------|----------------------------|-----------------------------|--------------------------|-----------------------------|
| Cs7D+5g7D                |                            | 6.1                         | 3                        | 2                           |
| Cs7M+Sg7M                |                            | 1.8                         | 2                        | 2                           |
| Cs7D+Sg5D                | 2                          | 1.3                         | 2                        | 2                           |
| Cs7M+Sg5M                | 1.2                        | 0.6                         | 2                        | 2                           |
| Cs7D+Sg2D                | 3                          | 1.1                         | 2                        | 2                           |
| Cs7M+Sg2M                | 1.2                        | 1.3                         | 2                        | 2                           |
| Cs7M+Sg7M+Sg5M           | 47.8                       | 2.2                         | 3                        | 3                           |
| Cs7M+Sg7M+Sg5M+Sg2M      | 14.7                       | 20.5                        | 5                        | 4                           |
| Cs5D+Sg7D                | 38.5                       | 1.1                         | 2                        | 2                           |
| Cs5M+Sg7M                | 0.5                        | 1.2                         | 2.5                      | 2                           |
| Cs5D+Sg5D                | 38                         | 0.5                         | 2                        | 2                           |
| Cs5M+Sg5M                | 0.7                        | 2.2                         | 3                        | 2                           |
| Cs5D+Sg2D                | 38.5                       | 0.6                         | 2                        | 2                           |
| Cs5M+Sg2M                | 1.2                        | 0.9                         | 2                        | 2                           |
| Cs5M+Sg7M+Sg5M           | 10.4                       | 3.5                         | 4                        | 3                           |
| Cs5M+Sg7M+Sg5M+Sg2M      | 28.4                       | 20.4                        | 5                        | 4                           |
| Cs2D+Sg7D                | 1.5                        | 1.6                         | 2                        | 2                           |
| Cs2M+Sg7M                | 2.2                        | 3.4                         | 1                        | 2                           |
| Cs2D+Sg5D                | 5.2                        | 1.6                         | 2                        | 2                           |
| Cs2M+Sg5M                | 19.2                       | 19.8                        | 1.5                      | 2                           |
| Cs2D+Sg2D                | 1.4                        | 0.3                         | 1.5                      | 2                           |
| Cs2M+Sg2M                | 2.2                        | 10.6                        | 2.2                      | 2                           |
| Cs2M+Sg7M+Sg5M           | 69                         | 1.1                         | 3                        | 3                           |
| Cs2M+Sg7M+Sg5M+Sg2M      | 15.9                       | 2.2                         | 5                        | 4                           |

Key: + = combination, D=Dichloromethane crude extracts, M=Methanol crude extracts, Cs7 Commiphora swynnertonii leaves extract, Cs5 Commiphora swynnertonii stem bark extracts, Cs2 Commiphora swynnertonii root bark extracts, Sg7= Synadenium glaucescens leaves extract, Sg5=Synadenium glaucescens stem bark extracts, Sg2 Synadenium glaucescens root bark extracts

Discussion

Antibacterial activity of individual and combined crude extracts

Herbal medicines are normally prepared either singly or in combination with several plant species (Vuuren and Viljoen, 2011). In this study, crude extracts from leaves, stem barks, and root barks of C. swynnertonii and S. glaucescens were screened for antibacterial properties both individually and in combinations against selected bacteria. The findings of this study for the individual plant parts of C. swynnertonii are in agreement with previous studies reported by Bakari et al. (2011) and Mkangara et al. (2014).

Bakari et al. (2011) confirmed antibacterial and anti-Candida activities of the methanol extracts of the leaves from stem and root barks of C. swynnertonii, and Mkangara et al. (2014) reported the activity of the same parts of the plant against pathogenic bacterial and fungal species. Hence, the results of this study together with those previously reported supporting the traditional uses of these plant parts for the management of bacterial and fungal infections.

Furthermore, a previous study conducted by Max et al. (2014) for the crude root extract of S. glaucescens reported antibacterial activity against S. aureus and moderate activity against P. aeruginosa. Similarly, in the current study individual methanol extracts of the parts of S. glaucescens showed strong activity against S. aureus and E. faecalis.
In this study, however, the individual extracts of these plant parts displayed weak activity against Gram-negative bacteria tested. The difference in susceptibility for Gram-positive bacteria and Gram-negative bacteria may be associated with differences in their cell wall structure. Gram-negative bacteria are reported to be more resistant due to impermeability/eflux of their outer membrane/cell wall which acts as a barrier to many environmental substances including herbal drugs or antibiotics (Rawat and Nair, 2010).

Moreover, this study reports the antibacterial effects of combined crude extracts of *S. glaucescens* and *C. swynnertonii*. It is clear from Table 4 that there is a greater antibacterial activity in some combined extracts than individual extracts. The combined extracts which showed synergistic effects may be promising alternatives for antibacterial therapy in the future, and their effects should be investigated further. Several synergistic effects of herb-herb combinations done in different plants have been reported in previous studies. Rapper *et al.* (2016) substantiated this point of synergy in the combinations of *Schkuhria pinnata* and *Commelina africana*, *Dombeya rotundifolia*, and *Schkuhria pinnata* against *P. aeruginosa* with $\sum \text{FIC}$ values $\leq 0.5$. Another synergic effects were demonstrated in the combinations of *Bidens pilosa* and *Leonotis nepetifolia* extracts against *Candida albicans* (Mbunde *et al.*, 2019). The synergistic effects observed in some combinations (Table 4) imply that there is an increase in antibacterial activity of the combined crude extracts against Gram-positive bacteria as a result of the summation of their individual effects.

However, in this study additive effects were also demonstrated in several combinations (Table 4). This effect occurs when the activity of the combined extracts is equivalent to the sum of the activity of each extract when used individually (Adams *et al.*, 2006). This effect signifies that the biological actions of the combined extracts interact with similar molecular targets or metabolic pathways (Vuuren and Viljoen, 2011). Antagonistic effects were also observed in some combinations against the tested bacteria (Table 4). This indicates that, the extracts have conflicting effect that may block or reduce the effectiveness of one or both extracts. Usually, this type of effect is discouraged for therapeutic application (Bassolé and Juliari, 2012).

**Conclusion**

Combined extracts of *S. glaucescens* and *C. swynnertonii* have additive effects against gram-positive bacteria tested. Further, combined extracts of root barks of *C. swynnertonii* and stem barks of *S. glaucescens* have synergistic effect against gram-positive bacteria tested, suggesting that it can be advantageous to combine such extracts to form their products.

Therefore, based on the combinations which showed synergistic effects against some of the tested bacteria, this study provides promising alternative herbal antimicrobials from plants. However, it is recommended that further studies on the combinations that showed synergistic effects should be carried out on their toxicity and mode of action to optimize their use.

**Conflict of interest statement**
The authors declare that they have no conflict of interest associated with this study.

**Acknowledgements**

The authors wish to thank the Green Resources Innovations for Livelihood Improvement (GRILI) project from the Danish International Development Agency (DANIDA) for funding this study. We thank Microbiology laboratory in the Department of Biosciences, (SUA), and Muhimbili University of Health and Allied Sciences (MUHAS), for providing bacterial strains used in this study.

**References**

1. Adams, L. S., Seeram, N. P., Hardy, M. L., Carpenter, C. and Heber, D. (2006). Analysis of the interactions of botanical extract combinations against the viability of prostate Cancer Cell Lines. *Evidence-Based Complementary and Alternative Medicine*, 3(1), 117–124.
2. Ayukekpong A James, Michel, N. and N., A. A. (2017). The threat of antimicrobial resistance in developing countries: Causes and control strategies. *Antimicrobial Resistance and Infection Control*, 6(1), 1–8.
3. Bakari, G. G., Max, R. A., Mdegea, R. H., Phiri, E. C. J. and Mtambo, M. A. (2012). Antiviral activity of crude extracts from Commiphora swynnertonii against Newcastle disease virus in ovo. *Tropical Animal Health and Production*, 44, 1389–1393.
4. Bakari, G. G., Max, R. A., Mdegea, R. H., Phiri, E. C. J. and Mtambo, M. A. (2011). Antibacterial and Antifungal Activity of Commiphora swynnertoni Against Selected Pathogens of Public Health Importance, *Research Journal of Biological Sciences* 6, 175–179.
5. Bassolé, I. H. N. and Juliani, H. R. (2012). Essential oils in combination and their antimicrobial properties. *Molecules*, 17(4), 3989–4006.
6. Bhardwaj, M., , Singh, B. R., Sinha, D., V. K. and Vadhana, P. (2016). Potential of Herbal Drug and Antibiotic Combination Therapy: A New Approach to Treat Multidrug Resistant Bacteria. *Pharmaceutica Analytica Acta*, 15
7(11), 1–14.

7. Gupta, R. C., Chang, D., Nammi, S., Bensoussan, A., Bilinski, K. and Roufogalis, B. D. (2017). Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action and clinical implications. *Diabetology & Metabolic Syndrome*, 9(59), 1–12.

8. Jain, S. N., Vishwanatha, T., Reena, V., Divyashree, B. C., Sampath, A., Siddhalingeshwara, K. G., Venugopal, N. and Ramesh, I. (2011). Antibiotic Synergy Test: Checkerboard Method on Multidrug Resistant Pseudomonas Aeruginosa. *International Research Journal of Medicinal Plant Research*, 2(12), 196–198.

9. Kalala, W., Magadula, J. and Mdegele, R. (2014). Ethnobotanical use of *Commiphora swynertonii*, amongst Dorobo people in Tanzania. *Journal of Medicinal Plant Research*, 8(23), 820–828.

10. Kununamoothi, K., Jegajeevanram, K., Vijayalakshmi, J. and Mengistie, E. (2013). Traditional Medicinal Plants: A Source of Phytotherapeutic Modality in Resource-Constrained Health Care Settings. *Journal of Evidence-Based Complementary and Alternative Medicine*, 18(1), 67–74.

11. Kassim, A., Omuse, G., Premji, Z. and Revathi, G. (2016). Comparison of Clinical Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing guidelines for the interpretation of antibiotic susceptibility at a University teaching hospital in Nairobi, Kenya: a cross-sectional. *Annals of Clinical Microbiology and Antimicrobials*, 15(1), 1–7.

12. Kudumela, R. G., Mcgaw, L. J. and Masoko, P. (2018). Antibacterial interactions, anti-inflammatory and cytotoxic effects of four medicinal plant species. *BMC Complementary and Alternative Medicine*, 18(1), 1–7.

13. Mabiki, F. P., Mdegele, R. H., Mosha, R. D. and Magadula, J. J. (2013). Antiviral activity of crude extracts of Synadenium glaucescens (Pax) against infectious bursal disease and fowlpox virus. *Journal of Medicinal Plant Research*, 7(14), 871–876.

14. Masimba, P. J., Magadula, J. J., Msengwa, Z., Tarimo, R. B., Mbwanzo, Z. H., Heydenreich, M., Breard, D., Richomme, P. and Alla Fadal Fadal, E. (2014). Biological Potentials of Extracts and Compounds. *Journal of Advanced Scientific Research*, 5(3), 07–12.

15. Max, R. A., Mwageni, C. and Bakari, G. G. (2014). Effect of crude root extract from Synadenium glaucescens on selected bacterial infections in albino mice (Mus musculus). *Journal of Medicinal Plant Research*, 8(26), 915–923.

16. Mbunde, M., Mabiki, F., Andersson, P. G. and Innocent, E. (2019). Antifungal activity of single and combined extracts of medicinal plants from Southern Highlands of Tanzania. *Journal of Pharmacognosy and Phytochemistry*, 8(1), 181–187.

17. Meletiadis, J., Pournaras, S., Roilides, E. and Walsh, T. J. (2010). Defining Fractional Inhibitory Concentration Index Cutoffs for Additive Interactions Based on Self-Drug Additive Combinations, Monte Carlo Simulation Analysis, and In Vitro-In Vivo Correlation data for Antifungal Drug Combinations against Aspergillus fumi. *Antimicrobial Agents and Chemotherapy*, 54(2), 602–609.

18. Mkangara, M., Chacha, M. and Kazyoba, P. (2014). Antimicrobial and Cytotoxicity Efficacy of *Commiphora swynertonii* Extracts. *International Journal of Science and Research*, 3(7), 1611–1615.

19. Odds, F. C. (2003). Synergy, antagonism, and what the checkerboard puts between them. *Journal of Antimicrobial Chemotherapy*, 52(1), 1.

20. Rapper, de S., Avaro, V. and Sandy, V. van. (2016). The in vitro Antimicrobial Effects of Lavandula angustifolia Essential Oil in Combination with conventional Antimicrobial Agents. *Evidence-Based Complementary and Alternative Medicine*, 2016(1), 1–9.

21. Rawat, D. and Nair, D. (2010). Extended-spectrum β-lactamases in gram negative bacteria. *Journal of Global Infectious Diseases*, 2(3), 263–273.

22. Rubaka, C., Ndakidemi, P., Malebo, H. M. and Francis, S. (2014). Individual and Combined Antibacterial Activity of Crude Extracts from Medicinal Plants Carissa spinarum Linn and Carica papaya Linn. *European Journal of Medicinal Plants*, 4(12), 1514–1523.

23. Sartoratto, A., Machado, A. L. M., Delarmelina, C., Figueira, G. M., Duarte, M. C. T. and Rehder, V. L. G. (2004). Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Brazilian J Microbiol*. 2004;35(4):275–80. Composition and antimicrobial activity. *Brazilian Journal of Microbiology*, 35(4), 275–280.

24. Sheng, Z., Sun, Y., Yin, Z., Tang, K. and Cao, Z. (2018). Advances in computational approaches in identifying synergistic drug combinations. *Briefings in Bioinformatics*, 19(6), 1172–1182.

25. Shi, S. and Klotz, U. (2012). Drug interactions with herbal medicines. *Clinical Pharmacokinetics*, 51(2), 77–104.

26. Stanifer, J. W., Lunyera, J., Boyd, D., Karia, F., Maro, V., Omolo, J. and Patel, U. D. (2015). Traditional medicine practices among community members with chronic kidney disease in northern Tanzania: An ethnomedical survey. *BMC Nephrology*, 16(1), 1–11.

27. Vuuren, S. Van, and Viljoen, A. (2011). Plant-based antimicrobial studies methods and approaches to study the interaction between natural products. *Planta Medica*, 77(11), 1168–1182.

28. Wang, Y., Yang, H., Chen, L., Jafari, M. and Tang, J. (2021). Network-based modeling of herb combinations in traditional Chinese medicine. In *Briefings in Bioinformatics* 00(00).

29. WHO. (2002). World Health Organization. In Prevention of hospital-acquired infections: a practical guide: Vol. No. 12, WHO/CD. World Health Organization.