INVESTIGATION OF ANTIBACTERIAL EFFECTS OF HERB-HERB AND HERB-ANTIBIOTIC COMBINATIONS

Thesis submitted to Sokoine University of Agriculture in Fulfilment of the Requirements for the Degree of Masters of Science in Phytochemistry

By

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EXTENDED ABSTRACT

Introduction

Several studies have reported the failure of single-drug treatment regimens for bacterial infections due to emergence of bacterial resistance. Hence, there has been a growing interest in researching and developing new antibacterial drugs, containing several combined ingredients as one of the means to combat bacterial resistance. Among the successful reported antibacterial combination treatments include herb-herb and herb-antibiotic. This study investigated the antibacterial effects of single and combined extracts from leaves, stems and root barks of *Commiphora swynnertonii* and *Synadenium glaucescens.* In addition, the study evaluated antibacterial effects of combined crude extracts of two medicinal plants, also the extracts were combined with selected three antibiotics namely ciprofloxacin, ampicillin, and erythromycin.

Methods

C. swynnertonii and *S. glaucescens* were respectively collected from Manyara and Njombe regions of Tanzania. The standard antibiotics used as positive control in this study were gentamicin, ciprofloxacin, ampicillin, and erythromycin. Extraction of plant materials was done by soxhlet and macerations methods using dichloromethane and methanol solvents respectively. The extracts were assessed for their effects on 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 micro dilution method while Fractional inhibitory concentration (FIC) indices were calculated from MIC values of individual and combined crude extracts to determine combination effects.

Findings

Strong antibacterial activities were demonstrated by all extracts of *S. glaucescens* (MIC 0.01-0.37mg/mL) and methanol extracts of *C. swynnertonii* (MIC 0.04-0.37mg/mL) against Gram-positive bacteria Synergistic effect was observed in combination of methanol extracts of *C. swynnertonii* stem barks and *S. glaucescens* leaves against *S.aureus* (∑FIC 0.5), other synergistic effects were observed against *E. faecalis* in combination of 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 (FIC index 0.3). For the remaining combinations of crude extracts, mainly additive effects were observed. The combinations of crude extracts with antibiotics revealed synergism 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 *S. 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*. Nevertheless, antagonism was observed between the combinations of ampicillin and erythromycin with all tested crude extracts against Gram-negative bacteria (ΣFIC of 4-8).

Conclusions

When two or more plant parts are combined among themselves or with antibiotics, the resulting antibacterial effects may either be synergistic, additive or antagonistic depending on the phytochemical contribution of each plant or part and also the bacteria or diseases intended.

Recommendations

Synergistic effects demonstrated in this study provide promising good combinations to be considered as alternative antimicrobials. Toxicity studies of the combinations of *S. glaucescens* stem barks of and *C. swynnertonii* leaves and *S. glaucescens* root barks and *C. swynnertonii* root barks which showed synergistic effects against Gram-positive bacteria are recommended in the future.

Keywards: *Synadenium glaucescens*, *Commiphora swynnertonii* Antibacterial activity, Crude extracts Synergy, Additive and Antagonism

IKISIRI KUU

Mada ya utafiti

Kuchunguza uwezo wa mchanyiko wa dawa za asili pekee, pia, dawa za asili na viuavijasumu katika kudhibiti ukuaji wa vimelea jamii ya bakteria.

Utangulizi

Tafiti za hivi karibuni zimeripoti kushindwa kwa mfumo wa kutumia dawa ya aina moja katika kudhibiti au kutibu maambukizi ya magonjwa yanayosababishwa na vimelea jamii ya bakteria. Hivyo, kumekuwa na shauku kubwa ya kugundua madawa yatakayojumuisha viambata kadhaa kwa pamoja kama njia mojawapo ya kupambana na usugu wa vimelea vya magonjwa dhidi ya madawa tajwa. Miongoni mwa dawa jumuishi zilizoripotiwa kuwa na matokeo mazuri juu ya hili tazizo ni pamoja na zile zenye mchanganyiko wa mimea pekee na zile zenye mchanganyiko wa mimea na viuavijasumu.

Malengo ya utafiti

Utafiti huu ulijikita katika kuchunguza uwezo wa viambata asilia vilivyotokana na majani, magome ya mizizi na mashina ya Mvunjakongwa na Mponda katika kupambana na bakteria vinavyosababisha vimelea vya magonjwa ya kuambukiza kwa binadamu. Aidha, utafiti huu pia uliweza kutathimini uwezo wa kudhibiti bakteria tajwa ikiwa mchanganyiko wa viambata vitokanavyo na mimea-dawa tajwa na viuavijasumu vitatu, zitachanganywa kwa pamoja. Viuavijasumu tulivyovitumia kwenye utafiti huu ni *gentamicin, ciprofloxacin, ampicillin* na *erythromycin*.

Methodolojia

Tulikusanya majani, magome ya mizizi na ya mashina ya Mponda na Mvunjakongwa kutoka mikoa ya Manyara na Njombe mtawalia. Viuavijasumu tulizotumia kwenye utafiti huu ni *gentamicin, ciprofloxacin, ampicillin* na *erythromycin*. Sehemu tatu za mimea zilikusanywa na kuandaliwa kwa njia ya kuchemsha sampuli (Soxhlet) na kuloweka (maceration) kwa kutumia kemikali aina ya *dichloromethane* na *methanol*. Viambata vyote vilichunguzwa kuangalia ufanisi wa mimea-dawa dhidi ya bakteria umbichanya na umbihasi kwa njia ya *broth micro dilution,* kwa kutumia *Minimum Inhibitory Concentrations (MIC)* wakati *Fractional inhibitory concentration* (*FIC*) indices zilipatikana kwa kukokotoa thamani ya *MIC* ya kila kiambata ghafi ili kuona uwezo wa michanganyo husika.

Matokeo

Matokeo ya maabara yalionyesha kuwa viambata vya Mvunjakongwa vimeweza kuzuia ukuaji wa bakteria umbichanya katika *MIC* kuanzia 0.01-37 mg/mL, ikifuatiwa na viambata vya Mponda ambavyo vimeweza kuzuia ukuaji wa aina hiyo ya bakteria kuanzia 0.04-0.37 mg/mL. Hata hivyo, matokeo haya yanaonyesha kwamba viambata vyote vya mimea yote miwili vilishindwa kudhibiti ukuaji wa bakteria umbihasi. Uwezo mkubwa (*synergistic effect*) ulionekana tulipochanganya viambata vya magome ya shina la Mponda na majani ya Mvunjakongwa dhidi ya *S. aureus* kwa kiwango cha *FIC index* 0.5. Mchanganyiko wa magome ya mashina na mizizi ya mimea yote miwili ulikuwa na mafanikio makubwa dhidi ya *E. faecalis* (*FIC index* 0.5 na 0.3). Mchanganyiko wa viambata ghafi na *ciprofloxacin* ulikuwa wa mafanikio makubwa dhidi ya *E. coli, S. aureus* na *E. faecalis* (*FIC index* 0.02-0.5). Mchanganyiko wa *ampicillin* na viambata vya mimea ulikuwa na mafanikio makubwa dhidi ya *E. facalis* (*FIC index* 0.03-0.1). Mwisho, udhaifu ulijionesha katika mchanganyiko wa viambata vya mimea-dawa na *erythromycin* (*FIC* 4-8). Kiujumla matokeo ya michanganyiko ya mimeadawa pekee na ile ya mimeadawa na viuavijasumu ilionesha uwezo wa kawaida katika kudhibiti bakteria.

Hitimisho

Kutokana na tasnifu hii, tunajifunza kwamba, tunapochanganya sehemu mbili au zaidi za mimea tofautitofauti, uwezo wa mchanganyiko unaweza kuwa na mafanikio makubwa, ya wastani au dhaifu. Matokeo yatategemea aina za mimea iliyochanganywa au sehemu ya mimea iliyochanganywa (mfano, majani, magome au mizizi), na aina za vimelea vya magonjwa yaliyolengwa kudhibitiwa.

Mapendekezo

Utafiti huu unashauri kuangalia usalama kwenye mchanganyiko ulioonyesha matokeo mazuri zaidi kwasababu dawa inaweza kuwa na uwezo wa kudhibiti ukuaji wa bakteria lakini isiwe salama kwa matumizi ya binadamu na wanyama wengine.

Maneno muhimu: Mvunjakongwa, Mponda, Viuavijasumu, Viziduzi Uwezo mkubwa, Uwezo wa kawaida na uwezo dhaifu

DECLARATION

I, Mary George Ochollah, do hereby declare to the Senate of the Sokoine University of Agriculture that this thesis is my original work done within a period of registration and that it has neither been submitted nor being concurrently submitted in any other institutions.

…….. …….

Mary George Ochollah **Date**
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The above declaration confirmed by:

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LIST PAPER AND MANUSCRIPT

I. **Mary G. Ochollah, Zaituni S. Msengwa, Faith P. Mabiki, Lughano J.M. Kusiluka, Robinson H. Mdegela and John E. Olsen.** ANTIBACTERIAL EFFECTS OF SINGLE AND COMBINED CRUDE EXTRACTS OF *SYNADENIUM GLAUCESCENS* AND *COMMIPHORA SWYNNERTONII. African Journal of Infectious Diseases.* **2022; 16 (2S):9-16**

II. **Mary G. Ochollah, Faith P. Mabiki, Lughano J.M. Kusiluka, Robinson H. Mdegela and John E. Olsen.** ANTIBACTERIAL EFFECTS OF COMBINED CRUDE EXTRACTS OF *SYNADENIUM GLAUCESCENS* AND *COMMIPHORA SWYNNERTONII* WITH ANTIBIOTICS*. Journal of Medecinal Plant Research JMPR-12.05.22-7241*

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DEDICATION

This work is dedicated to my family, my mother Dorice A. Ochollah, my sister Dorca, my brothers Gordon, Boniface, James, and Daniel.

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LIST OF ABBREVIATIONS AND ACRONYMS

FIC	Fractional Inhibitory Concentration
MIC	Minimum Inhibitory Concentration
ATCC	American Type Culture Collection
Sg	*Synadenium glaucescens*
*Sg*7	Leaves extracts of *Synadenium glaucescens*
*Sg*5	Stem barks extracts of *Synadenium glaucescens*
*Sg*2	Root barks extracts of *Synadenium glaucescens*
Cs	*Commiphora swynnertonii*
*Cs*7	Leaves extracts of *Commiphora swynnertonii*
*Cs*5	Stem barks extracts of *Commiphora swynnertonii*
Cs2	Root extracts of *Commiphora swynnertonii*
mg/ml	Milligram per millilitre
DMSO	Dimethyl sulfoxide
CFU mL^{-1}	Colon Forming Unit per milliliter
S. aureus	*Staphylococcus aureus*
E. faecalis	*Enterococcus faecalis*
E. coli	*Escherichia coli*
K. pneumoniae	*Klebsiella pneumoniae*
P. aeruginosa.	*Pseudomonas aeruginosa*
DCM/D	Dichloromethane
MeOH/M	Methanol
UK	United Kingdom

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background information

Medicinal plants and their products have been used for treatment of various diseases including bacterial infectious diseases since time immemorial (Sofowora *et al*., 2013). The different plant species produce phytochemical compounds such as alkaloids, flavonoids, phenols, terpenoids, steroids, glycosides, tannins, carotenoids, essential oil, phenolic and anthraquinones that function as defense substances against pathogenic microbes (Sabandar *et al*., 2013).

Medicinal plants and their products have been used either individually (herb), combination of herbs, or combination of herb(s) and drug(s) (Che *et al*., 2013). The use of herbal products has increased dramatically in recent years particularly in developing countries (Ekor, 2014). The demand for medicinal plants is likely to continue rising due to the belief that they are safe and environmentally friendly (Thomford *et al*., 2015). Herbal medicines are cheap, easily available and very effective and easier incorporated into the body since they contain more than one compound/ingredient (Sam, 2019).

For decades of use, conventional drugs such as antibiotics have proven to be effective therapy against bacterial infection that remain the standard for healthcare today (North and Brown, 2021). However, extensive use of these antibiotics not only inhibits bacterial growth but also promotes the emergence and spread of antimicrobial resistance (English and Gaur., 2010). The problem of antibiotic resistance in bacteria is increasing throughout the world (Frieri *et al*., 2017). This rise is accompanied by a sharp decline in the discovery of new antibiotics (Carlet *et al*., 2012).

Resistance mechanisms in bacteria include, the ability to utilize an influx mechanism to remove antibiotics (Abushaheen *et al*., 2020) and an ability to block the antibiotic mode of action (Sheard *et al*., 2019). A common mechanism of resistance is modification of the target site, which allows the bacteria to literally metabolize the drug by modifying its binding site (Wright, 2011) or produce an enzyme to degrade the antibiotic (Guo *et al*., 2011).

In environments where bacteria are continuously exposed to antibiotics such as health care facilities or livestock keeping facilities, therapeutic

combinations of either herb-herb, herb-antibiotic, or antibiotic-antibiotic may be advantageous for treatments of infectious diseases (Chanda and Rakholiya, 2011). Researchers have found that, the use of therapeutic combinations may remove the selective advantage of bacterial resistance (Baym *et al.*, 2016) or impose a high cost on resistance evolution by exertion of multiple selection pressure targeting different cellular processes simultaneously (Baloch *et al*., 2018).

In addition, therapeutic combinations exert strong genetic background of antibacterial effects and evolutionary stability against bacterial resistance, and this condition is termed as synergism (Torres *et al*., 2017). Therefore, the therapeutic combinations of herb-herb and herb-antibiotic could be used as weapons for fighting bacterial infectious diseases in different communities where antimicrobial resistance is a challenge.

1.2 Antibacterial effects of herb-herb and herb-antibiotic combinations

Drug combination therapy, refers to treatment regimens that involve the administration of two or more drugs together or sequentially to patients (Sheng *et al*., 2018). When two or more drugs are administered together, there is a possibility of causing chemical or pharmacological effects that might increase or decrease the effectiveness or severity of adverse effects (Shi and Klotz, 2012).

Drug combinations, may affect clinical safety and efficacy via synergistic, additive, or antagonistic effects of herbal components and drug molecules (Sheng *et al*., 2018). There have been several studies on the antibacterial activities for herbal and antibiotic combinations. For instance, a study conducted by Kudumela *et al*. (2018), involved a combination of *Schkuhria pinnata*, *Commelina africana*,*Dombeya rotundifolia* and *Elephantorrhiza elephantine* plants that exhibited synergistic effects against *Pseudomonas aeruginosa*. Another synergistic effect was exhibited by combination of *Bidens pilosa* and *Leonotis nepetifolia* extracts against *Candida albicans* pathogen (Mbunde *et al*., 2019).

A study conducted by Che *et al*., (2013), combined root extracts of *Astragalus membranaceus* and *Rehmannia glutinosa* and significantly reduced the wound area of rats in a foot ulcer animal model. No wound-healing effect was observed when an individual herb was applied to the wound. The huge experience accumulated from clinical observations of herb-herb and herbal-antibiotics combinations may provide a lead for

future exploration of new therapeutic benefits and products development from *S. glaucescens* and *C. swynnertonii* combination that could be more effective antimicrobial agents than individual plant alone.

1.3 Synergistic, additive and antagonistic effects in drugs combinations

With the increasing of antibiotic resistances, the combined effects of herbal drugs and antibiotics is a new approach to treat multidrug-resistant bacteria (Bhardwaj *et al*., 2016). This is because combination of two or more drugs can have synergistic, additive or antagonistic effects (Bhardwaj *et al*., 2016). Synergy is the combined effort of resources to generate outcomes that are greater than the additive impact of each resource (Someh and Shanks, 2013).

Whenever the effects observed after applying a mixture exceed an expectation, the action of the agents is called synergistic (Schindler, 2017). Synergy is complementary among resources applied together and it creates super-additive value, referring to the joint effect of resources that is greater than the sum of the effects from each individual i.e. A+B > (A) or (B) alone (Schindler, 2017). Additive effects are observed when the combined effect is equal to the sum of the individual effects while antagonism is observed when the effect of two or more compounds applied together is less than the individual effects (Kohanski *et al*., 2010).

1.4 Ethnobotany, phytochemistry and antimicrobial activity of *Synadenium glaucescens*

The *Euphorbiaceae* family, is complex and heterogeneous and contains approximately 300 genera and 7000 species that have been identified worldwide including *Synadenium* species are shrubs or trees with sub-fleshy cylindrical branches, when stem is cut copious a milky latex is produced which has an unpleasant taste and is toxic when ingested in significant quantities (Dawidar *et al*., 2011). Many chemical ingredients and bioactive compounds have been reported from the family *Euphorbiaceae (Sabandar et al., 2013).* The family *Euphorbiaceae* contains flavonoids, saponins, diterpenes and phorbol esters (Hassan *et al*., 2012). Dichloromethane extracts from *S. glaucescens* contains lanosterol and cycloartenol sterols as main compounds while its ethanol extracts possess phenolic compounds (Mabiki *et al.*, 2013). Some studies have reported *S. glaucescens* to possess antimicrobial activity against skin infections, worms, sores, Human Immuno deficiency Virus (HIV), tuberculosis ailments as well as exhibiting pesticidal effects when used in

post-harvest storage of grains (Max *et al*., 2014; Nyigo *et al*., 2016). Furthermore, it has been reported to contains antiviral chemical constituents that act against infectious bursal disease virus and fowl pox virus (Mabiki *et al*., 2013).

Plate 1: Photograph of *Synadenium glaucescens* upper shoot (Njombe)

1.5 Ethnobotany, phytochemistry and comparable bioactivity of *Commiphora swynnertonii*

C. swynertonii, dispersed in tropical and sub-tropical parts of Asia, North-eastern Africa, and South America is characterized by shrubs or small trees with spinescent branches (Mkangara *et al*., 2014). The chemistry of the genus *Commiphora* has been studied extensively and more than 300 compounds have been isolated and identified (Kalala *et al*., 2014; Credo *et al.*, 2022). The reported secondary metabolites associated with antimicrobial activities in *Commiphora species* are flavonoids, terpenoids, steroids and phytosterols and other constituents include carbohydrates, lignans, and long-chain aliphatic alcohol derivatives (Su *et al*., 2012). Myrrh, a traditional herbal medicine in China is derived from *Commiphora* (*Burseraceae*).

It has been used widely for the treatment of fever, stomach complaints, diseases of the gall bladder, skin infections, ache, dysmenorrhea or amenorrhea, tumors, chest ailments and snake bites in India, China, Rome and Greece (Su *et al*., 2012) This medicinal plant is reported to contains anti-inflammatory, anti-microbial and anti-cancer properties and is used to clean the body, and protect against sexually transmitted infections, skin infections, worm infestation, coughs and chest problems in both humans

and livestock, its bark exudates are also used as repellants for ticks and other insects in livestock and poultry (Kalala *et al*., 2014).

Plate 2: Photograph of *Commiphora swynnertonii* branch (Manyara)

1.6 Justification

There is increased failure of most single drug or medicine treatments in many infectious diseases. Among the root causes of this failure are reported to be antimicrobial resistance, narrowing of antimicrobial spectrum and limited activityof these single drugs. As a result of the increase several problems such as morbidity, mortality, disability and socioeconomic costs in different communities. Those effects have led to the search for combination therapies of either herb-herb or herb-antibiotics. Despite the evidence of the potential observed in many drug combinations, there are limited studies on antimicrobial effects of combined crude extracts from the *S. glaucescens* and *C. swynnertonii* medicinal plants with antibiotics. This study aimed to investigate the antibacterial effects of the crude extracts from two medicinal plants in combination with antibiotics. The results obtained from this study contributs the knowledge on better combinations that can enhance the efficacy of single herbs as well as combinations with conventional antibiotics, thereby reducing the emergence of antimicrobial resistance.

1.7 Objectives

1.7.1 Overall objective

To evaluate the antibacterial effects of herb-herb and herb-antibiotic combinations.

1.7.2 Specific objectives

i. To determine the antibacterial activity for combinations of *S. glaucescens* and *C. swynnertonii* crude extracts against selected bacteria.

ii. To assess the combined effects of *S. glaucescens* and *C. swynnertonii* crude extracts with antibiotics against selected bacteria.

1.8 Thesis Organization

This thesis is developed in the format of published papers, comprising of five main chapters. Chapter one is the general introduction, chapter two comprise published paper in *African Journal of Infectious Diseases* (AJID) and chapter three comprise accepted paper in *Journal of Medicinal Plants Research*. Chapter four is the general discussions and chapter five is the general conclusions and recommendations of this research study.

CHAPTER TWO

PUBLISHED PAPER[1]

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

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

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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 *pneumonia=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 (Karunamoorthi *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* (Mvunjakongwa in Swahili) and a tropical tree *Commiphora Swynnertonii* (Oltemwai in Maasai) 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; Ayukekbong *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 Labortetechnik, 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: 1:1v/v and 1:1:1:1v/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 (1.5×106 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:

$$\text{FIC } Cs = \frac{\text{MIC value of } Cs \text{ in combination with } Sg \text{ crude extract}}{\text{MIC value of } Cs \text{ independently}} \quad \text{and}$$

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

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).

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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	Minimum inhibitory concentration (mg/mL)				
	Gram-positive bacteria		Gram-negative bacteria		
	S. aureus ATCC 29213	*E. coli* ATCC 25922	*E. faecalis* ATCC 1559	*K. pneumoniae* ATCC 1145	*P. aeruginc* ATCC 27853
*Cs*7D	0.37	3	0.75	3	3
*Cs*7M	0.09	3	0.37	3	3
*Cs*5D	0.75	3	1.5	3	3
Cs5M	0.18	1.5	0.75	3	3
*Cs*2D	1.5	3	0.37	3	3
*Cs*2M	0.04	3	0.37	3	3
Gentamicin	0.002	0.004	0.002	0.008	0.004

Key: D= Dichloromethane extract, M= Methanol extract, *Cs= Commiphora swynnertonii, Cs7 leaves* extracts, *Cs*5 stem bark extracts, *Cs*2 root bark extracts.

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

Extract/Gentamicin	Minimum inhibitory concentration (mg/mL)				
	Gram-positive bacteria		Gram-negative bacteria		
	S. aureus ATCC 29213	*E. faecalis* ATCC 1559	*E. coli* ATCC 1559	*K. pneumoniae* ATCC 1145	*P. aeruginosa* ATCC 27853
*Sg*7D	0.75	0.75	3	3	1.5
*Sg*7M	0.37	0.37	3	3	0.75
*Sg*5D	0.37	0.75	3	3	3
*Sg*5M	0.02	1.5	3	3	3
*Sg*2D	0.02	0.37	3	3	1.5
*Sg*2M	0.01	0.02	1.5	3	1.5
Gentamicin	0.002	0.002	0.004	0.008	0.004

Key: D= Dichloromethane extract, M= Methanol extract, *Sg=Synadenium glaucescens, Sg7*=leaves extracts, *Sg*5=stem bark extracts, *Sg*2= 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 FIC Index values 6, 19, and 38 (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 1:1:1:1:1v/v showed different antagonistic effects against Gram-negative bacteria with FIC Index values 4, 5, 6 and 8 (Table 4).

Table 3: Minimum inhibitory concentration (mg/mL) of combined crude extracts from *Commiphora swynnertonii* and *Synadenium glaucescens* tested against selected bacteria

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
*Cs*7D+*Sg*7D	1.5	0.75	3	3	3
*Cs*7M+*Sg*7M	0.07	0.75	3	3	3
*Cs*7D+*Sg*5D	0.37	0.37	3	3	3
*Cs*7M+*Sg*5M	0.02	0.07	3	3	3
*Cs*7D+*Sg*2D	0.75	0.28	3	3	3
Cs7M+*Sg*2M	0.01	0.21	3	3	3
Cs7M+*Sg*7M+*Sg*5M	0.75	0.37	3	3	3
*Cs*7M+*Sg*7M+*Sg*5M+*Sg*2M	0.09	0.37	3	3	3
Cs5D+*Sg*7D	0.75	0.75	3	3	3
*Cs*5M+*Sg*7M	0.07	0.14	3	3	3
*Cs*5D+*Sg*5D	0.37	0.28	3	3	3
*Cs*5M+Sg5M	0.11	0.14	3	3	3
*Cs*5D+*Sg*2D	0.75	0.11	3	3	3
*Cs*5M+*Sg*2M	0.19	0.39	1.5	3	1.5
Cs5M+*Sg*7M+*Sg*5M	0.18	0.75	3	3	3
*Cs*5M+*Sg*7M+*Sg*5M+*Sg*2M	0.18	0.37	3	3	3
*Cs*2D+*Sg*7D	0.75	0.56	3	3	3
Cs2M+*Sg*7M	0.05	0.37	1.5	3	3
*Cs*2D+*Sg*5D	0.37	0.56	3	3	3
*Cs*2M+*Sg*5M	0.008	0.14	1.5	3	3
Cs2D+*Sg*2D	0.75	0.18	3	3	3
*Cs*2M+*Sg*2M	0.01	0.05	0.75	3	3
Cs2M+*Sg*7M+*Sg*5M	0.09	0.18	3	3	3
*Cs*2M+*Sg*7M+*Sg*5M+*Sg*2M	0.09	0.04	3	3	3

Key: + = combination, D= Dichloromethane crude extracts, M= Methanol crude extracts, *Cs*7 *Commiphora swynnertonii* leaves extracts, *Cs*5 *Commiphora swynnertonii* stem bark extracts, *Cs*2 *Commiphora swynnertonii* root bark extracts, *Sg*7 *Synadenium glaucescens* leaves extracts, *Sg*5=*Synadenium glaucescens* stem bark extracts, *Sg*2 *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.

	Fractional inhibitory concentration (FIC) Index				
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
*Cs*7D+*Sg*7D	6.1	2.1	2	2	3
*Cs*7M+*Sg*7M	1.8	2.5	2	2	2
*Cs*7D+*Sg*5D	2	1.3	2	2	3
*Cs*7M+*Sg*5M	1.2	0.6	2	2	2
*Cs*7D+*Sg*2D	3	1.1	2	2	3
Cs7M+*Sg*2M	1.2	1.3	2	2	2
Cs7M+*Sg*7M+*Sg*5M	47.8	2.2	3	3	6
*Cs*7M+*Sg*7M+*Sg*5M+*Sg*2M	14.7	20.5	5	4	8
Cs5D+*Sg*7D	38.5	1.1	2	2	3
*Cs*5M+*Sg*7M	0.5	1.2	2.5	2	5
*Cs*5D+*Sg*5D	38	0.5	2	2	3
*Cs*5M+Sg5M	0.7	2.2	3	2	5
*Cs*5D+*Sg*2D	38.5	0.6	2	2	3
*Cs*5M+*Sg*2M	1.2	0.9	2	2	4.5
Cs5M+*Sg*7M+*Sg*5M	10.4	3.5	4	3	6
*Cs*5M+*Sg*7M+*Sg*5M+*Sg*2M	28.4	20.4	5	4	8
*Cs*2D+*Sg*7D	1.5	1.6	2	2	2
*Cs*2M+*Sg*7M	2.2	3.4	1	2	3
*Cs*2D+*Sg*5D	5.2	1.6	2	2	2
*Cs*2M+*Sg*5M	19.2	19.8	1.5	2	2
Cs2D+*Sg*2D	1.4	0.3	1.5	2	3
*Cs*2M+*Sg*2M	2.2	10.6	2.2	2	3
*Cs*2M+*Sg*7M+*Sg*5M	69	1.1	3	3	6
*Cs*2M+*Sg*7M+*Sg*5M+*Sg*2M	15.9	2.2	5	4	8

Key: + = combination, D=Dichloromethane crude extracts, M=Methanol crude extracts, *Cs*7 *Commiphora swynnertonii* leaves extract, *Cs*5 *Commiphora swynnertonii* stem bark extracts, *Cs*2 *Commiphora swynnertonii* root bark extracts, *Sg*7= *Synadenium glaucescens* leaves extracts, *Sg*5=*Synadenium glaucescens* stem bark extracts, *Sg*2 *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 Makangara *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/efflux 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 $\sum$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 Juliani, 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.

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CHAPTER THREE

ACCEPTED MANUSCRIPT[2]:

In-vitro Assessment of Antibacterial Effects of Combined Crude Extracts of *Synadenium glaucescens* and *Commiphora Swynnertonii* with Antibiotics

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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 (1.5×10^8 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*
Cs7D	0.38	0.75	3	3	3
Cs7M	0.09	0.38	3	3	3
Cs5D	0.75	1.5	3	3	3
Cs5M	0.18	0.75	1.5	3	3
Cs2D	1.5	0.38	3	3	3
Cs2M	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*, *Cs7*= leaf extract, *Cs5*= stem bark extracts, *Cs2*= root bark extract.

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*
Sg7D	0.75	0.75	3	3	1.5
Sg7M	0.38	0.38	3	3	0.75
Sg5D	0.38	0.75	3	3	3
Sg5M	0.02	1.5	3	3	3
Sg2D	0.02	0.38	3	3	1.5
Sg2M	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*, *Sg7*= leaf extract, Sg5= stem bark extract, Sg2= root bark extract.

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*
Cs7M+CIPRO	0.02	1.50	0.002	0.09	0.02
Cs5M+CIPRO	0.09	1.50	0.001	0.04	0.02
Cs2M+CIPRO	0.02	1.50	0.002	0.38	0.02
Cs7D+CIPRO	0.18	1.50	0.001	0.04	0.02
Cs5D+CIPRO	0.37	1.50	0.001	0.04	0.02
Cs2D+CIPRO	0.75	1.50	0.001	0.04	0.02
Sg7M+CIPRO	0.09	0.75	0.002	0.09	0.19
Sg5M+CIPRO	0.01	0.75	0.001	0.09	0.04
Sg2M+CIPRO	0.001	0.77	0.001	0.09	0.02
Sg7DC+IPRO	0.37	0.75	0.001	0.04	0.02
Sg5D+CIPRO	0.18	0.75	0.001	0.04	0.02
Sg2D+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* root bark extract, Sg7, *Synadenium glaucescens* leaf extract, Sg5= *Synadenium glaucescens* stem bark extract, Sg2= *Synadenium glaucescens* root bark extract.

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* root bark extract, Sg7= *Synadenium glaucescens* leaf extract, Sg5=*Synadenium glaucescens* stem bark extract, Sg2=*Synadenium glaucescens* root bark extract.

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 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

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

Extracts/erythromycin	MIC mg/mL				
	S. aureus	*E. faecalis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
Cs7M + ERYT	0.76	0.375	1.55	0.38	1.55
Cs 5M + ERYT	0.38	0.75	1.55	1.55	1.55
Cs 2M + ERYT	0.02	0.01	1.55	0.76	1.55
Cs7D + ERYT	0.04	0.18	1.55	0.38	1.55
Cs 5D + ERYT	0.09	0.38	1.55	1.55	0.38
Cs 2D + 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* root bark extract, Sg7= *Synadenium glaucescens* leaf extract, Sg5= *Synadenium glaucescens* stem bark extract, Sg2= *Synadenium glaucescens* root bark extract.

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*
Cs7M+CIPRO	15.50	12.12	0.05	1.93	0.48
Cs5M+CIPRO	15.32	481.26	0.02	0.96	0.48
Cs2M+CIPRO	0.50	3.78	0.05	7.75	0.48
Cs7D+CIPRO	7.84	12.12	0.02	0.96	0.48
Cs5D+CIPRO	31.31	15.50	0.02	0.96	0.48
Cs2D+CIPRO	60.12	2.13	0.02	0.96	0.48
Sg7M+CIPRO	127.47	60.49	0.05	1.93	3.98
Sg5M+CIPRO	1.95	250.5	0.05	1.93	0.96
Sg2M+CIPRO	0.15	8.01	0.02	1.93	0.48
Sg7DC+IPRO	240.49	125.5	0.02	0.96	0.96
Sg5D+CIPRO	120.49	125.5	0.029	0.96	0.96
Sg2D+CIPRO	2.13	60.49	0.029	0.96	0.96

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

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

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 5M + ERYT	15.32	481.26	8.01	8.01	8.01
Cs 2M + 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 5D + ERYT	31.31	484.13	8.01	4.00	4.00
Cs 2D + 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

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 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 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 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.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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CHAPTER FOUR

4.0 GENERAL DISCUSSIONS

The combination therapy of either herb-herb or herb-antibiotic is an important step towards minimizing bacterial resistance against standard antibiotics. The current research which reports the evaluation of antibacterial effects of herb-herb and herb-antibiotic combinations of *S. glaucescens* and *C. swynnertonii* crude extracts with three standard antibiotics namely, ciprofloxacin, ampicillin and erythromycin against bacteria opened an important knowledge base as it registers some useful combinations of these two medicinal plants and with the studied antibiotics. This research work reports new findings on antibacterial effects of combined crude extracts of *S. glaucescens* and *C. swynnertonii* (herb-herb) and with antibiotics, it adds value to science. This study, opens an avenue for further studies in combinations of these two medicinal plant species as indicated in tables in papers chapters and additional information are provided in supplementary figure S1 in appendences.

The antibacterial activity of individual crude extracts of plants was observed against Gram-positive bacteria tested (*S. aureus and E. faecalis*). Moreover, the crude extracts of *S. glaucescens* showed strong activity against Gram-positive bacteria which is higher compared to those of *C. swynnertonii*. In this study, we observed the weak activities of the crude extracts were observed against gram-negative bacteria (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*). Similar results were reported by Max *et al*., (2014) in *S. glaucescens* Bakari *et al.*, (2011) ; Mkangara *et al*., (2014) in *C. swynnertonii.*

In this study, the results of combined crude extracts and combination which involved antibiotics showed activities against Gram-positive bacteria. This concurs with a *Che et al*., (2013), that when herbs are used in combination, the effects can be complicated as various interactions can occur among individual components. In combination therapy, the potential interactions may be higher due to many components (Alsanad *et al*., 2016). The use of the drug in combination can minimize the cost of resistance and suppress resistance evolution (North and Brown, 2021). A review study reported that, the drug combinations that address multiple targets simultaneously are better in controlling complex disease systems and are less prone to drug resistance (Merzenich, 2014). Combinations of

traditional and modern medicine provide several active agents that are used in treating different diseases (Foucquier and Guedj, 2015). In this work, the combination between crude extracts and antibiotics demonstrated weak activity against gram-negative bacteria. The difference in activities between Gram-positive and gram-negative bacteria is due to the difference in their cell structure. Gram-negative bacteria have an impermeable outer membrane that restricts the ability of antibacterial action (Cloutier, 2017). Urinary tract infections, intra-abdominal infections, and ventilator-associated pneumonia are the major hospital-acquired infections commonly caused by Gram-negative bacteria with *E. coli*, *K. pneumoniae* and *P. aeruginosa* (WHO, 2002). These bacteria can utilize an efflux mechanism to remove the administered drugs that result indicate primary impact of that significantly higher economic burden with treatment costs and losses attributed to decreased productivity of affected people or animals (Sheard *et al.*, 2019).

Due to the emergence of drug resistance, affected communities turn to combinations of the drugs which cause synergistic antimicrobial remedies against diverse infections resulting from known causative agents (Akinyele *et al.,* 2017). This study has demonstrated synergistic effects between crude extracts against gram-positive bacteria. The synergistic action may add effectiveness of the medicines under use (Weibl, 2019). The synergistic effect is considered to be the primary rationale for significantly greater activity when the agents are combined (Jain *et al*., 2011). Synergistic actions improve efficacy, minimize toxicity, cure faster, reduce the occurrence of drug resistance and broaden the antibacterial spectrum (Mbunde *et al*., 2019).

It is important not to confuse synergistic effect with additive effect. Synergy occurs when two or more drugs/compounds are combined to produce a total effect that is greater than the sum of the individual agents while an additive effect is an add up of individual effects where each agent is not affecting the other (Chou, 2010). A review study conducted by Bassolé and Juliani, (2012) interpreted additive effects as combined effects that are equal to the sum of individual effects. In this study, although some crude extracts in combinations showed antagonistic effects using the two-fold microdilution method, additive effects were mainly observed against five bacteria tested. This effect is also known as the non-interactive effect where the pure summation of the two substances is observed (Caesar and Cech, 2019).

CHAPTER FIVE

5.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The minimum inhibitory concentration was used in this study to determine the antibacterial effects of combined crude extracts alone and with antibiotics. The strong activities observed when two, three, or four crude extracts were combined with standard antibiotics, and tested against Gram-positive bacteria may be due to their synergistic effects. Weak activities observed against Gram-negative bacteria in the combination of crude extracts may be due to impermeability of their cell wall structure while the antagonism which was observed in several combinations may be due to the weak activities herb-herb or herb-antibiotic combinations.

5.2 Limitations of the study and Recommendations

Based on the findings from the current study, the following are recommended:

1. The study involved only standard bacteria; there is a need for testing of the combination of crude extracts from two medicinal plants against clinical bacterial isolates.

2. The plant parts which inhibited the growth of bacteria through synergism are a promising good alternative therapy in the future.

3. Since all combinations of the crude extracts exhibited the highest inhibitory effects against gram-positive bacteria, further studies are recommended to determine their effectiveness against clinical isolates and toxicity when applied to humans and vet medicine.

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APPENDICES

Appendix 1: Supplementary Figure S1

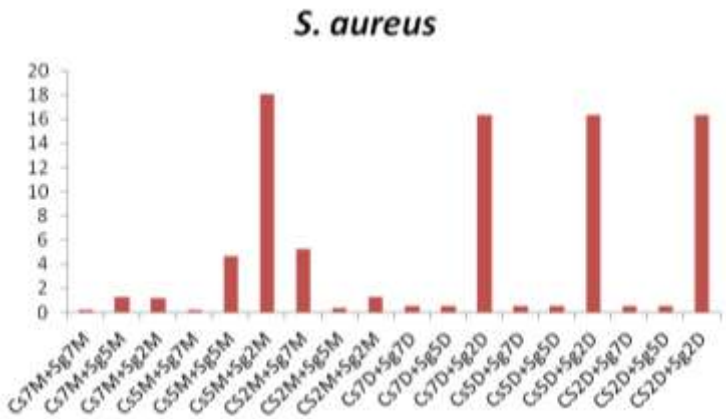

Figure 1: Antibacterial effects of combined crude extracts of *Synadenium glaucescens* and *Commiphora swynnertonii* against *Staphylococcus aureus* (FIC Index values 0.5) =synergistic effects and antagonistic effects = (FIC Index values 5, 20, and 38), M=methanol extract, D=dichloromethane extract, No 7, 5 and 2= leaves, stem barks and root barks respectively

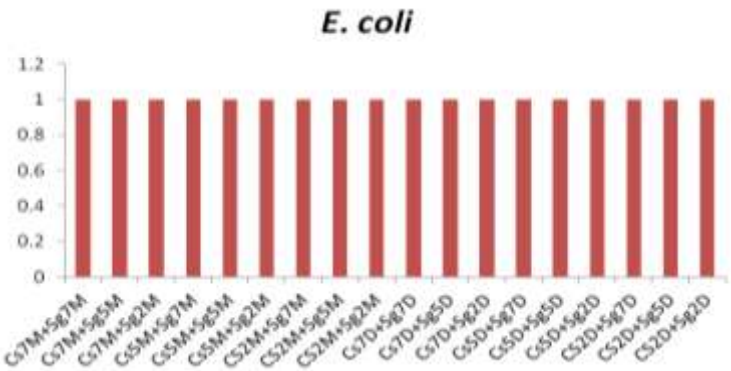

Figure 2: Antibacterial effects of combined crude extracts of *Synadenium glaucescens* and *Commiphora swynnertonii* against *Escherichia coli* and *Klebsiella pneumoniae* (FIC Index values 2) =additive effects, M=methanol extract, D=dichloromethane extract No 7, 5 and 2= leaves, stem barks and root barks respectively

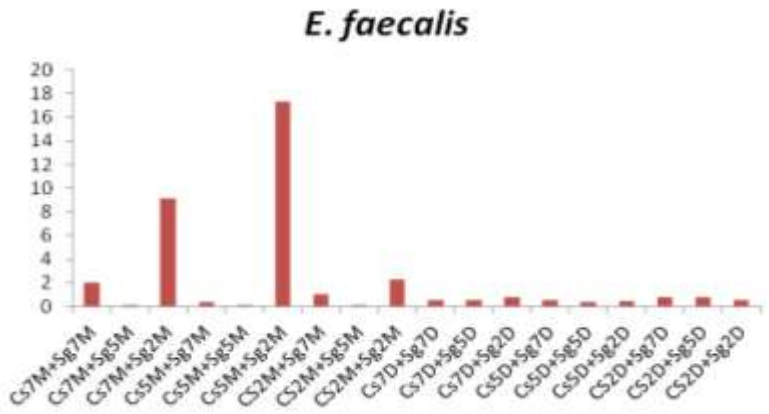

Figure 3: Antibacterial effects of combined crude extracts of *Synadenium glaucescens* and *Commiphora swynnertonii* against *Enterococcus faecalis* (FIC Index values 0.5 and 0.3) = synergistic effects and antagonistic effects = (FIC Index values 4, 10 and 18), M=methanol extract, D=dichloromethane extract, No 7, 5 and 2= leaves, stem barks and root barks respectively

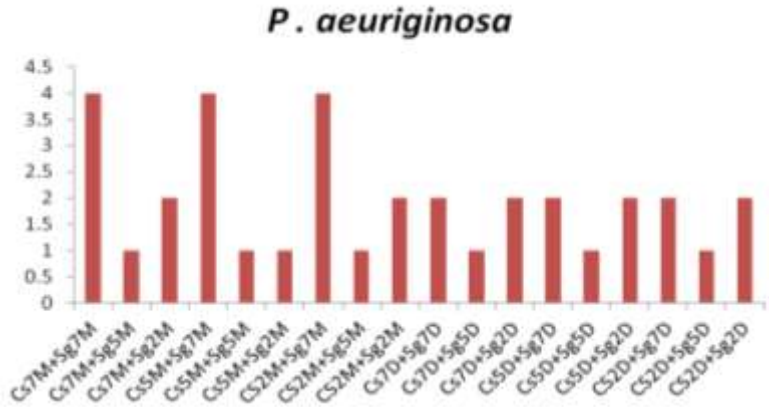

Figure 4: Antibacterial effects of combined crude extracts of *Synadenium glaucescens* and *Commiphora swynnertonii* against *Pseudomonas aeruginosa* (FIC Index values 2-3) =additive effects and antagonistic effects = (FIC Index values 5), M=methanol extract, D=dichloromethane extract, No 7, 5 and 2= Leaves, stem barks and root barks respectively

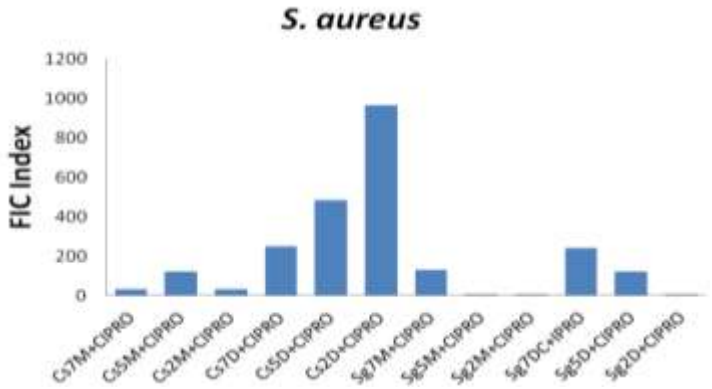

Figure 5: Antibacterial effects of combed crude extracts of Synadenium *glaucescens* leaves, stem barks and root barks of *Synadenium glaucescens* and *Commiphora swynnertonii* with ciprofloxacin against *Synadenium glaucescens* and *Commiphora swynnertonii* with ciprofloxacin against S*taphylococcus aureus* (FIC Index values 0.1 and 0.5)= synergistic effects and antagonistic effects = (FIC Index values 7, 15, 30, 250)M= methanol extract, D=dichloromethane extract. No 7, 5 and 2=leaves, stem barks and root barks respectively

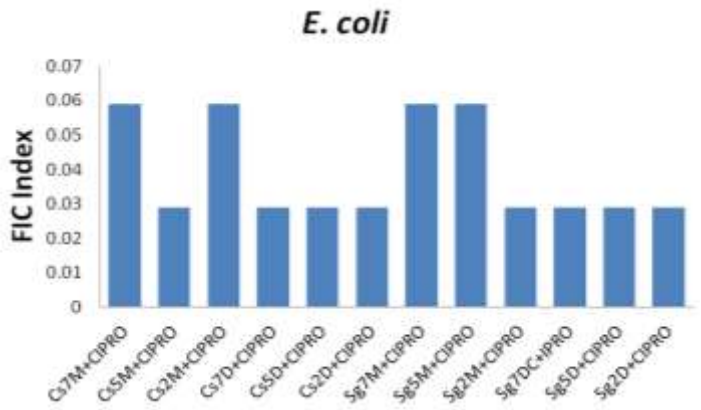

Figure 6: Antibacterial effects of combined crude extracts of *Synadenium glaucescens* and *Commiphora swynnertonii* with ciprofloxacin against *Escherichia coli* (FIC Index values 0.03- 0.06) =synergistic effects, M=methanol extract, D=dichloromethane extract, No 7, 5 and 2= leaves, stem barks and root barks respectively

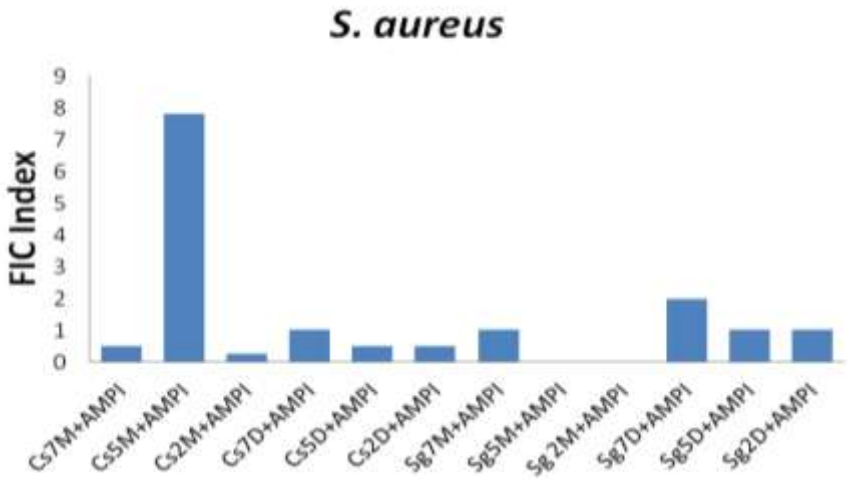

Figure 7: Antibacterial effects of combined crude extracts of *Synadenium glaucescens* and *Commiphora swynnertonii* with ampicillin against *Staphylococcus aureus* (FIC Index values 0.03- 0.5) =synergistic effects, (FIC Index values 8) = antagonistic effects. M=methanol extract, D=dichloromethane extract, No 7, 5 and 2= Leaves, stem barks and root barks respectively

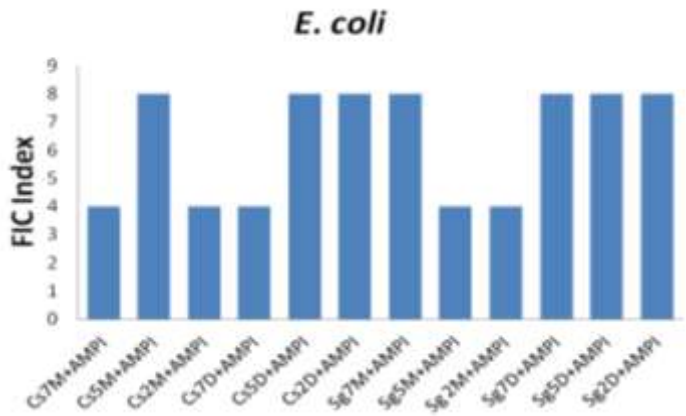

Figure 8: Antibacterial effects of combined crude extracts of *Synadenium glaucescens* and *Commiphora swynnertonii* with ampicillin against *Escherichia coli* (FIC Index values 4 and 8) = antagonistic effects, M=methanol extract, D=dichloromethane extract, No 7, 5 and 2= leaves, stem barks and root barks respectively

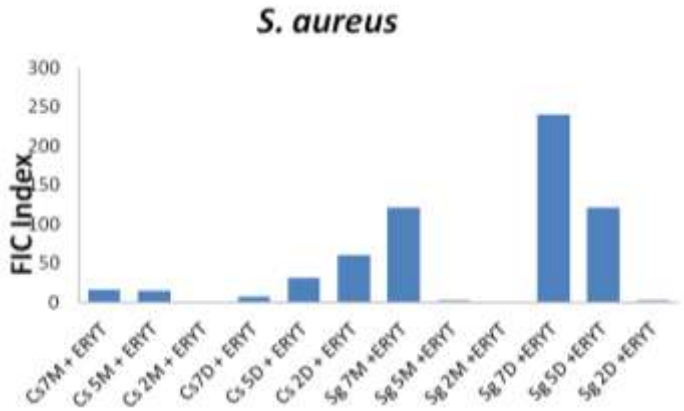

Figure 9: Antibacterial effects of combined crude extracts of Synadenium *glaucescens* and *Commiphora swynnertonii* with erythromycin against *Staphylococcus aureus* (FIC Index values 0.1 and 0.) = synergistic effects (FIC Index values 4 and 8) = antagonistic effects. M=methanol extract. D=dichloromethane extract. No 7, 5 and 2= leaves, stem barks and root barks respectively

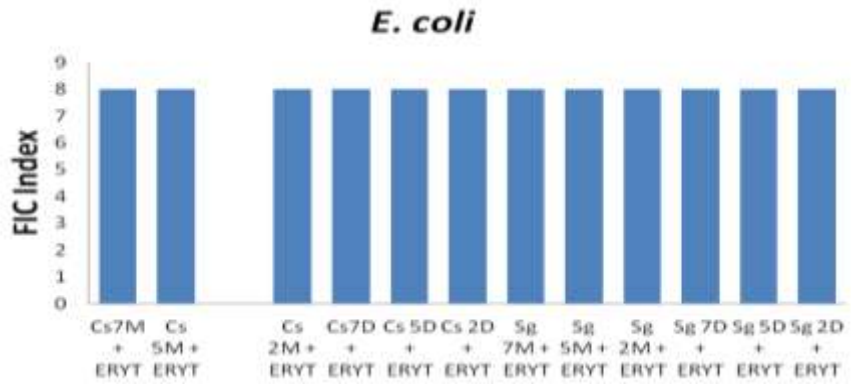

Figure 10: Antibacterial effects of combined crude extracts of *S*ynadenium *glaucescens* and *Commiphora swynnertonii* with erythromycin against *Escherichia coli* (FIC Index values 8) = antagonistic effects. M=methanol extract, D=Dichloromethane extract, No 7, 5 and 2= leaves, stem barks and root barks respectively