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Traditional medicine use in two public South African hospitals: Prevalence and interactive antimicrobial studies for combinations with conventional antimicrobials

THESIS BY PUBLICATION



Zelna Booth

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

30 May 2025

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Declaration: Student's contribution to publications and agreement of co-authors

I, Zelna Booth, student number 300896, declare that this thesis is my own work and that I was responsible for the development of study findings drafted as publications included in this thesis, with primary authorship hereby confirmed. Co-authors declare ample contribution toward conceptualisation and the reviewing of publications included herein.

This thesis is being submitted in fulfilment for the degree of Doctor of Philosophy in Pharmacy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for examination for any other degree at this or any other University.

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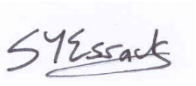
Signature:  **Date:** 30 May 2025

Agreement by co-authors:

By signing this declaration, the co-authors listed agree to the use of the articles by the candidate as part of the thesis. All co-author affiliations and contact details are provided in Appendix A.

Ethics declaration

I, Zelna Booth, declare that I am registered with the University of the Witwatersrand for the degree of Doctor of Philosophy, and have read the University's current research ethics guidelines.

I obtained ethical approval from Human Research Ethics Committee (HREC) – Medical (Appendix C) to conduct my research. Responsibility for ethical conduct in accordance with stipulated HREC requirements was accepted and applied.



Zelna Booth

30 May 2025

Date

List of publications

Publication 1: Book chapter

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Contributions per author:

Zelna Booth

- Conceptualised the review.
- Collation of the studies.
- Analysis of data.
- Drafting of the written chapter.

Professor Sandy F. van Vuuren

- Conceptualised the review.
- Supervision.
- Editing of the written chapter.

Both authors reviewed the drafts and contributed towards editing before submission for publication.

	Author Name	Signature	Date
1st Author	Zelna Booth		30 May 2025
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- Analysis of results.
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3rd Author	Prof. Sandy F. van Vuuren		30 May 2025

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Professor Sandy F. van Vuuren

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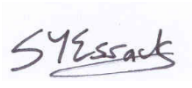
2nd Author	Prof. Sabiha Y. Essack		30 May 2025
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3. Booth, Z., Essack, S.Y., van Vuuren, S.F. Prevalence of traditional medicine use among patients in two public academic hospitals in South Africa. The 43rd Annual Academy of Pharmaceutical Sciences of South Africa (APSSA) Conference, Rhodes University, Makhanda, South Africa, 2 August 2022 (Podium presentation).

4. Booth, Z., Essack, S.Y., van Vuuren, S.F. Combining traditional and conventional medicines: A prevalence and interactive antimicrobial profiling study in South Africa. The 71st International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA), Dublin, Ireland, 03 July 2023 (Podium presentation).

5. Booth, Z., Khumalo, G., Essack, S.Y., van Vuuren, S.F. Integrative medicine: combining conventional antimicrobials and traditional, medicinal plants from Durban and Johannesburg *muthi* markets. The 26th Indigenous Plant Use Forum Conference (IPUF), Cape Town, South Africa, 12 August 2024 (Podium presentation – 3rd place award).

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Dedication

This thesis is dedicated to my husband, daughter, sister, and dearest mom. All the love and support sustained me throughout this journey. Without you all, this achievement would not have been possible.

To my dearest mommy, I wish I could share this final stage with you – I just hope I have made you proud!

Love always.

Abstract

Traditional medicines are entrenched in the cultural heritage of many South Africans. Integrative medicine involves the harmonised use of traditional medicine and conventional healthcare. The diverse availability of traditional medicinal plants and accessibility to many commercial *muthi* markets in South Africa lends itself to a noteworthy, alternate source of healthcare. A three-phase study was thus designed to explore integrative medicine in South Africa. Phase 1 of this study aimed to document existing literature on antimicrobial synergism or antagonism between natural products and conventional antimicrobials in a systematic framework to provide a valuable resource highlighting antimicrobial synergism or to draw attention to harmful interactions to be avoided in clinical practice (antagonism). Phase 2 of this study aimed at investigating the prevalence of use of traditional medicine, amongst patients at two large public hospitals in South Africa, namely Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in Johannesburg (JHB) and King Edward Hospital VIII (KEH) in KwaZulu-Natal (KZN) and further discern the extent of concurrent use with conventional medicines. Phase 3 aimed to determine the *in vitro* interactive antimicrobial and toxicity profiles of conventional antimicrobials in combination with traditional, medicinal plants most prevalently used for infectious diseases, purchased from two major *muthi* markets in South Africa (Faraday market in JHB and Warwick market in KZN).

Phase 1 of the study involved a systematic review of the literature, according to PRISMA methodology, with the inclusion of all interactions of synergy and antagonism documented within the past 20 years. In phase 2 of the study, a descriptive, self-administered, quantitative survey, consisting of demographic; and traditional medicine use was undertaken. Data analysis included quantitative frequencies and percentages. Ethical clearance (M190534) was obtained for the study. For phase 3, plant material was collected from the *muthi* markets, botanically identified and thereafter minimum inhibitory concentration (MIC) assays were conducted. Antimicrobial interactions were assessed by calculating the fractional inhibitory concentration (ΣFIC). Interactive toxicity profiles of the synergistic combinations were evaluated using the brine shrimp lethality assay (BSLA).

In phase 1, *in vitro* combination studies were found to focus mainly on essential oils and isolated plant compounds with conventional antimicrobials. This indicates a clear gap in research pertaining to plant extract combinations with conventional antimicrobials, which more closely aligns with traditional methods of consumption of medicinal plant products in South Africa. Furthermore, synergistic interactions were more frequently reported than antagonistic interactions.

In phase 2, a total of 263 and 307 surveys were conducted at the CMJAH-JHB and KEH-KZN sites, respectively. A higher prevalence of traditional medicine use was evident amongst KZN participants (94.1% of n = 307), as opposed to participants in JHB (76.8%, of n = 263), with indicated combination use of traditional and conventional medicine of 31.6% and 11.0%, respectively. Furthermore, medicinal plants were revealed as the most common source of traditional remedies (JHB - 49.1%; KZN - 88.3%), with JHB (34.6%) and KZN (26.4%) participants using medicinal plants regularly for infections. The five most common medicinal plants used amongst study participants from both study sites included *Acorus calamus* L., *Hydnora africana* Thunb., *Rauvolfia caffra* Sond., *Senecio serratuloides* DC., and *Warburgia salutaris* (G.Bertol.) Chiov.

For phase 3 of the study, a total of 1 632 antibiotic: plant combinations were tested against the ESKAPE (*Enterococcus faecium* ATCC 27270; *Staphylococcus aureus* ATCC 29212; *Klebsiella pneumoniae* ATCC 13883; *Acinetobacter baumannii* ATCC 19606; *Pseudomonas aeruginosa* ATCC 27853; *Enterobacter cloacae* ATCC 13460) pathogens. Furthermore, 272 antifungal: plant combinations were tested against two yeasts (*Candida albicans* ATCC 10231 and *Candida glabrata* ATCC 90030). Plant (from both JHB and KZN *muthi* markets): antibiotic combinations with definitive ΣFIC values, reflected synergistic (5.6%), antagonistic (49.6%), additive (7.5%) and non-interactive (37.3%) profiles. Furthermore, plant (from both JHB and KZN *muthi* markets): antifungal combinations, exhibited synergistic (0.7%), antagonistic (60.8%), additive (3.5%) and non-interactive (35.0%) profiles. Notably, *S. serratuloides* (KZN *muthi* market) with ciprofloxacin against *K. pneumoniae* (ΣFIC 139.9) demonstrated the most antagonistic interaction. *Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall. (JHB *muthi* market), in combination with azithromycin against *E. faecium* proved most synergistic (ΣFIC 0.06). The most toxic antimicrobial: JHB plant combination was *Synaptolepis oliveriana* Gilg with azithromycin (mortality of 54.5%) and *W. salutaris* with

azithromycin (mortality of 100.0%). The only toxic antimicrobial: KZN plant combination was fluconazole with *A. calamus* against *C. albicans* (mortality of 78.8%).

Synergistic interactions observed in phase 3 provide impetus for further investigations allowing for future clinical translations. The high antagonism noted in this study, in addition to a few toxic combinations, warrants safety concerns. Furthermore, the frequent antagonism identified in this study contradicts the overarching commonalty of synergy in published *in vitro* combination research. This requires further interpretation and a need to create awareness for standardisation in reporting of findings so as not to only depict positive results in publications. The study has provided valuable groundwork to outline beneficial and harmful impacts of integrative medicine in the South African context. The study has additionally provided useful information to bring the South African healthcare system closer to realising universal healthcare, as set out by the World Health Organisation which has mandated integrative medicine as a priority.

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Chapter 2

Equation 1: Sample size calculation

The formula below (Naing et al., 2006) was used to calculate the sample size for the survey studies:

$$n = \frac{\mathrm{Z}^2 P(1 - \mathrm{P})}{\mathrm{d}^2}$$

Where,

n = sample size,

Z = Z statistic for level of confidence

P = expected prevalence or proportion (in proportion of one; if 80%, P = 0.8), and

d = precision (in proportion of one, if 5%, d = 0.05)

Chapter 3 and 4

Equation 2: ΣFIC calculation

To determine the interactive antimicrobial activity of combinations, minimum inhibitory concentration (MIC) values were used in the fractional inhibitory concentration (FIC) calculation (van Vuuren and Viljoen, 2011), where "a" represents the antimicrobial sample and "b" represents the plant sample:

$$\mathrm{FIC}^{(i)} = \frac{\mathrm{MIC_a \text{ in combination with } MIC_b}}{\mathrm{MIC_a \text{ independently}}}$$

$$\mathrm{FIC}^{(ii)} = \frac{\mathrm{MIC_a \text{ in combination with } MIC_b}}{\mathrm{MIC_b \text{ independently}}}$$

The sum of the fractional inhibitory concentration index (ΣFICI) was calculated:

$$\Sigma\mathrm{FICI} = \mathrm{FIC}^{(i)} + \mathrm{FIC}^{(ii)}$$

List of abbreviations

AIDS	Acquired immunodeficiency syndrome
ATCC	American type culture collection
A	Antibiotic / antifungal (conventional antimicrobial)
AQ	Aqueous extract
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CLSI	Clinical and Laboratory Standards Institute
CFU	Colony forming unit
COVID-19	Coronavirus 2019
°C	Degree Celsius
DMSO	Dimethyl sulfoxide
ESKAPE	*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp.
FIC	Fractional inhibitory concentration
ΣFIC	Fractional inhibitory concentration index
HIV	Human immunodeficiency virus
JHB	Johannesburg
KEH	King Edward VIII Hospital
KZN	KwaZulu-Natal
µg	Micrograms
µl	Microliters
Mg	Milligram
Ml	Millilitres
MIC	Minimum inhibitory concentration
MDR	Multi-drug resistant
ND	Not determined
ORG	Organic extract
%	Percent
INT	*p*-Iodonitrotetrazolium violet
B	Plant sample (aqueous or organic extract)
SANBI	South African National Biodiversity Institute
WHO	World Health Organisation

Preview and description of thesis

This submission is a thesis by publication that incorporates research divided into five chapters, as outlined in Figure A. To attend to the aim of the study, a three-phased approach was followed. Phase 1 of the study, presented in Chapter 1, introduces the integrative medicine concept and its' use in infectious disease management and against antimicrobial resistance threats. Thereafter, Chapter 1 includes literature reviews (*Publication 1* and Section 1.2) on natural products in combination with conventional antimicrobials, to identify interactions of benefit (synergy) or those that may pose a threat to patient treatment outcomes (antagonism) for the treatment of infectious diseases.

Phase 2 of the study (Chapter 2) involves descriptive survey studies that were undertaken at two public hospitals in South Africa, namely Charlotte Maxeke Johannesburg Academic Hospital CMJAH), in Johannesburg (JHB), Gauteng and King Edward VIII Hospital (KEH) in Durban, KwaZulu-Natal (KZN), to determine traditional medicine use and practices amongst patients visiting the pharmacy to receive conventional medicines. Furthermore, phase 2 set out to determine the prevalence of concurrent use of traditional and conventional medicines in these two hospitals, which are both respectively, close in proximity to the two largest *muthi* markets in South Africa (Faraday market in JHB and Warwick market in KZN). Phase 3 of the study (Chapters 3 and 4) focused on *in vitro* based interactive antimicrobial evaluation of frequently used conventional antimicrobials (amoxicillin, azithromycin, ciprofloxacin, doxycycline, nystatin and fluconazole) with 34 traditional medicinal plant samples (aqueous and organic extracts) purchased from the Faraday (Chapter 3) and Warwick (Chapter 4) *muthi* markets, against the ESKAPE pathogens (*Enterococcus faecium* (ATCC 27270); *Staphylococcus aureus* (ATCC 29212); *Klebsiella pneumoniae* (ATCC 13883); *Acinetobacter baumannii* (ATCC 19606); *Pseudomonas aeruginosa* (ATCC 27853); *Enterobacter cloacae* (ATCC 13460); and yeasts (*Candida albicans* ATCC 10231; *Candida glabrata* ATCC 90030). Findings to the JHB plant: antimicrobial combinations are reflected in *Publication 2* (Chapter 3), and KZN plant: antimicrobial findings in *Publication 3* (Chapter 4). Furthermore, findings of the toxicity analysis of synergistic interactions are presented in both *Publication 2* and *3*. Chapter 5 provides final conclusions, study limitations and future recommendations.

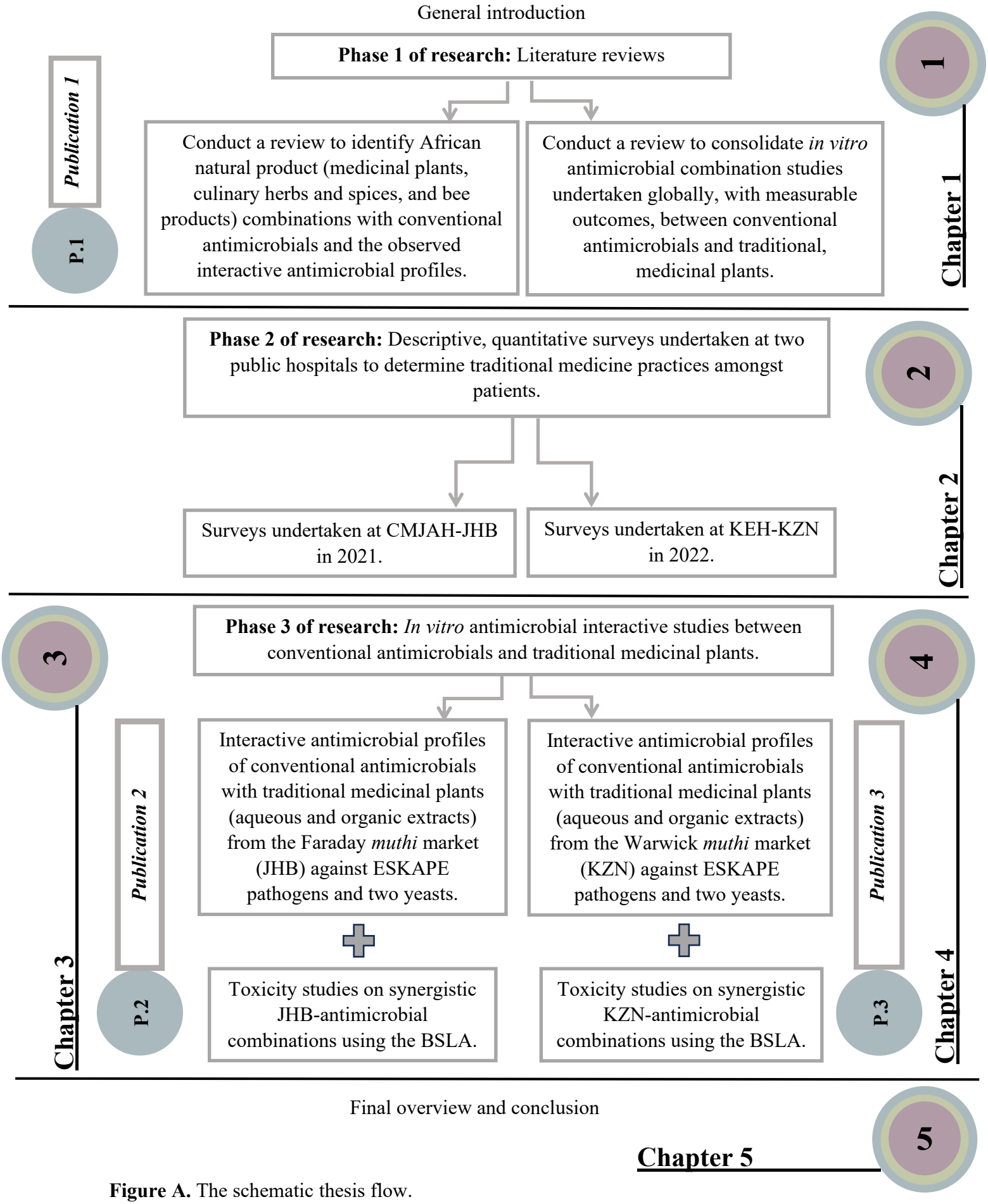


Figure A. The schematic thesis flow.

General introduction

This section of the thesis explores the theoretical framework underpinning the research, providing a foundation for understanding the interplay between integrative medicine practices and infectious disease therapies. Key elements, theories and concepts weaved throughout this thesis have briefly been introduced in this section. Furthermore, this section outlines the problem statement, the purpose of the study, along with the aim and respective objectives to ensure the research topic is achievable.

i. South African healthcare systems

Healthcare systems constitute the interplay of resources, to improve the health outcomes within a country (WHO, 2000). The South African healthcare system has been shaped by historial developments (Mayes, 2004), and is the reflection of the country's cultural, political, financial and economic capabilities (van Rensburg, 2004). A resultant parallel public and private healthcare framework, with high healthcare costs and poor service delivery (National Consultative Health Forum, 2011), has seen many seeking alternative healthcare avenues. South Africans have exposure to various informal traditional medicine markets, also known as *muthi* markets in South Africa, an isiZulu term. These markets provide consumers with various traditional medicine products, mostly inclusive of herbal, medicinal plant products, to treat or prevent disease (Williams et al., 2011). Three of the largest *muthi* markets in South Africa, are situated in provinces, Gauteng (Johannesburg Faraday market, Johannesburg KwaMai-Mai market and Pretoria Marabastad market), KwaZulu-Natal (Durban Warwick Triangle market) (Williams et al., 2011, Maroyi, 2016; Khumalo et al., 2018), and Limpopo (Ga-Maja market) (Matotoka and Masoko, 2017; Maroyi, 2016).

ii. Integrative medicine

Integrative medicine refers to a healthcare model, whereby conventional medicine and traditional, complementary and alternative medicine (TCAM) are practised interchangeably and

harmoniously, rather than in isolation (Lee et al., 2022). The TCAM framework is described as all medicines or healing practices that fall outside of conventional, biomedical healthcare models. It is a set of healthcare practices encompassing a variety of treatment approaches (James et al., 2018; Lee et al., 2022). The coordination of this TCAM framework with modern medicine systems, allows for the adoption of patient-centred care approaches to healthcare and has proven to optimise patient health outcomes (Lee et al., 2022). Furthermore, there is growing evidence that integrative medicine may be useful in the prevention and treatment of non-communicable diseases and more so, infectious diseases (Seetharaman et al., 2021).

The World Health Organisation (WHO, 2014), in its Traditional Medicines Strategy (2014 – 2023) highlighted the importance of integrative medicine in developing countries, with the aim to extend healthcare coverage. In Africa, integration practices of traditional medicine with modern healthcare could harness centuries of indigenous knowledge to treat patients more holistically (WHO, 2014). Integrative medicine is, however, faced with legislative and policy frameworks in Africa. South Africa's National Development Plan (NDP) recognises the widespread use of traditional medicine and advocates for integrative medicine practices in South Africa, to support poverty alleviation, economic development, and the preservation of indigenous knowledge. The NDP policy aims to ensure that the role of traditional medicine is acknowledged and harmonised with the conventional, national, healthcare system (National Planning Commission, 2025), with the Nagoya Protocol additionally adopted, to ensure biodiversity conservation, socioeconomic development and equitable benefit sharing amongst local, indigenous communities. with the Nagoya Protocol, a primary example. This protocol arose when *Aspalathus linearis* (Rooibos) commercialisation gained traction in South Africa. Although the protocol focuses on traditionally used plant material, it has since expanded its' scope to wild fauna, animal derivatives, and other natural resources (Resource Africa, 2025). The Indigenous Knowledge Systems Act (Act No. 6 of 2019) provides a legal framework for the protection and promotion of indigenous knowledge, including traditional medicinal practices in South Africa. It enables the development of policies that support the sustainable use and commercialization of indigenous knowledge, ensuring equitable benefit-sharing with local communities (National Planning Commission, 2025).

iii. Infectious diseases

Infectious diseases have consistently ranked among the top threats to global health. The National Institute of Health has classified infectious diseases as the second leading cause for worldwide morbidity. Furthermore, the WHO stipulated that infectious diseases were responsible for 5.9 million deaths in the sub-Saharan African region (WHO, 2014; Ugboko et al., 2020). In southern Africa, infectious diseases continue to contribute to morbidity and mortality, despite medical advances in antimicrobial therapies. In the second South African National Burden of Diseases study, 33.6% of more than 500 000 deaths and 13.5% of deaths in South Africa alone, were caused HIV and TB, or by other infectious diseases, respectively (Pillay-van Wyk et al., 2016). One of the most concerning contributing factors to this continuation in communicable disease-related deaths in South Africa, is the rapid development of antimicrobial resistance in bacterial, fungal, viral and parasitic pathogens (Govender and Reubenson, 2018).

iv. Medicinal plants and infectious diseases

Among the new approaches to combating infectious diseases, medicinal plants present an appealing alternative. They are a readily available source of medicine and widely accepted. Medicinal plants have served as a medicinal source in traditional healing practices for centuries, despite advances in modern medicine. This strong reliance not only signifies the intricate role of medicinal plant use in various cultures, but also to an extent legitimises indigenous knowledge and therapeutic efficacy claims, despite the lack of scientific validation (Ugboko et al., 2020). More than half of the conventional drugs currently approved and used in modern healthcare facilities were initially derived from or inspired by natural products (Cragg and Newman, 2013). Although only 3% are approved antibacterial natural products (Patridge et al., 2016).

v. Antimicrobial resistance

With the introduction of conventional antimicrobials, infectious diseases were rendered a negligible public health threat, however, the inappropriate use of these agents resulted in the

emergence of antimicrobial resistance (Cheesman et al., 2017; Ayaz et al., 2019). It is estimated by the Centre for Disease Control and Prevention (CDC), that antimicrobial resistant infections directly inflict five million mortalities annually (CDC, 2019). It is further projected that by 2050, this could escalate to 300 million. Infectious micro-organisms constantly develop ways to counteract the effects of conventional antimicrobials (Munita and Aries, 2016). Consequently, the WHO has labelled antimicrobial resistance as one of the three most pertinent public health threats of the 21st century. It is evident that new or alternative treatments for infectious diseases are urgently needed (Bhardwaj et al., 2016; Cheesman et al., 2017; Ugboko et al., 2020; WHO, 2024).

vi. Antimicrobial combinations

Combination therapy is a therapeutic concept employed in western medicine where certain antimicrobials are combined with other substances to enhance their efficacy. For example, the second agent protects the antimicrobial from degradation by resistant micro-organisms or alternatively, additional agents carry their own antimicrobial potential over and above that of the original single antimicrobial (Cheesman et al., 2017). Combination therapy is also practised in traditional medicine frameworks. Traditional healers often use combinations of plants to treat or cure diseases (Kamatou et al., 2006). Therefore, these principles could apply to combinations consisting of agents from each of the healthcare systems and warrants further investigation.

vii. Advantages and disadvantages to integrative medicine for infectious diseases

In the integrative medicine for infectious diseases context, both synergistic and antagonistic interactions between conventional and TCAM antimicrobial therapies are of clinical relevance, as synergy has the potential to increase the efficacy of conventional antimicrobials, resulting in therapeutic success (Malczak and Gajda, 2023). Plants have often been found to be synergistic enhancers and could potentiate the antimicrobial activity of existing antimicrobials rendering them more effective against resistant pathogens (Kamatou et al., 2006). Furthermore, a study by Stermitz et al. (2000), further found that natural products with antimicrobial potential, could be effective against pathogens exhibiting multi-drug resistance (MDR). A particular example, with the *Berberis* genus, producing berberine, was found to synethise an inhibitor of a MDR pump in *S.*

aureus, neutralising the pathogens resistance mechanisms and restoring antimicrobial potency (Stermitz et al., 2000). Furthermore, plants, constantly exposed to pathogens, have developed defence mechanisms to curb the occurrence and spread of infectious conditions, which could be harnessed (Sakkas and Papadopoulou, 2017). Of additional proposed benefit of combining existing antimicrobials with natural products, is reduced toxicity and side effects (van Vuuren et al., 2008)

Antagonism, however, has the potential to exacerbate antimicrobial resistance through the reduction in efficacy of antimicrobials, hence providing an adequate environment for micro-organisms to flourish and develop resistance, establishing the basis for therapeutic failure (Booth and van Vuuren, 2023).

viii. Problem statement

There is a lack of exploration of prevalence rates of the traditional medicine use amongst patients visiting western public healthcare facilities in South Africa for accurate interactive exposure risk analysis. Furthermore, scientific evidence of interactive antimicrobial and toxicity profiles of traditional and conventional medicine combinations are lacking. With the high infectious disease burden in South Africa, along with rising antimicrobial resistance, factors impacting on antimicrobial therapies need to be closely monitored. Interactive profiles of combinations of traditional, South African medicinal plant extracts and conventional antimicrobials have sorely been neglected in scientific investigations.

ix. Purpose of the study

Integrative medicine presents promising potential to address the WHO Traditional Medicine Strategy (2014 – 2023) of achieving extended healthcare coverage in developing countries, however, integration poses an array of concerns to be addressed to ensure patient wellbeing. The prevalence of traditional medicine use amongst patients visiting conventional healthcare facilities needs to be investigated, to discern the extent to which patient populations may be exposed to potential interactions between the two forms of healthcare. Thereafter, for safe integration, interactions between traditional and conventional medicines are to be explored and well defined.

The rapidly rising antimicrobial resistance in Africa poses a serious public health threat. Many African traditional medicinal plants are documented as possessing antimicrobial activity, hence presenting a worthy alternative source for antimicrobial therapy. South Africa has rich cultural practices embracing traditional medicinal plant use. Furthermore, South African medicinal plant: conventional antimicrobial combination therapies could present a less toxic approach to attend to antimicrobial resistance.

x. Aim and objectives of the study

Objectives for phase 1:

a. To review and collate the interactive synergistic and antagonistic profiles of *in vitro* combination studies where African natural products were investigated in combination with conventional antibiotics and antifungals.
b. To review and collate the interactive synergistic and antagonistic profiles of *in vitro* combination studies where medicinal plant extracts were investigated in combination with conventional antibiotics and antifungals.

Figure B represents the procedure followed to achieve the abovementioned objectives.

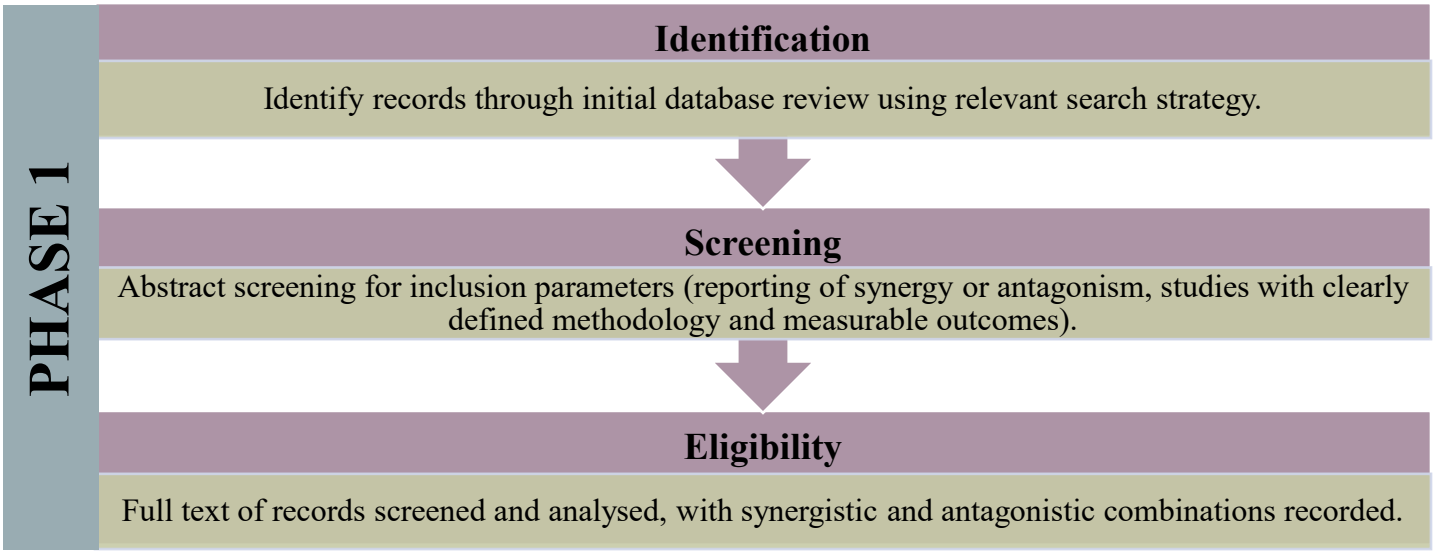


Figure B. A schematic representation of the workflow, for phase 1 (Chapter 1: *Publication 1* and Section 1.2) of the study.

Objectives for phase 2:

a. To determine the prevalence of use of traditional medicine in patients visiting two large tertiary public hospitals (CMJAH-JHB and KEH-KZN) in South Africa, using a quantitative survey provided to all patients in hospital pharmacy waiting areas.
b. To determine the prevalence of concurrent use of traditional medicine and conventional drugs (with the focus on infectious diseases) in patients visiting the two public hospitals in South Africa, through analysis of data collected.

These objectives were addressed through the workflow represented in Figure C.

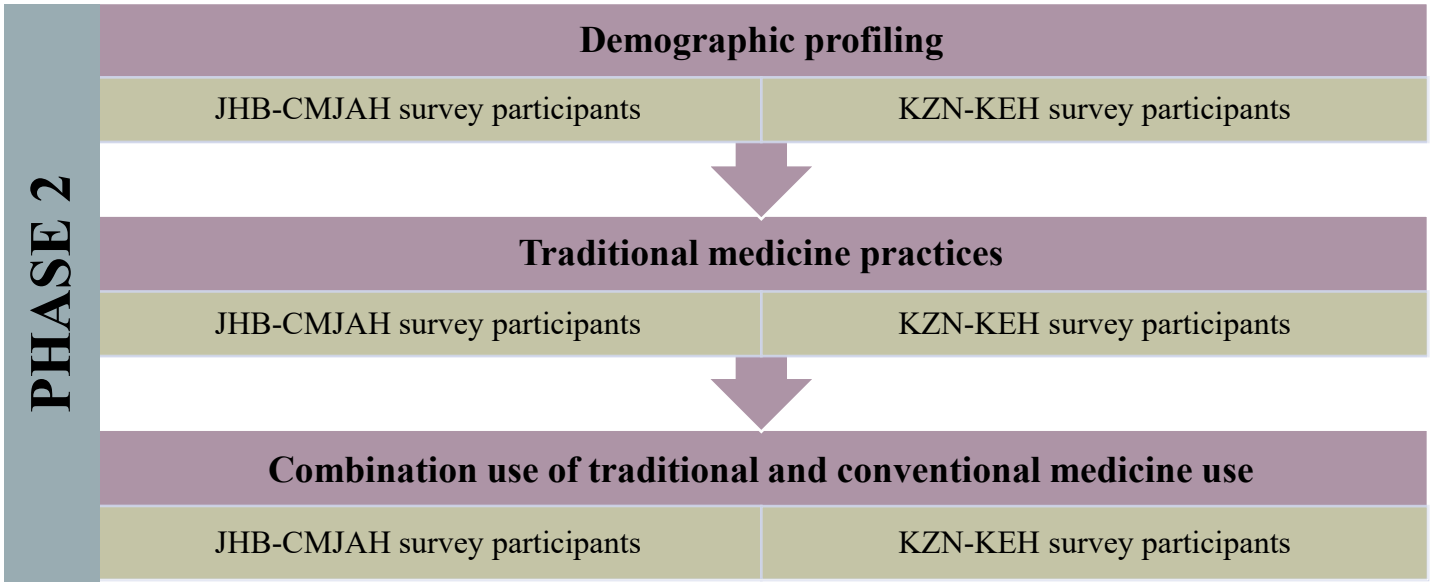


Figure C. A schematic representation of the workflow, for phase 2 (Chapter 2) of the study.

Objectives for phase 3:

a. To source the relevant traditional medicinal plant samples for *in vitro* evaluation.
b. To prepare the organic solvent and aqueous plant extracts for testing, as well as plant samples using the expertly recommended traditional methods of preparation.
c. To determine the *in vitro* interactive profiles between commonly identified combinations of traditional medicinal plants and conventional antimicrobials by:

- To determine the antimicrobial activity of the plant samples alone, using minimum inhibitory concentration (MIC) assays.
- To determine the antimicrobial activity of selected conventional antimicrobial agents in combination with plant samples, using the minimum inhibitory concentration (MIC) assay.
- To determine the sum of the fractional inhibitory concentration (ΣFIC), to assess interactions between the selected plant samples in combination with a selection of conventional antimicrobial agents.

d. To determine the toxicity profiles of notable combinations, with the use of the brine shrimp lethality assay (BSLA) by:

- To evaluate the independent toxicity of the plant samples shown to exhibit synergistic potential.
- To evaluate the toxicity of the synergistic plant sample: conventional antimicrobial combinations.

These objectives were attended to through undertaking the stepwise approach in Figure D.

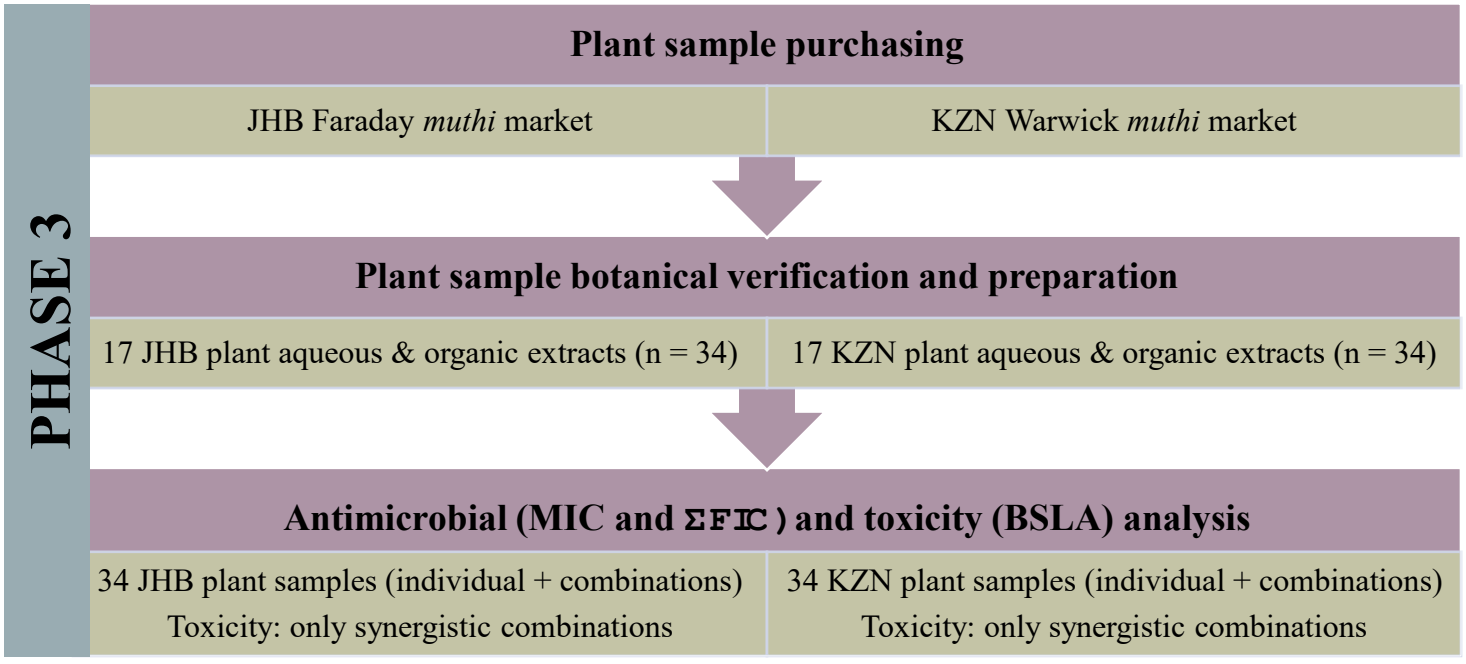
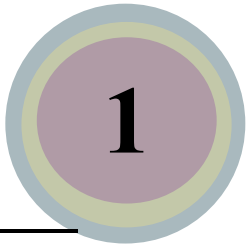


Figure D. A schematic representation of the workflow, for phase 3 (Chapter 3: *Publication 2* and Chapter 4: *Publication 3*) of the study.

Chapter 1
Evaluation of existing literature on combination studies



1.1. Chapter introduction

Chapter 1 serves to address objectives (a) and (b) outlined in phase 1 of the study, to further contextualise integrative medicine through examination of existing literature on *in vitro* interactive combination studies with conventional antimicrobials (antibiotics and antifungals) and natural products used traditionally for infectious conditions and to identify key gaps requiring attention.

This chapter includes a review on *in vitro* combination studies between conventional antibiotics and antifungals with African natural anti-infective products (*Publication 1*), and additional research focused on collating and evaluating *in vitro* combination findings for plant extracts with conventional antibiotics or antifungals. By collating existing research, this chapter not only clarifies the conceptual landscape but also frames the approach to be adopted in subsequent chapters.

P.1

Publication 1

Booth, Z., van Vuuren, S., 2023. The combined use of African natural products and conventional antimicrobials: an alternative tool against antimicrobial resistance in Abia, K.A.L., and Essack, S.Y., In Antimicrobial Resistance and One Health in Africa (ed 1), Springer International Publishing. https://doi.org/10.1007/978-3-031-23796-6 (Switzerland).

*Proof of publication and permission for re-use in this thesis is provided in Appendix B. A graphical abstract reflecting this publication has been presented in Figure 1.1.

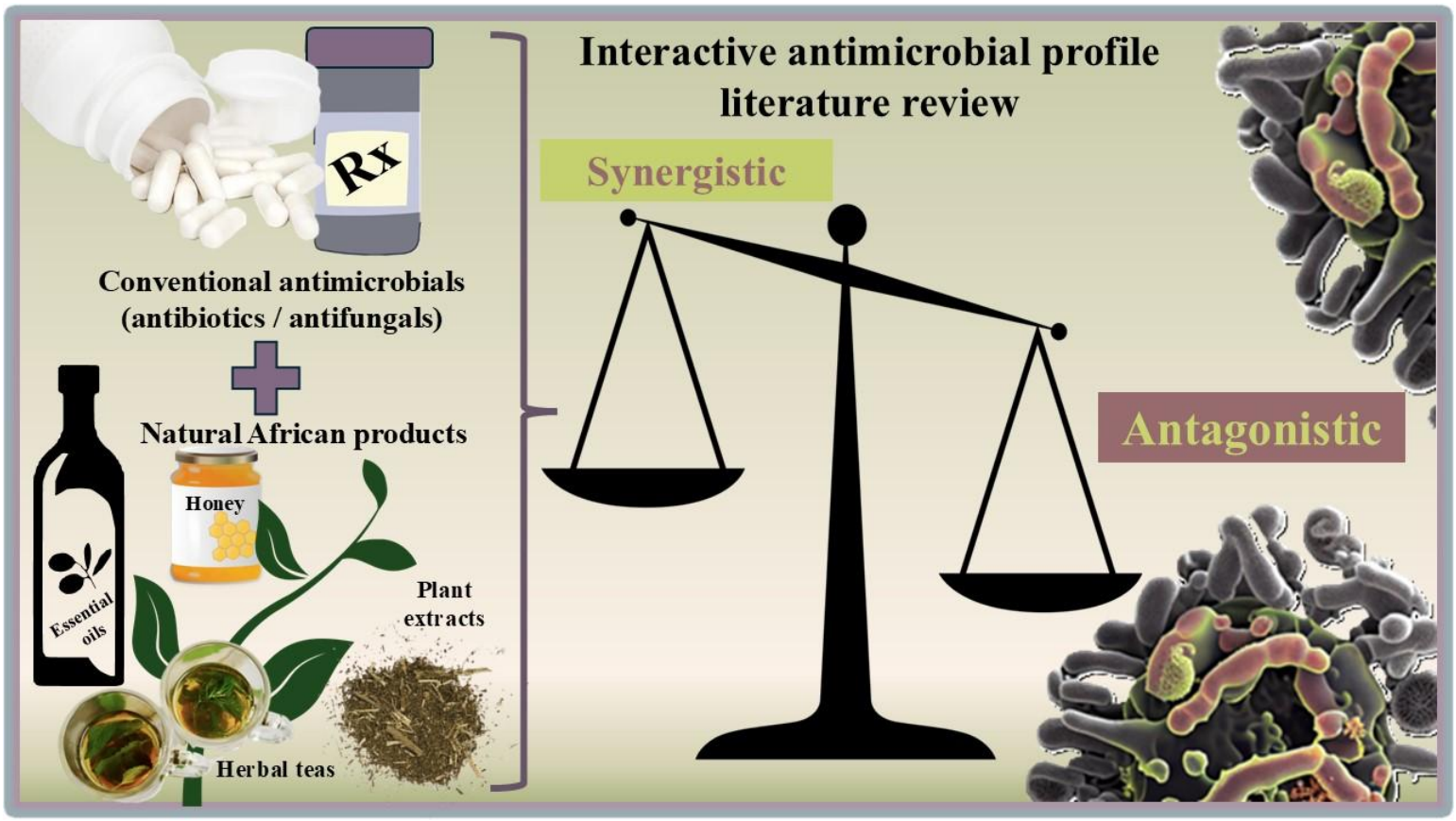


Figure 1.1. A graphical abstract for *Publication 1*.

The combined use of African natural products and conventional antimicrobials: an alternative tool against antimicrobial resistance.

Z. Booth, S.F. van Vuuren

Chapter synopsis

The challenges associated with antimicrobial resistance (AMR) has compelled researchers to identify alternative means of treating infectious diseases exhibiting resistance to conventional antimicrobials. One such approach, is the use of combination therapy, in particular, the use of natural products in combination with existing conventional antimicrobials. A few well documented natural products demonstrating synergistic effects with conventional antimicrobials include selected herbal teas, bee products, herbs, spices, and more frequently medicinal plant containing products, essential oils, plant extracts and pure compounds. Antagonism associated with combination use also needs to be considered, as these interactions could lead to adverse conditions and may contribute to the ever-rising occurrence of AMR. The ultimate goal would be to identify promising combinations of natural products with conventional antimicrobials, ultimately assisting in addressing the surge in AMR currently evident in conventional antimicrobial therapies.

This chapter explores the potential of combining conventional antimicrobial agents with natural products of interest in the African context and illustrates a few notable interactions between natural products available in Africa and conventional antibiotic and antifungal agents. Most of the natural products to be discussed in this chapter are indigenous to the African continent, however, some are invasive plant species, of which many have been naturalized or commercialized in Africa and hence included.

1. Introduction

1.1. Natural product classification

Natural products are groups of non-synthetic molecules, exhibiting biological activity and hence often used for their medicinal properties. Many products feature under this broad classification of “natural products”. Traditional medicines, herbal or supplementary products, are often derived from medicinal plants in various forms (essential oils, extracts, tinctures, teas and isolated

phytochemicals), along with commonly consumed foodstuff (most often culinary herbs, spices, seeds, fruit, and bee products), containing medicinal compounds (Cragg and Newman, 2013). These natural products used for medicinal purposes are often referred to as traditional, complementary, and/or alternative medicines (TCAMs). The TCAM framework is described as all medicines or healing practices that fall outside of standard, biomedical healthcare models (James *et al*., 2018).

Traditional medicine comprises of local herbal medicines or products, indigenous healthcare practices, and local or imported complementary and alternative medicine products and practices (James *et al*., 2018). Traditional medicine specifically is defined as "*the use of theories, beliefs, and native experiences from different cultures, in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness*" (WHO, 2019). Complementary and alternative medicines both refer to a wide range of healthcare products and techniques, that are not part of a country's traditional medicine framework and are not fully incorporated into the mainstream (conventional) healthcare system (WHO, 2019). The TCAM practices are geographically varied, giving rise to predominantly known, African traditional medicine (ATM), traditional Chinese medicine (TCM) and Ayurvedic medicine or traditional Indian medicine (TIM). Although some forms of traditional medicine and practices are specific to certain countries, many well-known types of traditional remedies are being adopted in other countries around the world (Mahomoodally, 2013). For the purposes of this chapter, a strong focus is given to ATM.

In many countries, natural products are not classified as drugs requiring regulatory authority registration and approval before entering the market. This is another contentious subject. More than half of the conventional drugs currently approved and used in modern healthcare facilities, were initially derived from or inspired by natural products (Newman and Cragg, 2016). Thereby, emphasizing the medicinal potential of natural products, and inadvertently, similarly with conventional drugs, the potential for harmful effects, if not used correctly. The World Health Organisation (WHO) has set out guidelines in advancing the acceptance and integration of TCAM with conventional health systems worldwide, to legitimize TCAM and also facilitate the drive

towards scientifically validating and profiling these products for the safety of patients (WHO, 2017).

1.2. Global natural product use and traditional health systems

Naturally derived products have become increasingly popular around the world (James *et al*., 2018). An estimated one-third of the United States of America (USA) population, 20 – 50% of the European population, and 80% of the African and Asian population, make use of natural products. Reasons for the high prevalence of use pertains to natural product accessibility, availability and affordability being more favourable than that of conventional medicine (WHO, 2019), particularly for those accessing healthcare in the public sector in Africa.

The WHO global report on TCAM (2019), specifies that in the American region, medicinal plants and herbal medicines constitute the main forms of traditional medicine practices. However, other forms of traditional, alternative and complementary medicines, like acupuncture and homeopathy, are also notably prevalent, with many clinics offering these services throughout the American region (Ong *et al*., 2005). Mordinez (2019) mentions that back in 2005, the National Institute of Health (NIH) already estimated their expenditure on herbal medicine in America, to be approximately 33 million dollars, reflecting the vast use of this form of healthcare in the region.

Asian countries like China, Korea, Japan, and India have been known for their well-developed traditional medicine systems, with East Asian medicines originating approximately 3000 years ago. Over the years, however, each region in Asia has developed its own form of TCAM that are uniquely practiced such as TCM, traditional Korean medicine (TKM), traditional Japanese medicine (TJM) and TIM (Mordeniz, 2019). Another commonly known form of Asian traditional medicine is Ayurveda. Ayurveda is a healthcare system founded by ancient Hindu healers which has been practiced throughout India. Ayurveda has even been recognized by the Indian government as a complete form of healthcare, together with western medicine (Mordeniz, 2019).

Europe possesses a large and respectable history of natural product use; however, the most dominant healthcare system remains allopathic in nature (Firenzuoli and Gori, 2007). In Germany, an estimated 600 – 700 plant-derived and plant-based medicines are accessible to the public and

are prescribed by nearly 70% of German physicians (Mordeniz, 2019). Germany also classifies herbal medicines as an element of naturopathy, a medical system that encompasses healthcare and traditional practices, which was popular in Europe in the 19th century (NCCIH, 2017). Jacobsen *et al*. (2015), determined through a study undertaken at 80 hospitals in Norway, that 64% of hospital staff offered their patients natural products or alternative therapies.

1.3. Natural product use in the African healthcare system

It is widely documented that the use of TCAM is widespread throughout sub-Saharan Africa, with a considerable population relying on it to maintain their health or prevent and treat communicable and non-communicable diseases (James *et al*., 2018). African traditional healers in rural Africa are far more accessible and affordable as healthcare practitioners, resulting in local communities consulting these healers first before reaching out to formalized healthcare facilities, such as public clinics and hospitals. African TCAM use is perhaps one of the oldest and most varied (Mahomoodally, 2013).

The WHO estimates that 80% of the African population makes use of natural, traditional products as their primary source of health (WHO, 2017). However, a literature review undertaken by James *et al*. (2018), reported a varied prevalence rate for natural product use. James *et al*. (2018) focused solely on literature documenting natural product use in sub-Saharan Africa during the period of 1 January 2006 to 28 February 2017 and found prevalence rates ranging from 4.3% to 69.4% (mean = 30.5%) in the various developing countries in sub-Saharan Africa. This discrepancy warrants further validation and hence there still remains a dearth of research necessary to further explore these statistics and patterns of natural product use.

In 2017, the WHO stated that the most common type of traditional medicine takes the form of natural, herbal products of plant origin. In Africa, this is most certainly true, with medicinal plants forming the largest portion of treatment options available from traditional healers or from “muthi” markets (WHO, 2017). In southern Africa, there is approximately 3400 indigenous plant species that have been documented as being used traditionally. Of these plants, 2062 plant species are traded in South Africa alone, for medicinal purposes. The most prevalent trading of medicinal

plants for traditional medicine use occurs in KwaZulu-Natal, Gauteng, Eastern Cape, Mpumalanga and Limpopo (James *et al*., 2018).

Medicinal plants that are most popularly traded in South Africa include *Agathosma betulina* (buchu), *Aloe ferox* (bitter aloe), *Artemisia afra* (African wormwood), *Aspalathus linearis* (rooibos), *Bulbine frutescens* (burn jelly plant); *Cyclopia genistoides* (honeybush), *Harpagophytum procumbens* (devil's claw), *Hoodia gordonii* (hoodia), *Hypoxis hemerocallidea* (African potato), *Lippia javanica* (fever tea), *Mesembryanthemum tortuosum* (kanna), *Pelargonium sidoides* (African geranium), *Siphonochilus aethiopicus* (African ginger), *Sutherlandia frutescens* (cancer bush), *Warburgia salutaris* (pepperbark tree) and *Xysmalobium undulatum* (milk bush). Many of these commonly used medicinal plants have been commercialized in the form of standardized formulations, rather than the crude, unprocessed plant material purchased at traditional "muthi" markets. The commercialization of these natural products further emphasizes the efficacy of these products, even with limited regulatory and scientific backing (van Wyk, 2011).

1.4. Natural products with antimicrobial properties

Before the development of conventional antimicrobials, natural products, most commonly traditional medicinal plant preparations (commonly referred to as teas, herbs and spices), were consumed to fight infection. It has also been noted that these natural remedies resulted in minimal side effects. Van Vuuren and Holl (2017), Khameneh *et al*. (2019), Chassagne *et al*. (2021) and Stan *et al*. (2021) are just some reviews conducted on medicinal plants (indigenous, invasive, naturalized or commercialized species), as natural products exhibiting noteworthy antimicrobial activity against a range of pathogens.

Mahomoodally (2013), highlighted important medicinal plants in Africa that have short and long-term potential to manage and treat infectious or chronic conditions, and the possibility for further development as phytopharmaceuticals in the future. The African medicinal plants most commonly identified in the reviewed literature by Mahomoodally (2013), for their anti-infective properties were *Acacia senegal* (gum arabic), *Aloe ferox* (bitter aloe or Cape aloe), *Artemisia afra* (wormwood), *Aspalathus linearis* (rooibos), *Centella asiatica* (centella), *Catharanthus roseus*

(Madagascan periwinkle), *Cyclopia genistoides* (honeybush), *Harpagophytum procumbens* (devil's claw), and *Pelargonium sidoides* (African geranium).

Culinary herbs and spices such as *Rosmarinus officinalis* (rosemary), *Piper nigrum* (black pepper) and *Cinnamomum zeylanicum* (cinnamon) have also demonstrated antimicrobial potential (Abascal and Yarnell, 2013). In the review by Abascal and Yarnell (2013), it was claimed that the antimicrobial action of *Allium sativum* (garlic) is comparable to that of standard antibiotics. Liu *et al*. (2017) conducted a review on the antimicrobial activity of common household herbs and spices and reported that many exhibited notable antimicrobial activities against a variety of pathogens. Those with the most noteworthy antimicrobial activity were *Eugenia caryophyllata* (clove), *Origanum vulgare* (oregano), *Thymus vulgaris* (thyme), *C. zeylanicum* and *Cuminum cyminum* (cumin), against various bacterial (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Salmonella typhimurium*) and fungal (*Aspergillus niger* and *Aspergillus flavus*) micro-organisms. Interestingly, these herbs and spices also exhibited activity against resistant micro-organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Liu *et al*., 2017).

Some gallic acid rich culinary herbs such as *Eupatorium perfoliatum* (boneset) and *Marrubium vulgare* (horehound), exhibit antimicrobial properties (Livestrong, 2020). Certain foods that are consumed, are also rich in gallic acid, such as *Allium sativum* (garlic) and *Grifola frondosa* (maitake mushrooms) (Abascal and Yarnell, 2013).

Many natural products have demonstrated promising antimicrobial and immunomodulatory properties against highly infectious diseases, such as influenza, tuberculosis and malaria (Pandey *et al*., 2020) and addressed the role of traditional medicine in the coronavirus disease (COVID-19) pandemic. Based on the urgency of the situation, immune-boosting approaches using natural products have been promoted widely to reduce the number of SARS-CoV-2 infections worldwide (Ang *et al*., 2020).

2. Exploring the combination of natural products in combination with conventional antimicrobial agents to address antimicrobial resistance (AMR)

With the high incidence of infectious diseases in Africa, AMR is a major threat to the country's public health, further exacerbating the insurmountable burden already placed on the healthcare system (Dalal *et al*., 2011; Avert, 2020).

New conventional antimicrobial discovery has proven a challenge in pharmaceutical research and development. With the rapid increase in AMR and many existing antimicrobials rendered ineffective, the slow research and development process is worrisome. Researchers are needing to adapt approaches and consider alternative means of enhancing existing antimicrobials potency to ward off AMR.

One such approach would be the use of combination therapies, in particular with natural products that have demonstrated effective antimicrobial activity. Combination therapies using existing conventional antimicrobials and natural products, could be a simpler, faster and more cost-effective way of overcoming AMR, as opposed to formulating new antimicrobial compounds (Cock *et al*., 2017). In 2016, the WHO announced a favorable response toward the use of combination therapies as opposed to monotherapy, particularly for the treatment of potentially fatal infectious diseases, such as HIV/AIDS, malaria and tuberculosis. This was based on evidence that combination therapy has the ability to limit resistance and allows for multiple targets of the causative organism and/or its host to be targeted in a holistic manner (WHO, 2017).

It is proposed that the term "natural" confers a sense of safety, and so an identified effective natural product combination with existing conventional antimicrobials, may be safer in addressing drug-resistant infections (Barbosa *et al*., 2020). With Ma *et al*. (2019), stating that combination therapy with traditional and western medicine can lead to synergy and improved outcomes, this approach appears even more favorable. Natural products have commonly been found to enhance or potentiate conventional antimicrobial activity, even if the product itself does not possess antimicrobial activity (Sibanda and Okoh, 2007; Adwan *et al*., 2010).

Mahomoodally (2013) emphasizes that medicinal plants, as natural products, contain different secondary metabolites that have the potential to interact in a variety of ways. It is described that with the complexity in constituency of medicinal plants, several chemicals may work synergistically to improve health outcomes, such as enhanced antimicrobial potency to fight off infection, but may also present further advantages. Secondary metabolites of plant origin have demonstrated the potential to speed up or slow down pharmacokinetic activities, resulting in fewer undesirable adverse effects or toxicity of conventional drugs when consumed concurrently (Mahomoodally, 2013; Kahraman *et al*., 2020. Furthermore, it has been noted that with the complex constituents within plant materials, these may very well offer more than one mechanism of action and assist not only in treating the infection but also providing symptomatic relief through possessing anti-inflammatory or anti-pyretic properties. It is for this reason that Mahomoodally (2013) suggests the preference for research on crude or standardized plant extracts as opposed to isolated plant compounds for medical use.

Viljoen and Vermaak (2013), also highlights an additional concern related to natural products of plant origin, and that is the challenge associated with standardization and quality control of plant products. The phytochemical complexity and species variability are dependent on cultivation methods, geographical harvesting, soil and temperature differences. These are all factors that impact on standardization of any plant product.

Mahomoodally (2013) and Eman (2014) stress that the vast number of compounds in medicinal plants creates much uncertainty in predicting potential interactive profiles with conventional drugs within the human body and could also prove to negatively impact conventional medicine, through antagonism. Many natural products target drug transporters or metabolizing enzymes, with another commonly identified effect being that of drug protein binding alterations, affecting the metabolism or excretion of a conventional drugs. The most documented interaction results from natural products inhibiting or promoting cytochrome P450 enzymes, which are liver enzymes responsible for the metabolism of most conventional drugs in the human body. More than 78 natural products have demonstrated potential to affect cytochrome P450 system activity (Eman, 2014). Hence, there is much still to be investigated, before any form of implementation or formulation studies can be undertaken.

2.1. Interactive antimicrobial profiling of combinations

Natural products combinations with conventional antimicrobials may interact on a pharmacodynamic level, allowing for four interactive antimicrobial profile classifications, namely synergistic, antagonistic, additive or non-interactive effects. Pharmacodynamic interactions, therefore, relate to natural product potential to either potentiate or reduce the pharmacological activity of a conventional antimicrobial (Eman, 2014).

Interactive antimicrobial profiles for natural product-conventional antimicrobial combinations can be determined through undertaking *in vitro* minimum inhibitory concentration (MIC) assays for each of the components of the combination individually, and thereafter, in combination. The antimicrobial interaction can then be evaluated through a calculation to determine the sum of the fractional inhibitory concentration (ΣFIC). Interactive antimicrobial activities of combinations are classified as either being synergistic (ΣFIC values less than; and equal to 0.5), additive (ΣFIC values above 0.5; but less than or equal to 1.0), indifferent (ΣFIC values greater than 1.0; but less than or equal to 4.0), and antagonistic (ΣFIC values greater than 4.0) (van Vuuren and Viljoen, 2011).

2.1.1. Synergistic interactions

Synergy is a word derived from the Greek word, *synergos*, which means to work together. A synergistic interaction can be classified as an interaction that results between two components, producing a final effect that is greater than each of the individual components. In relation to antimicrobial activity, a synergistic effect signifies increased potency in antimicrobial activity of that combination, thereby allowing for a dose reduction and possibly fewer side effects (Abreu *et al*., 2012). Although most studies consider the ΣFIC of less than or equal to 0.5, as a synergistic interaction, some studies have extended synergism from ΣFIC values to 0.75, classifying them as partial synergistic interactions (Salem *et al*., 2018).

Synergistic interactions between natural products and conventional antimicrobials could potentially assist increasing solubility or membrane transport of conventional agents (Gurley, 2012). This would result in increased susceptibility of micro-organisms to conventional antimicrobials (Stefanovic *et al*., 2011; Ovais *et al*., 2018), thereby presenting a further avenue to

addressing the surge in AMR (Sanhueza *et al*., 2017). It has been documented that through synergistic interactions posed by natural products on conventional antimicrobials, antimicrobial resistance may be overcome (Abascal and Yarnell, 2013).

2.1.2. Antagonistic interactions

Antagonism refers to the result when two agents in combination oppose one another's effect, thereby producing a final effect that is less than that of the individual agents. In the case of antimicrobial interactions, a reduced antimicrobial efficacy or potency against a micro-organism will be observed in combination.

Antagonistic interactions between natural products and conventional antimicrobials could reduce antimicrobial efficacy, thereby increasing the burden placed on healthcare systems with returning patients (Eman, 2014). The reduction in efficacy could also translate further into exacerbating the AMR issues that the healthcare system faces. If patients do not disclose the use of natural products with their healthcare professional, healthcare professionals may assume that a treatment failure may be due to insufficient dosages. The cascade would filter down to higher dose administration and an increased risk for toxic or adverse effects from conventional drugs (Adwan *et al*., 2010; Chanda and Rakholiya, 2011).

2.1.3. Additive and non-interactive interactions

Having less impact are additive and non-interactive effects. Additive interactions provide an effect that is equivalent to the combined effect of the individual agents, that is no less nor greater in combination, than when tested separately. Therefore, there is no advantage or disadvantage of the combination. Additive interactions usually occur between agents with similar mechanisms or sites of action (van Vuuren and Viljoen, 2011).

With the lack of advantages or disadvantages arising from non-interactive profiles and additive interactions, these combinations do not form the center of discussion in most literature, as they are not considered to be of any clinical relevance (Eman, 2014). A non-interactive or additive profile identification could, however, provide necessary confirmation that certain combinations would not cause detrimental adverse therapeutic outcomes for the patient. For this chapter, synergistic and

antagonistic interactions will be reflected on supporting the efficacy of such combinations in an attempt to curb antimicrobial resistance.

2.2. Conventional antimicrobials in synergistic combinations with essential oils

Essential oils are natural products derived via distillation from aromatic plants. Essential oil use has existed for decades, from ancient healing practices, to the most frequent aromatherapeutic use today (Langeveld, 2013). Research is primarily focused on scientifically evaluating the antimicrobial activity of essential oils, or alternatively, identifying phytochemicals from the essential oils and testing the antimicrobial activity of those alone, and then in combination with conventional antimicrobials to identify any potential synergism (Sibanda and Okoh, 2007; Adwan *et al*., 2010).

Essential oils, derived from a variety of plant species, have been found to synergistically enhance conventional antimicrobials. Interestingly, some essential oils, when tested individually do not exhibit antimicrobial activity, however, still possess the ability to enhance the potency of conventional antimicrobials when tested in combination.

Langeveld (2013) conducted a comprehensive review on the synergistic interactions between various essential oils distilled from commonly consumed culinary herbs (e.g. coriander, cinnamon, lemongrass, oregano, peppermint, rosemary and thyme) in combination with common conventional antimicrobials (e.g. ciprofloxacin, gentamicin, ceftriaxone, norfloxacin, erythromycin, ampicillin and vancomycin). These combinations were tested against a range of both Gram-positive and Gram-negative pathogens, and synergistic interactions were evident. Langeveld (2013), also studied some phytochemicals, derived from these essential oils, in combination with the aforementioned antimicrobials and tested these against some pathogens. Again, synergistic interactions were evident. Further evidence of synergistic interactions between conventional antimicrobials and essential oils derived from medicinal plants predominantly focusing on an African context is given in Table 1.

2.3. Conventional antimicrobials in synergistic combinations with plant extracts

Both Adwan *et al*. (2010) and Van Vuuren and Viljoen (2011) proposed that the potentiating effect of plant extracts on conventional antimicrobials has been neglected and requires further investigation. Hubsch *et al*. (2014[a]) studied various commercially available traditional medicinal plant extracts in combination with commonly used conventional antimicrobials, against a range of pathogens. Even though most interactions were found to be non-interactive in nature, there were a few notable synergistic interactions identified. It was found that both the aqueous and organic extract of *Agathosma betulina* (buchu) when combined with ciprofloxacin, against *E. coli*, provided synergistic interactions. A further notable synergistic interaction was identified between *Sutherlandia frutescens* (cancer bush) organic extract (a 1:1 mixture of dichloromethane: methanol) and ciprofloxacin against *E. coli* (ΣFIC of 0.28). Urinary tract infections, where *E. coli* is the most common causative pathogen, has shown resistance to ciprofloxacin. With *S. frutescens* used traditionally in the treatment of urinary tract infections, the combination with ciprofloxacin demonstrating synergy against *E. coli* could very well be an avenue of addressing the ciprofloxacin resistance (Hubsch *et al*., 2014[a]). It was also found that *P. sidoides* extracts demonstrated the highest synergistic interaction in comparison to other medicinal plants investigated. This finding could provide clinical benefit, in that *P. sidoides* is found in many natural remedies used to boost the immune system to fight off infection.

A South African study conducted on the combination of the ethanol extract of *Ziziphus mucronata* (buffalo thorn) with conventional antibiotics (tetracycline, chloramphenicol, amoxicillin and ciprofloxacin), found that more synergistic interactions (54.17%) occurred between the combinations than antagonism (1.39%). This study evaluated clinically relevant bacteria (*Bacillus cereus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *E. coli*) (Olajuyigbe and Afolayan, 2013). Some of the most noteworthy synergistic interactions identified in combination studies with conventional antimicrobials and plant extracts have been documented in Table 2.

Table 1. Synergistic interactions between conventional antimicrobials and African medicinal plant (indigenous/invasive/naturalized/ commercialized) essential oils (ΣFIC below or equal to 0.5 is considered a synergistic interaction)

Conventional antimicrobial	Natural product	Test micro-organism	ΣFIC	Reference
Amphotericin B	*Thymus vulgaris*	*Candida albicans* (Clinical isolate)	0.38	Jafri and Ahmad, 2019
		Candida tropicalis (Clinical isolate)	0.25	
	Cymbopogon citratus	*Candida albicans* (Clinical isolate 1)	0.16	Khan *et al*., 2012
		Candida albicans (Clinical isolate 2)	0.38	
	Coriandrum sativum	*Candida albicans* (ATCC 90028)	0.38	Silva *et al*., 2011
		Candida albicans (ATCC 24433)	0.19	
	Myrtus communis	*Aspergillus niger* (ATCC 16404)	0.26	Mahboubi and Bidgoli, 2010
		Aspergillus parasiticus (ATCC 15517)	0.26	
		Candida albicans (ATCC 10231)	0.25	
	Citrus aurantium	*Candida albicans* (ATCC 90028)	0.24	Nidhi *et al*., 2020
		Candida albicans (MTCC 277)	0.05	
Cefixime	*Thymus broussonetia*	*Salmonella typhimurium* (Laboratory isolate $CCMMB_{17}$)	0.18	Fadli *et al*., 2012
	Thymus maroccanus	*Staphylococcus aureus* (Laboratory isolate $CCMMB_3$)	0.18	
		Salmonella typhimurium (Laboratory isolate $CCMMB_{17}$)	0.18	
Ciprofloxacin	*Artemisia afra*	*Escherichia coli* (ATCC 25922)	0.27	Hubsch *et al*., 2014[a]
	Lippia javanica	*Escherichia coli* (ATCC 25922)	0.14	
		Enterococcus faecalis (ATCC 29212)	0.44	
	Thymus broussonetia	*Bacillus cereus* (ATCC 14579)	0.15	Fadli *et al*., 2012
		Bacillus subtilis (ATCC 9524)	0.26	
		Escherichia coli (Laboratory isolate $K_{12}W_{113}$)	0.37	
		Micrococcus luteus (ATCC 10240)	0.26	
		Pseudomonas aeruginosa (Clinical isolate)	0.14	
		Vibrio cholera (Environmental isolate)	0.14	

Conventional antimicrobial	Natural product	Test micro-organism	ΣFIC	Reference
Ciprofloxacin	*Thymus maroccanus*	*Bacillus cereus* (ATCC 14579)	0.15	Fadli *et al*., 2012
		Bacillus subtilis (ATCC 9524)	0.09	
		Enterobacter cloacae (Clinical isolate)	0.37	
		Escherichia coli (Laboratory isolate $K_{12}W_{113}$)	0.12	
		Klebsiella pneumoniae (Clinical isolate)	0.37	
		Micrococcus luteus (ATCC 10240)	0.28	
		Pseudomonas aeruginosa (Clinical isolate)	0.15	
		Salmonella typhimurium (Laboratory isolate $CCMMB_{17}$)	0.37	
		Staphylococcus aureus (Laboratory isolate $CCMMB_3$)	0.26	
		Vibrio cholera (Environmental isolate)	0.18	
	Calamintha sylvatica	*Escherichia coli* (ATCC 10536)	0.25	Sienkiewicz *et al*., 2016
		Salmonella abony (ATCC 6017)	0.25	
	Calamintha vardarensis	*Escherichia coli* (ATCC 10536)	0.25	
		Salmonella abony (ATCC 6017)	0.25	
	Calamintha nepeta	*Pseudomonas aeruginosa* (ATCC 9027)	0.50	
		Salmonella abony (ATCC 6017)	0.25	
	Plectranthus amboinicus	*Staphylococccus aureus* (ATCC 25923)	0.09	Jugreet and Mahomoodally, 2020
		Methicillin-resistant *Staphylococcus aureus* (Clinical isolate)	0.50	
		Bacillus spizizenni (ATCC 6633)	0.31	
Cefixime	*Thymus broussonetia*	*Salmonella typhimurium* (Laboratory isolate $CCMMB_{17}$)	0.18	Fadli *et al*., 2012
	Thymus maroccanus	*Staphylococcus aureus* (Laboratory isolate $CCMMB_3$)	0.18	
		Salmonella typhimurium (Laboratory isolate $CCMMB_{17}$)	0.18	
Clotrimazole	*Curcuma longa*	*Candida albicans* (Clinical isolate)	0.50	Ogidi *et al*., 2021
Fluconazole	*Thymus vulgaris*	*Candida albicans* (Clinical isolate)	0.28	Jafri and Ahmad, 2019
		Candida tropicalis (Clinical isolate)	0.14	
	Matricaria recutita	*Candida albicans* (Clinical isolate)	0.31	Goger *et al*., 2018

Conventional antimicrobial	Natural product	Test micro-organism	ΣFIC	Reference
Fluconazole	*Cinnamomum verum*	*Candida albicans* (Clinical isolate)	0.31	Khan *et al*., 2012
	Syzygium aromaticum		0.38	
	Cymbopogon citratus		0.38	
	Citrus aurantium	*Candida albicans* (ATCC 90028)	0.24	Nidhi *et al*., 2020
		Candida albicans (MTCC 277)	0.36	
Gentamicin	*Thymus broussonetia*	*Bacillus cereus* (ATCC 14579)	0.12	Fadli *et al*., 2012
		Bacillus subtilis (ATCC 9524)	0.09	
		Escherichia coli (Laboratory isolate K_{12}W113)	0.37	
		Micrococcus luteus (ATCC 10240)	0.12	
		Pseudomonas aeruginosa (Clinical isolate)	0.28	
		Staphylococcus aureus (Laboratory isolate $CCMMB_3$)	0.50	
		Vibrio cholera (Environmental isolate)	0.28	
	Thymus maroccanus	*Bacillus cereus* (ATCC 14579)	0.25	
		Bacillus subtilis (ATCC 9524)	0.50	
		Enterobacter cloacae (Clinical isolate)	0.19	
		Escherichia coli (Laboratory isolate K_{12}W113)	0.28	
		Klebsiella pneumoniae (Clinical isolate)	0.50	
		Pseudomonas aeruginosa (Clinical isolate)	0.18	
		Salmonella typhimurium (Laboratory isolate $CCMMB_{17}$)	0.62	
		Staphylococcus aureus (Laboratory isolate $CCMMB_3$)	0.50	
	Cinnammomum zeylanicum	*Enterococcus faecalis* (ATCC 29212)	0.08	Rosato *et al*., 2020
	Mentha piperita		0.08	
	Origanum vulgare		0.08	
	Thymus vulgaris		0.08	

Conventional antimicrobial	Natural product	Test micro-organism	ΣFIC	Reference
Gentamicin	*Cinnammomum zeylanicum*	*Staphylococcus aureus* (ATCC 29213)	0.08	Rosato *et al*., 2020
	Mentha piperita		0.08	
	Origanum vulgare		0.11	
	Thymus vulgaris		0.08	
	Calamintha sylvatica	*Salmonella abony* (ATCC 6017)	0.50	Milenkovic *et al*., 2018
	Calamintha vardarensis		0.50	
	Calamintha nepeta	*Staphylococcus aureus* (ATCC 6538)	0.25	
		Salmonella abony (ATCC 6017)	0.50	
	Calamintha glandulosa	*Staphylococcus aureus* (ATCC 6538)	0.25	
		Escherichia coli (Laboratory isolate K_{12}W113)	0.28	
		Pseudomonas aeruginosa (Clinical isolate)	0.50	
		Salmonella typhirium (Laboratory isolate $CCMMB_{17}$)	0.18	
Ketoconazole	*Melaleuca alternifolia*	*Trichophytum rubrum* (Clinical isolate)	0.37	Roana *et al*., 2021
Norfloxacin	*Cinnammomum zeylanicum*	*Enterococcus faecalis* (ATCC 29212)	0.08	Rosato *et al*., 2020
	Mentha piperita		0.11	
	Origanum vulgare		0.08	
	Thymus vulgaris		0.08	
	Cinnammomum zeylanicum	*Staphylococcus aureus* (ATCC 29213)	0.08	
	Mentha piperita		0.08	
	Origanum vulgare		0.18	
	Thymus vulgaris		0.08	
Oxacillin	*Cinnammomum zeylanicum*	*Enterococcus faecalis* (ATCC 29212)	0.08	
	Mentha piperita		0.11	
	Origanum vulgare		0.08	
	Thymus vulgaris		0.08	
	Cinnammomum zeylanicum	*Staphylococcus aureus* (ATCC 29213)	0.08	
	Mentha piperita		0.11	
	Origanum vulgare		0.11	

Conventional antimicrobial	Natural product	Test micro-organism	ΣFIC	Reference
Oxacillin	*Thymus vulgaris*	*Staphylococcus aureus* (ATCC 29213)	0.17	Rosato *et al*., 2020
Terbinafine	*Curcuma longa*	*Aspergillus niger* (Clinical isolate)	0.50	Ogidi *et al*., 2021
		Candida albicans (Clinical isolate)	0.40	
Tetracycline	*Agathosma betulina*	*Bacillus cereus* (ATCC 11778)	0.46	Hubsch *et al*., 2014[a]
	Artemisia afra		0.48	
	Lippia javanica		0.32	
	Salvia fruticose	*Staphylococcus epidermidis* (Clinical isolates)	0.12 to 0.37	Chovanova *et al*., 2015
	Salvia officinalis		0.31 to 0.49	
	Salvia sclarea		0.12 to 0.49	

Table 2. Synergistic interactions between conventional antimicrobials and African medicinal plant (indigenous/invasive/naturalized/ commercialized) extracts (ΣFIC of below or equal to 0.5 is considered a synergistic interaction)

Conventional antimicrobial	Natural product	Extract type	Test micro-organism	ΣFIC	Reference
Amoxicillin	*Acacia mearnsii*	Methanol	*Enterococcus faecalis* (ATCC 29212)	0.07	Olajuyigbe and Afolayan, 2012
			Escherichia coli (ATCC 25922)	0.50	
			Enterococcus faecalis (Clinical isolate)	0.31	
			Shigella sonnei (ATCC 29930)	0.02	
	Salvia officinalis	Acetone	*Escherichia coli* (ATCC 25922)	0.38	
			Staphylococcus aureus (ATCC 25923)	0.35	Stefanovic *et al*., 2012
			Pseudomonas aeruginosa (ATCC 27853)	0.35	
			Bacillus subtilis (Clinical isolate)	0.50	
			Enterobacter cloacae (Clinical isolate)	0.35	
			Klebsiella pneumoniae (Clinical isolate)	0.35	
			Staphylococcus aureus (Clinical isolate)	0.35	
			Proteus mirabilis (Clinical isolate)	0.49	
	Salvia officinalis	Ethyl acetate	*Escherichia coli* (ATCC 25922)	0.42	
			Bacillus subtilis (Clinical isolate)	0.35	
			Enterobacter cloacae (Clinical isolate)	0.49	
			Klebsiella pneumoniae (Clinical isolate)	0.35	
			Staphylococcus aureus (Clinical isolate)	0.49	
Amikacin	*Thymbra spicata*	Aqueous	*Staphylococcus aureus* (Clinical isolate)	0.26	Haroun and Al-Kayali, 2016
		Ethanol	*Staphylococcus aureus* (Clinical isolate)	0.50	
			Klebsiella pneumoniae (Clinical isolate)	0.50	
		Petroleum	*Staphylococcus aureus (*Clinical isolate)	0.38	
Ampicillin	*Vitis vinifera*	Ethanol	*Escherichia coli (*Clinical isolate)	0.28	Sanhuenza *et al*., 2017
Cefotaxime	*Thymbra spicata*	Aqueous	*Staphylococcus aureus* (Clinical isolate)	0.26	Haroun and Al-Kayali, 2016
		Ethanol	*Staphylococcus aureus* (Clinical isolate)	0.19	
		Petroleum	*Staphylococcus aureus* (Clinical isolate)	0.26	
Cephalexin	*Clinopodium vulgare*	Ethanol	*Bacillus subtilis* (Clinical isolate)	0.44	Stefanovic *et al*., 2011
		Ethyl acetate	*Bacillus subtilis* (Clinical isolate)	0.44	
		Acetone	*Klebsiella pneumoniae* (Clinical isolate)	0.50	
Chloramphenicol	*Acacia mearnsii*	Methanol	*Escherichia coli* (ATCC 25922)	0.38	Olajuyigbe and Afolayan, 2012

Conventional antimicrobial	Natural product	Extract type	Test micro-organism	ΣFIC	Reference
Chloramphenicol	*Acacia mearnsii*	Methanol	*Bacillus subtilis* (Clinical isolate)	0.14	Olajuyigbe and Afolayan, 2012
			Shigella sonnei (ATCC 29930)	0.38	
			Proteus vulgaris (ATCC 6830)	0.50	
	Salvia officinalis	Ethyl acetate	*Bacillus subtilis* (Clinical isolate)	0.40	Stefanovic *et al*., 2012
	Vitis vinifera	Ethanol	Methicillin-resistant *Staphylococcus aureus* (Clinical isolate)	0.06	Sanhuenza *et al*., 2017
			Escherichia coli (Clinical isolate)	0.05	
Ciprofloxacin	*Acacia mearnsii*	Methanol	*Proteus vulgaris* (Clinical isolate)	0.50	Olajuyigbe and Afolayan, 2012
	Vitis vinifera	Ethanol	Methicillin-resistant *Staphylococcus aureus* (Clinical isolate)	0.06	Sanhuenza *et al*., 2017
			Escherichia coli (Clinical isolate)	0.03	
Clarithromycin	*Hibiscus sabdarariffa*	Aqueous	*Helicobacter pylori* (Clinical isolate HP06)	0.21	Hassan *et al*., 2016
Erythromycin	*Acacia mearnsii*	Methanol	*Shigella sonnei* (ATCC 29930)	0.08	Olajuyigbe and Afolayan, 2012
	Aloe ferox	Dichloromethane methanol	*Bacillus cereus* (ATCC 11778)	0.34	Hubsch *et al*., 2014[a]
	Pelargonium sidoides	Dichloromethane methanol		0.32	
Metronidazole	*Acacia mearnsii*	Methanol	*Enterococcus faecalis* (ATCC 29212)	0.25	Olajuyigbe and Afolayan, 2012
			Escherichia coli (ATCC 25922)	0.31	
Nalidixic acid	*Vitis vinifera*	Ethanol	Methicillin-resistant *Staphylococcus aureus* (Clinical isolate)	0.05	Sanhuenza *et al*., 2017
			Escherichia coli (Clinical isolate)	0.05	
Norfloxacin			Methicillin-resistant *Staphylococcus aureus* (Clinical isolate)	0.05	
			Escherichia coli (Clinical isolate)	0.06	
Oxacillin	*Daphne genkwa*		Methicillin-resistant *Staphylococcus aureus* (Clinical isolate)	0.38	Kuok *et al*. ,2017
	Vitis vinifera		Methicillin-resistant *Staphylococcus aureus* (Clinical isolate)	0.05	Sanhuenza *et al*., 2017

Conventional antimicrobial	Natural product	Extract type	Test micro-organism	ΣFIC	Reference
Penicillin G	*Pelargonium sidoides*	Dichloromethane methanol	*Enterococcus faecalis* (ATCC 29212)	0.24	Hubsch *et al*., 2014[a]
	Aloe ferox		*Bacillus cereus* (ATCC 11778)	0.35	
	Pelargonium sidoides		*Enterococcus faecalis* (ATCC 29212)	0.24	
	Sutherlandia frutescens			0.38	
	Pelargonium sidoides		*Staphylococcus aureus* (ATCC 25923)	0.20	
Tetracycline	*Pelargonium sidoides*	Aqueous	Methicillin-resistant *Staphylococcus aureus* (Clinical isolate)	0.06	Sanhueza *et al*., 2017
			Escherichia coli (Clinical isolate)	0.09	
			Staphylococcus aureus (ATCC 25923)	0.48	Hubsch *et al*., 2014[a]

2.4. Conventional antimicrobials in synergistic combinations with culinary herbs, spices and other plant-based foods

Culinary herbs and spices, usually in the form of essential oils, extracts or isolated phytochemical compounds, have also demonstrated antimicrobial activity. Liu *et al*. (2017) even reported that household herbs and spices may be considered for alternative antimicrobial treatments for infectious diseases, due to their proven antimicrobial efficacy against a variety of micro-organisms. It has been found that most often these natural products not only interact synergistically with antibiotics, but also with conventional antifungals. Although fungal infections are not as prevalent as those caused by bacteria or viruses, physicians have very limited antifungal agents at their disposal. It has been noted that one of the approaches to developing more antifungal treatments, is to consider the combination of phytochemicals derived from culinary herbs and spices, with existing conventional antimicrobial agents (Movahed *et al*., 2016).

Palaniappan and Holley (2010) investigated isolated compounds thymol, cinnamaldehyde and carvacrol, derived from culinary herbs and spices, with antibiotics (ampicillin, penicillin, tetracycline, erythromycin, bacitracin and novobiocin). Thymol was found to be synergistic with all antibiotics tested, except for erythromycin. Similarly, carvacrol was found to be synergistic with most antibiotics, except for ampicillin and erythromycin. Cinnamaldehyde interacted synergistically with all the antibiotics tested, except for penicillin.

Various plant-based foods can also be medicinal in nature. Fruits and other consumed plant-based foods often contain berberine, which is a compound well known for exhibiting antimicrobial activity. It has been noted that the mechanism of action of berberine on pathogens could very much lend itself to potentiating conventional antimicrobial activity (Bhandari *et al*., 2000). Cock *et al.* (2017) explored a further compound, a-mangostin, isolated from the mangosteen fruit, and found it to synergistically interact when combined with beta-lactam antibiotics.

2.5. Conventional antimicrobials in synergistic combinations with herbal teas

Another interesting natural product that may be considered for its potentiating antimicrobial efficacy properties, include various teas (black, white, green tea, oolong and rooibos). Many teas are prepared from household herbs and spices, which are then classified according to the plant of

origin. Examples include, but are not limited to, that of chamomile, ginseng, ginger, peppermint, cinnamon and rosemary tea (Malongane *et al*., 2017). Malongane *et al*. (2017) provides a review of the various teas, with the classification, derived plant material and preparation thereof. Teas are well-known for containing phytochemicals that exhibit antimicrobial, anti-cancer and anti-diabetic characteristics. There are many teas derived from indigenous South African herbal plants, such as *Athrixia phylicoides* (bush tea), *Aspalathus linearis* (rooibos tea), *Cyclopia* species (honeybush tea) and *Monsonia burkeana* (special tea), exhibiting medicinal properties, inclusive of antimicrobial activity (Malongane *et al*., 2017). Tea has also been noted as being effective in the treatment of dermal infections (Pazyar *et al*., 2012; Malongane *et al*., 2017).

Chan *et al*. (2011) investigated the antimicrobial activity of green, black and various other herbal teas, against a range of pathogens. It was found that the tea samples exhibited antimicrobial activity against Gram-positive *S. aureus, B. cereus* and *Micrococcus luteus*. Interestingly though, these tea samples did not demonstrate the same antimicrobial efficacy against Gram-negative bacteria, such as *E. coli*, *P. aeruginosa* and *Salmonella enterica*. It was stated that plant extracts, in this case being in the form of a tea, are less effective against fighting Gram-negative micro-organisms, as a result of the bacteria having an outer membrane, which limits access of these compounds into the micro-organism.

The catechins present in green tea have been found to inhibit bacterial growth in mice that have been infected with *E. coli.* When green tea was combined with the antibiotic levofloxacin, the mice receiving both were protected from organ damage (Setiawan *et al*., 2001). Tea constituents have abundantly been documented in the literature to be useful adjunctive agents to conventional antimicrobial agents, such as beta-lactams, carbapenems, norfloxacin, and tetracycline.

A study conducted by Hubsch *et al*. (2014[b]), demonstrated that the popular beverage, rooibos tea, derived from *Aspalathus linearis* (rooibos), demonstrated synergistic effects with conventional antimicrobials, particularly penicillin. Chovanova *et al*. (2013), reported a promising synergistic effect displayed by certain species of *Salvia* (sage), as well as *Matricaria recutita* (German chamomile), when used in combination with the conventional antibiotic, oxacillin. The medicinal plant samples were found to damage the cytoplasmic membrane of *S. epidermidis*, causing the loss

of intracellular components and allowing entrance of the antibiotic, resulting in increased efficacy of oxacillin in eradicating bacteremia in patents infected with methicillin-resistant *S. epidermidis* (Chovanova *et al*., 2013).

Da Silva *et al*. (2014) studied isolated tea compounds in combination with the antifungal, fluconazole, against resistant isolates of *Candida tropicalis*, where synergistic interactions were demonstrated through enhanced antimicrobial activity of fluconazole. A number of studies (Tiwari *et al*., 2005; Erbil *et al*., 2013; Haghjoo *et al*., 2013; Koech *et al*., 2014; Mbuthia *et al*., 2014; Ning *et al*., 2015), also investigated compounds extracted from teas in combination with conventional antimicrobials, such as doxycycline, erythromycin, fluconazole, miconazole, penicillin G, and promisingly, most often synergistic interactions were identified. A few of the notable synergistic interactions identified in studies conventional antimicrobials in combination with teas have been summarized in Table 3.

2.6. Conventional antimicrobials in synergistic combinations with bee products

Honey has been used for centuries, particularly for the treatment of skin wounds, respiratory and gastrointestinal diseases (Visavadia *et al*., 2006). Anyanwu (2012), Moussa *et al*. (2012) and Shenoy *et al*. (2012), amongst others investigated the *in vitro* antimicrobial activity of honeys. In particular a study 42 South African honey samples demonstrated efficacy that in some cases were better than the gold standard Manuka honey (Khan *et al.*, 2014). There are also clinical studies that have demonstrated that honey and other bee products exhibit the potential to treat infections. Manuka honey has received attention in respect to its effective antimicrobial treatment of skin infections. It has even demonstrated activity against MRSA (Mandal *et al*., 2010). A few of the synergistic interactions identified in studies on bee products in combination with conventional antimicrobials have been documented in Table 4.

Table 3. Synergistic interactions between conventional antimicrobials and common herbal teas available in Africa (ΣFIC of below or equal to 0.5 is considered a synergistic interaction)

Conventional antimicrobial	Natural product	Extract type	Test micro-organism	ΣFIC	Reference
Ampicillin	Pomegranate blossom tea	Aqueous	*Staphylococcus aureus* (Clinical isolate)	0.30	Hacioglu *et al*., 2017
	Thyme tea			0.50	
	Wormwood tea			0.50	
	Rosehip tea			0.60	
Fluconazole	Epigallocatechin 3-o-gallate (compound from green tea)	Pure compound	*Candida dupliniensis* (Clinical isolate 2)	0.38	Behbehani *et al*., 2019
			Candida albicans (Clinical isolate 1)	0.38	
			Candida glabrata (Clinical isolate)	0.50	
			Candida dupliniensis (Clinical isolate 2)	0.50	
			Candida albicans (ATCC 24433)	0.31	
			Candida glabrata (ATCC 15126)	0.50	
	Flavonoid catechin		*Candida tropicalis* (Clinical isolate)	0.38	Da Silva *et al*., 2014
	Quercetin		*Candida tropicalis* (Clinical isolate)	0.25	
	Epigallocatechin 3-o-gallate (compound from green tea)		*Candida tropicalis* (Clinical isolate)	0.25	
Gentamicin	*Aspalathus linearis* (rooibos tea)	Dichloromethane methanol	*Staphylococcus aureus* (ATCC 25923)	0.50	Hubsch *et al*., 2014[b]
Ketoconazole	Epigallocatechin 3-o-gallate (compound from green tea)	Pure compound	*Candida albicans* (Clinical isolates)	0.38 to 0.50	Behbehani *et al*., 2019
			Candida glabrata (ATCC 15126)	0.38	
			Candida dupliniensis (Clinical isolate)	0.38	
			Candida albicans (ATCC 24433)	0.50	
	Flavonoid catechin		*Candida tropicalis* (Clinical isolate)	0.25	
	Quercetin			0.25	
Nystatin	Black tea	Aqueous	*Candida albicans* (Clinical isolate)	0.50	Hacioglu *et al*., 2017
	Green tea		*Candida albicans* (Clinical isolate)	0.30	
Penicillin G	*Aspalathus linearis* (rooibos tea)	Dichloromethane methanol	*Bacillus cereus* (ATCC 11778)	0.08	Hubsch *et al*., 2014[b]
		Aqueous	*Enterococcus faecalis* (ATCC 29212)	0.46	
Tetracycline		Dichloromethane methanol	*Staphylococcus aureus* (ATCC 25923)	0.01	
			Bacillus cereus (ATCC 11778)	0.48	

Table 4. Synergistic interactions between conventional antimicrobials and African bee products (ΣFIC of below or equal to 0.5 is considered a synergistic interaction)

Conventional antimicrobial	Natural product	Test micro-organism	ΣFIC	Reference
Ciprofloxacin	South African Honey 18-(MIXEDGUM/FS0)	*Pseudomonas aeruginosa* (ATCC 9027)	0.38	Khan, 2014
	South African Honey 19-(CITYMIX/FS)		0.50	
Clindamycin	Mellitin (pure compound) from bee venom	*Staphylococcus aureus* (ATCC 33591)	0.38	Mahmoudi *et al*., 2020
		Methicillin-resistant *Staphylococcus aureus* (Clinical isolate 1)	0.38	
		Methicillin-resistant *Staphylococcus aureus* (Clinical isolate 2)	0.19	
		Methicillin-resistant *Staphylococcus aureus* (Clinical isolate 3)	0.28	
		Methicillin-resistant *Staphylococcus aureus* (Clinical isolate 4)	0.26	
		Methicillin-resistant *Staphylococcus aureus* (Clinical isolate 5)	0.08	
		Methicillin-resistant *Staphylococcus aureus* (Clinical isolate 6)	0.26	
		Enterococcus faecalis (ATCC 51299)	0.40	
		Streptococcus pyogenes (ATCC 12344)	0.50	
Gentamicin	South African Honey 16-(FYNBOS/WC)	*Staphylococcus aureus* (ATCC 25923)	0.27	Khan, 2014
	South African Honey 18-(MIXEDGUM/FS0)		0.27	
	South African Honey 19-(CITYMIX/FS)		0.27	
	South African Honey 26-(FYNBOS/WC)		0.27	
	South African Honey 41-(INDIGENOUS/WC)		0.27	
	South African Honey 16-(FYNBOS/WC)	*Pseudomonas aeruginosa* (ATCC 9027)	0.31	
	South African Honey 19-(CITYMIX/FS)		0.47	
Nystatin	South African Honey 16-(FYNBOS/WC)	*Candida albicans* (Clinical isolate)	0.31	

3. Conventional antimicrobials in antagonistic interactions with natural products

While synergistic interactions are frequently reported, less attention has been given to antagonistic interactions among researchers, which is equally important, especially when considering clinical combination therapy. An interesting common thread resonating through many of the combination studies presenting in this chapter, is the lack of reporting on antagonistic interactions. Demonstrated in this section are some of the studies that had documented antagonism.

Melaleuca alternifolia (tea tree)*, T. vulgaris, Mentha piperita* (peppermint)*,* and *R. officinalis* (rosemary) essential oils have demonstrated antagonistic interactions with ciprofloxacin against *S. aureus, K. pneumoniae*, and with amphotericin B against *C. albicans* (Van Vuuren *et al*., 2008). Olajuyigbe and Afolayan (2013) and Hubsch *et al*. (2014[a]) conducted studies on various combinations of natural products with conventional antimicrobials, demonstrating antagonistic profiles. Hubsch *et al*. (2014) further found that *A. afra* extract in combination with ciprofloxacin, provided a notable antagonistic interaction (ΣFIC of 8.55), when tested against *E. coli*. Furthermore, conventional antimicrobials were found to demonstrate antagonistic interactions more often when in combination with *A. ferox*.

Peng *et al*. (2010) studied the antagonistic effects of the green tea extracts from *C. sinesis* on modulating the resistant mechanism of MRSA toward beta-lactam antibiotics, namely amoxicillin, ampicillin, oxacillin and cefazolin. Under investigation, in mice, it was found that green tea extract, alone or in combination with amoxicillin, does not have protective benefits in MRSA-infected mice. The study suggested that tea drinking is cautioned in combination with amoxicillin treatment. A few natural products demonstrating noteworthy antagonistic interactions with conventional antimicrobials have been indicated Table 5.

Table 5. Antagonistic interactions between conventional antimicrobials and African natural products (indigenous/invasive/naturalized/ commercialized) (ΣFIC value greater than 4.0 is considered antagonistic)

Conventional antimicrobial	Natural product	Extract type	Test micro-organism	ΣFIC	Reference
Amphotericin B	*Artemisia afra*	Dichloromethane methanol	*Candida albicans* (ATCC 10231)	6.76	Hubsch *et al*., 2014[a]
		Aqueous		5.34	
Chloramphenicol	*Acacia mearnsii*	Methanol	*Enterobacter cloacae* (Clinical isolate)	4.50	Olajuyigbe and Afolayan, 2012
Ciprofloxacin	*Artemisia afra*	Aqueous	*Escherichia coli* (ATCC 25922)	8.55	Hubsch *et al*., 2014[a]
	Pomegranate blossom tea		*Staphylococcus aureus* (Methicillin-resistant ATCC 43300)	9.00	Hacioglu *et al*., 2017
	Rosehip tea		*Staphylococcus aureus* (ATCC 29213)	5.00	
	Pomegranate blossom tea			5.00	
	Rosehip tea		*Staphylococcus aureus* (Methicillin-resistant ATCC 43300)	8.00	
	Rosehip tea		*Enterococcus faecalis* (ATCC 29212)	5.00	
	Pomegranate blossom tea			9.00	
	Acacia mearnsii	Methanol	*Staphylococcus aureus* (ATCC 6538)	4.25	Olajuyigbe and Afolayan, 2012
			Bacillus subtilis (Clinical isolate)	4.06	
			Shigella sonnei (ATCC 29930)	18.00	
Erythromycin	Pomegranate blossom tea	Aqueous	*Staphylococcus aureus* (ATCC 29213)	5.00	Hacioglu *et al*., 2017
	Rosehip tea			5.00	
Kanamycin	*Acacia mearnsii*	Methanol	*Enterococcus faecalis* (Clinical isolate)	8.13	Olajuyigbe and Afolayan, 2012
Penicillin G	*Artemisia afra*	Dichloromethane methanol	*Staphylococcus aureus* (ATCC 25923)	4.25	Hubsch *et al*., 2014[a]
			Bacillus cereus (ATCC 11778)	4.40	

4. Discussion and conclusion

With the rise in AMR to existing conventional antimicrobial therapies, and the limited conventional antimicrobial drugs at the disposal of healthcare professionals in Africa, it is critical that alternative avenues to enhance antimicrobial activity of existing agents are rapidly discovered, to avoid a modern healthcare system being deemed incapable of treating infectious diseases (Bhardwaj *et al*., 2016).

Findings identified in the scientific interactive studies considered in this chapter indicate that synergistic interactions are more frequently reported on, compared to antagonistic interactions. This could be an indication that natural products may show more synergistic potential in addressing AMR in combination with conventional antimicrobials. However, this warrants further exploration to determine whether the increased synergistic interaction reporting is a true reflection; or merely as result of antagonistic identification being less of a research focus. Even though fewer antagonistic interactions are documented, this should not provide a false sense of complacency on the potential of medicinal plants reducing the efficacy of conventional antimicrobials. Any antagonistic potential reported, even if it be a single combination occurrence, should be a cause for concern.

This chapter has highlighted that many natural products exhibit considerable antimicrobial activity. This is also evident through the extensive history of use of natural products, not just in Africa, but globally, in the treatment of various infections. There still, however, remains a dearth of research necessary to further understand the interactive profiles of combination therapies, however, natural product combinations with conventional antimicrobials is arguably a potential avenue of research to assist in discovering new medicines that prove effective against resistant micro-organisms.

One of the advantages of combination therapy, includes decreased costs of development and reduced time to achieve clinical use in treating infections (Cock *et al*., 2017). Developing and marketing new antimicrobials involves extensive research, clinical trials and evaluation, whereas combination therapy would require a less extensive process subsequently costing less. With the vast availability to natural products in Africa, harnessing the medicinal properties of these products to enhance the efficacy of conventional drugs should not be underestimated. With the drive to

integrate the two forms of healthcare, and with regulation amendments and stricter controlling of natural products, this is a definite alternative to be considered in the fight against AMR.

Although natural product combinations with conventional antimicrobials provide a promising alternate approach to addressing AMR, it is important to note that in order to exploit synergistic combinations, it is vital that favorable ratios and doses of antimicrobials and traditional medicine combinations are determined (Bhardwaj *et al*, 2016), together with the necessary quality control and standardization of medicinal plant products to ensure uniformity in therapy (Viljoen and Vermaak, 2013).

With the high estimate of TCAM / natural product use in Africa, it is essential that policy makers, researchers and healthcare professionals recognize that these products are legitimate forms of healthcare. Furthermore, also recognizing that these aforementioned combination therapies still require an immense amount of research and further in-depth analysis, with development based on scientific research and regulatory mechanisms, on par with that of allopathic medicines (Pandey *et al*., 2020). Natural product integration in modern healthcare has long been under contention, due to the lack of scientific validation and the skepticism with regard to the efficacy of natural products, which needs to be addressed.

The ever-increasing occurrence of AMR in Africa may very well be due to not only the misuse and abuse of conventional antimicrobials, but also as a result of antagonistic interactions occurring between conventional antimicrobials and certain natural products being consumed concurrently. This may be going undetected, due to the lack of the above-mentioned aspects still not being definitively outlined and understood. The enquiry of natural product use in modern healthcare facilities prior to prescribing or dispensing has also been noted as a challenge, since many do not consider the need to disclose TCAM use with other healthcare professionals. Government legislation and regulations regarding natural products and traditional remedies are thus necessary, in order to avoid future adverse interactions and exacerbation of AMR. These regulations may include more comprehensive enquiry in medical history taking practices, being more inclusive of TCAM use, or including more labelling of natural product remedies to include warnings of interactions with conventional drugs; or informing and educating traditional healers on these interactions, so that the information can be relayed to the patients. Furthermore, healthcare

professionals in conventional health settings in Africa need to be trained on TCAM and its potential to interact with conventional medicines (James *et al*., 2018).

Africa, with its rich diversity in flora, offers the ideal opportunity for researchers to identify and develop innovative products and advantageous combination therapies to treat infections (van Wyk, 2011). Certainly, the options for future integration of natural products with conventional antimicrobials demonstrates promise and a viable option of combatting the global onslaught of AMR.

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1.2. Additional research exploring combination studies - *in vitro* combination studies of conventional antimicrobials with traditionally used medicinal plant extracts

1.2.1. Introduction

The literature review conducted in *Publication 1* looked at all forms of African natural products in combination with conventional antimicrobials, inclusive of essential oils, plant compounds, bee products, culinary herbs and spices, fruits and commercial teas. Furthermore, in *Publication 1*, it was evident, that essential oils of African medicinal plants represented most of the literature for combination studies with antimicrobials, with medicinal plant extract combinations sorely neglected.

Medicinal plant extracts are being proposed to potentiate antimicrobial efficacy, where the preparation of plant samples using various solvents allows for the concentration of plant antimicrobial compounds (Abubaker and Haque, 2020; Malczak and Gajda, 2023). Previous reviews by Chanda and Rakholiya (2011), Mundy et al. (2016) Saini et al. (2018) and Nitsch-Velasquez (2020), indicated plant extracts to be more effective as antimicrobial agents when used in their complete composition rather than as isolated compounds. It is also important to be cognisant that medicinal plant extracts are more closely aligned with the traditional methods of consumption of plant material. Thus, a global search on plant extracts in combination with conventional antimicrobials was undertaken in this section to phase 1 of the study.

1.2.2. Methodology

1.2.2.1. Study design

A systematic literature review was conducted according to PRISMA guidelines (Figure 1.2), to synthesize a compilation of global medicinal plant extracts used for infectious conditions, investigated *in vitro* in combination with conventional antimicrobials.

1.2.2.2. Search strategy

A comprehensive search was conducted in a variety of electronic databases including PubMed, Science Direct, Scopus, and Google Scholar, to ensure broad coverage of relevant literature. The search strategy was structured around three main thematic domains: (1) plant extracts combined with antibiotics; (2) plant extracts combined with antifungals; and (3) synergism and antagonism.

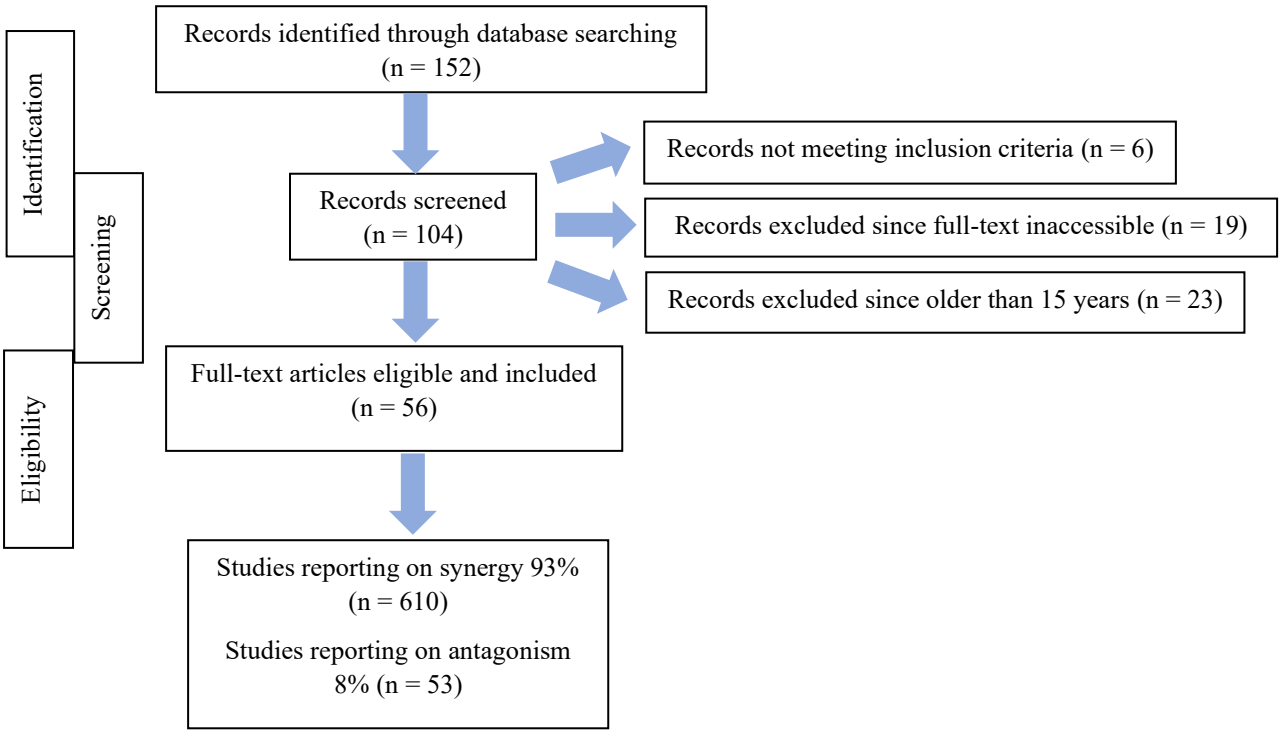


Figure 1.2. The PRISMA search and selection processes applied.

1.2.2.3. Inclusion and exclusion parameters

No sources other than scientifically published articles were included in the study. Gray literature was therefore, excluded from the review. Only *in vitro*, peer-reviewed, full-text journal articles, in English, were included. Articles having no measurable antimicrobial interactive outcomes were excluded.

1.2.2.4. Data extraction and synthesis

Title and abstract screening of the collected studies were done to verify relevance in relation to the study inclusion criteria. Date of publication was noted to ensure that only current literature was reviewed as antimicrobial resistance patterns are continuously changing. Excluded studies were recorded. Literature that met all inclusion criteria were downloaded and stored on a personal computer, with back-up. Literature was saved in pdf format and renamed according to the author, date, and key words for referencing ease. All articles were further organised into folders titled according to specific medicinal plant product (essential oil, aqueous or organic extract) and conventional antimicrobials evaluated in combination.

To retrieve relevant data from literature, a tabulated format was used for information synthesis. Full-text review of the studies was undertaken for data extraction and tabulation. Tables consisted of several columns with headings that are easy to comprehend. The characteristics of the data collected consisted of antimicrobial specification, plant scientific name, region of study, method of plant sample preparation, interactive profile (synergistic or antagonistic), authors' names and year of publication. Furthermore, pie charts were used to represent some of the numerical data in a visual form. The number of the recorded combinations were counted, and the total was ascertained. Thereafter the percentages of synergism and antagonism was calculated with the total number of combinations as the denominator.

1.2.3. Results

The data collected, with the total number of retrieved articles at 152, and using the exclusion criteria, only 56 articles were suitable for this review (Figure 1.2). Combinations demonstrating synergy or antagonism are, effectively, of most relevance in impacting on possible antimicrobial clinical outcomes. Literature reporting on these two interactive profiles have been tabulated in this section of the chapter (Synergy – Table 1.1 and 1.2; Antagonism – Table 1.3 and 1.4). A total of 425 combinations were recorded, of which 77.88% reported synergy and only 22.12% antagonism. Majority (n = 314) of the synergistic combinations consisted of plant: conventional antibiotic combinations, as opposed to plant: conventional antifungal combinations (n = 17). This variation was also noted for antagonistic interactions (n = 55 for plant: conventional antibiotic and n = 39 for plant: conventional antifungal), however, the difference was not as substantial.

Table 1.1. Combinations of conventional antibiotics with medicinal plant extracts demonstrating synergy.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Amoxicillin	*Helichrysum pedunculatum*	Methanol	*Klebsiella pneumoniae* (ATCC 10031)	-2.85 ± 0.36 (log_{10} CFU/ml)	Aiyegoro, Afolayan and Okoh, 2009
	Helichrysum pedunculatum	Methanol	*Staphylococcus epidermidis* (Environmental strain)	-2.85 ± 0.36 (log_{10} CFU/ml)	
	Lannea barteri	Aqueous	*Staphylococcus aureus* (Clinical strain)	ΣFIC 0.16	Donkor et al., 2024
	Lannea barteri	Aqueous	*Escherichia coli* (Clinical strain)	ΣFIC 0.24	
	Lannea barteri	Ethanol	*Escherichia coli* (Clinical strain)	ΣFIC 0.45	
	Senna alata	Aqueous	*Pseudomonas aeruginosa* (Clinical strain)	ΣFIC 0.48	
	Senna alata	Ethanol	*Escherichia coli* (Clinical strain)	ΣFIC 0.27	
	Senna alata	Ethanol	*Pseudomonas aeruginosa* (Clinical strain)	ΣFIC 0.45	
	Ocimum basilicum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	Lakshmi et al., 2013
	Plumbago auriculata	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC value increase	
	Punica granatum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Vitis vinifera	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Boesenbergia pandurata	Ethanol	*Streptococcus mutans* (ND)	ΣFIC 0.375	Sukandar et al., 2016
	Salvia officinalis	Acetone	*Escherichia coli* (ATCC 25922)	ΣFIC 0.38	Stefanovic et al., 2012
	Salvia officinalis	Acetone	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.35	
	Salvia officinalis	Acetone	*Pseudomonas aeruginosa* (ATCC 27853)	ΣFIC 0.35	
	Salvia officinalis	Acetone	*Bacillus subtilis* (Clinical strain)	ΣFIC 0.50	
	Salvia officinalis	Acetone	*Enterobacter cloacae* (Clinical strain)	ΣFIC 0.35	
	Salvia officinalis	Acetone	*Klebsiella pneumoniae* (Clinical strain)	ΣFIC 0.35	
	Salvia officinalis	Acetone	*Staphylococcus aureus* (Clinical strain)	ΣFIC 0.35	
Amoxicillin	*Salvia officinalis*	Acetone	*Proteus mirabilis* (Clinical strain)	ΣFIC 0.49	
	Salvia officinalis	Ethyl acetate	*Escherichia coli* (ATCC 25922)	ΣFIC 0.42	

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Amoxicillin	*Salvia officinalis*	Ethyl acetate	*Bacillus subtilis* (Clinical strain)	ΣFIC 0.35	Stefanovic et al., 2012
	Salvia officinalis	Ethyl acetate	*Enterobacter cloacae* (Clinical strain)	ΣFIC 0.49	
	Salvia officinalis	Ethyl acetate	*Klebsiella pneumoniae* (Clinical strain)	ΣFIC 0.35	
	Salvia officinalis	Ethyl acetate	*Staphylococcus aureus* (Clinical strain)	ΣFIC 0.49	
Ampicillin	*Artemisia afra*	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.1	Hacioglu et al., 2017
	Camillia sinensis assamica	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.3	
	Camillia sinensis	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.1	
	Echinacea purpurea	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.1	
	Mentha piperita	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.1	
	Garcinia kola	Methanol	*Escherichia coli* (AG102)	2-fold MIC reduction	Lacmata et al., 2012
	Garcinia kola	Methanol	*Pseudomonas aeruginosa* (PA124)	>8-fold MIC reduction	
	Picralima nitida	Methanol	*Escherichia coli* (AG102)	4-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA3)	8-fold MIC reduction	
	Picralima nitida	Methanol	*Pseudomonas aeruginosa* (PA124)	>32-fold MIC reduction	
	Punica granatum	Methanol	Methicillin-sensitive *Staphylococcus aureus* (Clinical strains)	38-73% increase in antimicrobial activity	Braga et al., 2005
	Punica granatum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strains)	38-73% increase in antimicrobial activity	
	Punica granatum	Aqueous	*Enterococcus faecalis* (Clinical strain)	ΣFIC 0.5	Hacioglu et al., 2017
	Rosa canina	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.3	
	Thymus vulgaris	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.1	
Azithromycin	*Averrhoa bilimbi*	Methanol	*Enterobacter* spp. (ND)	5 – 10 mm increase in zone of inhibition	Acharjee et al., 2023
	Chenopodium album	Methanol	*Enterobacter* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Enhydra fluctuans	Methanol	*Enterobacter* spp. (ND)	5 – 10 mm increase in zone of inhibition	

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Azithromycin	*Mentha arvensis*	Methanol	*Enterobacter* spp. (ND)	5 – 10 mm increase in zone of inhibition	Acharjee et al., 2023
	Mimosa diplotricha	Methanol	*Enterobacter* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Sphagneticola calendulacea	Methanol	*Enterobacter* spp. (ND)	5 – 10 mm increase in zone of inhibition	
Cefepime	*Picralima nitida*	Methanol	*Escherichia coli* (AG102)	>4-fold MIC reduction	Lacmata et al., 2012
	Picralima nitida	Methanol	*Enterobacter aerogenes* (CM64)	4-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA3)	2-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA27)	2-fold MIC reduction	
	Picralima nitida	Methanol	*Klebsiella pneumoniae* (KP63)	2-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (CM64)	>2-fold MIC reduction	
	Garcinia kola	Methanol	*Klebsiella pneumoniae* (KP63)	2-fold MIC reduction	
Cefixime	*Terminalia chebula*	Ethyl acetate	*Staphylococcus aureus* (3.1)	ΣFIC 0.375	Atta et al., 2023
	Chamomilla amada	Methanol	*Staphylococcus aureus* (3.1)	ΣFIC 0.375	
	Nigella sativa	Ethyl acetate	*Staphylococcus aureus* (3.3)	ΣFIC 0.375	
	Chamomilla amada	Methanol	*Staphylococcus aureus* (3.4)	ΣFIC 0.500	
	Terminalia chebula	Methanol	*Staphylococcus aureus* (3.4)	ΣFIC 0.500	
	Nigella sativa	Methanol	*Acinetobacter baumannii* (29)	ΣFIC 0.375	
	Chamomilla amada	Methanol	*Acinetobacter baumannii* (29)	ΣFIC 0.500	
	Terminalia chebula	Methanol	*Escherichia coli* (80-A)	ΣFIC 0.500	
	Momordica charantia	Ethyl acetate	*Escherichia coli* (80-A)	ΣFIC 0.375	
	Nigella sativa	Ethyl acetate	*Escherichia coli* (7C)	ΣFIC 0.500	
	Chamomilla amada	Methanol	*Klebsiella pneumoniae* (K)	ΣFIC 0.250	
	Nigella sativa	Ethyl acetate	*Pseudomonas aeruginosa* (Resistant clinical strain)	ΣFIC 0.375	
Cefuroxime	*Plumbago auriculata*	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	Lakshmi et al., 2013
	Ocimum basilicum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Vitis vinifera	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Cefuroxime	*Punica granatum*	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	Lakshmi et al., 2013
Cephalosporin	*Punica granatum*	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Vitis vinifera	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Ocimum basilicum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Plumbago auriculata	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
Cephalexin	*Rhus coriaria*	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>2000-fold MIC reduction	Adwan et al. 2010
	Sacropoterium spinosum	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>160-fold MIC reduction	
	Rosa damascene	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>160-fold MIC reduction	
Chloramphenicol	*Helichrysum pedunculatum*	Methanol	*Streptococcus faecalis* (ATCC 29212)	-2.29 ± 0.3 (log_{10} CFU/ml)	Aiyegoro, Afolayan and Okoh, 2009
	Helichrysum pedunculatum	Methanol	*Staphylococcus epidermidis* (Environmental strain)	-2.01 ± 0.07 (log_{10} CFU/ml)	
	Punica granatum	Methanol	Methicillin-sensitive *Staphylococcus aureus* (Clinical strains)	38-73% increase in antimicrobial activity	Braga et al., 2005
	Punica granatum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strains)	38-73% increase in antimicrobial activity	
	Picralima nitida	Methanol	*Escherichia coli* (AG102)	8-fold MIC reduction	Lacmata et al., 2012
	Picralima nitida	Methanol	*Enterobacter aerogenes* (CM64)	8-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA3)	8-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA27)	8-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA289)	8-fold MIC reduction	
	Picralima nitida	Methanol	*Klebsiella pneumoniae* (KP55)	2-fold MIC reduction	
	Picralima nitida	Methanol	*Klebsiella pneumoniae* (KP63)	4-fold MIC reduction	
	Picralima nitida	Methanol	*Pseudomonas aeruginosa* (PA124)	8-fold MIC reduction	
Chloramphenicol	*Garcinia kola*	Methanol	*Escherichia coli* (AG102)	8-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (CM64)	2-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (EA27)	8-fold MIC reduction	

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Chloramphenicol	*Garcinia kola*	Methanol	*Enterobacter aerogenes* (EA289)	4-fold MIC reduction	Lacmata et al., 2012
	Garcinia kola	Methanol	*Klebsiella pneumoniae* (KP63)	4-fold MIC reduction	
	Garcinia kola	Methanol	*Pseudomonas aeruginosa* (PA124)	2-fold MIC reduction	
	Salvia officinalis	Ethyl acetate	*Bacillus subtilis* (Clinical strain)	ΣFIC 0.40	Stefanovic et al., 2012
	Vitis vinifera	Ethanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.056	Sanhuenza et al., 2017
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8298)	ΣFIC 0.047	
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8275)	ΣFIC 0.062	
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8275)	ΣFIC 0.047	
Ciprofloxacin	*Vitis vinifera*	Ethanol	*Staphylococcus aureus* (ATCC 6538)	ΣFIC 0.028	
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8275)	ΣFIC 0.062	
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8298)	ΣFIC 0.063	
	Terminalia prunioides	Aqueous	*Klebsiella pneumoniae* (ATCC 13883)	ΣFIC 0.31	Zai et al., 2024
	Terminalia prunioides	Methanol	*Klebsiella pneumoniae* (ATCC 13883)	ΣFIC 0.15	
	Terminalia gazensis	Aqueous	*Klebsiella pneumoniae* (ATCC 13883)	ΣFIC 0.15	
	Terminalia gazensis	Methanol	*Klebsiella pneumoniae* (ATCC 13883)	ΣFIC 0.15	
	Terminalia prunioides	Aqueous	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.25	
	Terminalia prunioides	Methanol	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.18	
	Terminalia gazensis	Aqueous	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.37	
	Terminalia gazensis	Methanol	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.37	
	Terminalia gazensis	Methanol	Methicillin-resistant *Staphylococcus aureus* (ATCC 43300)	ΣFIC 0.50	
	Pelargonium sidoides	Aqueous	*Bacillus cereus* (ATCC11778)	ΣFIC 0.50	Hubsch et al., 2014[a]
	Aspalathus linearis	Dichloromethane methanol	*Klebsiella pneumoniae* (ATCC 13883)	ΣFIC 0.16	Hubsch et al., 2014[b]
Ciprofloxacin	*Pelargonium sidoides*	Dichloromethane methanol	*Bacillus cereus* (ATCC11778)	ΣFIC 0.44	Hubsch et al., 2014[a]
	Helichrysum pedunculatum	Methanol	*Staphylococcus epidermidis* (Environmental strain)	-2.11 ± 0.55 (log_{10} CFU/ml)	Aiyegoro, Afolayan and Okoh, 2009

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Ciprofloxacin	*Picralima nitida*	Methanol	*Escherichia coli* (AG102)	2-fold MIC reduction	Lacmata et al., 2012
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA3)	4-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA289)	4-fold MIC reduction	
	Picralima nitida	Methanol	*Klebsiella pneumoniae* (KP55)	2-fold MIC reduction	
	Picralima nitida	Methanol	*Klebsiella pneumoniae* (KP63)	2-fold MIC reduction	
	Picralima nitida	Methanol	*Pseudomonas aeruginosa* (PA124)	4-fold MIC reduction	
	Garcinia kola	Methanol	*Escherichia coli* (AG102)	4-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (EA3)	2-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (EA289)	2-fold MIC reduction	
	Garcinia kola	Methanol	*Klebsiella pneumoniae* (KP55)	2-fold MIC reduction	
	Garcinia kola	Methanol	*Klebsiella pneumoniae* (KP63)	4-fold MIC reduction	
	Vitis vinifera	Ethanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.063	Sanhuenza et al., 2017
Cloxacillin	*Garcinia kola*	Methanol	*Enterobacter aerogenes* (EA27)	4-fold MIC reduction	Lacmata et al., 2012
	Garcinia kola	Methanol	*Pseudomonas aeruginosa* (PA124)	>4-fold MIC reduction	
	Picralima nitida	Methanol	*Escherichia coli* (AG102)	8-fold MIC reduction	
Doxycycline	*Vangueria spinosa*	Ethanol	*Staphylococcus aureus* (ND)	ΣFIC 0.315	Chatterjee et al., 2009
	Vangueria spinosa	Ethanol	*Escherichia coli* (ND)	ΣFIC 0.405	
	Vangueria spinosa	Ethanol	*Pseudomonas aeruginosa* (ND)	ΣFIC 0.321	
	Vangueria spinosa	Ethanol	*Klebsiella pneumoniae* (ND)	ΣFIC 0.446	
Enrofloxacin	*Rhus coriaria*	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>2000-fold MIC reduction	Adwan et al. 2010
	Sacropoterium spinosum	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>64-fold MIC reduction	
	Rosa damascene	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>64-fold MIC reduction	
Erythromycin	*Helichrysum pedunculatum*	Methanol	*Staphylococcus epidermidis* (Environmental strain)	-2.29 ± 0.13 (log_{10} CFU/ml)	Aiyegoro, Afolayan and Okoh, 2009
	Helichrysum pedunculatum	Methanol	*Klebsiella pneumoniae* (ATCC 10031)	-2.70 ± 0.30 (log_{10} CFU/ml)	
	Helichrysum pedunculatum	Methanol	*Streptococcus faecalis* (ATCC 29212)	-2.15 ± 0.5 (log_{10} CFU/ml)	
	Enhydra fluctuans	Methanol	*Pseudomonas* spp. (ND)	5 – 10 mm increase in zone of inhibition	Acharjee et al., 2023

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Erythromycin	*Sphagneticola calendulacea*	Methanol	*Pseudomonas* spp. (ND)	5 – 10 mm increase in zone of inhibition	Acharjee et al., 2023
	Chenopodium album	Methanol	*Pseudomonas* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Averrhoa bilimbi	Methanol	*Pseudomonas* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Mentha arvensis	Methanol	*Pseudomonas* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Mimosa diplotricha	Methanol	*Pseudomonas* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (CM64)	8-fold MIC reduction	Lacmata et al., 2012
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA3)	4-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA27)	2-fold MIC reduction	
	Picralima nitida	Methanol	*Klebsiella pneumoniae* (KP55)	4-fold MIC reduction	
	Picralima nitida	Methanol	*Klebsiella pneumoniae* (KP63)	2-fold MIC reduction	
	Picralima nitida	Methanol	*Pseudomonas aeruginosa* (PA124)	16-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (CM64)	16-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (EA3)	4-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (EA27)	2-fold MIC reduction	
	Garcinia kola	Methanol	*Klebsiella pneumoniae* (KP63)	8-fold MIC reduction	
	Garcinia kola	Methanol	*Pseudomonas aeruginosa* (PA124)	8-fold MIC reduction	
	Plumbago auriculata	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	Lakshmi et al., 2013
	Ocimum basilicum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Aloe ferox	Dichloromethane: methanol	*Bacillus cereus* (ATCC11778)	ΣFIC 0.34	Hubsch et al., 2014[a]
	Lippia javanica	Dichloromethane methanol	*Enterococcus faecalis* (ATCC 29212)	ΣFIC 0.32	
	Pelargonium sidoides	Aqueous	*Bacillus cereus* (ATCC11778)	ΣFIC 0.39	
	Pelargonium sidoides	Dichloromethane methanol	*Bacillus cereus* (ATCC11778)	ΣFIC 0.32	
	Vitis vinifera	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	Lakshmi et al., 2013

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Erythromycin	*Punica granatum*	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	Lakshmi et al., 2013
	Mangifera indica	Ethanol	*Staphylococcus aureus* (RN4220)	4-fold MIC reduction	Souto de Oliveira et al., 2011
Gentamicin	*Averrhoa bilimbi*	Methanol	*Proteus* spp. (ND)	5 – 10 mm increase in zone of inhibition	Acharjee et al., 2023
	Chenopodium album	Methanol	*Proteus* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Enhydra fluctuans	Methanol	*Proteus* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Mentha arvensis	Methanol	*Proteus* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Sphagneticola calendulacea	Methanol	*Proteus* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Mimosa diplotricha	Methanol	*Proteus* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Punica granatum	Methanol	Methicillin-sensitive *Staphylococcus aureus* (Clinical strains)	38-73% increase in antimicrobial activity	Braga et al., 2005
	Punica granatum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strains)	38-73% increase in antimicrobial activity	
	Pelargonium sidoides	Aqueous	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.34	Hubsch et al., 2014[a]
	Pelargonium sidoides	Dichloromethane: methanol	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.42	
	Aspalathus linearis	Aqueous	*Staphylococcus aureus* (ATCC6538)	ΣFIC 0.50	Hubsch et al., 2014[b]
	Camellia sinensis	Aqueous	*Staphylococcus aureus* (ATCC25923)	0.1 ± 0.77 mg/ml zone of inhibition increase	Mbuthia et al., 2014
	Terminalia gazensis	Aqueous	*Klebsiella pneumoniae* (ATCC 13883)	ΣFIC 0.18	Zai et al., 2024
	Terminalia gazensis	Methanol	*Klebsiella pneumoniae* (ATCC 13883)	ΣFIC 0.09	
	Terminalia prunioides	Aqueous	*Klebsiella pneumoniae* (ATCC 13883)	ΣFIC 0.18	
	Terminalia prunioides	Methanol	*Klebsiella pneumoniae* (ATCC 13883)	ΣFIC 0.18	
Kanamycin	*Garcinia kola*	Methanol	*Klebsiella pneumoniae* (KP55)	8-fold MIC reduction	Lacmata et al., 2012
	Garcinia kola	Methanol	*Klebsiella pneumoniae* (KP63)	2-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (EA289)	8-fold MIC reduction	
	Garcinia kola	Methanol	*Pseudomonas aeruginosa* (PA124)	>8-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA27)	>2-fold MIC reduction	

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Kanamycin	*Picralima nitida*	Methanol	*Enterobacter aerogenes* (EA289)	>8-fold MIC reduction	Lacmata et al., 2012
	Picralima nitida	Methanol	*Klebsiella pneumoniae* (KP55)	4-fold MIC reduction	
	Picralima nitida	Methanol	*Klebsiella pneumoniae* (KP63)	4-fold MIC reduction	
	Picralima nitida	Methanol	*Pseudomonas aeruginosa* (PA124)	2-fold MIC reduction	
Levofloxacin	*Vitis vinifera*	Ethanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.061	Sanhuenza et al., 2017
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8275)	ΣFIC 0.047	
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8298)	ΣFIC 0.031	
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 6538)	ΣFIC 0.078	
Methicillin	*Plumbago auriculata*	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	Lakshmi et al., 2013
	Ocimum basilicum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Punica granatum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Vitis vinifera	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Sphagneticola calendulacea	Methanol	*Staphylococcus* spp. (ND)	5 – 10 mm increase in zone of inhibition	Acharjee et al., 2023
	Mimosa diplotricha	Methanol	*Staphylococcus* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Enhydra fluctuans	Methanol	*Staphylococcus* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Chenopodium album	Methanol	*Staphylococcus* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Averrhoa bilimbi	Methanol	*Staphylococcus* spp. (ND)	5 – 10 mm increase in zone of inhibition	
	Mentha arvensis	Methanol	*Staphylococcus* spp. (ND)	5 – 10 mm increase in zone of inhibition	
Nalidixic acid	*Vitis vinifera*	Ethanol	*Staphylococcus aureus* (ATCC 6538)	ΣFIC 0.078	Sanhuenza et al., 2017
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8275)	ΣFIC 0.065	
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8298)	ΣFIC 0.062	
	Vitis vinifera	Ethanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.047	
Norfloxacin	*Picralima nitida*	Methanol	*Escherichia coli* (AG102)	4-fold MIC reduction	Lacmata et al., 2012
	Picralima nitida	Methanol	*Enterobacter aerogenes* (CM64)	2-fold MIC reduction	

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Norfloxacin	*Picralima nitida*	Methanol	*Enterobacter aerogenes* (EA3)	4-fold MIC reduction	Lacmata et al., 2012
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA27)	2-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA289)	2-fold MIC reduction	
	Picralima nitida	Methanol	*Klebsiella pneumoniae* (KP55)	2-fold MIC reduction	
	Picralima nitida	Methanol	*Klebsiella pneumoniae* (KP63)	8-fold MIC reduction	
	Picralima nitida	Methanol	*Pseudomonas aeruginosa* (PA124)	4-fold MIC reduction	
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 6538)	ΣFIC 0.078	Sanhuenza et al., 2017
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8275)	ΣFIC 0.047	
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8298)	ΣFIC 0.047	
	Vitis vinifera	Ethanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.047	
Ofloxacin	*Vangueria spinosa*	Ethanol	*Staphylococcus aureus* (ND)	ΣFIC 0.440	Chatterjee et al., 2009
	Vangueria spinosa	Ethanol	*Escherichia coli* (ND)	ΣFIC 0.405	
	Vangueria spinosa	Ethanol	*Klebsiella pneumoniae* (ND)	ΣFIC 0.455	
Oxacillin	*Punica granatum*	Methanol	Methicillin-sensitive *Staphylococcus aureus* (Clinical strains)	38-73% increase in antimicrobial activity	Braga et al., 2005
	Punica granatum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strains)	38-73% increase in antimicrobial activity	
	Plumbago auriculata	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	Lakshmi et al., 2013
	Punica granatum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Ocimum basilicum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Vitis vinifera	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 6538)	ΣFIC 0.047	Sanhuenza et al., 2017
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8298)	ΣFIC 0.031	
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8275)	ΣFIC 0.063	
	Vitis vinifera	Ethanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.047	

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Oxytetracycline	*Helichrysum pedunculatum*	Methanol	*Klebsiella pneumoniae* (ATCC 10031)	-2.70 ± 0.30 (log_{10} CFU/ml)	Aiyegoro, Afolayan and Okoh, 2009
	Helichrysum pedunculatum	Methanol	*Staphylococcus epidermidis* (Environmental strain)	-2.70 ± 0.30 (log_{10} CFU/ml)	
	Rhus coriaria	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>1024-fold MIC reduction	Adwan et al. 2010
	Sacropoterium spinosum	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>4-fold MIC reduction	
	Rosa damascene	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>4-fold MIC reduction	
Penicillin G	*Helichrysum pedunculatum*	Methanol	*Streptococcus faecalis* (ATCC 29212)	-2.04 ± 0.07 (log_{10} CFU/ml)	Aiyegoro, Afolayan and Okoh, 2009
	Helichrysum pedunculatum	Methanol	*Klebsiella pneumoniae* (ATCC 10031)	-2.85 ± 0.36 (log_{10} CFU/ml)	
	Rhus coriaria	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>2000-fold MIC reduction	Adwan et al. 2010
	Sacropoterium spinosum	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>8-fold MIC reduction	
	Rosa damascene	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>8-fold MIC reduction	
	Catha edulis	Aqueous	*Fusobacterium nucleatum* 9911	4-fold potentiation	Al-hebshi et al., 2006
	Aspalathus linearis	Dichloromethane methanol	*Staphylococcus aureus* (ATCC6538)	ΣFIC 0.01	Hubsch et al., 2014[b]
	Aspalathus linearis	Dichloromethane methanol	*Enterococcus faecalis* (ATCC 29212)	ΣFIC 0.46	
	Aspalathus linearis	Dichloromethane methanol	*Bacillus cereus* (ATCC11778)	ΣFIC 0.08	
	Pelargonium sidoides	Dichloromethane: methanol	*Enterococcus faecalis* (ATCC 29212)	ΣFIC 0.24	Hubsch et al., 2014[a]
	Aloe ferox	Dichloromethane: methanol	*Bacillus cereus* (ATCC11778)	ΣFIC 0.35	
	Pelargonium sidoides	Aqueous	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.32	
	Pelargonium sidoides	Dichloromethane: methanol	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.20	
	Camellia sinensis	Aqueous	*Escherichia coli* (ATCC 25922)	0.1 ± 0.08 mg/ml zone of inhibition increase	Mbuthia et al., 2014
	Plumbago auriculata	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	Lakshmi et al., 2013
	Ocimum basilicum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Penicillin G	*Punica granatum*	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	Lakshmi et al., 2013
	Vitis vinifera	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
Streptomycin	*Enhydra fluctuans*	Methanol	*Escherichia coli* (ND)	5 – 10 mm increase in zone of inhibition	Acharjee et al., 2023
	Chenopodium album	Methanol	*Escherichia coli* (ND)	5 – 10 mm increase in zone of inhibition	
	Averrhoa bilimbi	Methanol	*Escherichia coli* (ND)	5 – 10 mm increase in zone of inhibition	
	Mentha arvensis	Methanol	*Escherichia coli* (ND)	5 – 10 mm increase in zone of inhibition	
	Mimosa diplotricha	Methanol	*Escherichia coli* (ND)	5 – 10 mm increase in zone of inhibition	
	Sphagneticola calendulacea	Methanol	*Escherichia coli* (ND)	5 – 10 mm increase in zone of inhibition	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (CM64)	2-fold MIC reduction	Lacmata et al., 2012
	Garcinia kola	Methanol	*Enterobacter aerogenes* (EA27)	2-fold MIC reduction	
	Garcinia kola	Methanol	*Klebsiella pneumoniae* (KP55)	2-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (EA289)	2-fold MIC reduction	
	Garcinia kola	Methanol	*Pseudomonas aeruginosa* (PA124)	>32-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (CM64)	2-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA3)	8-fold MIC reduction	
Sulfadimethoxine	*Rhus coriaria*	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>256-fold MIC reduction	Adwan et al. 2010
	Sacropoterium spinosum	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>8-fold MIC reduction	
	Rosa damascene	Ethanol	*Pseudomonas aeruginosa* (3 Clinical strains)	>8-fold MIC reduction	
Tetracycline	*Agathosma betulina*	Dichloromethane: methanol	*Bacillus cereus* (ATCC11778)	ΣFIC 0.46	Hubsch et al., 2014[a]
	Pelargonium sidoides	Aqueous	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.48	
	Aspalathus linearis	Dichloromethane methanol	*Bacillus cereus* (ATCC11778)	ΣFIC 0.48	Hubsch et al., 2014[b]
	Picralima nitida	Methanol	*Escherichia coli* (AG102)	8-fold MIC reduction	Lacmata et al., 2012
	Picralima nitida	Methanol	*Enterobacter aerogenes* (CM64)	4-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA3)	8-fold MIC reduction	
	Picralima nitida	Methanol	*Enterobacter aerogenes* (EA27)	4-fold MIC reduction	

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Tetracycline	*Picralima nitida*	Methanol	*Enterobacter aerogenes* (EA289)	4-fold MIC reduction	Lacmata et al., 2012
	Picralima nitida	Methanol	*Klebsiella pneumoniae* (KP55)	2-fold MIC reduction	
	Picralima nitida	Methanol	*Klebsiella pneumoniae* (KP63)	2-fold MIC reduction	
	Picralima nitida	Methanol	*Pseudomonas aeruginosa* (PA124)	4-fold MIC reduction	
	Garcinia kola	Methanol	*Escherichia coli* (AG102)	4-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (CM64)	2-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (EA3)	2-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (EA27)	4-fold MIC reduction	
	Garcinia kola	Methanol	*Klebsiella pneumoniae* (KP55)	4-fold MIC reduction	
	Garcinia kola	Methanol	*Klebsiella pneumoniae* (KP63)	4-fold MIC reduction	
	Garcinia kola	Methanol	*Enterobacter aerogenes* (EA289)	4-fold MIC reduction	
	Garcinia kola	Methanol	*Pseudomonas aeruginosa* (PA124)	2-fold MIC reduction	
	Catha edulis	Aqueous	*Streptococcus oralis* SH-2	4-fold potentiation	Al-hebshi et al., 2006
	Catha edulis	Aqueous	*Streptococcus sanguis* TH-13	2-fold potentiation	
	Helichrysum pedunculatum	Methanol	*Staphylococcus epidermidis* (Environmental strain)	-2.70 ± 0.30 (log_{10} CFU/ml)	Aiyegoro, Afolayan and Okoh, 2009
	Punica granatum	Methanol	Methicillin-sensitive *Staphylococcus aureus* (Clinical strains)	38-73% increase in antimicrobial activity	Braga et al., 2005
	Punica granatum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strains)	38-73% increase in antimicrobial activity	
	Plumbago auriculata	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	Lakshmi et al., 2013
	Ocimum basilicum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Punica granatum	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Vitis vinifera	Methanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	2-fold MIC reduction	
	Terminalia gazensis	Aqueous	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.37	Zai et al., 2024
	Terminalia gazensis	Methanol	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.37	

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Tetracycline	*Terminalia prunioides*	Aqueous	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.25	Zai et al., 2024
	Terminalia prunioides	Methanol	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 0.18	
	Terminalia gazensis	Aqueous	Methicillin-resistant *Staphylococcus aureus* (ATCC 43300)	ΣFIC 0.50	
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8275)	ΣFIC 0.047	Sanhuenza et al., 2017
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 8298)	ΣFIC 0.047	
	Vitis vinifera	Ethanol	*Staphylococcus aureus* (ATCC 6538)	ΣFIC 0.094	
	Vitis vinifera	Ethanol	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	ΣFIC 0.062	
	Mangifera indica	Ethanol	*Staphylococcus aureus* (IS-58)	4-fold MIC reduction	Souto de Oliveira et al., 2011
Vancomycin	*Stevia rebaudiana*	Ethanol	*Streptococcus mutans* (ND)	ΣFIC 0.062	Sukandar et al., 2016
	Boesenbergia pandurata	Ethanol	*Streptococcus mutans* (ND)	ΣFIC 0.500	

ND – strain / quantitative result not recorded in original study.

Table 1.2. Combinations of conventional antifungals with medicinal plant extracts demonstrating synergy.

Conventional antifungal	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Amphotericin B	*Sutherlandia frutescens*	Dichloromethane: methanol	*Candida albicans* (ATCC 10231)	ΣFIC 0.22	Hubsch et al., 2014[a]
Chlorhexidine	*Bauhinia holophylla*	Ethanol	*Candida albicans* (ATCC 90028)	ΣFIC 0.55	de Oliveira Rosa et al., 2025
Fluconazole	*Bauhinia holophylla*	Aqueous	*Candida albicans* (ATCC 90028)	ΣFIC 0.99	
	Bauhinia holophylla	Ethanol	*Candida albicans* (ATCC 90028)	ΣFIC 0.74	
	Senna alata	Aqueous	*Candida albicans* (Clinical strain)	ΣFIC 0.27	Donkor et al., 2024
	Senna alata	Ethanol	*Candida albicans* (Clinical strain)	ΣFIC 0.03	
	Lannea barteri	Aqueous	*Candida albicans* (Clinical strain)	ΣFIC 0.04	
	Lannea barteri	Ethanol	*Candida albicans* (Clinical strain)	ΣFIC 0.31	
	Ricinus communis	Aqueous	*Candida albicans* (Clinical strain)	ΣFIC 0.13	
	Ricinus communis	Ethanol	*Candida albicans* (Clinical strain)	ΣFIC 0.06	
Ketoconazole	*Zingiber officinale*	Ethanol	*Candida albicans* (ND)	ΣFIC 0.281	Sukandar et al., 2016
	Stevia rebaudiana	Ethanol	*Candida albicans* (ND)	ΣFIC 0.197	
	Boesenbergia pandurata	Ethanol	*Candida albicans* (ND)	ΣFIC 0.312	
Nystatin	*Bauhinia holophylla*	Aqueous	*Candida albicans* (ATCC 90028)	ΣFIC 0.62	de Oliveira Rosa et al., 2025
	Bauhinia holophylla	Ethanol	*Candida albicans* (ATCC 90028)	ΣFIC 0.62	
	Camellia sinensis assamica	Aqueous	*Candida albicans* (ATCC 10231)	ΣFIC 0.30	Hacioglu et al., 2017
	Lippia javanica	Aqueous	*Candida albicans* (ATCC 10231)	ΣFIC 0.50	Hubsch et al., 2014[a]

ND – strain / quantitative result not recorded in original study.

Table 1.3. Combinations of conventional antibiotics with medicinal plant extracts demonstrating antagonism.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Ampicillin	*Senna alata*	Aqueous	*Klebsiella pneumoniae* (Clinical strain)	ΣFIC 4.12	Donkor et al., 2024
	Lannea barteri	Ethanol	*Klebsiella pneumoniae* (Clinical strain)	ΣFIC 4.28	
Amoxicillin	*Lannea barteri*	Ethanol	*Klebsiella pneumoniae* (Clinical strain)	ΣFIC 4.28	
Ciprofloxacin	*Rosa canina*	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 9.0	Hacioglu et al., 2017
	Punica granatum	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 9.0	
	Rosa canina	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (ATCC 29213)	ΣFIC 5.0	
	Punica granatum	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (ATCC 29213)	ΣFIC 5.0	
	Black tea	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (ATCC 29213)	ΣFIC 5.0	
	Rosa canina	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 9.0	
	Rosa canina	Aqueous	*Enterococcus faecalis* (Clinical strain)	ΣFIC 9.0	
	Punica granatum	Aqueous	*Enterococcus faecalis* (Clinical strain)	ΣFIC 5.0	
	Punica granatum	Aqueous	*Enterococcus faecalis* (ATCC 29212)	ΣFIC 9.0	
	Rosa canina	Aqueous	*Enterococcus faecalis* (ATCC 29212)	ΣFIC 5.0	
	Punica granatum	Aqueous	Methicillin-resistant *Staphylococcus aureus* (ATCC 43300)	ΣFIC 9.0	
	Rosa canina	Aqueous	Methicillin-resistant *Staphylococcus aureus* (ATCC 43300)	ΣFIC 8.0	
	Punica granatum	Aqueous	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	ΣFIC 9.0	
	Rosa canina	Aqueous	Methicillin-resistant *Staphylococcus aureus* (Clinical strain)	ΣFIC 9.0	
Erythromycin	*Rosa canina*	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 5.0	
	Punica granatum	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 5.0	
	Rosa canina	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 5.0	
	Punica granatum	Aqueous	Methicillin-sensitive *Staphylococcus aureus* (Clinical strain)	ΣFIC 5.0	

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Penicillin G	*Artemisia afra*	Dichloromethane: methanol	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 4.25	Hubsch et al., 2014[a]
	Artemisia afra	Dichloromethane: methanol	*Bacillus cereus* (ATCC 11778)	ΣFIC 4.40	
Ciprofloxacin	*Artemisia afra*	Aqueous	*Escherichia coli* (ATCC 25922)	ΣFIC 8.55	Hubsch et al., 2014[a]
Gentamicin	*Phyllanthus niruri*	Aqueous	*Bacillus cereus* (ND)	ΣFIC 9.81	Tiwana et al., 2024
	Phyllanthus niruri	Aqueous	*Klebsiella pneumoniae* (ND)	ΣFIC >4.00	
Polymyxin B	*Phyllanthus niruri*	Aqueous	*Klebsiella pneumoniae* (ND)	ΣFIC 63.50	
	Phyllanthus niruri	Aqueous	*Shigella sonnei* (ND)	ΣFIC 63.00	
	Phyllanthus niruri	Aqueous	*Shigella flexneri* (ND)	ΣFIC 15.62	
Chloramphenicol	*Phyllanthus niruri*	Methanol	*Bacillus cereus* (ND)	ΣFIC 4.51	
	Phyllanthus niruri	Methanol	*Shigella sonnei* (ND)	ΣFIC >4.00	
Tetracycline	*Phyllanthus niruri*	Methanol	*Shigella flexneri* (ND)	ΣFIC 4.53	
Polymyxin B	*Phyllanthus niruri*	Methanol	Extended-spectrum beta-lactamase *Escherichia coli* (ND)	ΣFIC 63.00	
	Phyllanthus niruri	Methanol	*Klebsiella pneumoniae* (ND)	ΣFIC 32.25	
	Phyllanthus niruri	Methanol	*Shigella sonnei* (ND)	ΣFIC >4.00	
	Phyllanthus niruri	Methanol	*Shigella flexneri* (ND)	ΣFIC 15.63	
Erythromycin	*Terminalia prunioides*	Methanol	Methicillin-resistant *Staphylococcus aureus* (ATCC 43300)	ΣFIC 5.00	Zai et al., 2024
	Terminalia sericea	Methanol	Methicillin-resistant *Staphylococcus aureus* (ATCC 43300)	ΣFIC 4.50	
Gentamicin	*Terminalia prunioides*	Aqueous	Extended-spectrum ß-lactamase resistant *Klebsiella pneumoniae* (ATCC 700603)	ΣFIC 17.00	
	Terminalia prunioides	Methanol	Extended-spectrum ß-lactamase resistant *Klebsiella pneumoniae* (ATCC 700603)	ΣFIC 16.25	
	Terminalia gazensis	Aqueous	Extended-spectrum ß-lactamase resistant *Klebsiella pneumoniae* (ATCC 700603)	ΣFIC 16.50	
	Terminalia gazensis	Methanol	Extended-spectrum ß-lactamase resistant *Klebsiella pneumoniae* (ATCC 700603)	ΣFIC 32.50	

ND – strain / quantitative result not recorded in original study.

Conventional antibiotic	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Gentamicin	*Terminalia prunioides*	Aqueous	Extended-spectrum ß-lactamase resistant *Escherichia coli* (Clinical strain)	ΣFIC 8.30	Zai et al., 2024
	Terminalia prunioides	Methanol	Extended-spectrum ß-lactamase resistant *Escherichia coli* (Clinical strain)	ΣFIC 7.93	
	Terminalia gazensis	Aqueous	Extended-spectrum ß-lactamase resistant *Escherichia coli* (Clinical strain)	ΣFIC 8.51	
	Terminalia gazensis	Methanol	Extended-spectrum ß-lactamase resistant *Escherichia coli* (Clinical strain)	ΣFIC 8.06	
	Terminalia gazensis	Methanol	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 8.50	
	Terminalia prunioides	Aqueous	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 8.26	
	Terminalia prunioides	Methanol	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 8.13	
	Terminalia gazensis	Aqueous	*Staphylococcus aureus* (ATCC 25923)	ΣFIC 8.51	
	Terminalia prunioides	Aqueous	Methicillin-resistant *Staphylococcus aureus* (ATCC 43300)	ΣFIC 8.26	
	Terminalia prunioides	Methanol	Methicillin-resistant *Staphylococcus aureus* (ATCC 43300)	ΣFIC 16.25	
	Terminalia gazensis	Aqueous	Methicillin-resistant *Staphylococcus aureus* (ATCC 43300)	ΣFIC 4.25	
	Terminalia gazensis	Methanol	Methicillin-resistant *Staphylococcus aureus* (ATCC 43300)	ΣFIC 17.02	
	Terminalia sericea	Methanol	Methicillin-resistant *Staphylococcus aureus* (ATCC 43300)	ΣFIC 16.27	

ND – strain / quantitative result not recorded in original study.

Table 1.4. Combinations of conventional antifungals with medicinal plant extracts demonstrating antagonism.

Conventional antifungal	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Amphotericin B	*Acmella caulirhiza*	Methanol	*Candida famata* (ATCC 2037476)	ΣFIC 16.3	Igunza et al., 2019
	Acmella caulirhiza	Hexane	*Candida famata* (ATCC 2037476)	ΣFIC 5.0	
	Acmella caulirhiza	Hexane	*Candida haemulonii* (ATCC 1609496)	ΣFIC 4.5	
	Artemisia afra	Aqueous	*Candida albicans* (ATCC 10231)	ΣFIC 6.76	Hubsch et al., 2014[a]
	Artemisia afra	Dichloromethane: methanol	*Candida albicans* (ATCC 10231)	ΣFIC 5.34	
	Sutherlandia frutescens	Dichloromethane: methanol	*Candida neoformans* (ATCC 14116)	ΣFIC 6.76	
	Senna didymobotrya	Methanol	*Candida famata* (ATCC 2037476)	ΣFIC 5.0	Igunza et al., 2019
	Senna didymobotrya	Methanol	*Candida orientaris* (ATCC 6258)	ΣFIC 4.1	
	Senna didymobotrya	Hexane	*Candida orientaris* (ATCC 6258)	ΣFIC 4.3	
	Senna didymobotrya	Hexane	*Candida.daubus haemulonii* (ATCC 2052030)	ΣFIC 5.0	
	Senna didymobotrya	Methanol	*Candida haemulonii* (ATCC 1609496)	ΣFIC 16.1	
	Senna didymobotrya	Methanol	*Candida krusei* (ATCC 14243)	ΣFIC 16.3	
	Senna didymobotrya	Hexane	*Candida krusei* (ATCC 14243)	ΣFIC 8.0	
	Senna didymobotrya	Methanol	*Candida albicans* (ATCC 14053)	ΣFIC 8.5	
	Senna didymobotrya	Methanol	*Candida auris* (ATCC 2050582)	ΣFIC 16.1	
	Senna didymobotrya	Hexane	*Candida auris* (ATCC 2050582)	ΣFIC 4.1	
	Acmella caulirhiza	Methanol	*Candida albicans* (ATCC 14053)	ΣFIC 4.5	
Clotrimazole	*Acmella caulirhiza*	Methanol	*Candida albicans* (ATCC 14053)	ΣFIC 4.0	Igunza et al., 2019
	Acmella caulirhiza	Hexane	*Candida auris* (ATCC 2050582)	ΣFIC 8.0	
	Acmella caulirhiza	Hexane	*Candida.daubus haemulonii* (ATCC 2052030)	ΣFIC 32.0	
	Acmella caulirhiza	Hexane	*Candida haemulonii* (ATCC 1609496)	ΣFIC 8.0	
	Acmella caulirhiza	Hexane	*Candida famata* (ATCC 2037476)	ΣFIC 8.0	
	Senna didymobotrya	Methanol	*Candida auris* (ATCC 2050582)	ΣFIC 32.0	
	Senna didymobotrya	Hexane	*Candida auris* (ATCC 2050582)	ΣFIC 8.0	
	Senna didymobotrya	Methanol	*Candida krusei* (ATCC 14243)	ΣFIC 64.0	
	Senna didymobotrya	Methanol	*Candida duabus haemulonii* (ATCC 2052030)	ΣFIC 64.0	
	Senna didymobotrya	Methanol	*Candida orientaris* (ATCC 6258)	ΣFIC 4.0	
	Senna didymobotrya	Hexane	*Candida orientaris* (ATCC 6258)	ΣFIC 32.0	
	Senna didymobotrya	Methanol	*Candida.daubus haemulonii* (ATCC 2052030)	ΣFIC 64.0	

ND – strain / quantitative result not recorded in original study.

Conventional antifungal	Medicinal plant	Extract type	Pathogen (strain number)	Quantitative result	Reference
Clotrimazole	*Senna didymobotrya*	Hexane	*Candida.daubus haemulonii* (ATCC 2052030)	ΣFIC 16.0	Igunza et al., 2019
Ketoconazole	*Acmella caulirhiza*	Methanol	*Candida haemulonii* (ATCC 1609496)	ΣFIC 16.0	
	Acmella caulirhiza	Methanol	*Candida albicans* (ATCC 14053)	ΣFIC 16.1	
	Acmella caulirhiza	Hexane	*Candida auris* (ATCC 2050582)	ΣFIC 4.1	
	Senna didymobotrya	Methanol	*Candida albicans* (ATCC 14053)	ΣFIC 16.3	
	Senna didymobotrya	Methanol	*Candida krusei* (ATCC 14243)	ΣFIC 256.0	
	Senna didymobotrya	Methanol	*Candida orientaris* (ATCC 6258)	ΣFIC 32.0	
	Senna didymobotrya	Hexane	*Candida krusei* (ATCC 14243)	ΣFIC 4.1	
	Senna didymobotrya	Hexane	*Candida auris* (ATCC 2050582)	ΣFIC 64.0	
	Senna didymobotrya	Hexane	*Candida.daubus haemulonii* (ATCC 2052030)	ΣFIC 16.0	

ND – strain / quantitative result not recorded in original study.

1.2.4. Discussion

These findings demonstrate that combination research is geared toward plant extracts in combination with conventional antibiotics, whilst combinations of plant extracts with antifungals are sorely neglected. This is an avenue that should be pursued, particularly since it is proposed that natural products could reduce toxic effects of conventional antimicrobials, as explored by Nasrollahi and Yadegari (2016). Many antifungal agents, particularly the azole antifungals, present unfavourable side effect profiles (Behbehani et al., 2019) and hence the benefit of antifungal synergism and reduced toxicity is promising.

A further pattern clearly indicated through conducting of this literature review, is the predominant reporting of synergistic interactions, with antagonism rarely documented. Due to the urgency that arises from the rate of development of resistance among pathogens, researchers might be tempted to report synergy as it is deemed the most clinically relevant, while ignoring or not reporting antagonistic interactions. In a review by Mundy et al. (2016) it was also conveyed that most research findings lean more on the synergistic side rather than being equally distributed between synergism and antagonism. However, continued and further exploration of traditional medicinal plant extracts in combination with conventional antimicrobials, needs to occur and the importance for antagonism to be emphasised to ensure there is no complacency toward the potential harmful interactions that integrative medicine may pose (Ugboko et al., 2020).

Table 1.5 summarizes findings from the literature review, to further classify the research methodologies applied and identify gaps requiring attention. Only 22.73% of the studies undertook phytochemical analysis of the plant extracts. Medicinal plants have complex chemical profiles, with specific phytochemicals known to exert antimicrobial activity. Conducting phytochemical analysis provides the chemical profile of the plant and identification of any key compounds known to exert antimicrobial activity. Therefore, these studies provide insight to the inherent antimicrobial potential of the plant, which could translate into synergism when combined with conventional antimicrobials (Vaou et al., 2021). Phytochemistry lends itself to the mechanism of antimicrobial action. Mechanism of action studies were rarely studied (9.09%) in the literature reviewed. It is necessary to understand the mechanism of action of these interactions as it would further enable the clinical translation of noteworthy synergistic and harmful antagonistic interactions. With many of the studies reviewed referring

Table 1.5. Evaluation of literature on plant extract: antimicrobial combinations.

Plant: antimicrobial study detail	Quantitative antimicrobial analysis	Chemical analysis	Mechanism of action studies	Toxicity analysis	Anti-biofilm analysis	Reference
6 plants; 5 antibiotics; 5 pathogens	Yes – agar well diffusion	No	No	No	No	Acharjee et al., 2023
3 plants; 5 antibiotics; 1 pathogen	Yes - MIC dose reduction	No	No	No	No	Adwan et al. 2010
1 plant; 8 antibiotics; 11 pathogens;	Yes – time kill assay	No	No	No	No	Aiyegoro, Afolayan and Okoh, 2009
1 plant; 2 antifungals; 1 pathogen	Yes - MIC dose reduction	No	No	No	No	Al-hebshi et al., 2006
9 plants; 5 antibiotics; 7 pathogens	Yes – ΣFIC	Yes – polyphenol concentration	Yes – bacterial synthesis	Yes – time kill assays	No	Atta et al., 2023
1 plant; 5 antibiotics; 1 pathogen	Yes – time kill assay	No	No	No	No	Braga et al., 2005
1 plant; 2 antibiotics; 4 pathogens	Yes - ΣFIC and time-kill	No	No	No	No	Chatterjee et al., 2009
1 plant; 3 antibiotics; 1 pathogen	Yes – ΣFIC	Yes – saponins, flavonoids, phenols, tannins	No	Yes – haemolytic toxicity	Yes	de Oliveira Rosa et al., 2025
3 plants; 2 antibiotics and 1 antifungal; 4 pathogens	Yes – ΣFIC	No	No	No	No	Donkor et al., 2024
31 plants; 9 antibiotics and 3 antifungals; 8 pathogens	Yes – ΣFIC and time-kill	No	No	No	No	Hacioglu et al., 2017
1 plant; 5 antibiotics and 2 antifungals; 8 pathogens	Yes – ΣFIC	None	No	Yes – brine shrimp lethality assay	No	Hubsch et al., 2024[a]
6 plants; 5 antibiotics and 2 antifungals; 8 pathogens	Yes – ΣFIC	None	No	Yes – brine shrimp lethality assay	No	Hubsch et al., 2024[b]
15 plants; 5 antibiotics; 1 pathogen	Yes – disc diffusion	Yes - steroids, terpenoids, flavonoids, tannins, anthraquinones and cardiac glycosides.	No	No	No	Igunza et al., 2019
9 plants; 10 antibiotics; 6 pathogens	Yes - MIC dose reduction	Yes - alkaloids, flavonoids, phenols, saponins, tannins, anthocyanins, anthraquinones, sterol, and triterpenes	Yes – efflux pump activity	No	No	Lacmata et al., 2012
4 plants; 8 antibiotics; 1 pathogen	Yes – MIC dose reduction	No	No	No	No	Lakshmi et al., 2013
2 plants; 2 antibiotics; 2 pathogens	Yes – agar well diffusion	No	No	No	No	Mbuthia et al., 2014
1 plant; 7 antibiotics; 2 pathogens	Yes - ΣFIC	Yes	No	Yes – cytotoxicity	No	Sanhuenza et al., 2017
1 plant; 3 antibiotics; 1 pathogen	Yes – MIC dose reduction	No	No	No	No	Souto de Oliveira et al., 2011
2 plants; 2 antibiotics; 7 pathogens	Yes – ΣFIC	No	No	No	No	Stefanovic et al., 2012
3 plants; 2 antibiotics and 1 antifungal; 3 pathogens	Yes - ΣFIC	Yes - flavonoid, alkaloid, quinone, steroid/triterpenoids	No	No	No	Sukandar et al., 2016
1 plant; 12 antibiotics; 3 pathogens	Yes – ΣFIC	No	No	Yes – brine shrimp lethality assay	No	Tiwana et al., 2024
3 plants; 2 antibiotics; 3 pathogens	Yes – ΣFIC	No	No	Yes – alpha lipoic acid assay	No	Zai et al., 2024

to the advantage of reduced side effect profiles due to reduced toxicity, it is of concern that only 31.82% of the studies reviewed have *in vitro* antimicrobial combination studies supplemented by toxicity assays. Furthermore, anti-biofilm analysis was only undertaken in one of the studies reviewed. Pathogens producing biofilms are of grave public health concern, as these pathogens render most conventional antimicrobials ineffective (Ampofo et al., 2020). For this reason, *in vitro* antimicrobial combination studies should be supplemented by anti-biofilm studies against appropriate pathogens.

1.2.5. Conclusion

Although *in vitro* combination studies are not always translated effectively in clinical practice, being exposed to a myriad of differing conditions, these studies most definitely provide valuable baseline data to highlight combinations worth noting and thus allows for streamlining of future research endeavours.

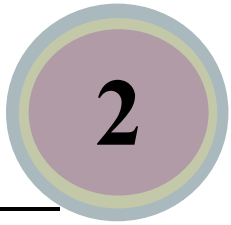
1.3. Chapter summary

Chapter 1 sought to achieve objectives (a) and (b) of phase 1 of the study, providing context to integrative medicine by reviewing existing literature on *in vitro* interactive combination studies involving conventional antimicrobials (antibiotics and antifungals) and traditionally used natural products for infectious conditions.

Findings in this chapter revealed that essential oils are the most predominantly featured form of medicinal plant in the literature reporting on combination studies with conventional antimicrobials. A lack of research on plant extract combinations with conventional antimicrobials was emphasized. A further common thread in this chapter was the neglect of antagonistic interaction reporting in the literature. This finding could qualm concerns related to harmful interactions resulting in reduced antimicrobial efficacy from dualism. However, concern to the preference for reporting of synergism in literature first needs to be elucidated. Therefore, findings from phase 1 of the current study, highlighted the necessity for research efforts to be directed at the investigation of interactions of medicinal plant extracts with conventional antimicrobials.

Chapter 2

Prevalence of traditional medicine use amongst patients – a comparative analysis between a Gauteng and KwaZulu-Natal public hospital



2.1. Chapter introduction

Chapter 2 sought to address objectives (a) and (b) outlined in phase 2 of the study. Whilst integrative medicine holds promise in aiding efforts focused on addressing antimicrobial resistance, as highlighted by the synergistic interactions documented in Chapter 1, there remains concern of harmful, antagonistic interactions. Prevention of these interactions will not only be achievable through reporting of scientific evidence but will also require a change in both conventional and traditional healthcare professional practice. Cognisance of interactions and the active enquiry of combination use amongst patients would be central to ensuring safe integrative medicine practices.

To explore this further, phase 2 of this study focused on examining the prevalence of combined use of traditional and conventional medicines among patients at two major public hospitals in South Africa: CMJAH-JHB and KEH-KZN. These academic hospitals are close in proximity to two of the largest *muthi* markets in South Africa, namely, Faraday *muthi* market in Johannesburg, Gauteng, and the Warwick *muthi* market in Durban, KwaZulu-Natal where medicinal plants are sold and used informally.

2.2. Background

The World Health Organisation (WHO) released a Traditional Medicine Strategy (2014 – 2023) advocating for integrative medicine use in national healthcare systems (WHO, 2019). This strategy, in alignment with the need to integrate and harmonise traditional and conventional therapies to expand healthcare coverage, however, raises concerns of potential harmful interactions between traditional and conventional medicines to be addressed. Integrative medicine refers to the model of medicine, whereby traditional medicine is incorporated in modern healthcare systems. It aims to harness all resources for the purposes of

meeting greater healthcare needs (WHO, 2019; Nsagha et al., 2020). This is relevant as South Africa's public healthcare sector is heavily burdened with chronic and infectious diseases (Boyles et al., 2019). The use of traditional medicine in developed countries is estimated to be around 50%, with a higher percentage reflected amongst developing countries, ranging from 60-80% (WHO, 2019; Haque et al., 2018; Sobiecki, 2014; Ekpor et al., 2023). In Africa, studies amongst patients visiting conventional healthcare facilities have indicated varied estimates, ranging from 20-80% (Nyeko et al., 2016; Shewamene et al., 2017; Ameade et al., 2018; James et al., 2018; Hill et al., 2022). Similar studies are somewhat limited in South African healthcare facilities (Nlooto and Naidoo, 2016; Weintraub et al., 2018; Zingela et al., 2019). Despite the availability of modern medicine, traditional medicine is still heavily relied on due to the cultural heritage of many South Africans (Williams and Whiting, 2016). This is supported by several avenues of traditional medicine practised in South Africa, from consultation with traditional healers to access of *muthi* medicines from various street market vendors (Williams and Whitings, 2016). Two of the largest *muthi* markets in South Africa are in Johannesburg (JHB) (Faraday market), Gauteng, and in Durban, KwaZulu-Natal (KZN) (Warwick Triangle market).

The substantial trade of traditional medicine at these markets, signifies the demand for traditional medicine amongst the respective populations in the two provinces (Williams and Whitings, 2016). Moreover, the use of traditional and conventional medicines in combination may mitigate interactive risks and this requires further investigation, to ensure holistic patient care and safety (WHO, 2023). Therefore, the aim of this chapter was to determine the prevalence of traditional medicine use amongst patients visiting two large public hospitals, located in two different provinces in South Africa. A further focus was to examine concurrent use of conventional and traditional medicines.

2.3. Methods to determine prevalence of traditional medicine and its concurrent use with conventional antimicrobials

2.3.1. Ethical clearance

The Human Research Ethics Committee (Medical) from the University of the Witwatersrand (M190534), South Africa, granted ethical clearance. Permissions study sites were sought and

clearance obtained at provincial level. All study procedures were undertaken in alignment with ethical research guidelines, and participant confidentiality maintained throughout the study.

2.3.2. Study design

A descriptive, cross-sectional, quantitative study was undertaken, using a self-administered questionnaire amongst patients providing voluntary informed consent, in the waiting areas of the out-patient department (OPD) pharmacies at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH-JHB) and King Edward VIII Hospital Durban, KZN (KEH-KZN), South Africa. Reliability and validity of the data collection tool was confirmed with a pilot study undertaken at the CMJAH-JHB study site in February 2021 amongst 15 patients (10% of calculated sample size).

2.3.3. Study site

The CMJAH-JHB and KEH-KZN sites are situated in two of the provinces with the largest population size amongst all nine provinces in South Africa. Furthermore, two of the largest *muthi* markets in South Africa are in Johannesburg (Faraday market) and Durban (Warwick Triangle market), each located 7.1 km and 4.1 km, respectively, from the selected hospitals.

2.3.4. Study population and sampling

The study population consisted of the hospital OPD pharmacy patients. The OPD pharmacies each attend to approximately 250 adult patients daily. Using the single population proportion formula (Raosoft©), with the assumption of 50% as a proportion (p) of patients, at 95% confidence interval (CI), margin of error (d) = 5%, a sample size was estimated at a minimum of 145 participants for each study site. Although purposive sampling was applied by ensuring that those surveyed were patients to receive conventional medicines from the OPD pharmacy on days of data collection (CMJAH-JHB 21 – 25 June 2021; KEH-KZN 14 – 18 February 2022), sampling of available study population was randomised by selecting every 3rd patient in the OPD pharmacy, irrespective of sociodemographic profile or known traditional medicine use (Viechtbauer et al., 2015). The assumed prevalence in the sample size calculation was based on the WHO estimate, as the benchmark statistic frequently quoted in literature. However, this statistic is related to the African continent, and not South Africa specifically.

There was no literature available quoting otherwise specific to the South African context that could have been used as the estimate. The omission of finite population correction (FPC) for hospital surveys (~1,000 patients) was not applied due to oversampling strategy. A total of 263 and 307 surveys were conducted at CMJAH-JHB and KEH-KZN, respectively. The calculated sample size was surpassed during data collection at both study sites, due to the greater availability of patients. The inclusion criteria consisted of volunteering, adults (18 years and older). Visitors in the pharmacy waiting areas accompanying patients and not receiving medication themselves were excluded.

2.3.5. Questionnaire and data collection

Surveys were accompanied by an information sheet providing participants with an outline of the study. Surveys were distributed amongst patients who met the inclusion criteria. The questionnaire consisted of closed-ended questions, divided into two sections. The first section focused on sociodemographics (age, sex, race, religion/belief system, level of education and employment status). The second section focused on traditional medicine practices. Completion of the questionnaire varied between 10 to 25 minutes, where assistance with translations was provided by research assistants when necessary. No personal identifiers were requested in the process of completing the questionnaire. Completed questionnaires were collected and coded for anonymity. Since the study was a self-administered survey, with either the principal investigator or data assistants available during participant completion of the survey, completeness of the surveys were confirmed upon participant return of the survey. Assistance with questions pertaining to the survey questions was always readily available throughout the data collection process.

2.3.6. Data analysis

Data from the questionnaires was transcribed onto Microsoft Excel spreadsheets. Data was stored on a password-protected computer and only accessible to the researcher and supervisors. Descriptive statistics were presented as frequencies, percentages and mean values between categorical variables compared.

2.4. Results and Discussion

2.4.1. Sociodemographic profiles and traditional medicine use amongst participants

Sociodemographic findings of participants from both study sites, exhibited similarities (Table 2.1). The majority of respondents were of the 18-to-35-year-old age bracket (CMJAH-JHB 48.7%; KEH-KZN 67.1%), female (CMJAH-JHB 71.1%; KEH-KZN 70.3%), black (CMJAH-JHB 93.9%; KEH-KZN 88.6%), Christian (CMJAH-JHB 78.3%; KEH-KZN 54.0%), and unemployed (CMJAH-JHB 59.7%; KEH-KZN 59.3%), with a secondary level of education (CMJAH-JHB 61.2%; KEH-KZN 82.7%). It has been argued that sociodemographic factors have a strong influence on traditional medicine use and as such the complexities should be acknowledged before integrated medicine can be practiced safely (Ezekwesili-Olifi and Ogbonna, 2022). Traditional medicine practices were found to be employed by 76.8% (n = 202) and a greater 94.1% (n = 289) participants from the CMJAH-JHB and KEH-KZN study site, respectively.

An 84% prevalence of traditional medicine use was previously documented amongst South Africans in KZN in 2007 (Mander et al., 2007). The current study surpassing this, at 94.1%, emphasises the need for continuous monitoring of traditional medicine in South Africa. There is a predominant reliance on these medicines, despite accessibility to free healthcare in the public conventional healthcare settings. At both study sites, the younger generation (18-to-35-years) were found to make use of traditional medicines more readily (CMJAH-JHB $p = 0.032$; KEH-KZN $p = 0.041$). Contrastingly, most other studies documented a higher prevalence in the older generations (Mander et al., 2007; Hughes et al., 2013; Logial et al., 2021). The predominant female reliance on traditional medicines reflected in this current study, has similarly been documented in other African studies amongst patient populations (Mander et al., 2006; Highes et al., 2013; Ekpor et al., 2023) and has been attributed to the pro-active, autonomous health seeking nature of females (Nethathe et al., 2016; Ameade et al., 2018). In a further study, amongst South African hypertensive patients, 82.1% of traditional medicine users were female (Williams et al., 2000). Furthermore, a previous study reported up to 77.1% of diabetic patients found to be using traditional medicine, with females primarily contributing to this high prevalence (Ekpor et al., 2023).

Most participants making use of traditional medicines were Christian (CMJAH-JHB 78.3% with $p = 0.042$; KEH-KZN 54.0% with $p = 0.013$), with 12.9% and 33.0% of CMJAH-JHB and KEH-KZN participants respectively, practising a traditional African belief system.

Another study also evaluated traditional medicine use amongst a patient population in South Africa, where 86% of users were Christian and 18.5% of the traditional African belief system (Mander and Ntuli, 2007). The traditional African belief system, often referred to as a religion, encompasses the spiritualism of communicating with ancestors and traditional medicinal plant use practices that vary amongst ethnic groups (Mander and Ntuli, 2007). Often, traditional medicine has been associated with witchcraft and superstition, and as such, certain Christian religious groups may discourage this practise (Hughes et al., 2013).

Although not explicitly explored in this study, the high prevalence of Christian traditional medicine users documented, dispels this notion, since religion did not evidently reduce traditional medicine use.

In a study investigating perceptions of conventional healthcare professionals on traditional medicine use, 66.7% of the participants indicated strong disagreement that Christian patients would consult traditional healers (Hughes et al., 2013; Nethathe et al., 2016; Logiel et al., 2021).

Mnguni (2021) explains the revitalisation of African culture after colonialism, and the Christian attitude toward indigenous medicine. Although colonialistic religion tends to distance itself from any issue that it deems as secular or cultural in viewpoint, this does not necessarily reflect compatably with South Africas. Although many followers meet some or all of these expectations, some individuals still struggle with significant internal conflict (Mnguni, 2021).

African traditions are often heavily entrenched in the Zulu population. There is a large population of traditional, black, Zulu individuals (approximately 78%) residing in KZN, more so than in JHB, Gauteng, with the KZN province often referred to as the "Zulu Kingdom" (Watts, 2010; Mokgobi, 2014; Stats SA, 2017). The results from this study indicated a greater reliance on traditional medicine in the Durban (KZN) (94.1%) study area compared to the JHB (Gauteng) (76.8%) study area.

Most participants (CMJAH-JHB 61.2%; KEH-KZN 82.7%) at both study sites had a secondary level of education, which is a finding that was supported previously, where a majority (53.8%) of South African hypertensive patients using traditional medicine had a secondary level of education [20]. In the current study, there was a stronger correlation between traditional medicine use and a tertiary level education amongst KEH-KZN participants ($p = 0.034$). In a previous study conducted in Durban (Mander and Ntuli, 2007), an inference was made that only the poorly or uneducated make use of traditional medicine (26.0% of participants reaching secondary levels of education), which contradicts the current study findings. With the high level of unemployment in South Africa, most private sector healthcare services may be unattainable (Mokgobi, 2014; Stats SA 2017). Most participants were unemployed (CMJAH-JHB 59.7%; KEH-KZN 59.3%). This was very similarly reflected in other studies, where 54.8% (Mander and Ntuli, 2007) and 96.0% (Hughes et al., 2013) of study participants were unemployed and found to readily make use of traditional medicines.

2.4.2. Traditional medicine practices amongst participants

Participants were further questioned on specific traditional medicine practices, to determine the indications and reasons for traditional medicine use. The types of traditional medicine used, the source, as well as the combination use with conventional medicines was noted (Table 2.2). The KEH-KZN participants most frequently made use of traditional medicines for the treatment or prevention of chronic diseases (60.3%), whereas the CMJAH-JHB participants (36.5%) were found to make use of traditional medicines for reasons other than for either disease prevention/treatment or infection prevention/treatment. In South Africa, traditional medicine encompasses many health practices, approaches, and beliefs, which can include the use of plant, animal, mineral-based medicine uses as well as spiritual therapies and techniques used both individually and in combination to maintain health and prevent disease and infections (Richter, 2003). Therefore, "other" indications amongst CMJAH-JHB participants (36.5%),

could encompass the cultural and spiritual healing included in traditional healthcare, which appeals to many due to the holistic treatment approach and spiritualistic ties. Respondents in the current study indicated preference for herbal-based products (CMJAH-JHB 97.3%; KEH-KZN 88.3%), supported by the rich flora accessible in South Africa (van Wyk, 2018). This finding also correlates with similar African studies reporting high herbal product use (Muoka, 2008; Opiyo, 2018; Hill et al., 2022). Plants can be employed to aid in divination healing, as they affect neurological activity or medicinally for certain therapeutic activity (Sobiecki, 2014). Medicinal plants most frequently used by the study participants included *umhlonyane* (*Artemisia afra*), *ughobha* (*Gunnera perpensa*), *inkomfe* (*Hypoxis hemerocallidea*), *isibhaha* (*Warburgia salutaris*), *umsuzwane* (*Lippia javanica*), and *ikalimuzi* (*Acorus calamus*). These plants are also strongly correlated with those listed as the most commercially relevant medicinal plants in South Africa (van Wyk, 2018). Furthermore, these plants have also been classified amongst some of those exhibiting the most promising therapeutic potential, inclusive of antimicrobial activity, for consideration in new conventional medicines (van Wyk, 2011). In addition, *Imbiza ephuzwato* was commonly mentioned at both study sites. This is a herbal tonic, prepared from the mixture of up to 21 medicinal plants and is available from both Faraday and Warwick *muthi* markets (Ndhlala et al., 2011). The tonic is used for treating a myriad of infections (skin, urinary, respiratory and gastrointestinal tract infections), functioning as a detoxifying and energising tonic. It is also used to relieve pain, reduce anxiety and boost vitality (Ndhlala et al., 2011). This mixture, however, can pose a serious threat of interacting with conventional medicines, and requires further investigating in combination with conventional medicines (Ndhlala et al., 2011). Only one participant indicated the use of animal products in traditional healing methods (Table 2). The animal product used is commonly known as "*umchamo wemfene"* (baboon urine mixed with various minerals) and is used traditionally for abortions or to initiate labour in pregnancy (Alabi et al. 2015). Animal-based traditional medicines are rarely documented, stemming from the cultural secrecy (Williams and Whiting, 2016), where there is a very high rate of non-disclosure (86%). Traditional medicine and "*muthi"* markets are often tainted with a negative reputation due to the use of animal products. Animal products are sometimes presented in the context of witchcraft and superstition (Alabi et al., 2015). However, it appears overzealous to categorise an entire *muthi* market negatively based on this, as the current study demonstrated that animal product use was not widely practiced amongst participants. However, it could also be that there still may be reluctance amongst participants to disclose their use of these types of products.

Most participants from both study sites (CMJAH-JHB 56.7%; KEH-KZN 58.3%) indicated the ease of access to traditional medicine as the most pertinent driver for medicinal plant use (Table 2). A few other studies determined contributing factors, such as accessibility, affordability, perceived safety ("natural"), alignment with sociocultural beliefs and practices, but also dissatisfaction with conventional medicine or healthcare facilities [questions 18, 36-38]. Only 4.9% and 8.8% of CMJAH-JHB and KEH-KZN participants, respectively, indicated a preference for traditional medicine due to it being a cheaper option than conventional medicine. It is therefore evident that cost-effectiveness of traditional medicine is not a major driving factor for its' use and dispels the notion that traditional medicine is a cheap form of healthcare. A previous study conducted a similar survey amongst clinic-going patients in Durban, KZN, where it was indicated by participants that traditional medicines are often more expensive than visiting local government clinics to obtain treatment (Mander et al., 2007). Furthermore, in urbanised areas, traditional medicine is often costly, due to logistics of transporting these medicines from rural areas where they are cultivated. Geographic variation could impact on the way in which traditional medicines are sourced, whether it be from self-harvesting or procurement from *muthi* markets (Williams, 2013). Harvesters are more prevalent in rural KZN (80%) than in the more urbanised JHB (20%), which directly impacts on accessibility and cost (Williams, 2013). Areas where traditional medicinal plant growth occurs naturally is far more evident in rural KZN than in JHB (van Wyk and Prinsloo, 2018). There are coastal forests, coastal grasslands, mangroves, swamp forests and other grasslands all offering potential cultivation and harvesting regions (Mander et al., 2007; Williams, 2013). This is supported by the findings from the current study, whereby traditional medicine procurement amongst KEH-KZN participants, was indicated as "other" (44.6%), and participants listed independent cultivation of medicinal plants or independent harvesting in grasslands, mountains, or riversides as their "other" reasons. In comparison, CMJAH-JHB study participants mostly purchased their traditional medicine from street markets (42.6%). Traditional medicine street markets served as the second most common source of traditional medicine (36.5%) amongst KEH-KZN participants. A similar study found that participants mostly purchased their medicinal plants from traditional healers in KZN (61.0%), as opposed to street markets (39.0%) (Mander et al., 2007). Traditional healers were indicated as a source of traditional medicine purchases more in the CMJAH-JHB (20.5%) participants compared to the KEH-KZN (7.2%) participants. This may be indicative of the cultural practices richly instilled in the Zulu population predominant in KZN, whereby practices and knowledge are passed down from one generation to another. This is also validated by KEH-KZN participants sourcing information

on traditional medicines from friends and family (88.3%), as opposed to only 52.1% of the CMJAH-JHB participants. Moreover, other studies in South America and southern Ethiopia, respectively, reported that most of the respondents had also been informed of traditional medicine from their family members or friends, demonstrating this consistency amongst different cultures and geographies (van Andel and Carvalheiro, 2013; Laelago et al., 2016).

Table 2.1. Sociodemographic profiles of participants and correlation with traditional medicine use.

Category	Frequency N* (%)	Past / current traditional medicine use (CMJAH-JHB *p*)	Frequency N** (%)	Past / current traditional medicine use (KEH-KZN *p*)
Age				
18 – 35 years	**128 (48.7)**	0.032	**206 (67.1)**	0.041
36 – 59 years	96 (36.5)	0.137	34 (11.1)	0.052
60 – 69 years	28 (10.6)	0.259	56 (18.2)	0.094
≥ 70 years	11 (4.2)	0.467	11 (3.6)	0.102
Sex				
Male	76 (28.9)	0.041	91 (29.7)	0.080
Female	**187 (71.1)**	0.041	**216 (70.3)**	0.032
Race				
Black	**247 (93.9)**	0.034	**272 (88.6)**	0.013
White	7 (2.7)	0.131	10 (3.3)	0.735
Coloured	6 (2.3)	0.095	4 (1.3)	0.460
Indian	2 (0.8)	0.054	21 (6.9)	0.565
Other	1 (0.4)	0.158	-	-
Religious / beliefs				
Christian	**206 (78.3)**	0.042	**166 (54.0)**	0.036
Muslim	3 (1.1)	0.109	20 (6.5)	0.010
African	34 (12.9)	0.134	101 (33.0)	0.028
Other	20 (7.7)	0.132	20 (6.5)	0.154
Education				
Primary	22 (8.3)	0.060	20 (6.5)	0.159
Secondary	**161 (61.2)**	0.038	**254 (82.7)**	0.146
Tertiary	78 (29.7)	0.047	23 (7.5)	0.034
Not stated	2 (0.8)	0.049	10 (3.3)	0.032
Employment				
Unemployed	**157 (59.7)**	0.073	**182 (59.3)**	0.037
Employed	87 (33.1)	0.113	99 (32.2)	0.053
Self-employed	19 (7.2)	0.053	26 (8.5)	0.023

N* = 263; N** = 307; Bold figures = highest percentage of respondents per variable.

When determining whether its common practice for patients to make use of both traditional and conventional medicines simultaneously, 23.6% of CMJAH-JHB participants were found to frequently practice combination use, whereas only 31.7% of KEH-KZN participants indicated that traditional and conventional medicines were regularly combined in their

healthcare practices (Table 2.1). A study amongst diabetic patients in Africa, reported concurrent traditional and conventional medicine use amongst 34.5% to 88.4% of participants (Ekpor et al., 2023). Studies specifically in South Africa, reported combination use varying between 20-to-50% among patients surveyed in a hospital setting (Mander et al., 2007; Weintraub et al., 2018; Zingela et al, 2019). Combination use is considered a public health threat due to potential interactions between the two forms of medicine use and requires in-depth safety analysis (Hubsch et al., 2014).

2.5. Conclusion

A high percentage of participants indicated past or current use of traditional medicine (CMJAH-JHB 76.8%; KEH-KZN 94.1%), with 23.6% (CMJAH-JHB) and 31.7% (KEH-KZN) of participants using traditional and conventional medicine in combination. This study provides a valuable examination of traditional medicine use and concurrent use with conventional medicines within two large public hospitals in South Africa.

Furthermore, the findings from this study provides beneficial insight into the complexities associated with traditional medicine practices amongst patients from the two different provinces in South Africa. When comparing traditional medicine use amongst participants visiting the CMJAH-JHB and KEH-KZN study sites, a greater reliance on traditional medicine was observed amongst KEH-KZN participants compared with CMJAH-JHB participants. The KEH-KZN participants have a greater tendency to practice combination therapies and are thus at greater risk of experiencing traditional: conventional medicine interactions. Additionally, with medicinal plants the most common type of traditional medicine used, the importance of studies on the interactions between medicinal plants and conventional medicines should be further explored. Furthermore, understanding site-specific drivers and sociodemographic predictors for traditional medicine use will assist in the development of culturally inclusive, safe integration of traditional medicines with conventional health systems, to achieve health system harmonisation and holistic, patient-centred healthcare in South Africa.

Table 2.2. Summary of traditional medicine (TM) practices for the CMJAH-JHB (2021) and KEH-KZN (2022) study respondents.

Variable	Category	CMJAH-JHB (N*)		KEH-KZN (N**)	
		Frequency (n)	**Respondents (%)**	**Frequency (n)**	**Respondents (%)**
TM indications	Prevent or treat chronic disease	31	11.8	**185**	**60.3**
	Prevent or treat infections	91	34.6	81	26.4
	Symptomatic treatment	45	17.1	91	29.6
	Other	**96**	**36.5**	19	6.2
Type of TM used	Herbal plant-based preparations	**256**	**97.3**	**271**	**88.3**
	Animal derived products	1	0.4	1	0.3
	Combination of plant and animal products	6	2.3	15	4.9
	Other	0	0	15	4.9
Reasons for using TM	Cheaper than conventional medicine	13	4.9	27	8.8
	Ease of accessibility	**149**	**56.7**	**179**	**58.3**
	Safer than conventional medicines	4	1.5	16	5.2
	Conventional medicines did not work	14	5.3	33	10.8
	Other	83	31.6	104	33.9
Source of TM purchases	Pharmacy	0	0	22	7.2
	Health shop	33	12.5	17	5.5
	Traditional healers' room	54	20.5	22	7.2
	Street markets	**112**	**42.6**	112	36.5
	Other	64	24.3	**137**	**44.6**
Source of knowledge about TM	Friends or family	**137**	**52.1**	**271**	**88.3**
	Traditional healers	19	7.2	29	9.5
	Media	12	4.6	2	0.7
	Pharmacist/health care workers	1	0.4	12	3.9
	Other	94	35.7	0	0

N* = 263; N** = 307; TM = traditional medicine; CMJAH-JHB = Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg; KEH-KZN = King Edward Hospital, KwaZulu-Natal; Bold figures = highest percentage of respondents per variable.

2.6. Chapter summary

Findings in this chapter highlight existing substantial reliance on traditional medicine amongst patients surveyed at CMJAH-JHB (76.8%) and KEH-KZN (94.1%), with 23.6% and 31.7% of patients, respectively, often combining traditional and conventional healthcare. These patients are at risk of exposure to interactive effects.

A comparison of traditional medicine use between participants at the CMJAH-JHB and KEH-KZN study sites revealed a greater reliance on traditional medicine among KEH-KZN participants than their CMJAH-JHB counterparts. The KEH-KZN participants were also more likely to use combination therapies, putting them at a higher risk of interactions between traditional and conventional medicines.

Medicinal plants (88.3%) were the most prevalently used form of traditional medicine, with *Artemisia afra* Jacq. ex Willd. (African wormwood), *Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall. ("African potato"), *Aloe ferox* Mill. (bitter Aloe), *Lippia javanica* (Burm.f.) Spreng (fever tea), *Gunnera perpensa* L. (river pumpkin) and *Acorus calamus* L. (sweet-flag) frequently indicated.

Chapter 3

Interactive antimicrobial and toxicity profiles of Johannesburg traditional medicinal plants with conventional antimicrobials

3

3.1. Chapter introduction

Chapter 3 sought to achieve objectives set out in phase 3 objectives “a” to “e” of this study, with focus on the Johannesburg sourced medicinal plant samples. In Chapter 2, it was revealed that 78.6% of patients at CMJAH-JHB, used traditional medicine alongside their prescribed conventional medicines.

The Faraday *muthi* market in Johannesburg, Gauteng, plays a key role in ensuring ease of accessibility to traditional medicinal plant material in the urbanised area of Johannesburg. Given the high prevalence of medicinal plant use amongst patients visiting a conventional hospital near the Faraday *muthi* market, and the significant infectious disease burden in South Africa, interactions between medicinal plants from Faraday and conventional antimicrobials raise critical concerns.

This chapter presents the findings to the *in vitro* investigation of antimicrobial interactions and toxicity profiles of commonly traded traditional medicinal plants (Table 3.1) from the Faraday *muthi* market in combination with various conventional antimicrobials.

Table 3.1. Traditional medicinal plant material purchased from the Faraday *muthi* market in Johannesburg, Gauteng.

Scientific plant name	Plant sample image	Scientific plant name	Plant sample image	Scientific plant name	Plant sample image
Acorus calamus L.		*Eucalyptus sideroxylon* A.Cunn.		*Rhoicissus tomentosa* (Lam.) Wild & R.B.Drumm.	
Alepidea amatymbica Eckl. & Zeyh.		*Hydnora africana* Thunb.		*Sclerocarya birrea* (A.Rich.) Hochst.	
Bauhinia bowkeri Harv.		*Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall.		*Senecio serratuloides* DC.	
Cinnamomum camphora (L.) Siebold		*Merwilla plumbea* (Lindl.) Speta		*Synaptolepis oliveriana* Gilg	
Cunonia capensis L.		*Pterocarpus angolensis* DC.		*Warburgia salutaris* (G.Bertol.) Chiov.	
Dombeya rotundifolia (Hochst.) Planch.		*Rauvolfia caffra* Sond.			

*Plant identification and verification by Dr. Gugulethu Khumalo. Images by Prof. Sandy van Vuuren.

P.2

Publication 2

Booth, Z., Khumalo, G., van Vuuren, S.F., 2024. Toxicity and antimicrobial interactions with conventional antimicrobials of commonly traded medicinal plants from Faraday *muthi* market (Johannesburg, South Africa). *South African Journal of Botany,* 175, 637-656. https://doi.org/10.1016/j.sajb.2024.10.048

*Proof of publication and permission for re-use in this thesis is provided in Appendix B. A graphical abstract reflecting this publication has been presented in Figure 3.1.

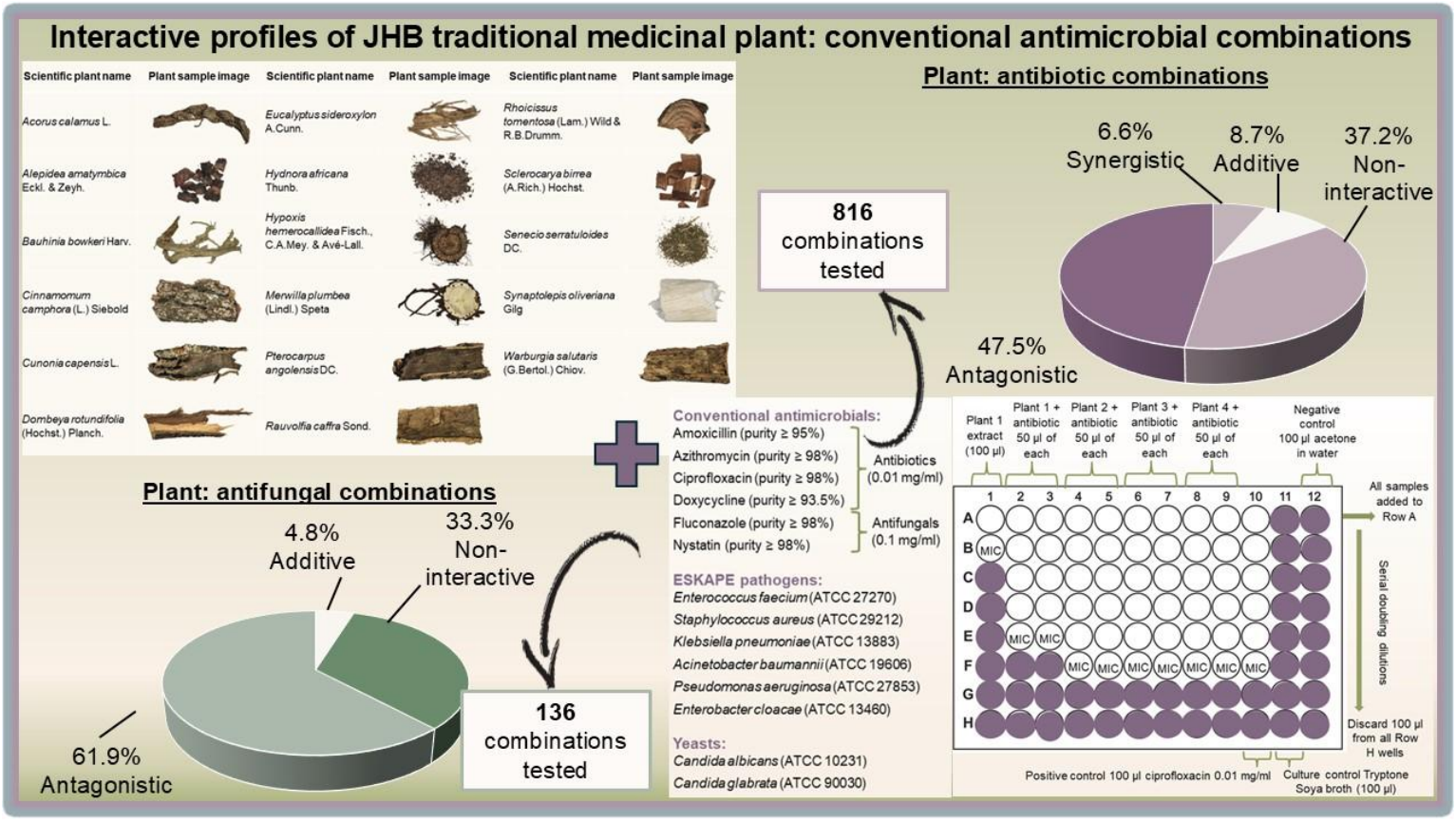


Figure 3.1. A graphical abstract for *Publication 3*.

Toxicity and antimicrobial interactions with conventional antimicrobials of commonly traded medicinal plants from Faraday *muthi* market (Johannesburg, South Africa)

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ABSTRACT

In South Africa, medicinal plants are frequently used to treat infections, with the Faraday *muthi* market in Johannesburg ensuring accessibility to these medicines. This study aimed to determine the *in vitro* interactive antimicrobial and toxicity profiles of commonly traded traditional medicinal plants at the Faraday *muthi* market, with a range of conventional antimicrobials. Minimum inhibitory concentration (MIC) assays were conducted to determine the *in vitro* interactive profiles and tested against the ESKAPE pathogens (*Enterococcus faecium* ATCC 27270, *Staphylococcus aureus* ATCC 29212, *Klebsiella pneumoniae* ATCC 13883, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloacae* ATCC 13460) and yeasts, *Candida albicans* ATCC 10231 and *Candida glabrata* ATCC 90030. The fractional inhibitory concentration index (ΣFIC) was determined to classify interactions as synergistic, additive, non-interactive or antagonistic. The brine-shrimp lethality assay (BSLA) was conducted to determine the toxicity of synergistic interactions. A total of 816 of plant: antibiotic combinations were tested against the ESKAPE pathogens, with 392 combinations exhibiting a definitive ΣFIC. Synergy (6.63%), antagonism (47.49%), additive (8.67%) and non-interactive (37.24%) profiles were observed. Medicinal plants were most widely synergistic with azithromycin against *S. aureus*. *Hypoxis hemerocallidea* with azithromycin against *S. aureus* (ΣFIC 0.06) was the most synergistic, with no toxicity evident in the BLSA (6.06% mortality). The most antagonistic interaction was with *Eucalyptus sideroxylon* and amoxicillin against *K. pneumoniae* (ΣFIC 138.12). Furthermore, a total of 136 plant: antifungal combinations were tested, where a definitive ΣFIC was obtained

for 84 of the combinations. No synergy was observed, but 61.91% of the combinations were antagonistic. The most antagonistic plant: antifungal combination was observed with *Senecio serratuoloides* and nystatin against *C. albicans* (ΣFIC 34.6). The vast antagonism against the ESKAPE pathogens (47.49%) and yeasts (61.91%) is cause for concern, particularly in antimicrobial resistance development. The most toxic combinations in the BSLA were when azithromycin was combined with *Synaptolepis oliveriana* (54.51% average mortality after 48 h) and with *Warburgia salutaris* (100% average mortality after 48 h). The synergy and antagonism observed in this study warrants further investigation for clinical translation, to determine potentially beneficial or harmful combinations where applicable.

Keywords:
Antimicrobial; combinations; traditional medicinal plants; Faraday *muthi* market; interactions; synergy; antagonism; toxicity.

1. Introduction

In South Africa, cultural heritage is intricately linked to the traditional use of medicinal plants. A previous study by Booth et al. (2024), found that 78.6% and 94.1% of patients surveyed at a public hospital in Johannesburg and Durban (South Africa), respectively used traditional medicine either with currently prescribed conventional medicines or used traditional medicine in the past.

With the high prevalence of medicinal plant use amongst patients visiting conventional hospitals, along with the high infectious disease burden in South Africa, interactions between medicinal plants and conventional antimicrobials are a concern. Patient safety and optimal health outcomes are a priority. Interactions could either increase (synergy) or decrease (antagonism) the potency of conventional antimicrobials (Zhang and Lui, 2020).

The Faraday *muthi* market, located in Johannesburg, South Africa, is a prominent marketplace for traditional medicinal plants. Vendors offer a wide range of medicinal plants to treat infections and/or other health concerns (Williams et al., 2000). Near (7.1 km) to one of the largest public hospitals in Johannesburg (Charlotte Maxeke Johannesburg Academic Hospital), prompted the concern of concurrent healthcare modalities (Booth et al., 2024).

Despite the common use of medicinal plants for traditional healing in South Africa, the interactive nature and impact on patient therapeutic outcomes and toxicity when these are administered with conventional medicine, is largely unknown (Tirona and Bailey, 2006). South

African medicinal plants not included in the current study, have to a limited extent been evaluated for interactive potential with conventional antimicrobials. Our previous study (Hubsch et al., 2014) investigated some popular South African plants (essential oils, aqueous and organic extracts), including *Agathosma betulina* (P.J.Bergius) Pillans, *Aloe ferox* Mill., *Pelargonium sidoides* DC. and *Sutherlandia frutescens* (L.) R.Br. ex W.T.Aiton, purchased from commercial traders, in combination with antimicrobials. Most often non-interactive profiles were noted in combination with ciprofloxacin, erythromycin and nystatin, among other commercial antimicrobials (Hubsch et al., 2014). While the previous study demonstrated some notable interactions, it did not evaluate the most traded medicinal plants directly purchased from Faraday *muthi* market. The medicinal plants evaluated in the current study have been extensively researched in combination with antiretrovirals (Mills et al., 2005; Van den Bout-Van den Beukel et al., 2006; Fasuna et al., 2013), however, combinations with conventional antibiotics and antifungals remain limited (Kumar et al., 2015). Therefore, the aim of this study was to determine the *in vitro* interactive antimicrobial and toxicity profiles of frequently used conventional antimicrobials with traditional medicinal plants, commonly traded at the Faraday *muthi* market.

2. Materials and methods

2.1. Sourcing and processing of plant material

A total of 23 plant samples were purchased from traders at the Faraday *muthi* market, in Johannesburg, Gauteng (co-ordinates -26.211, 28.045), in February 2022. Trading was undertaken in the local language between research assistant and vendor. Purchases were made based on plants that were most sold to customers for symptoms related to infectious diseases. After botanical identification and validation, six samples were eliminated. The remaining 17 medicinal plant samples (Table 1) were dried and macerated. Organic extracts were prepared by submerging plant material in equal parts dichloromethane and methanol, for 24 h at 37˚C. Aqueous extracts were prepared by submerging plant material in sterile water, for 24 h at 37˚ C. The subsequent liquids were filtered, and solvent placed in suitable containers for evaporation (organic extracts) or freeze-drying (aqueous extracts), using the Virtus lyophiliser. Plant extracts were prepared at a concentration of 32 mg/ml for antimicrobial screening, by dissolving in acetone and sterile water, for organic and aqueous extracts, respectively.

Table 1. Traditional medicinal plant material purchased from the Faraday *muthi* market in Johannesburg.

Scientific plant name	Vernacular and common name	Voucher number	Plant part	Traditional uses
Acorus calamus L.	*Ikalimuzi* (Z); sweet flag, flag root (E); *makkalmoes* (A)	PM294	Roots	Gastrointestinal complaints (Van Wyk and Gericke, 2000; Van Wyk et al., 2009; SANBI, 2021).
Alepidea amatymbica Eckl. & Zeyh.	*Ikhathazo* (Z); giant alepidea, larger tinsel flower (E); *kalmoes*, *slangwortel* (A)	PM292	Bark	Respiratory tract infections and gastrointestinal complaints (Van Wyk and Gericke, 2000; Van Wyk et al., 2009, SANBI, 2021).
Bauhinia bowkeri Harv.	Umdlandlovu (X); Kei Bauhinia, Kei White Bauhinia (E); *Keibeesklou* (A)	PM305	Bark	Skin ailments (Van Wyk and Gericke, 2000; Van Wyk et al., 2009, SANBI, 2021).
Cinnamomum camphora (L.) Siebold	*Uroselina* (Z); Camphor tree (E); *kanferboom* (A)	PM298	Bark	Respiratory tract infections, gastrointestinal complaints (Van Wyk and Gericke, 2000; Van Wyk et al., 2009; SANBI, 2021).
Cunonia capensis L.	*Umlulama* (Z); red alder, butterspoon tree (E); *rooiels* (A); *umqwashube* (X)	PM304	Bark	Symptomatic treatmeant of fever and headaches (Van Wyk and Gericke, 2000; Van Wyk et al., 2009) and strengthening the activity of other treatments (Hutching et al., 1996), as well as respiratory tract infections and to treat food poisoning (Khumalo, 2018).
Dombeya rotundifolia (Hochst.) Planch.	Unhliziyonkulu (Z); wild pear, wild plum (E); *blombos*, *blomhout* (A)	PM301	Bark	Gastrointestinal, skin and sexually transmitted infections (Van Wyk and Gericke, 2000; Van Wyk et al., 2009; SANBI, 2021).
Eucalyptus sideroxylon A.Cunn.	*Umdlavuza* (Z); red ironbark, eucalyptus, gum (E)	PM293	Bark	Respiratory tract complaints (SANBI, 2021).
Hydnora africana Thunb.	*Umavumbuka* (Z); jackal food (E); *jakkalskos*, *bobbejaankos* (A)	PM302	Bark	Gastrointestinal, urinary tract and skin infections (Van Wyk and Gericke, 2000; Van Wyk et al., 2009; SANBI, 2021).
Hypoxis hemerocallidea Fisch., C.A.Mey. & Avé-Lall.	*Inkomfe* (Z); star flower, "African potato" (E)	PM290	Bark	Urinary tract infections (Van Wyk and Gericke, 2000; Van Wyk et al., 2009; SANBI, 2021).
Merwilla plumbea (Lindl.) Speta	*Inguduza* (Z); wild squill, blue squill, blue hyacinth (E); *blouberglelie*, *blouslangkop* (A)	PM291	Roots	Wound treatment and other skin ailments (Van Wyk and Gericke, 2000; Van Wyk et al., 2009; Street and Prinsloo, 2013; SANBI, 2021).
Pterocarpus angolensis DC.	*Umvangazi* (Z); kiaat, bloodwood (E); kiaat, bloedhout, dolfhout, kajatenhout (A)	PM303	Bark	Skin and eye infections, gastrointestinal complaints (Van Wyk and Gericke, 2000; Van Wyk et al., 2009; SANBI, 2021).
Rauvolfia caffra Sond.	*Umhlambamanzi,* (Z); quinine tree (E); *kinaboom* (A); umjelo (X)	PM300	Bark	Skin, gastrointestinal and respiratory tract infections (Van Wyk and Gericke, 2000; Van Wyk et al., 2009; SANBI, 2021).
Rhoicissus tomentosa (Lam.) Wild & R.B.Drumm.	*Isinwazi* (Z); wild grape, bush grape, African grape, forest grape, wild vine (E); *bosdruif, wildedruif, bostou* (A)	PM299	Bark	Gastointestinal, skin and sexually transmitted infections and for blood purification (Van Wyk and Gericke, 2000; Van Wyk et al., 2009; Dube et al., 2021; SANBI 2021).
Sclerocarya birrea (A.Rich.) Hochst.	*Umganu* (Z); marula (E)	PM296	Bark	Skin, gastrointesinal and sexually transmitted infections (Van Wyk and Gericke, 2000; Van Wyk et al., 2009, SANBI 2021).
Senecio serratuloides DC.	*Unsukumbili* (Z); two-day cure (E)	PM289	Leaves and stems	Respiratory tract, skin and sexually transmitted infections (Van Wyk and Gericke, 2000; McGaw et al., 2003; Van Wyk et al., 2009; De Wet et al., 2013; SANBI, 2021).
Synaptolepis oliveriana Gilg	*Uvuma omhlophe* (Z); chalk-stick root (E)	PM297	Bark	Infected snakebites and neurological disorders (Van Wyk and Gericke, 2000; Van Wyk et al., 2009, SANBI, 2021), also to induce sleep, reduce anxiety, enhance efficacy of other medicines and induce visions, allowing for diviners to harness spiritual healing for many diseases when patient is entranced (SANBI, 2021). Also used to offer "good luck" to users in maintenance of health (Rasethe et al., 2019).
Warburgia salutaris (G.Bertol.) Chiov.	*Isibhaha* (Z); pepper-bark tree (E)	PM295	Bark	Respiratory tract, gastrointestinal and sexually transmitted infections (Van Wyk and Gericke, 2000; Van Wyk et al., 2009) and skin inflammation often resulting from infections (Leonard and Viljoen, 2015).

A = Afrikaans; E = English; X = Xhosa; Z = Zulu.

2.2. Antimicrobial analysis

2.2.1. Minimum inhibitory concentration (MIC) assays

The ESKAPE pathogens (American Type Culture Collection purchased from Davies Diagnostics) were selected due to the prominent infectious disease burden in South Africa, caused by these pathogens, some of which have been ranked as "critical or high priority" by the World Health Organisation (2017) on the global priority pathogen list. These included *Enterococcus faecium* (ATCC 27270), *Staphylococcus aureus* (ATCC 29212), *Klebsiella pneumoniae* (ATCC 13883), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853), and *Enterobacter cloacae* (ATCC 13460). Two yeasts, *Candida albicans* (ATCC 10231) and *Candida glabrata* (ATCC 90030), were also tested due to their prevalence in causing opportunistic infections. All micro-organisms were cultured in Tryptone Soya broth (TSB), a media supporting optimal growth for both bacteria and yeasts, for 24 h at 37˚C, and 48 h at 37˚C, respectively.

The conventional antimicrobials (amoxicillin, azithromycin, ciprofloxacin, doxycycline, nystatin and fluconazole), purchased from Sigma-Aldrich, were selected based on their association with resistance against selected pathogens and common use within the public healthcare setting (Supplementary Table S1). Antimicrobials were prepared at 0.01 mg/ml and 0.1 mg/ml for antibacterials and antifungals respectively, in appropriate solvents according to the manufacturer (Sigma-Aldrich) and Clinical and Laboratory Standards Institute (CLSI) guidelines (2021).

The CLSI guidelines (2021) were followed to ensure accuracy of microbiological assays and transfer techniques. All antimicrobials were screened for alignment with EUCAST-CLSI (2021) breakpoint ranges against the pathogens tested (Supplementary Table S2).

The antimicrobial activity of the plant samples were analysed individually (Supplementary Table S3 and Supplementary Table S4) and in combination with conventional antimicrobials, using the MIC assay. Individual test samples (100 µl) were added to sterile growth media, TSB (100 µl) in the first row of wells of a 96-well micro-titre plate. The

combinations were introduced to the first row, as 50 µl of plant extract (32 mg/ml) with 50 µl (0.01 mg/ml antibiotic or 0.1 mg/ml antifungal) of conventional antimicrobial (Van Vuuren et al., 2008).

The serial doubling dilution method commenced, after which, each well was inoculated with pathogen ($1x10^8$ colony forming units (CFU)/ml). Sealed plates were incubated

accordingly. A colour indicator, 0.4 mg/ml *p*-iodonitrotetrazolium violet (Sigma-Aldrich) was added (40 µl) to all the wells in the plates. Positive, negative and culture controls were included for each assay. The indicator turns purple/pink in the presence of microbial growth and remains clear in the absence of microbial growth. The lowest concentration of sample to inhibit microbial growth was deemed the endpoint MIC value (Adwan et al., 2010). All individual samples and combinations were tested in triplicate.

2.2.2. Fractional inhibitory concentration (FIC) assessment

To determine the interactive antimicrobial activity of the combinations, MIC values were used to calculate the ΣFIC (Van Vuuren and Viljoen, 2011).

$$FIC^{(i)} = \frac{MIC_a \text{ in combination with } MIC_b}{MIC_a \text{ independently}}$$

$$FIC^{(ii)} = \frac{MIC_a \text{ in combination with } MIC_b}{MIC_b \text{ independently}}$$

The sum of the fractional inhibitory concentration index (ΣFIC) was calculated as $\Sigma FIC = FIC^{(i)} + FIC^{(ii)}$, where "a" represents the conventional antimicrobial sample and "b" represents the plant sample. Combinations were classified as synergistic ($\Sigma FIC \leq 0.5$), additive ($\Sigma FIC > 0.5 - 1.0$), non-interactive ($\Sigma FIC > 1.0$ and ≤ 4.0) and antagonistic ($\Sigma FIC > 4.0$).

2.3. Toxicity analysis: Brine-shrimp lethality assay (BLSA)

Combinations demonstrating antimicrobial synergy were tested for toxicity. Artificial salt water was prepared by dissolving 16 g of Tropic Marine® Sea Salt in 500 ml of distilled water. Dried, brine-shrimp (*Artemia franciscana*) larvae (Ocean Nutrition™) weighed at 0.5 g was added to the salt water and exposed to a constant light, emitting diode bulb (220 – 240 V). Aeration was ensured throughout the incubation period, by using a rotary pump (Kiho) to promote the hatch rate of the larvae. Incubation conditions at 25˚C were maintained for 24 - 48 h. Upon hatching, a volume of 400 µl salt water containing approximately 40-60 live brine-shrimp was added to each well of a 48 well sterile micro-titre plate. Thereafter, 400 µl sample (individual plant samples or antimicrobials, followed by combinations, all diluted in sterile water for aqueous extracts or 2% v/v dimethylsulphoxide (DMSO) for organic extracts)

were prepared (2 mg/ml) and added to the wells in triplicate, resulting in a test concentration of 1 mg/ml (Bussmann et al., 2011). The negative control consisted of 32 g/l salt water and the positive control consisted of 1.6 mg/ml potassium dichromate (Sigma-Aldrich). The plates were observed under a light microscope (Leica DMi1) (40 × magnification) immediately after sample addition (at time 0), for dead brine-shrimp to be excluded from the mortality rate calculations. Brine-shrimp mortality was further screened for after 24 and 48 h. Thereafter, a lethal dose of 50 µl glacial acetic acid (100% v/v; Saarchem) was added to each of wells and mortality calculations undertaken, using methods outlined by Cock and Kalt (2010). A percentage mortality greater than 50% was considered toxic (Bussman et al., 2011). An ethics waiver was garnered from the University of the Witwatersrand research ethics committee (W-CBP-220314-01) for testing on brine-shrimp and micro-organisms.

3. Results and discussion

A total of 816 combinations of plant (aqueous and organic) extracts with conventional antibiotics were tested against the six ESKAPE pathogens. Furthermore, 136 plant: antifungal combinations were tested against the two yeasts (*C. albicans* and *C. glabrata*). A limitation was observed for 340 of the combinations, whereby a definitive ΣFIC could not be determined. This occurs due to the inability to obtain MIC endpoints, whereby the MIC value of individual plant samples equate to >8 mg/ml, or the combination exhibits an MIC value of > 4 mg/ml for plant sample and >1.25 µg/ml for antibiotics / >12.5 µg/ml for antifungals. Interaction classification can be undertaken based on tentative ΣFIC values in these instances, however, for absolute accuracy only those combinations with a definitive ΣFIC value were discussed in this study (n = 476). Although most of the medicinal plants investigated in the current study have been tested individually for antimicrobial activity (Eloff, 2001; Afolayan and Lewu, 2009; Devi and Ganjewala, 2009; Street et al., 2009; Elaissi et al., 2012; Njau et al., 2014; Nciki and Van Vuuren, 2016; Kudumela et al., 2018; Baloyi et al., 2019; Khumalo et al., 2019; Tran et al*.,* 2021; Chukwudozie and Ezeonu, 2022; Lee et al., 2022), there are very few studies (Van Vuuren et al., 2009; Van Vuuren and Viljoen, 2011; Hubsch et al., 2014) on the medicinal plants in combination with conventional antimicrobials against the pathogens similarly evaluated in this study.

Of the 476 plant: antimicrobial combinations tested with definitive ΣFIC, overall interactive plant: antibiotic (n = 392) antimicrobial activity against the ESKAPE pathogens is

reflected in Fig. 1. Furthermore, overall interactive plant: antifungal (n = 84) antimicrobial activity against the two yeasts (Fig, 18 and 19) is represented by Fig. 2, with no synergistic interactions observed.

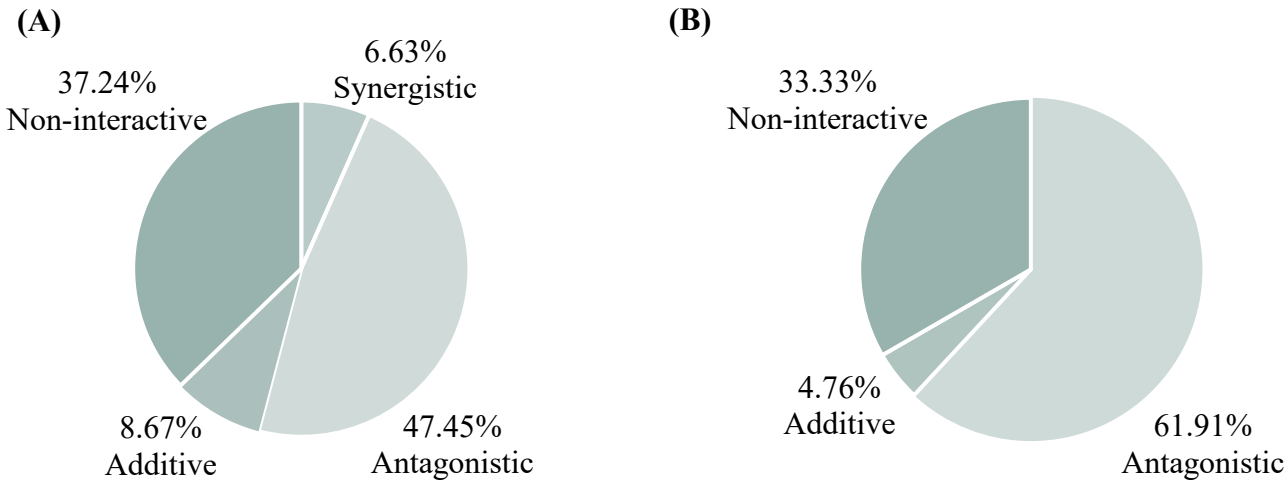

Fig. 1. Interactive antimicrobial profiles for plant: antibiotic (A) and plant: antifungal (B) combinations tested.

Our previous plant: antimicrobial study (Hubsch et al., 2014) focused on popular South African medicinal plants of commercial relevance, and findings were very different to the current study. Hubsch et al. (2014) found that most of the combinations (42.4%), were non-interactive in nature, with only 7.6% antagonistic and more synergy (14.3%) was observed. The variance could potentially be attributed to the sourcing of plant samples, as Hubsch et al. (2014) procured plant material from large formal commercial traders or were collected *in situ*, whereas plants in this current study were purchased directly from the Faraday *muthi* market.

3.1.Plant: antibiotic combinations

The specific medicinal plant: antibiotic combinations (Fig. 2 – Fig. 18) evaluated in this study are further discussed as follows:

The *Acorus calamus*: antibiotic combinations (Fig. 2), had only one synergistic interaction (organic extract *A. calamus*: azithromycin against *S. aureus* - ΣFIC of 0.50). The pathogen, *S. aureus*, can contaminate food and cause gastroenteritis due to enterotoxin development (Argudin et al., 2010). *Acorus calamus* is a traditional remedy commonly employed for gastrointestinal complaints (Table 1), with azithromycin (Supplementary Table S1) used to treat certain gastrointestinal infections in the clinical setting (SAMF, 2022; EMGuidance, 2024). Hence, this synergistic combination warrants further investigation in the

treatment of *S. aureus*-induced gastroenteritis. Four antagonistic interactions were noted for *A. calamus*: antibiotic combinations. Of potential clinical concern, is the antagonism observed against *Pseudomonas aeruginosa* with *A. calamus*: azithromycin (ΣFIC of 16.98) and *A. calamus*: ciprofloxacin combinations (ΣFIC of 4.23). Patients experiencing symptoms of *Pseudomonal* gastroenteritis may be receiving azithromycin or ciprofloxacin as conventional treatments (EMGuidance, 2024) and similarly using *A. calamus* traditionally (Table 1). This antagonism between conventional and traditional treatments of *Pseudomonal* gastroenteritis poses risks of treatment failure and could exacerbate *Pseudomonal* resistance (SAMF, 2022).

The *Alepidea amatymbica*: antibiotic combinations (Fig. 3) had two synergistic interactions (organic extract *A. amatymbica*: azithromycin against *E. faecium* - ΣFIC of 0.13 and organic extract *A. amatymbica*: azithromycin against *S. aureus* - ΣFIC of 0.25). *Alepidea amatymbica* is a medicinal plant used traditionally to treat both gastrointestinal and respiratory infections (Table 1), with azithromycin (Supplementary Table S1) conventionally used for gastroenteritis (SAMF, 2022; EMGuidance, 2024). Both combinations, therefore, warrant future antimicrobial consideration for the treatment of gastrointestinal and respiratory tract infections. Furthermore, *A. amatymbica*: antibiotic combinations had nine antagonistic interactions against a range of pathogens, with ΣFIC values ranging from 4.11 to as high as 136.45. The aqueous extract *A. amatymbica*: amoxicillin combination was highly antagonistic against *K. pneumoniae* (ΣFIC 136.37). With *A. amatymbica* (Table 1) and amoxicillin (Supplementary Table S1), traditionally and conventionally used respectively, for respiratory tract infections. Antagonism is thus a concern. Furthermore, the aqueous extract *A. amatymbica*: ciprofloxacin combination also proved highly antagonistic against *K. pneumoniae* (ΣFIC 136.45). Although ciprofloxacin (SAMF, 2022; EMGuidance, 2024), is an antibiotic more commonly known for its use in treating urinary tract infections, it can be used for respiratory and gastrointestinal infections (SAMF, 2022; EMGuidance, 2024). With *A. amatymbica* and ciprofloxacin used as a traditional and conventional treatment for respiratory tract infections, respectively, along with *K. pneumoniae* being the causative pathogen of many respiratory infections in hospitalised patients (EMGuidance, 2024), antagonism is worrisome.

The *Bauhinia bowkeri*; antibiotic combinations (Fig. 4) yielded one synergistic interaction (organic extract *B. bowkeri*: azithromycin against *S. aureus* - ΣFIC of 0.46), for future investigation. This is due to potential clinical relevance, whereby *Bauhinia bowkeri* is used traditionally to treat an array of skin ailments (Table 1), and azithromycin (Supplementary Table S1) used conventionally for similar skin infections, many of which are caused by *S. aureus* (SAMF, 2022; EMGuidance, 2024). The *B. bowkeri*: antibiotic combinations were

further found to exhibit antagonism in six of the ten combinations with a definitive ΣFIC and ΣFIC values ranging from 4.30 to 136.45, warranting concern for skin infection treatment efficacy should the traditional and conventional forms of treatment be combined.

The *Cinnamomum camphora*: antibiotic combinations (Fig. 5) had only one synergistic interaction (organic extract *C. camphora*: azithromycin against *S. aureus* - ΣFIC of 0.43). *Cinnamomum camphora* is well documented for its traditional use in treating respiratory tract infections (Table 1). Similarly, azithromycin is used conventionally for respiratory tract infections (Supplementary Table S1). Therefore, the synergistic interaction noted could be explored further for improved therapeutic outcomes, particularly since *S. aureus* poses substantial antimicrobial resistance risks (Rao et al., 2022). The *C. camphora*: antibiotic combinations yielded a concerning 12 antagonistic interactions (ΣFIC values ranging from 4.06 to 68.62).

The *Cunonia capensis*: antibiotic combinations (Fig. 6) had two synergistic interactions observed (organic extract *C. capensis*: azithromycin against *S. aureus* - ΣFIC 0.21; and aqueous extract *C. capensis*: ciprofloxacin against *P. aeruginosa* - ΣFIC 0.31). Although *C. capensis* is reported more for symptomatic treatment of headaches and fever (Table 1), it has been overlooked in literature for its' use in respiratory tract infections and treating food poisoning (Khumalo, 2018), hence these synergistic interactions should be further explored for improved antimicrobial treatments of respiratory and gastrointestinal infections. Furthermore, *C. capensis*: antibiotic combinations proved antagonistic in 15 of the combinations with a definitive ΣFIC (ΣFIC values ranging from 4.11 to 51.83 against the ESKAPE pathogens).

The *Dombeya rotundifolia*: antibiotic combinations (Fig. 7), had only one synergistic interaction (organic extract *D. rotundifolia*: azithromycin against *S. aureus* - ΣFIC 0.43). This interaction is notable, since *D. rotundifolia* (Table 1) and azithromycin (Supplementary Table S1) are used traditionally and conventionally respectively, in treating skin infections which may often result from *S. aureus*. Hence, the synergistic nature of this combination may prove beneficial to clinical outcomes for patients suffering from *S. aureus*-caused skin infections. Moreover, the *D. rotundifolia*: antibiotic combinations yielded 10 antagonistic interactions (ΣFIC ranging from 4.11 to the most antagonistic at 69.33 against ESKAPE pathogens). The most antagonistic being the *D. rotundifolia*: ciprofloxacin combination, against *A. baumannii* (ΣFIC of 69.33).

The *Eucalyptus sideroxylon*: antibiotic combinations (Fig. 8) had one synergistic interaction of notability, (organic extract *E. sideroxylon*: azithromycin against *S. aureus* - ΣFIC of 0.19). *Eucalyptus sideroxylon* (Table 1) and azithromycin (Supplementary Table S1) are

employed traditionally and conventionally, respectively, to treat respiratory tract infections. The synergy observed offers a worthy consideration for the treatment of *S. aureus* related respiratory tract infections, such as pneumonia and sinusitis (SAMF, 2022). Furthermore, antagonism was noted amongst 13 of the *E. sideroxylon*: antibiotic combinations with a definitive ΣFIC (ΣFIC ranging from 4.06 to 138.12). The most antagonistic interaction (ΣFIC of 138.12) was observed for the organic extract *E. sideroxylon*: amoxicillin combination against *K. pneumoniae*, with *E. sideroxylon* (Table 1) and amoxicillin (Supplementary Table S1). Additionally, the aqueous extract *E. sideroxylon*: amoxicillin combination was also antagonistic against *K. pneumoniae* (ΣFIC of 67.96).

Hydnora africana: antibiotic combinations against the ESKAPE pathogens (Fig. 9) presented only one synergistic interaction (organic extract *H. africana*: azithromycin against *S. aureus* - ΣFIC 0.50). This synergistic interaction may have relevance in treating skin infections caused by *S. aureus*, since both *H. africana* and azithromycin, are frequently used as traditional (Table 1) and conventional (Supplementary Table S1) therapeutic agents for skin infections, commonly arising from *S. aureus*. Furthermore, antagonism was noted in 11 combinations (ΣFIC values ranging from 4.06 to 69.92), raising concern for combination use of these two forms of healthcare.

The *Hypoxis hemerocallidea*: antibiotic combinations (Fig. 10), yielded the most synergistic interactions amongst all combinations tested. This synergy was observed with the organic extract of *H. hemerocallidea*: azithromycin against *S. aureus* (ΣFIC 0.06). Azithromycin is a broad-spectrum antibiotic, and although it is not the first line treatment for urinary tract infections, it can be a treatment option (SAMF, 2022). Some of the most antagonistic interactions overall were also observed with these combinations, with 11 antagonistic interactions documented, of which five had ΣFIC values of 100.0 or greater. Of notoriety is the *H. hemerocallidea*: ciprofloxacin combination, specifically against *A. baumannii* (ΣFIC of 135.93). *Acintebacter baumannii* is a pathogen associated with urinary tract infections (EMGuidance, 2024). Furthermore, *H. hemerocallidea* and ciprofloxacin are used as traditional (Table 1) and conventional (Supplementary Table S1) treatments for urinary tract infections, and so the antagonism noted is of concern amongst patients with urinary tract infections making use of both traditional and conventional treatments simultaneously.

The *Merwilla plumbea:* antibiotic combinations (Fig. 11), as with the previous combinations discussed, was synergistic against *S. aureus* (ΣFIC of 0.26). *Merwilla plumbea* is applied topically to treat skin infections (Table 1), with azithromycin (Supplementary Table S1), a macrolide antibiotic, often used for treating similar skin infections, often resulting from

S. aureus (SAMF, 2022; EMGuidance, 2024). From the seven combinations yielding a definitive ΣFIC, four were antagonistic (ΣFIC values ranging from ΣFIC of 4.1 to 69.06).

Pterocarpus angolensis: antibiotic combinations (Fig. 12), had two synergistic interactions (organic extract *P. angolensis*: azithromycin against *S. aureus* - ΣFIC 0.26; and aqueous extract *P. angolensis*: azithromycin against *E. cloacae* - ΣFIC 0.39). *Pterocarpus angolensis* is a medicinal plant used in many traditional remedies for various infections, inclusive of skin infections (Table 1). *Staphylococcus aureus* is a common causative pathogen for skin infections (SAMF, 2022; EMGuidance, 2024). Both *P. angolensis* and azithromycin are used traditionally (Table 1) and conventionally (Supplementary Table S1) for skin infections. Therefore, this synergistic combination is one to consider for clinical translation in treating *Staphylococcal* skin infections. A worrisome 13 antagonistic interactions were noted for *P. angolensis*: antibiotic combinations against a range of ESKAPE pathogens (ΣFIC values of 4.11 to 135.27).

The *Rauvolfia caffra*: antibiotic combinations (Fig. 13), had three synergistic interactions (organic extract *R. caffra*: ciprofloxacin against *E. cloacae* - ΣFIC 0.31; and aqueous extract *R. caffra*: ciprofloxacin against *A. baumannii* - ΣFIC 0.31 and organic extract *R. caffra*: azithromycin against *S. aureus* - ΣFIC 0.32). Since *R. caffra* is used traditionally to treat similar conditions where azithromycin and ciprofloxacin are used clinically, the synergistic interactions are to be considered in improving antimicrobial treatments for skin, gastrointestinal and respiratory tract infections. A further nine antagonistic combinations were noted, each of which occurred when *R. caffra* was combined with different antibiotics (amoxicillin, azithromycin, ciprofloxacin and doxycycline), with ΣFIC values ranging from 4.07 to 35.03, demonstrating a diverse span of antagonism.

For the *Rhoicissus tomentosa*: antibiotic combinations (Fig. 14), the most synergistic combination was found with the aqueous extract *R. tomentosa*: ciprofloxacin combination, against *P. aeruginosa* (ΣFIC 0.07). *Rhoicissus tomentosa* has been used traditionally for treating gastrointestinal, skin and sexually transmitted infections (Table 1), with ciprofloxacin employed conventionally for similar infections (Supplementary Table S1). Therefore, the shared indication for use of ciprofloxacin (conventional medicine) and *R. tomentosa* (traditional medicine), warrants further interactive studies. Furthermore, *R. tomentosa* is also traditionally used as a blood purification agent in cases of suspected sepsis. *Pseudomonas aeruginosa* is frequently associated with causing sepsis, particularly after surgery (CDC, 2019). Thus, the synergistic *R. tomentosa*: ciprofloxacin combination should be evaluated further for potential prophylactic avoidance of sepsis. Additional synergy was observed for the aqueous

extract of *R. tomentosa*: azithromycin and the organic extract of *R. tomentosa*: azithromycin combinations against *S. aureus* (ΣFIC 0.46 and 0.50 respectively). *Rhoicissus tomentosa* was one of very few plants studied, where the aqueous extract was found to exert notable synergy in combination with the antibiotics tested. *Rhoicissus tomentosa*: antibiotic combinations were antagonistic in 12 of the combinations tested against the ESKAPE pathogens (ΣFIC values ranging from 4.25 to 68.62).

The *Sclerocarya birrea*: antibiotic combinations (Fig. 15), yielded only one synergistic interaction with azithromycin against *S. aureus* (ΣFIC 0.50). *Staphylococcus aureus* prevalently causes skin infections (CDC, 2019). This synergism should be explored further for the treatment of Staphylococcal skin infections. The combination also demonstrated 13 combinations with a definitive ΣFIC value that was antagonistic (ΣFIC values ranging from 4.06 to 69.94).

Senecio serratuloides: antibiotic combinations (Fig. 16), yielded synergism in combination with azithromycin against *S. aureus*, in both aqueous (ΣFIC 0.13) and organic (ΣFIC 0.14) extracts. The *S. serratuloides*: antibiotic combinations in comparison to other plant: antibiotic combinations, yielded the most (41) interactions with a definitive ΣFIC. These combinations had a vast number (21) that were antagonistic, in combination with various antibiotics tested against the ESKAPE pathogens, with ΣFIC values ranging from 4.06 to 135.93. The most antagonistic interaction was noted when the aqueous extract was combined with ciprofloxacin against *E. faecium* (ΣFIC 135.93), and with the aqueous extract and azithromycin combination against *P. aeruginosa* (ΣFIC 132.56). With patients potentially using the traditional (*S. serratuloides*) and conventional (azithromycin) remedies for respiratory tract and skin infections, the antagonistic profile warrants concern, more so in those infections caused by *P. aeruginosa*. It is also interesting to note, that the same aqueous extract of *S. serratuloides* in combination with azithromycin, had opposing interactive profiles, when the tested pathogen was varied from *S. aureus* (synergistic) to *P. aeruginosa* (antagonistic).

The *Synaptolepis oliveriana*: antibiotic combinations (Fig. 17) had only one synergistic interaction noted for the organic extract in combination with azithromycin against *S. aureus* (ΣFIC 0.38). Furthermore, from the seven combinations with definitive ΣFIC values, five combinations were classified as antagonistic (ΣFIC values ranging from 4.30 to 34.51). Although *S. oliveriana* is not well documented for its medicinal purposes, it is one of the most frequently purchased medicinal plants at Faraday *muthi* market, due to its psycholeptic activity in treating insomnia, reducing anxiety and ability to reduce various types of epileptic seizures, but also to strengthen the activity of other medicines (Rasethe et al., 2019). Due to its'

popularity amongst South African users, this plant was still investigated for interactive potential with conventional antimicrobials.

The *Warburgia salutaris*: antibiotic combinations (Fig. 18) had only two synergistic interactions, where both the aqueous (ΣFIC 0.34) and organic (ΣFIC 0.46) extract of *W. salutaris* was synergistic with azithromycin against *S. aureus.* Both *W. salutaris* (Table 1) and azithromycin (Supplementary Table S1) are used for the treatment of respiratory and gastrointestinal infections. With *S. aureus* being the cause of many respiratory tract infections and less frequently, enterotoxin-induced gastroenteritis, the synergistic combinations should be examined further against *Staphylococcal* infections. The *W. salutaris*: antibiotic combinations were antagonistic in 18 of the 32 combinations with a definitive ΣFIC (ΣFIC values ranging from 4.06 to 135.49). The aqueous extract of *W. salutaris* with amoxicillin against *K. pneumoniae* was the most antagonistic (ΣFIC 135.49). With *W. salutaris* (Table 1) and amoxicillin (Supplementary Table S1) used to treat respiratory tract infections, which may be caused by *K. pneumoniae*, this antagonistic interaction is worrisome. Notably also, *W. salutaris* when combined as an organic extract with doxycycline and tested against *A. baumannii*, provided an antagonistic interaction (ΣFIC 27.69). *Acinetobacter baumannii* is an opportunistic pathogen, which has developed a concerning resistant trait toward antibiotics (Ahuatzin-Flores et al., 2024). The pathogen is known to cause respiratory tract and skin infections (SAMF, 2022). Doxycycline, a tetracycline antibiotic is employed for the conventional treatment of skin and respiratory tract infections (SAMF, 2022). With the overlapping uses of *W. salutaris* (Table 1) and doxycycline (Supplementary Table S1) for the treatment of both skin and respiratory tract infections, this antagonism may pose the risk of reduced antimicrobial treatment of *A. baumannii* caused by skin and respiratory infections.

In summary, there was a common thread of synergy documented for azithromycin in combination with all 17 plants investigated (*A. calamus*, *A. amatymbica*, *B. bowkeri*, *C. camphora*, *C. capensis*, *D. rotundifolia, E. sideroxylon, H. africana, H. hemerocallidea, M. plumbea, P. angolensis, R. caffra, R. tomentosa, S. birrea, S. serratuloides, S. oliveriana* and *W. salutaris*), when tested against *S. aureus.* Furthermore, the most synergistic interaction noted for all combinations tested against ESKAPE pathogens was that of *H. hemerocallidea* organic extract with azithromycin against *S. aureus* (ΣFIC 0.06) and *R. tomentosa* aqueous extract when combined with ciprofloxacin and tested against *P. aeruginosa* (ΣFIC 0.07). The most antagonistic interaction for all combinations with the antibiotics tested was reflected by *E. sideroxylon* organic extract in combination with amoxicillin against *K. pneumoniae* (ΣFIC 138.12).

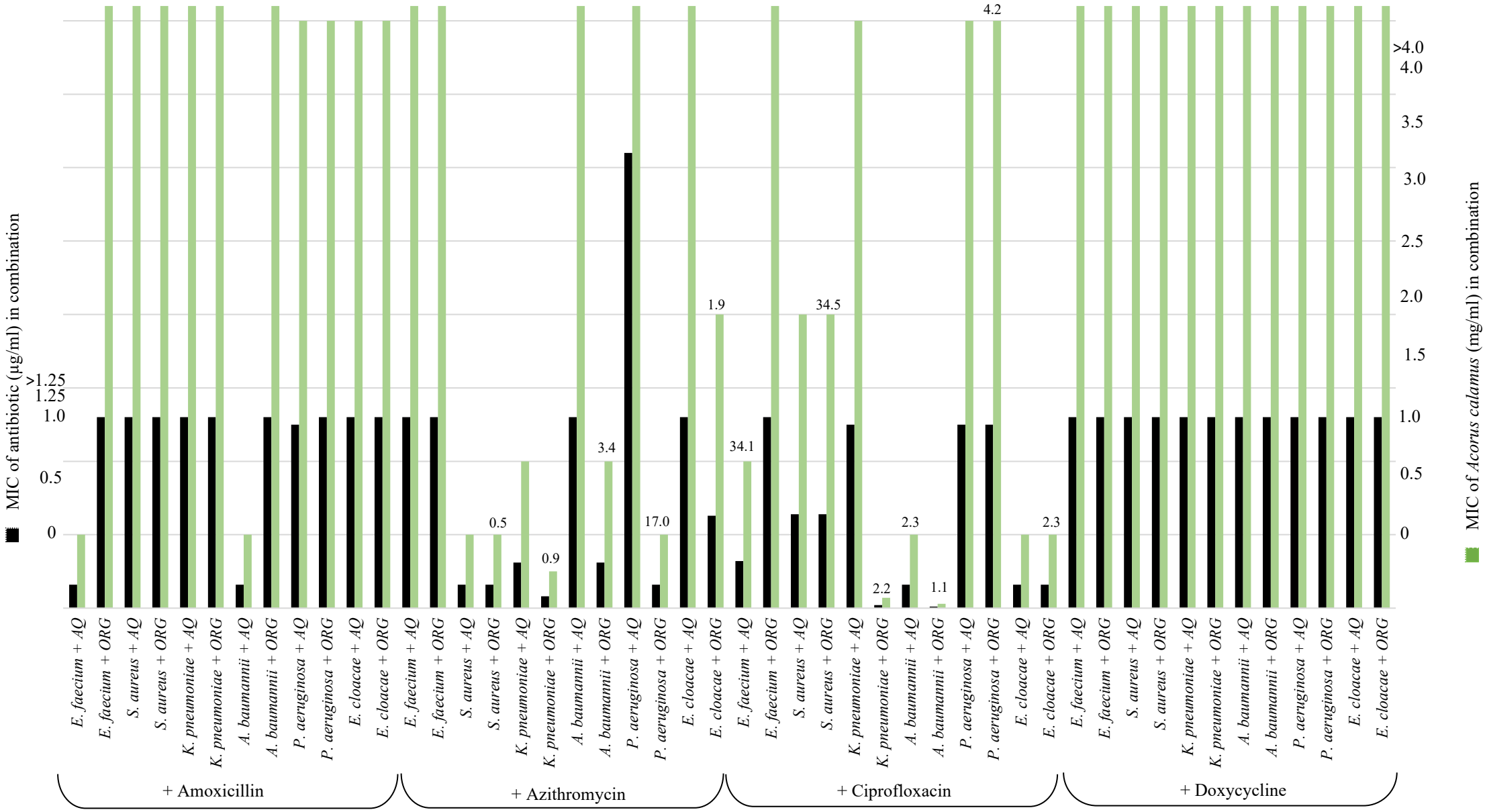

Fig.2. Interactive profiles of antibiotics with *Acorus calamus* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

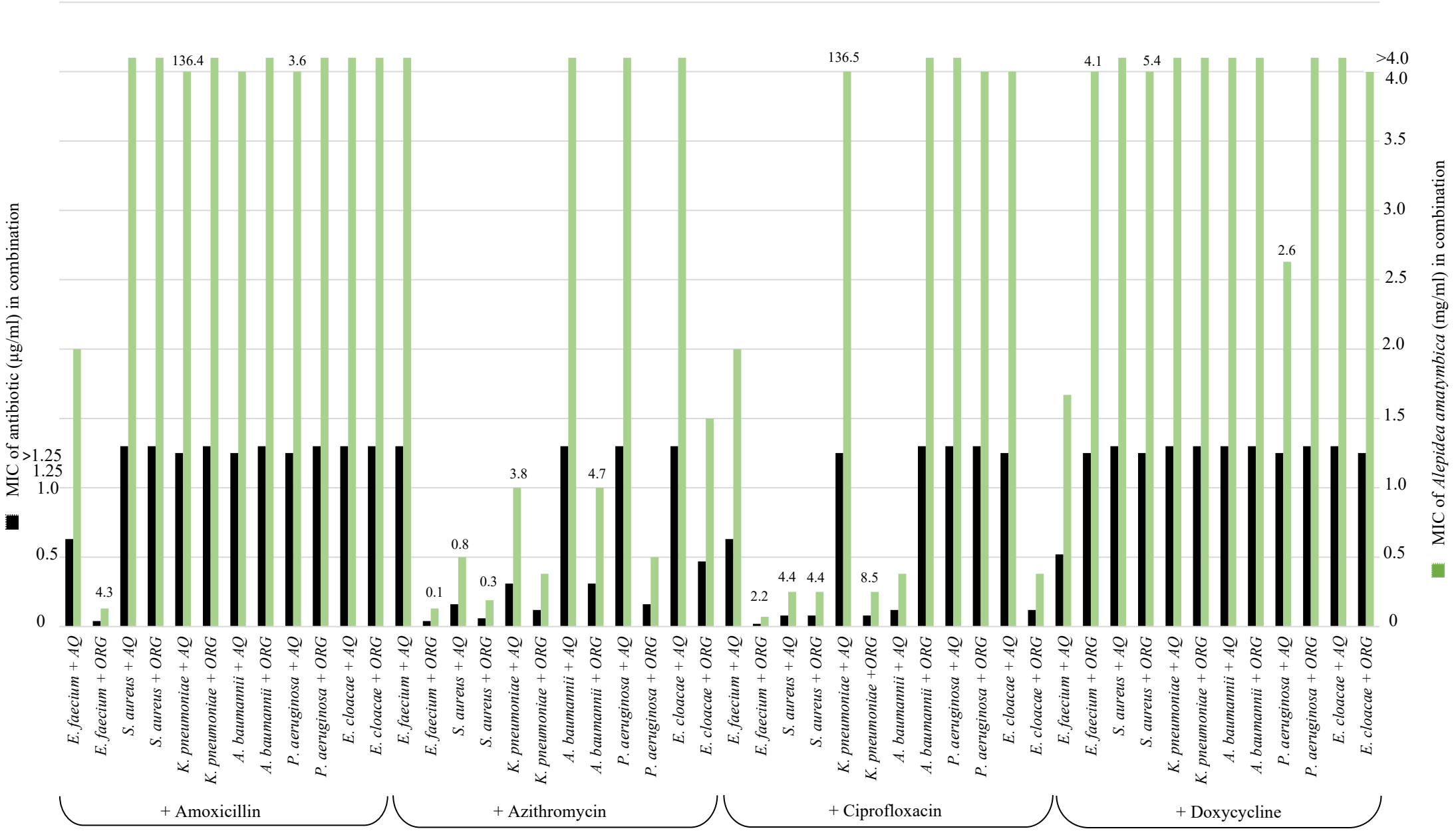

Fig.3. Interactive profiles of antibiotics with plant extracts of *Alepidea amatymbica* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

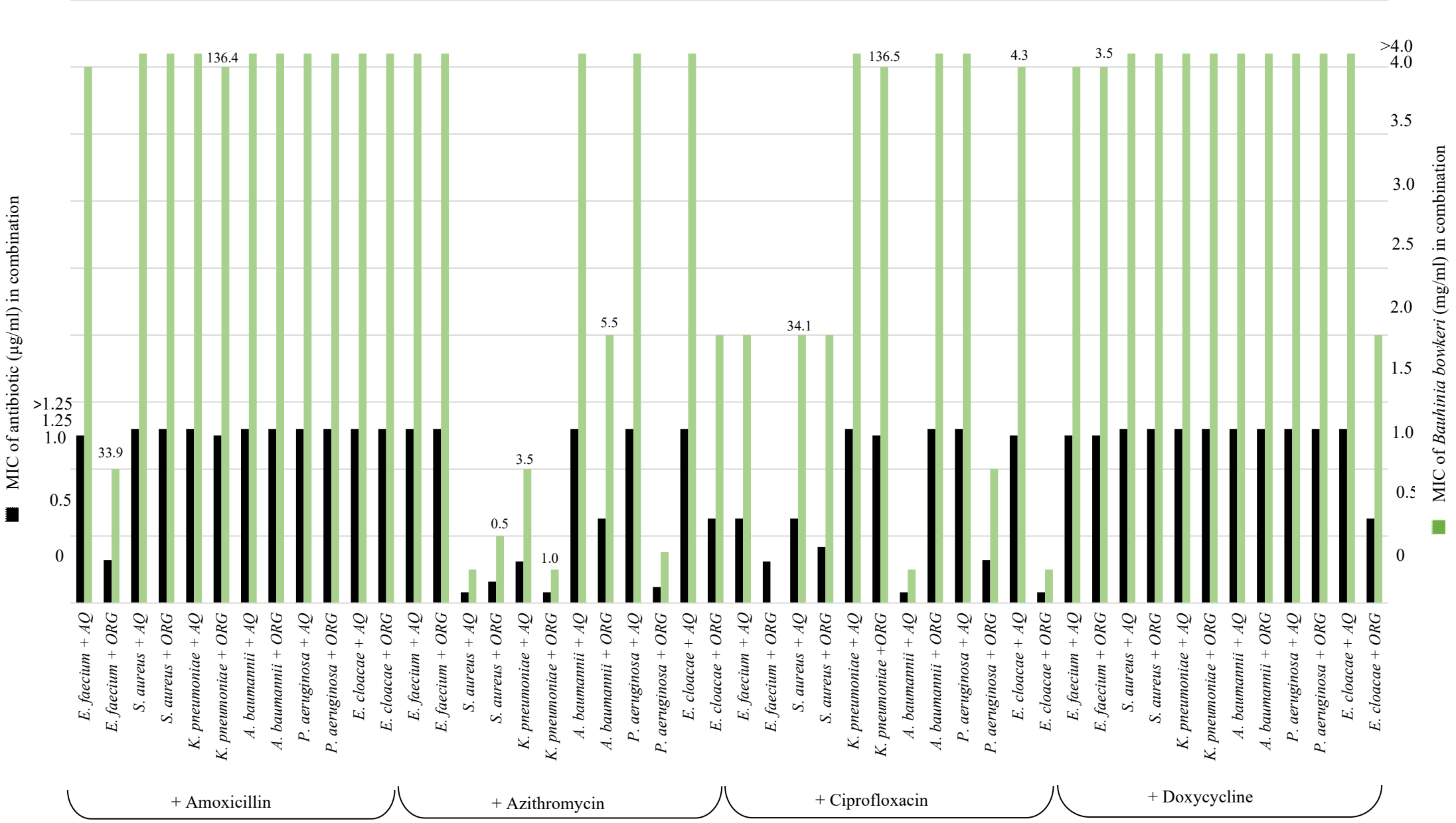

Fig.4. Interactive profiles of antibiotics with plant extracts of *Bauhinia bowkeri* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

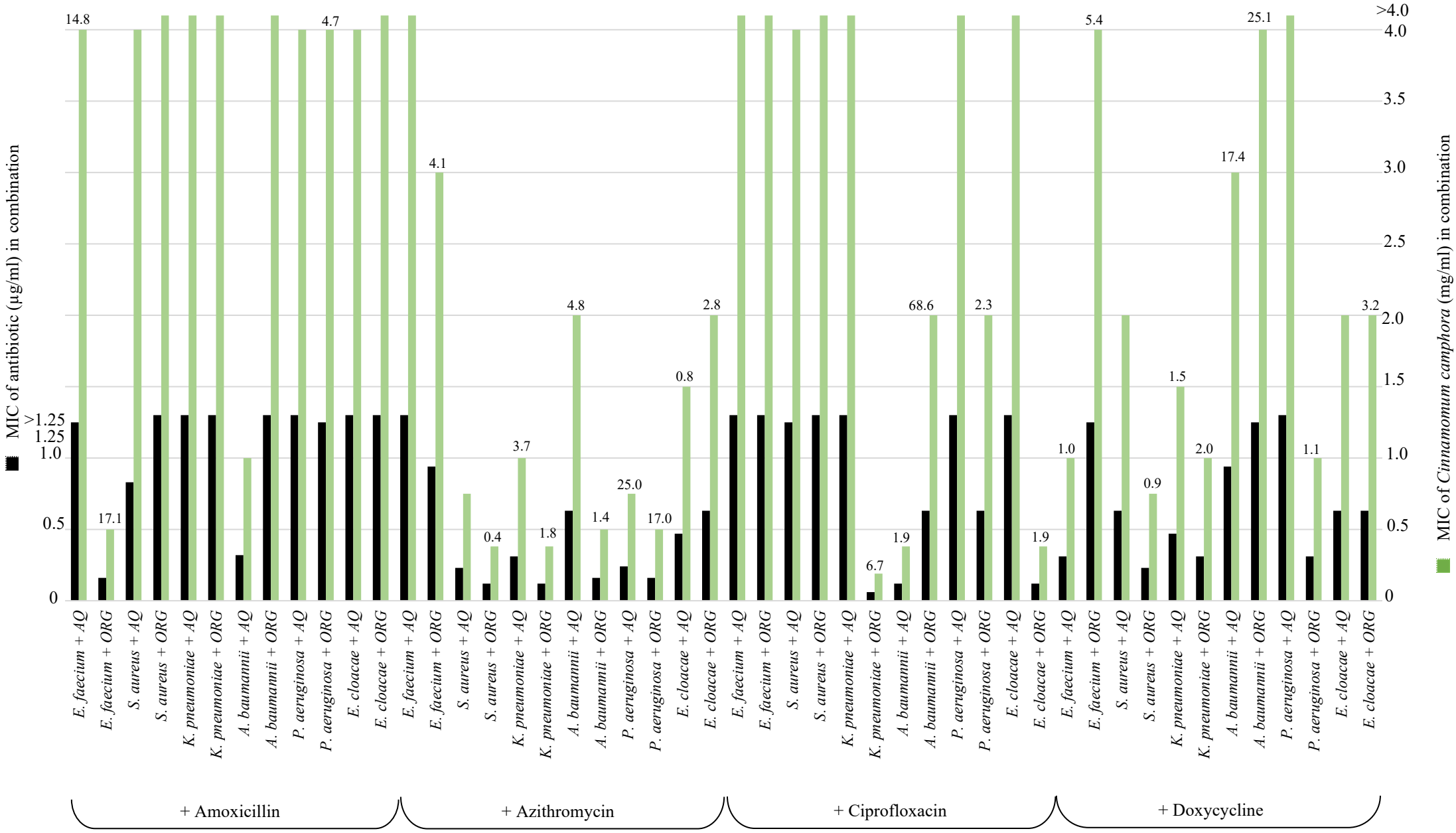

Fig.5. Interactive profiles of antibiotics with plant extracts of *Cinnamomum camphora* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

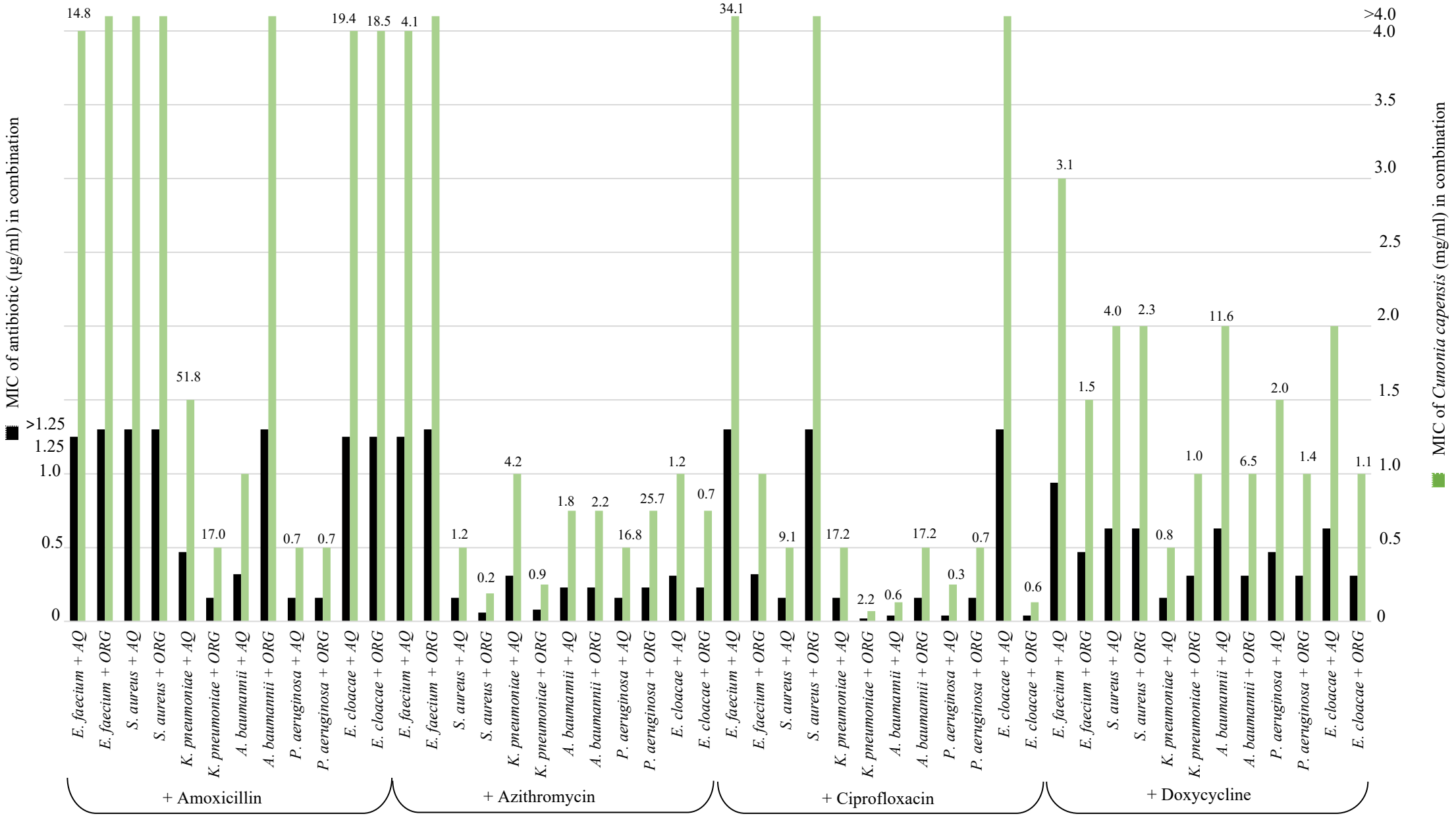

Fig.6. Interactive profiles of antibiotics with plant extracts of *Cunonia capensis* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

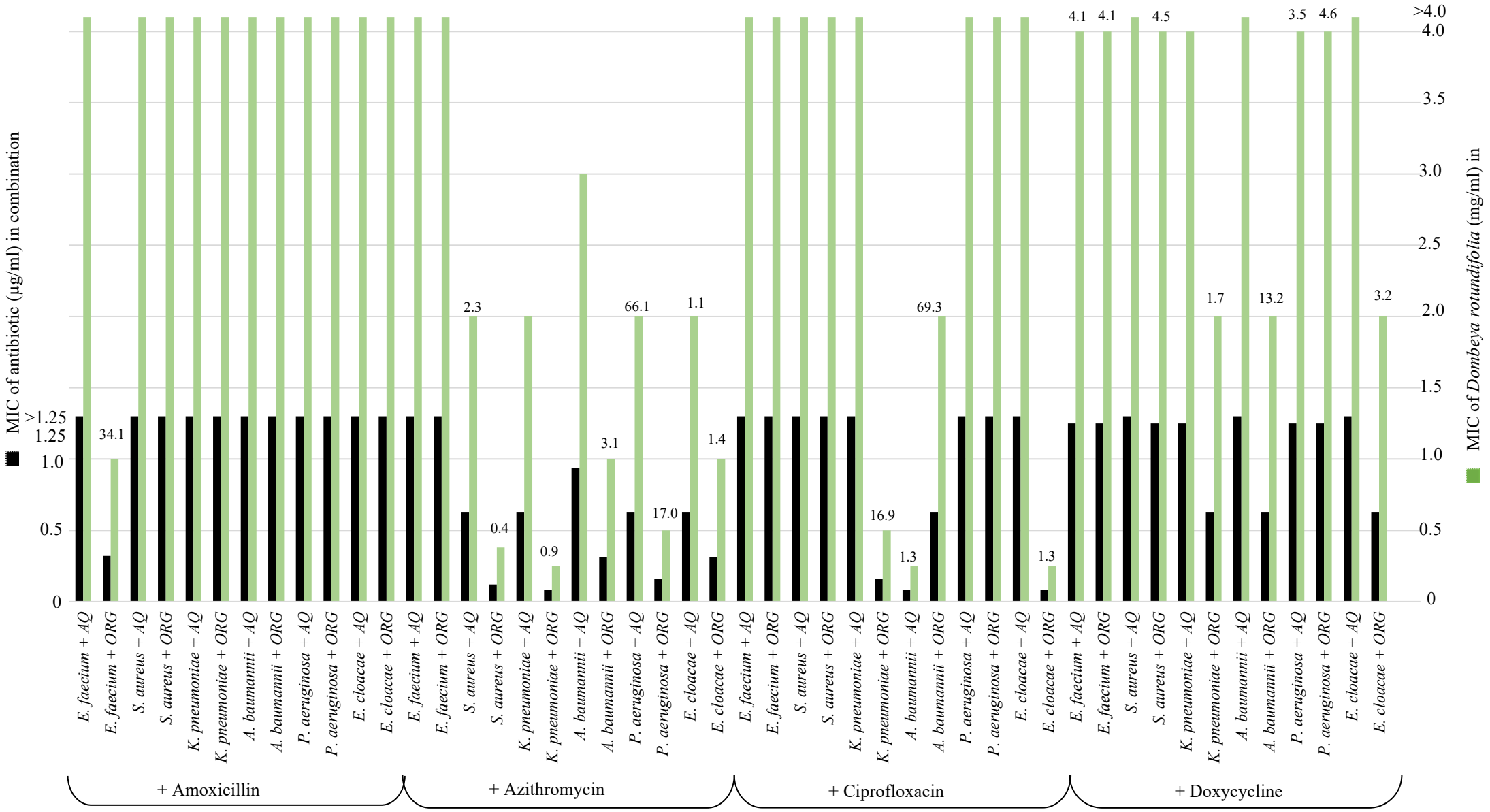

Fig.7. Interactive profiles of antibiotics with plant extracts of *Dombeya rotundifolia* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

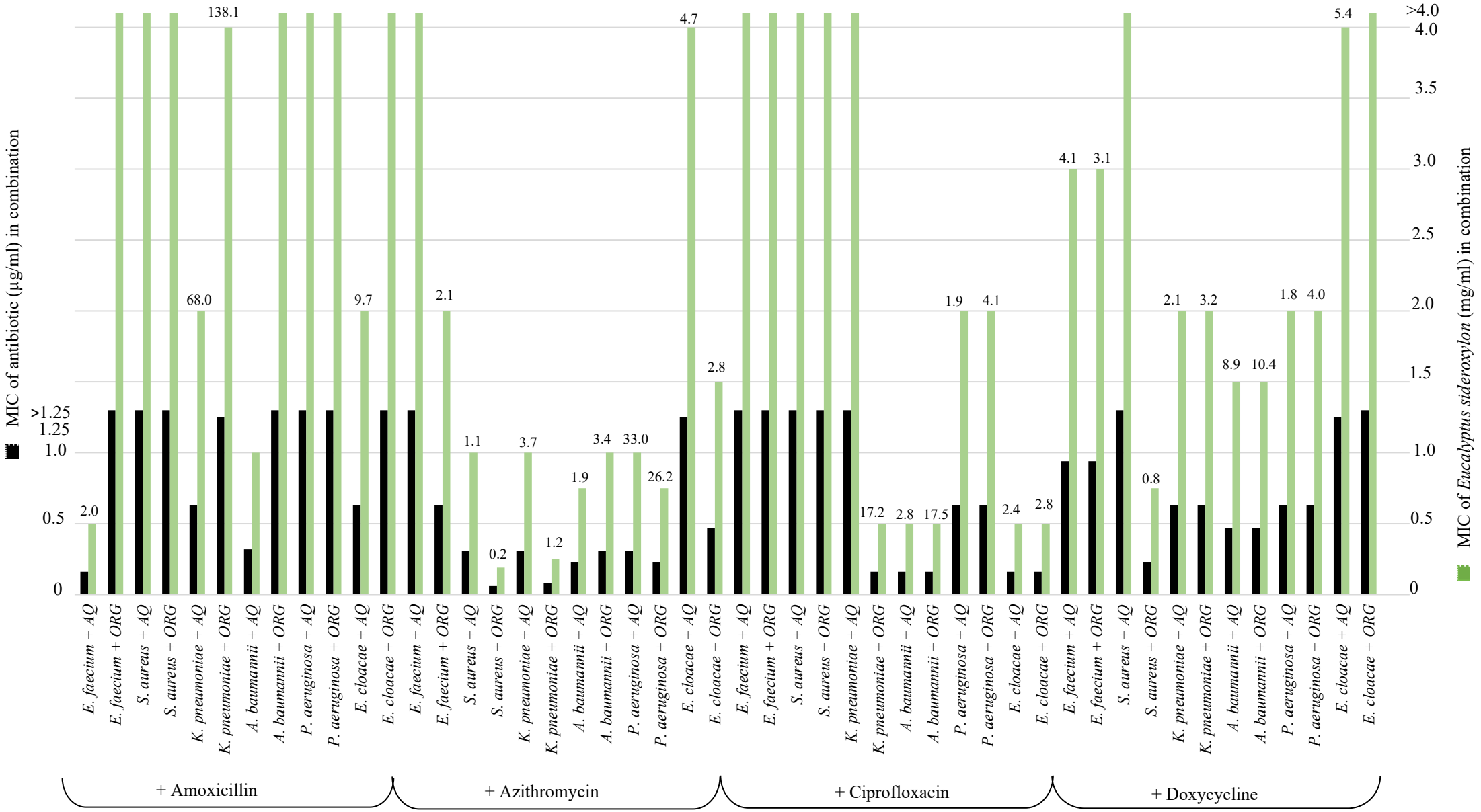

Fig.8. Interactive profiles of antibiotics with plant extracts of *Eucalyptus sideroxylon* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

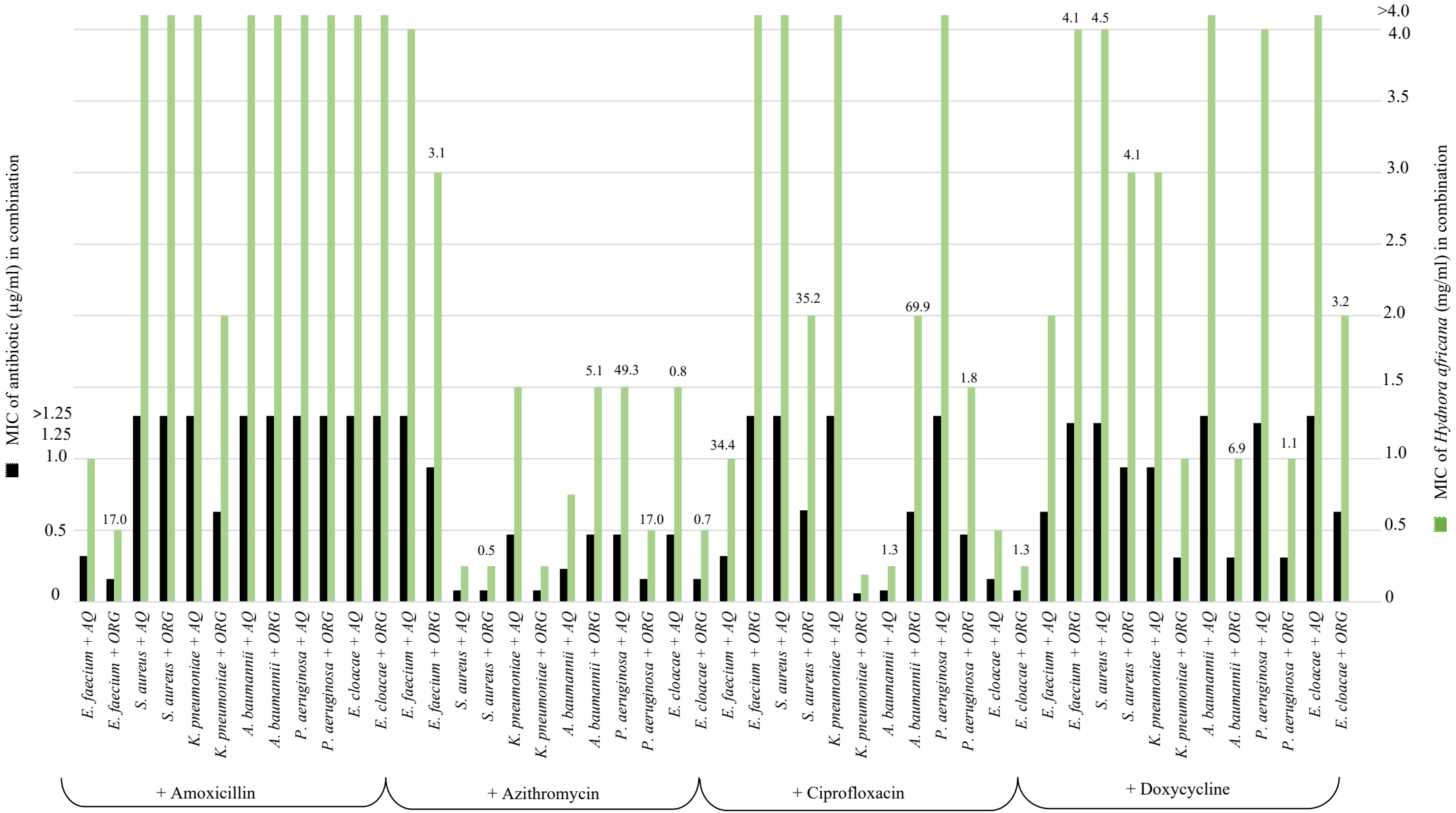

Fig.9. Interactive profiles of antibiotics with plant extracts of *Hydnora africana* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

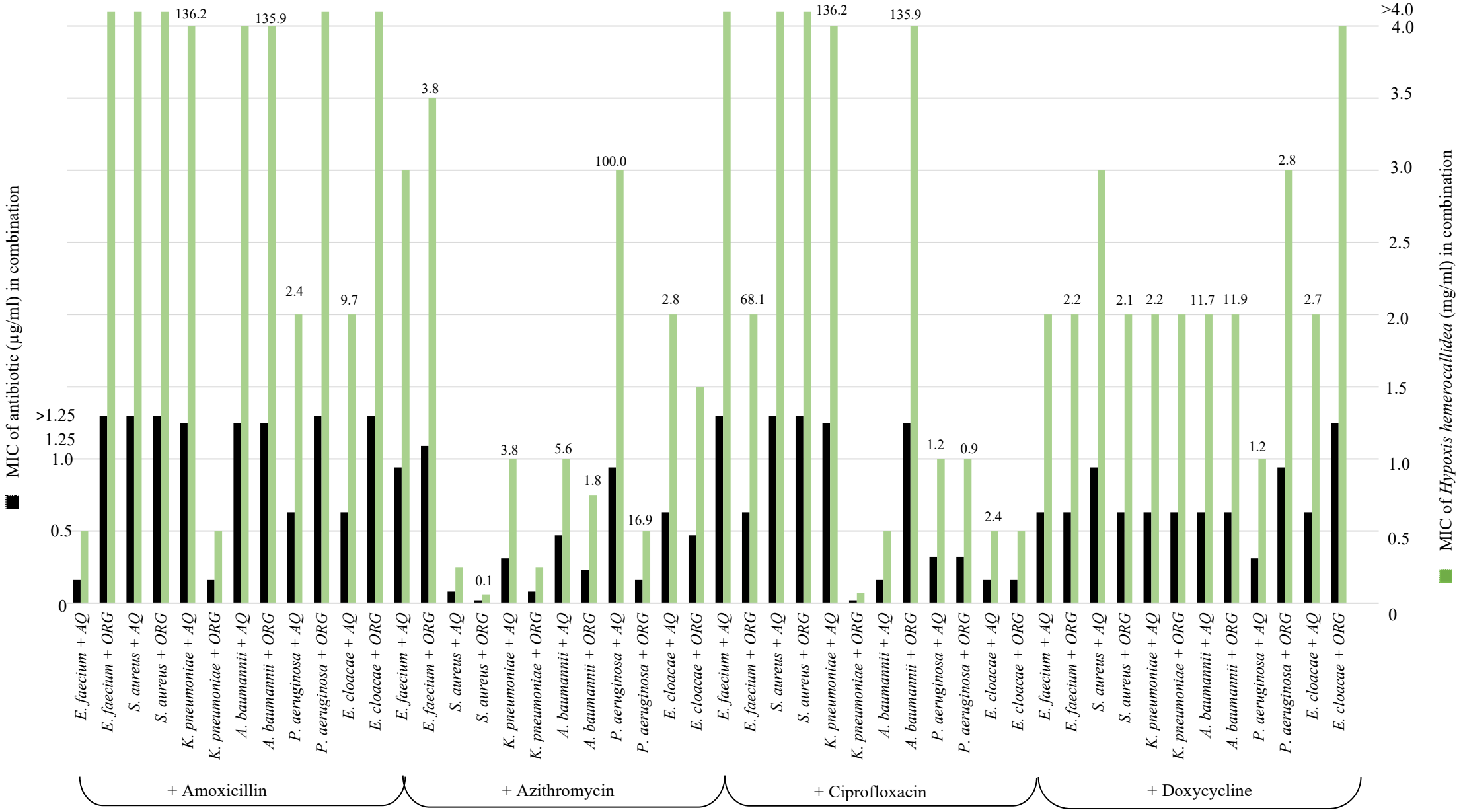

Fig.10. Interactive profiles of antibiotics with plant extracts of *Hypoxis hemerocallidea* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

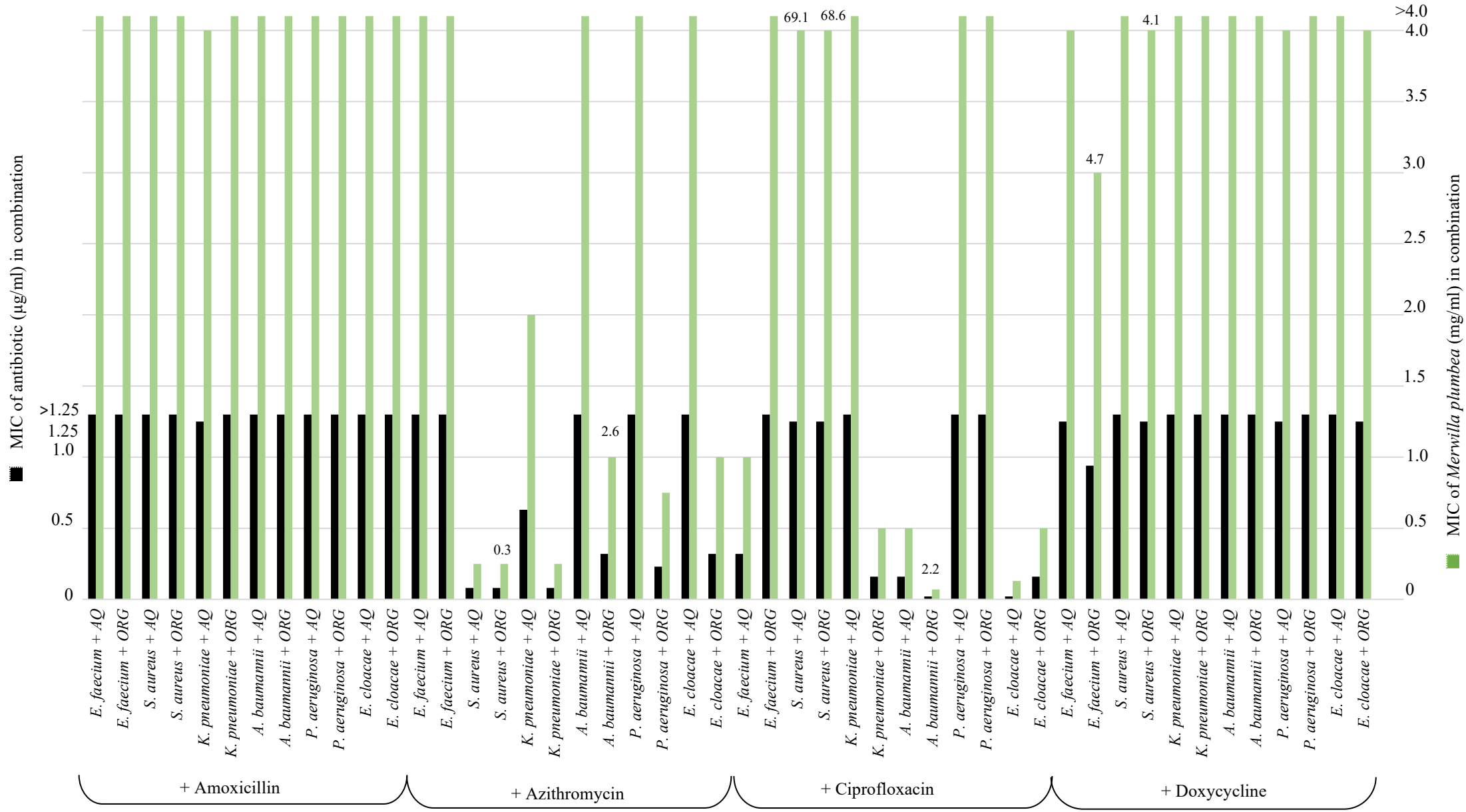

Fig.11. Interactive profiles of antibiotics with plant extracts of *Merwilla plumbea* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

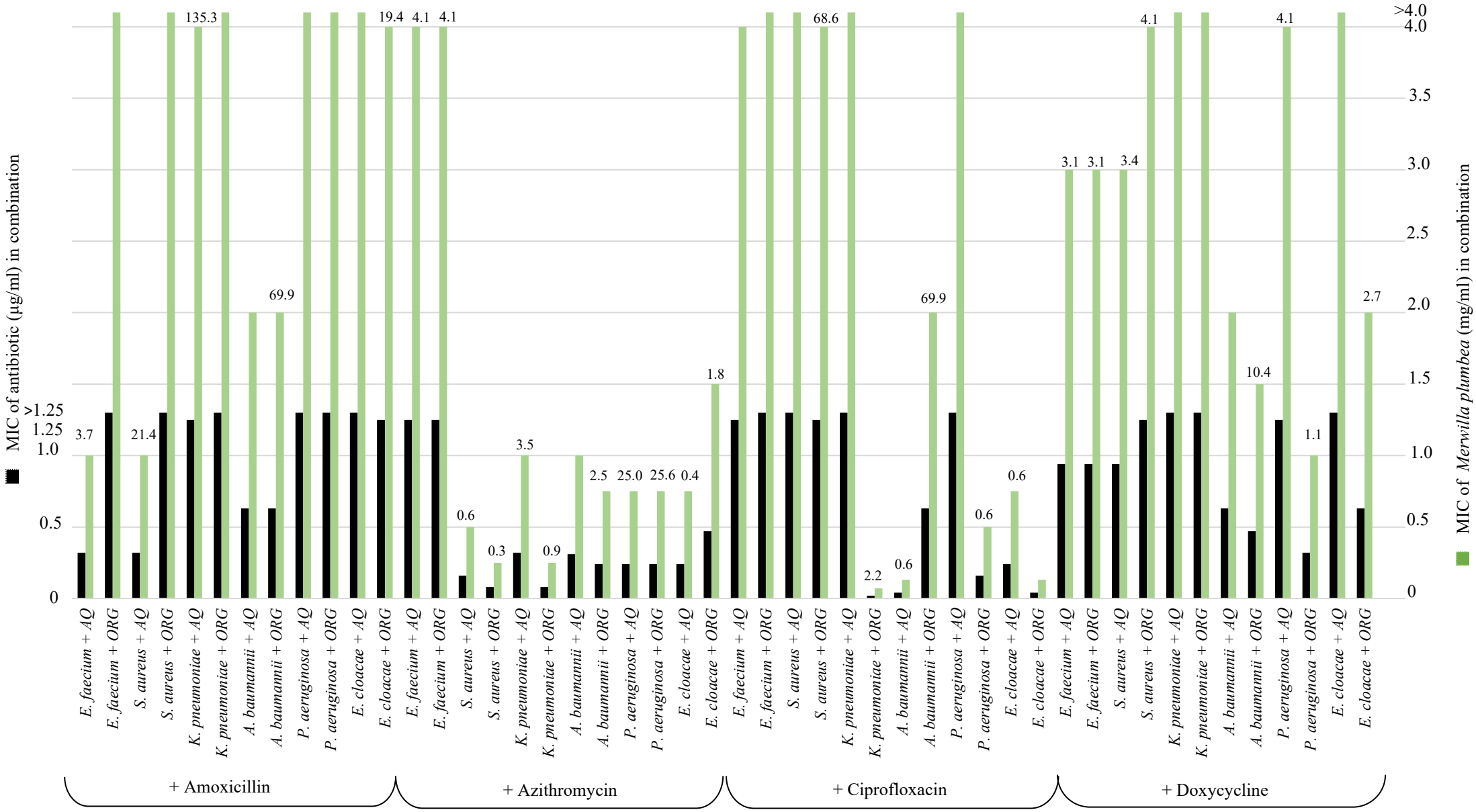

Fig.12. Interactive profiles of antibiotics with plant extracts of *Pterocarpus angolensis* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

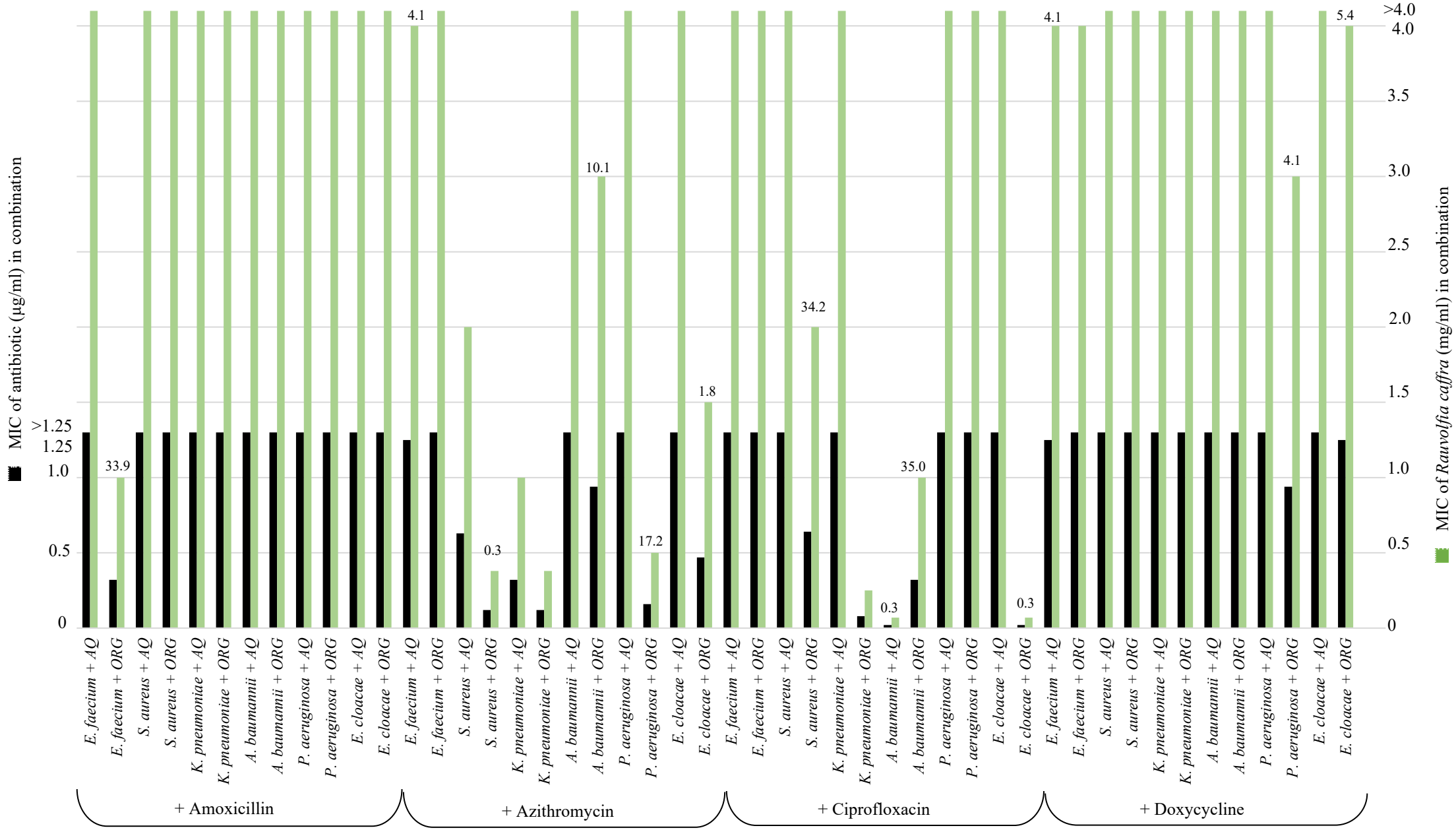

Fig.13. Interactive profiles of antibiotics with plant extracts of *Rauvolfia caffra* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

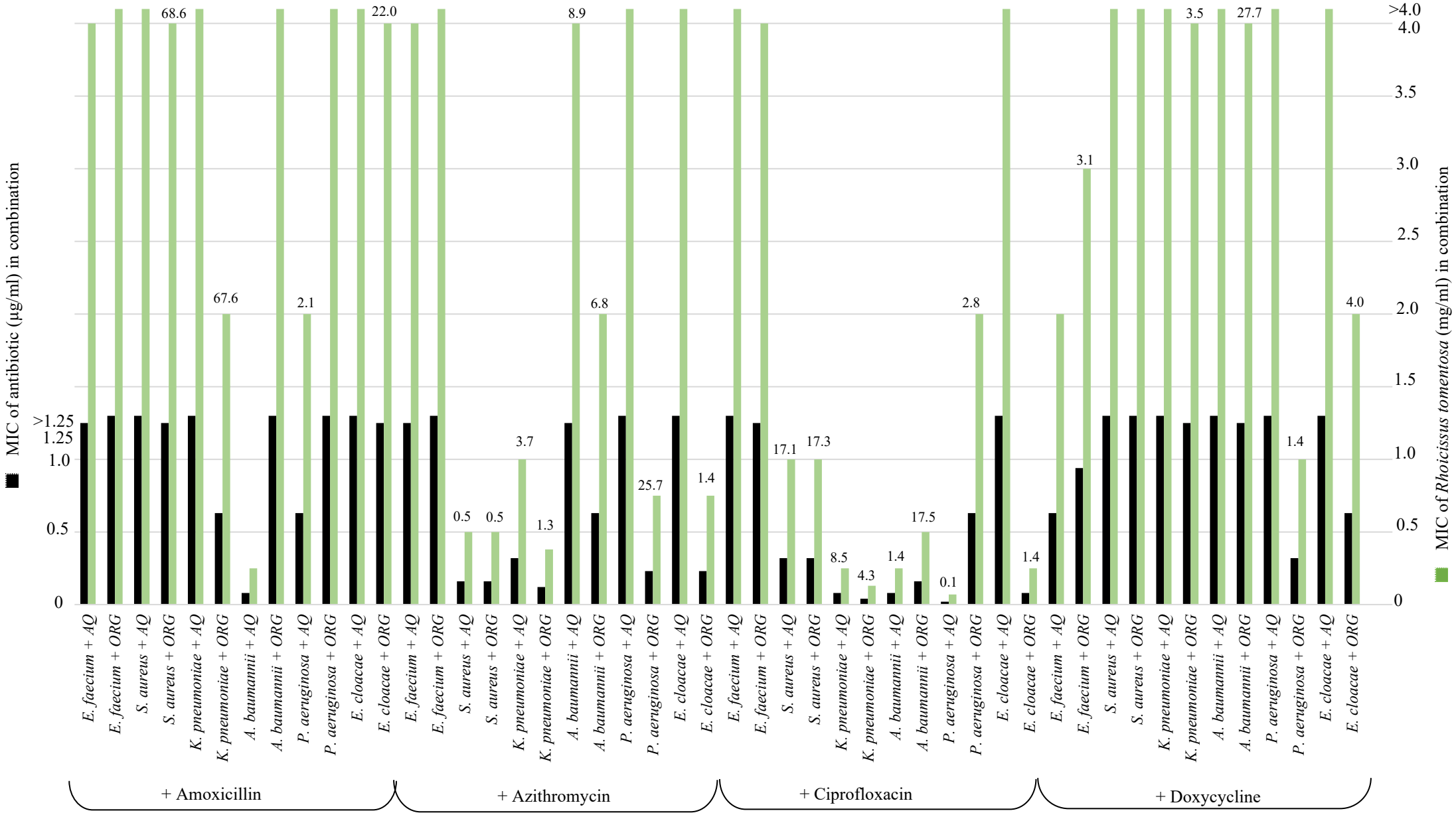

Fig.14. Interactive profiles of antibiotics with plant extracts of *Rhoicissus tomentosa* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

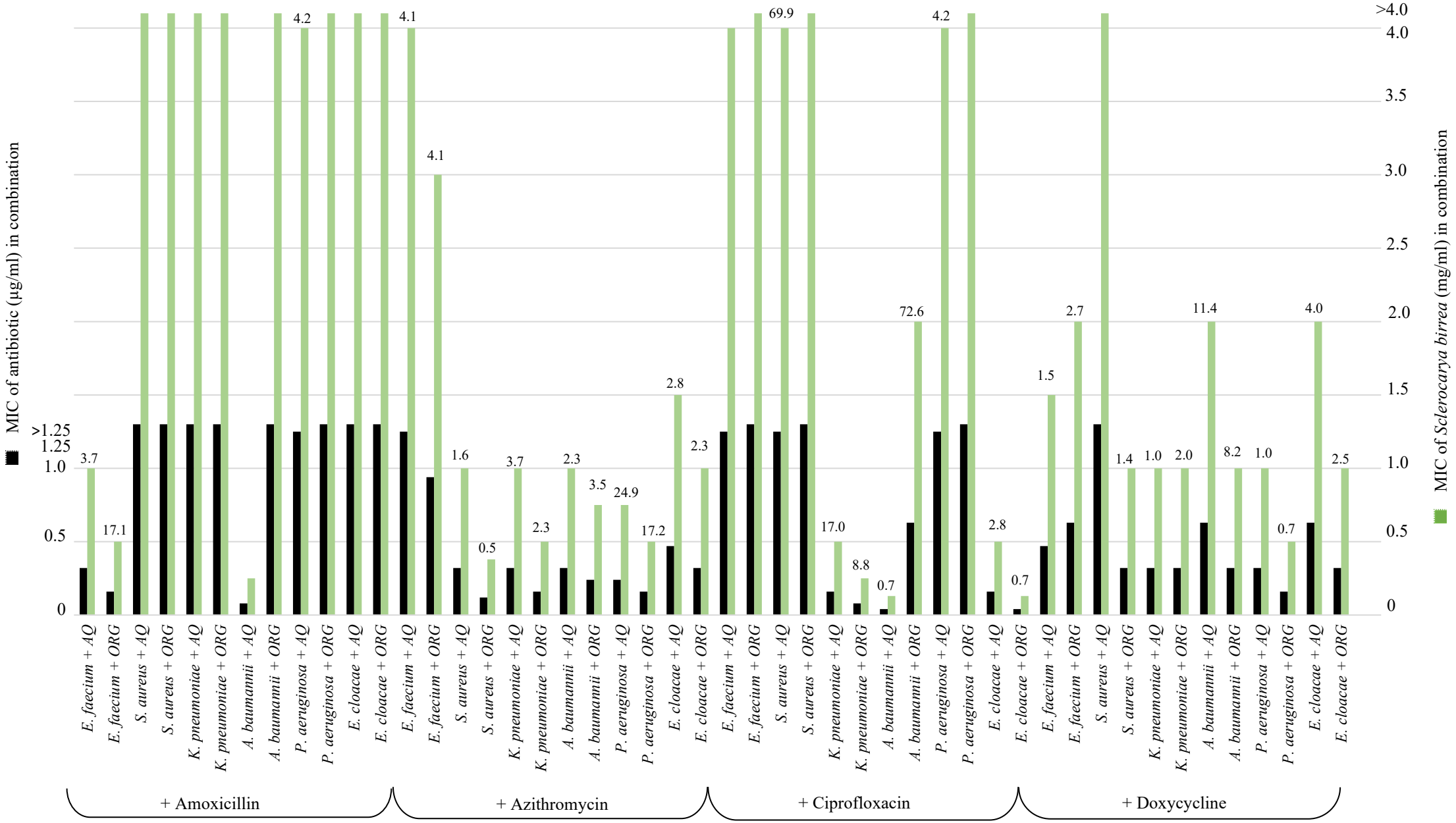

Fig.15. Interactive profiles of antibiotics with plant extracts of *Sclerocarya birrea* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

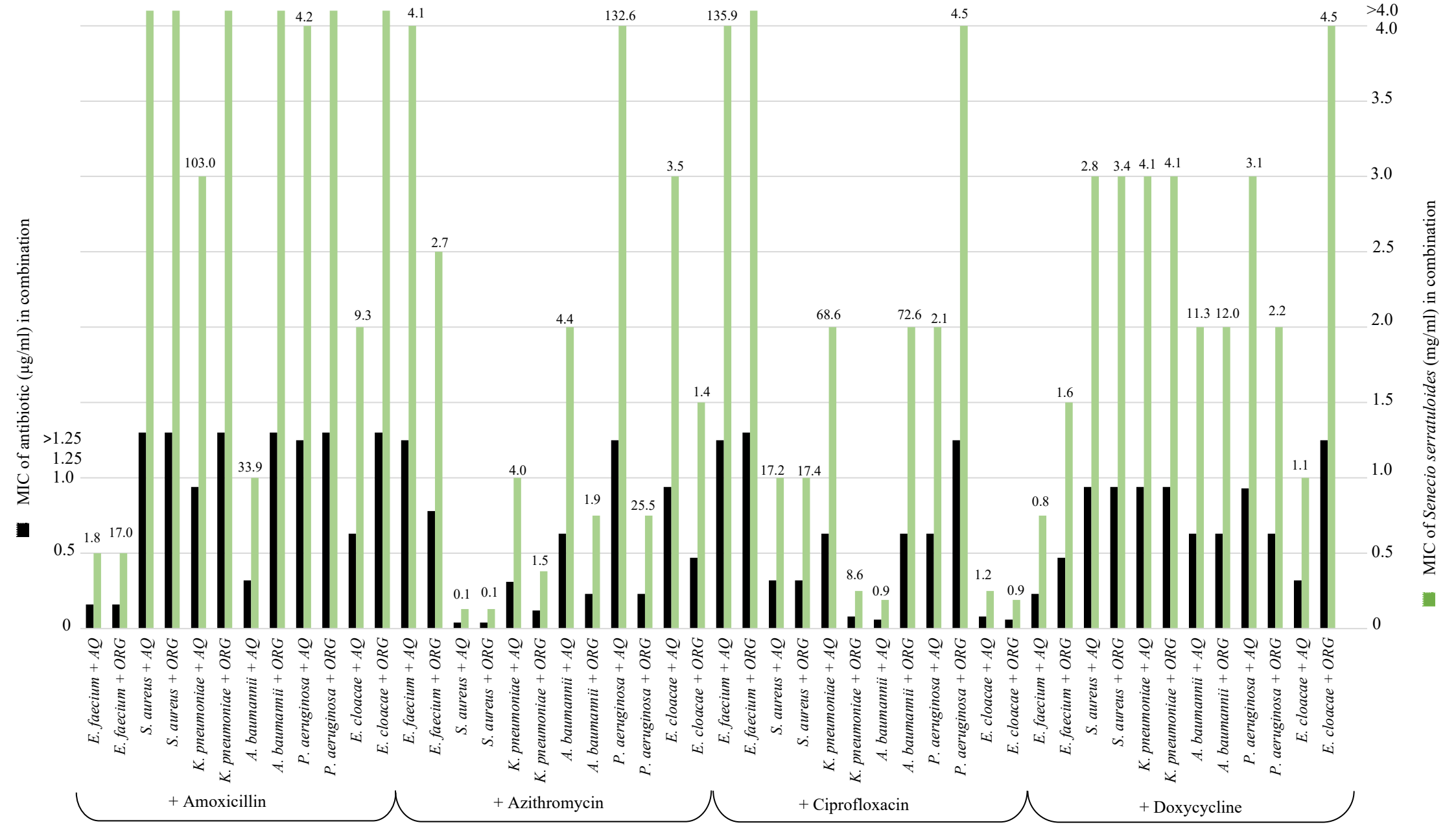

Fig.16. Interactive profiles of antibiotics with plant extracts of *Senecio serratuloides* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

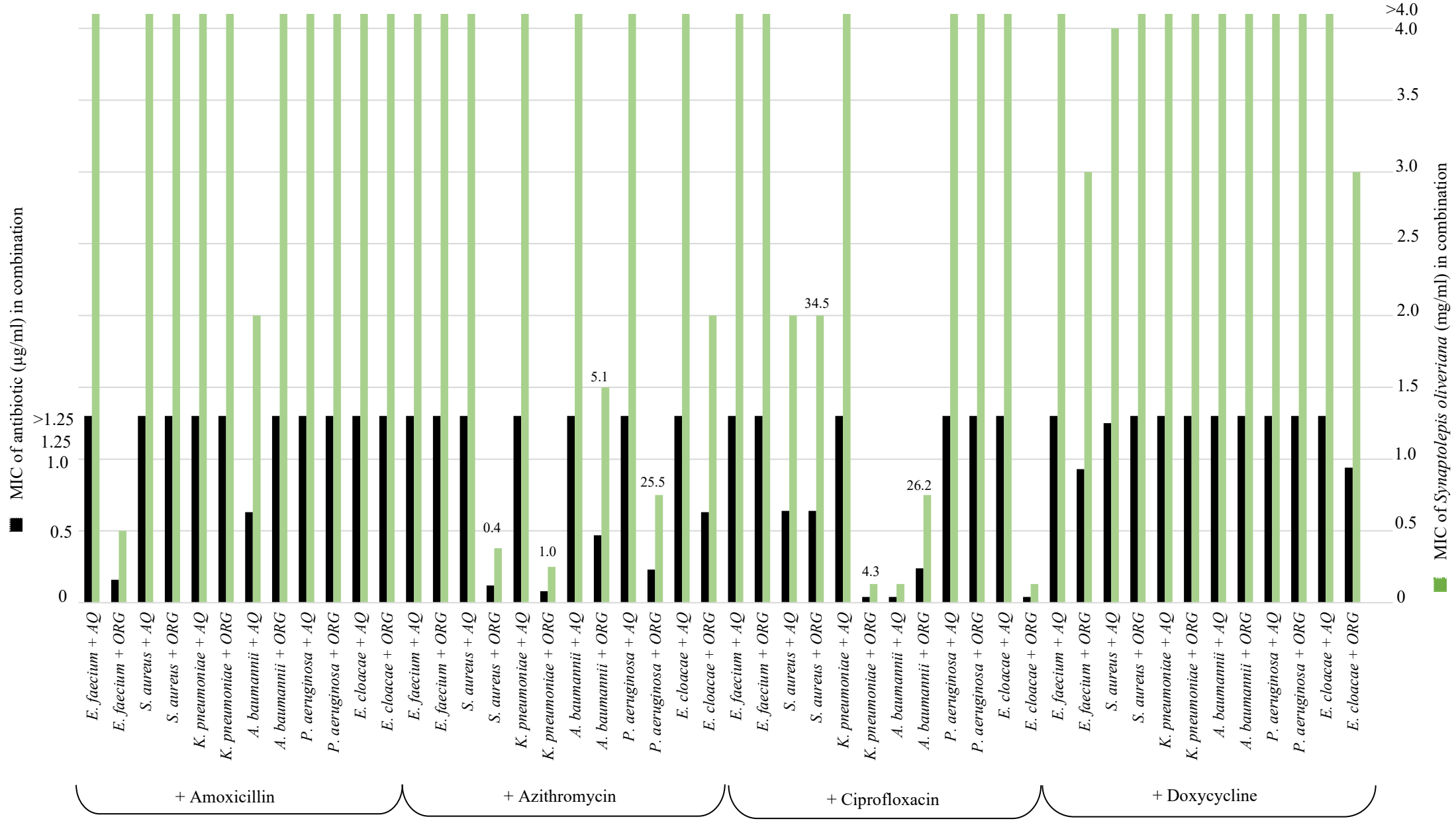

Fig.17. Interactive profiles of antibiotics with plant extracts of *Synaptolepis oliveriana* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

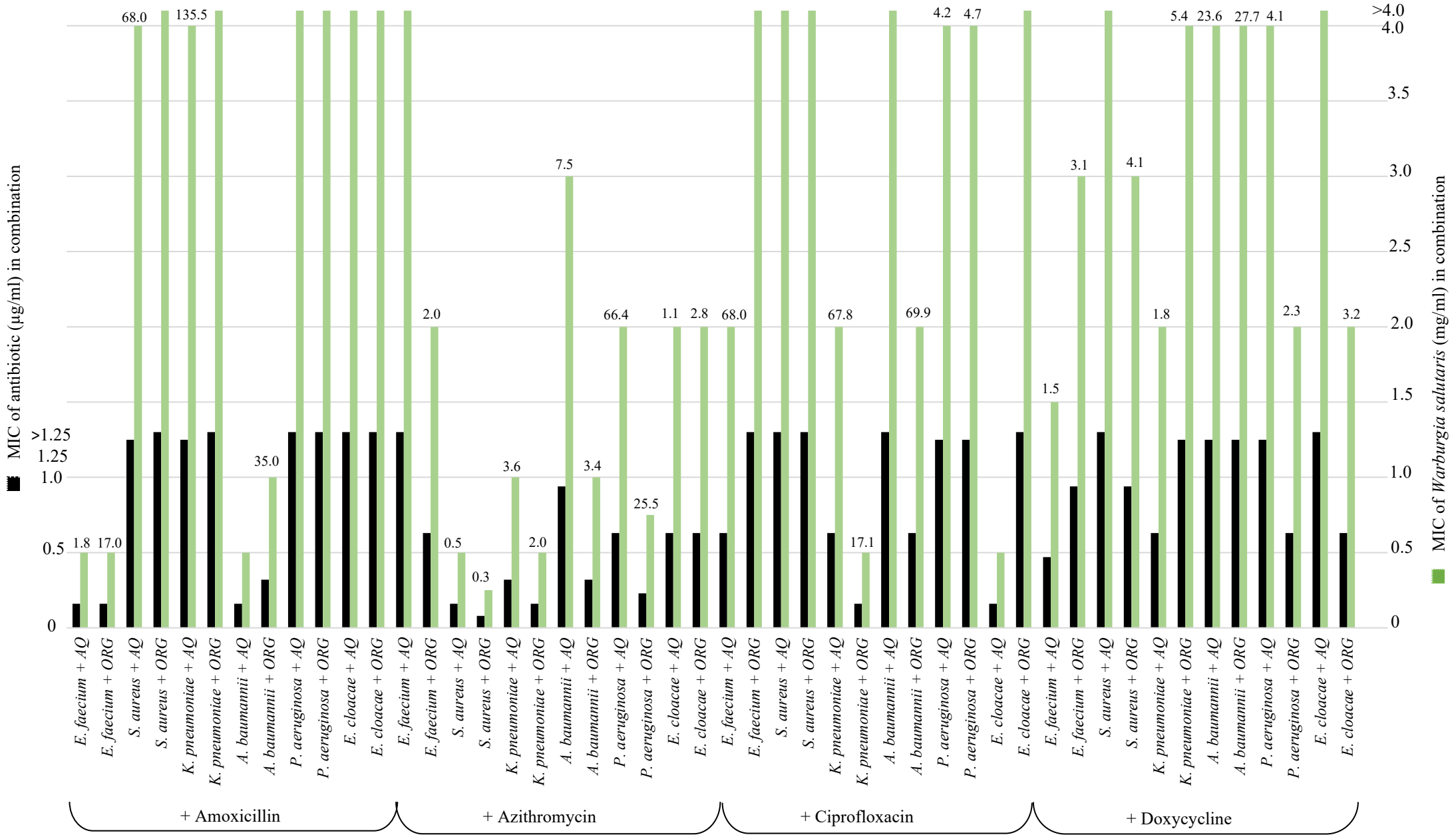

Fig.18. Interactive profiles of antibiotics with plant extracts of *Warburgia salutaris* when tested against the ESKAPE pathogens.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

Table 2 summarises the synergistic interactions identified in this study, categorised by conventional antimicrobials against ESKAPE pathogens. Both amoxicillin and doxycycline when combined with all the plants extracts (aqueous and organic), showed no synergistic interactions against any of the ESKAPE pathogens.

Table 2

Summary of the synergistic interactions classified according to antibiotic.

Azithromycin					
Plant		**Pathogen**	**MIC (A) mg/ml**	**MIC (B) mg/ml**	**ΣFIC**
Organic extracts	*A. amatymbica*	*E. faecium*	0.04	0.13	0.13
	A. calamus	*S. aureus*	0.16	0.50	0.50
	A. amatymbica		0.06	0.19	0.25
	B. bowkeri		0.16	0.50	0.46
	C. camphora		0.12	0.38	0.43
	C. capensis		0.06	0.19	0.21
	D. rotundifolia		0.12	0.38	0.43
	E. sideroxylon		0.06	0.19	0.19
	H. africana		0.08	0.25	0.50
	H. hemerocallidea		0.02	0.06	0.06
	M. plumbea		0.08	0.25	0.26
	P. angolensis		0.08	0.25	0.26
	R. caffra		0.12	0.38	0.32
	R. tomentosa		0.16	0.50	0.50
	S. birrea		0.12	0.38	0.50
	S. serratuloides		0.04	0.13	0.14
	S. oliveriana		0.12	0.38	0.38
	W. salutaris		0.08	0.25	0.34
Aqueous extracts	*R. tomentosa*		0.16	0.50	0.46
	S. serratuloides		0.04	0.13	0.13
	W. salutaris		0.16	0.50	0.46
	P. angolensis	*E. cloacae*	0.24	0.75	0.39
Ciprofloxacin					
Plant		**Pathogen**	**MIC (A) mg/ml**	**MIC (B) mg/ml**	**ΣFIC**
Organic extracts	*R. caffra*	*E. cloacae*	0.02	0.06	0.31
Aqueous extracts		*A. baumannii*	0.02	0.06	0.31
	C. capensis	*P. aeruginosa*	0.04	0.13	0.31
	R. tomentosa		0.02	0.06	0.07

*A = Antibiotic; B = Plant sample.

To note, azithromycin, in combination with the plant extracts (aqueous and organic) demonstrated the most synergistic interactions, particularly against *S. aureus* (ΣFIC values ranging from 0.06 to 0.50). The remaining synergistic interactions were identified when ciprofloxacin was combined with three of the plant extracts (*C. capensis*, *R. caffra* and *R. tomentosa*).

Table 3 shows the summary of the antagonism noted for antibiotics in combination with the various plant extracts (organic and aqueous), against the ESKAPE pathogens. All four antibiotics (amoxicillin, azithromycin, ciprofloxacin, and doxycycline) contributed to some extent to the total 47.49% antagonism noted amongst the 392 combinations with definitive ΣFIC values. Amongst the total of 187 antagonistic combinations recorded in this study, amoxicillin contributed the least toward antagonism, with 39 amoxicillin-plant combinations being antagonistic (20.86%), followed by doxycycline at 22.99% (n = 43) and azithromycin at 24.06% (n = 45). The ciprofloxacin: plant combinations contributed the most (32.09%; n = 60) to the overall antagonism represented in this study.

Table 3

Antagonistic interactions classified according to antibiotic.

Amoxicillin					
Plant		**Pathogen**	**MIC (A) mg/ml**	**MIC (B) mg/ml**	**ΣFIC**
Organic extracts	*A. amatymbica*	*E. faecium*	0.04	0.13	4.27
	B. bowkeri		0.32	1.00	33.90
	C. camphora		0.16	0.50	17.12
	D. rotundifolia		0.32	1.00	34.05
	H. africana		0.16	0.50	16.96
	R. caffra		0.32	1.00	33.94
	S. birrea		0.16	0.50	17.12
	S. serratuloides		0.16	0.50	16.99
	W. salutaris		0.16	0.50	16.96
	R. tomentosa	*S. aureus*	1.25	4.00	68.62
	B. bowkeri	*K. pneumoniae*	1.25	4.00	136.40
	C. capensis		0.16	0.50	16.96
	E. sideroxylon		1.25	4.00	138.12
	R. tomentosa		0.64	2.00	67.64
	H. hemerocallidea	*A. baumannii*	1.25	4.00	135.93
	P. angolensis		0.64	2.00	69.93
	W. salutaris		0.32	1.00	35.03
	C. camphora	*P. aeruginosa*	1.25	4.00	4.67
	C. capensis	*E. cloacae*	1.25	4.00	18.52
	P. angolensis		1.25	4.00	19.40
	R. tomentosa		1.25	4.00	22.02
Aqueous extracts	*C. camphora*	*E. faecium*	1.25	4.00	14.77
	C. capensis		1.25	4.00	14.77
	P. angolensis	*S. aureus*	0.32	1.00	21.40
	W. salutaris		1.25	4.00	67.96
	A. amatymbica	*K. pneumoniae*	1.25	4.00	136.37
	C. capensis		0.47	1.50	51.83
	E. sideroxylon		0.64	2.00	67.96
	H. hemerocallidea		1.25	4.00	136.19
	P. angolensis		1.25	4.00	135.27
	S. serratuloides		0.94	3.00	103.00
	W. salutaris		1.25	4.00	135.49
	S. serratuloides	*A. baumannii*	0.32	1.00	33.88
	S. birrea	*P. aeruginosa*	1.25	4.00	4.23

Amoxicillin					
Plant		**Pathogen**	**MIC (A) mg/ml**	**MIC (B) mg/ml**	**ΣFIC**
Aqueous extracts	*S. serratuloides*	*P. aeruginosa*	1.25	4.00	4.23
	C. capensis	*E. cloacae*	1.25	4.00	19.40
	E. sideroxylon		0.64	2.00	9.70
	H. hemerocallidea		0.64	2.00	9.70
	S. serratuloides		0.64	2.00	9.26
Azithromycin					
Plant		**Pathogen**	**MIC (A) mg/ml**	**MIC (B) mg/ml**	**ΣFIC**
Organic extracts	*C. camphora*	*E. faecium*	0.32	1.00	4.06
	P. angolensis		1.25	4.00	4.11
	S. birrea		0.94	3.00	4.06
	A. amatymbica	*A. baumannii*	0.32	1.00	4.69
	B. bowkeri		0.64	2.00	5.45
	H. africana		0.47	1.50	5.07
	R. caffra		0.94	3.00	10.14
	R. tomentosa		0.64	2.00	6.76
	S. oliveriana		0.47	1.50	5.07
	A. calamus	*P. aeruginosa*	0.16	0.50	16.98
	C. camphora		0.16	0.50	17.04
	C. capensis		0.24	0.75	25.72
	D. rotundifolia		0.16	0.50	17.04
	E. sideroxylon		0.24	0.75	26.21
	H. africana		0.16	0.50	17.04
	H. hemerocallidea		0.16	0.50	16.93
	P. angolensis		0.24	0.75	25.56
	R. caffra		0.16	0.50	17.15
	R. tomentosa		0.24	0.75	25.72
	S. birrea		0.16	0.50	17.15
	S. serratuloides		0.24	0.75	25.53
	S. oliveriana		0.24	0.75	25.48
	W. salutaris		0.24	0.75	25.48
Aqueous extracts	*C. capensis*	*E. faecium*	1.30	4.00	4.11
	P. angolensis		1.30	4.00	4.11
	R. caffra		1.30	4.00	4.11
	S. birrea		1.30	4.00	4.11
	S. serratuloides		1.30	4.00	4.11
	C. capensis	*K. pneumoniae*	0.32	1.00	4.24
	C. camphora	*A. baumannii*	0.64	2.00	4.76
	H. hemerocallidea		0.47	1.00	5.62
	R. tomentosa		1.25	4.00	8.86
	S. serratuloides		0.64	2.00	4.43
	W. salutaris		0.94	3.00	7.46
	C. camphora	*P. aeruginosa*	0.24	0.75	25.00
	C. capensis		0.16	0.50	16.83
	D. rotundifolia		0.64	2.00	66.08
	E. sideroxylon		0.32	1.00	32.97
	H. africana		0.47	1.50	49.25
	H. hemerocallidea		0.94	3.00	99.98
	P. angolensis		0.24	0.75	25.00
	S. birrea		0.24	0.75	24.87
	S. serratuloides		1.25	4.00	132.56
	W. salutaris		0.64	2.00	66.41
	E. sideroxylon	*E. cloacae*	1.25	4.00	4.73
Ciprofloxacin					
Plant		**Pathogen**	**MIC (A) mg/ml**	**MIC (B) mg/ml**	**ΣFIC**
Organic extracts	*H. hemerocallidea*	*E. faecium*	0.64	2.00	68.10
	A. calamus	*S. aureus*	0.64	2.00	34.51

Ciprofloxacin					
Plant		**Pathogen**	**MIC (A) mg/ml**	**MIC (B) mg/ml**	**ΣFIC**
Organic extracts	*A. amatymbica*	*S. aureus*	0.08	0.25	4.40
	H. africana		0.64	2.00	35.17
	M. plumbea		1.25	4.00	68.62
	P. angolensis		1.25	4.00	68.62
	R. caffra		0.64	2.00	34.18
	R. tomentosa		0.32	1.00	17.25
	S. serratuloides		0.32	1.00	17.36
	S. oliveriana		0.64	2.00	34.51
	A. amatymbica	*K. pneumoniae*	0.08	0.25	8.46
	B. bowkeri		1.25	4.00	136.45
	C. camphora		0.06	0.19	6.66
	D. rotundifolia		0.16	0.50	16.88
	E. sideroxylon		0.16	0.50	17.23
	R. tomentosa		0.04	0.13	4.25
	S. birrea		0.08	0.25	8.79
	S. oliveriana		0.04	0.13	4.31
	S. serratuloides		0.08	0.25	8.63
	W. salutaris		0.16	0.50	17.12
	C. camphora	*A. baumannii*	0.62	2.00	68.62
	C. capensis		0.16	0.50	17.23
	D. rotundifolia		0.62	2.00	69.33
	E. sideroxylon		0.16	0.50	17.45
	H. africana		0.62	2.00	69.93
	H. hemerocallidea		1.25	4.00	135.93
	P. angolensis		0.62	2.00	69.93
	R. caffra		0.32	1.00	35.03
	R. tomentosa		0.16	0.50	17.45
	S. birrea		0.62	2.00	72.56
	S. serratuloides		0.63	2.00	72.56
	S. oliveriana		0.32	0.75	26.24
	W. salutaris		0.62	2.00	69.93
	A. calamus	*P. aeruginosa*	1.25	4.00	4.23
	E. sideroxylon		0.62	2.00	4.08
	S. serratuloides		1.25	4.00	4.51
	W. salutaris		1.25	4.00	4.67
Aqueous extracts	*A. calamus*	*E. faecium*	0.32	1.00	34.05
	C. capensis		0.32	1.00	34.05
	H. africana		0.32	1.00	34.38
	S. serratuloides		1.25	4.00	135.93
	W. salutaris		0.63	2.00	67.96
	A. amatymbica	*S. aureus*	0.08	0.25	4.40
	B. bowkeri		0.63	2.00	34.05
	C. capensis		0.16	0.50	9.12
	M. plumbea		1.25	4.00	69.06
	R. tomentosa		0.32	1.00	17.09
	S. birrea		1.25	4.00	69.94
	S. serratuloides		0.32	1.00	17.17
	A. amatymbica	*K. pneumoniae*	1.25	4.00	136.47
	B. bowkeri		1.25	4.00	136.47
	C. capensis		0.16	0.50	17.23
	H. hemerocallidea		1.25	4.00	136.19
	R. tomentosa		0.08	0.25	8.54
	S. birrea		0.16	0.50	16.96
	S. serratuloides		0.62	2.00	68.62
	W. salutaris		0.62	2.00	67.75

Ciprofloxacin					
Plant		**Pathogen**	**MIC (A) mg/ml**	**MIC (B) mg/ml**	**ΣFIC**
Aqueous extracts	*S. birrea*	*P. aeruginosa*	1.25	4.00	4.23
	W. salutaris		1.25	4.00	4.23
	B. bowkeri	*E. cloacae*	1.25	4.00	4.30
Doxycycline					
Plant		**Pathogen**	**MIC (A) mg/ml**	**MIC (B) mg/ml**	**ΣFIC**
Organic extracts	*A. amatymbica*	*E. faecium*	1.25	4.00	4.11
	C. camphora		1.25	4.00	5.42
	D. rotundifolia		1.25	4.00	4.11
	H. africana		1.25	4.00	4.10
	M. plumbea		0.83	2.67	4.72
	A. amatymbica	*S. aureus*	1.25	4.00	5.42
	D. rotundifolia		1.25	4.00	4.54
	H. africana		0.94	3.00	4.06
	M. plumbea		1.25	4.00	4.11
	P. angolensis		1.25	4.00	4.11
	W. salutaris		0.94	3.00	4.06
	S. serratuloides	*K. pneumoniae*	0.94	3.00	4.07
	W. salutaris		1.25	4.00	5.43
	C. camphora	*A. baumannii*	1.25	4.00	25.06
	C. capensis		0.32	1.00	6.48
	D. rotundifolia		0.62	2.00	13.19
	E. sideroxylon		0.47	1.50	10.38
	H. africana		0.32	1.00	6.92
	H. hemerocallidea		0.62	2.00	11.87
	P. angolensis		0.47	1.50	10.38
	R. tomentosa		1.25	4.00	27.69
	S. birrea		0.32	1.00	8.23
	S. serratuloides		0.62	2.00	12.01
	W. salutaris		1.25	4.00	27.69
	D. rotundifolia	*P. aeruginosa*	1.25	4.00	4.55
	R. caffra		0.94	3.00	4.07
	R. caffra	*E. cloacae*	1.25	4.00	5.42
	S. serratuloides		1.25	4.00	4.54
Aqueous extracts	*D. rotundifolia*	*E. faecium*	1.25	4.00	4.11
	E. sideroxylon		0.94	3.00	4.06
	R. caffra		1.25	4.00	4.11
	H. africana	*S. aureus*	1.25	4.00	4.54
	S. serratuloides	*K. pneumoniae*	0.94	3.00	4.06
	C. camphora	*A. baumannii*	0.94	3.00	17.39
	C. capensis		0.62	2.00	11.59
	E. sideroxylon		0.47	1.50	8.86
	H. hemerocallidea		0.62	2.00	11.73
	S. birrea		0.62	2.00	11.38
	S. serratuloides		0.62	2.00	11.27
	W. salutaris		1.25	4.00	23.63
	P. angolensis	*P. aeruginosa*	1.25	4.00	4.11
	W. salutaris		1.25	4.00	4.11
	E. sideroxylon	*E. cloacae*	1.25	4.00	5.42

*A = Antibiotic; B = Plant sample.

3.2. Plant: antifungal combinations

A total of 136 plant (aqueous and organic extracts): antifungal (nystatin or fluconazole) combinations were tested against *C. albicans* and *C. glabrata*, of which 84 combinations demonstrated a definitive ΣFIC value (Fig.19 and Fig.20). No synergy was observed against either of the pathogens amongst any of the combinations tested. Other interactive profiles that were exhibited included additive interactions (4.76%, n = 4), non-interactive profiles (33.33%; n = 28) and antagonism (61.91%; n = 52), which was the most prevalent type of interaction exhibited for plant: antifungal combinations against *C. albicans* and *C. glabrata*. Table 4 summarizes the antagonistic interactions observed.

Table 4

Antagonistic interactions with ΣFIC values when plant extracts are combined with nystatin.

Nystatin					
Plant		**Pathogen**	**MIC (A) µg/ml**	**MIC (B) mg/ml**	**ΣFIC**
Organic extracts	*A. amatymbica*	*C. albicans*	2.3	0.8	6.8
	B. bowkeri		6.3	2.0	18.1
	C. camphora		3.1	1.0	9.1
	C. capensis		1.6	0.5	4.5
	D. rotundifolia		4.7	1.5	13.6
	E. sideroxylon		2.3	0.8	13.0
	H. africana		3.1	1.0	9.1
	H. hemerocallidea		3.1	1.0	17.3
	M. plumbea		3.1	1.0	17.3
	P. angolensis		3.1	1.0	9.1
	R. caffra		4.7	1.5	13.6
	R. tomentosa		1.6	0.5	4.5
	S. oliveriana		3.1	1.0	9.1
	S. serratuloides		6.3	2.0	34.6
	W. salutaris		3.1	1.0	9.1
Aqueous extracts	*S. birrea*		3.1	1.0	4.9
	S. serratuloides		6.3	2.0	5.7
	W. salutaris		6.3	2.0	9.8
Organic extracts	*D. rotundifolia*	*C. glabrata*	6.3	2.0	4.1
	R. caffra		12.5	4.0	5.4
	R. tomentosa		4.7	1.5	5.1
Aqueous extracts	*W. salutaris*		9.4	3.0	5.1
Fluconazole					
Plant		**Pathogen**	**MIC (A) µg/ml**	**MIC (B) mg/ml**	**ΣFIC**
Organic extracts	*A. calamus*	*C. albicans*	1.6	0.5	8.9
	A. amatymbica		1.6	0.5	4.8
	B. bowkeri		3.1	1.0	9.6
	C. camphora		1.6	0.5	4.8
	C. capensis		1.6	0.5	4.8
	D. rotundifolia		1.6	0.5	4.8
	E. sideroxylon		1.6	0.5	8.9
	H. africana		2.3	0.8	7.2

Fluconazole					
Plant		**Pathogen**	**MIC (A) µg/ml**	**MIC (B) mg/ml**	**ΣFIC**
Organic extracts	*P. angolensis*	*C. albicans*	1.6	0.5	4.8
	R. caffra		1.6	0.5	4.8
	R. tomentosa		1.6	0.5	4.8
	S. oliveriana		1.6	0.5	4.8
	S. serratuloides		1.6	0.5	8.9
Aqueous extracts	*W. salutaris*		3.1	1.0	5.4
Organic extracts	*A. amatymbica*	*C. glabrata*	6.3	2.0	8.0
	B. bowkeri		12.5	4.0	12.6
	C. camphora		6.3	2.0	6.4
	C. capensis		9.4	3.0	14.1
	D. rotundifolia		12.5	4.0	16.1
	E. sideroxylon		4.7	1.5	8.1
	H. africana		6.3	2.0	8.0
	H. hemerocallidea		6.3	2.0	8.0
	M. plumbea		12.5	4.0	14.7
	P. angolensis		6.3	2.0	8.0
	R. tomentosa		6.3	2.0	6.4
	S. serratuloides		4.7	1.5	6.0
	W. salutaris		6.3	2.0	8.0
Aqueous extracts	*E. sideroxylon*		4.7	1.5	6.0
	P. angolensis		4.7	1.5	8.1
	W. salutaris		6.3	2.0	7.4

*A = Antifungal; B = Plant sample.

The specific medicinal plant: antifungal combinations (Fig. 19 – Fig. 20) evaluated in this study are further discussed as follows;

Nystatin contributed less toward antagonism when combined with the plant extracts (42.3%, n = 22), than fluconazole (57.7%, n = 30). Furthermore, more antagonism was noted against *C. albicans* (32 combinations) than against *C. glabrata* (20 combinations). The most antagonistic interaction against the yeasts was noted with the organic extract of *S. serratuloides* in combination with nystatin, when tested against *C. albicans* (ΣFIC 34.6).

Given that fungal infections are often classified as opportunistic infections, occurring in immunocompromised patients, antagonism could pose a serious health concern, warranting further investigation. This is even more relevant in South Africa, where the population is plagued with human immunodeficiency virus (HIV)/ acquired immune-deficiency syndrome (AIDS), deeming those patients more susceptible to opportunistic fungal infections (Nlooto and Naidoo, 2016; Wanke, 2000). Furthermore, many studies have identified interactions between medicinal plants and antiretroviral treatments, where the conventional drugs are less effective, which further poses threats to a patients' HIV/AIDS treatment and exposure to opportunistic Candidal infections (Van den Bout-Van den Beukel et al., 2006).

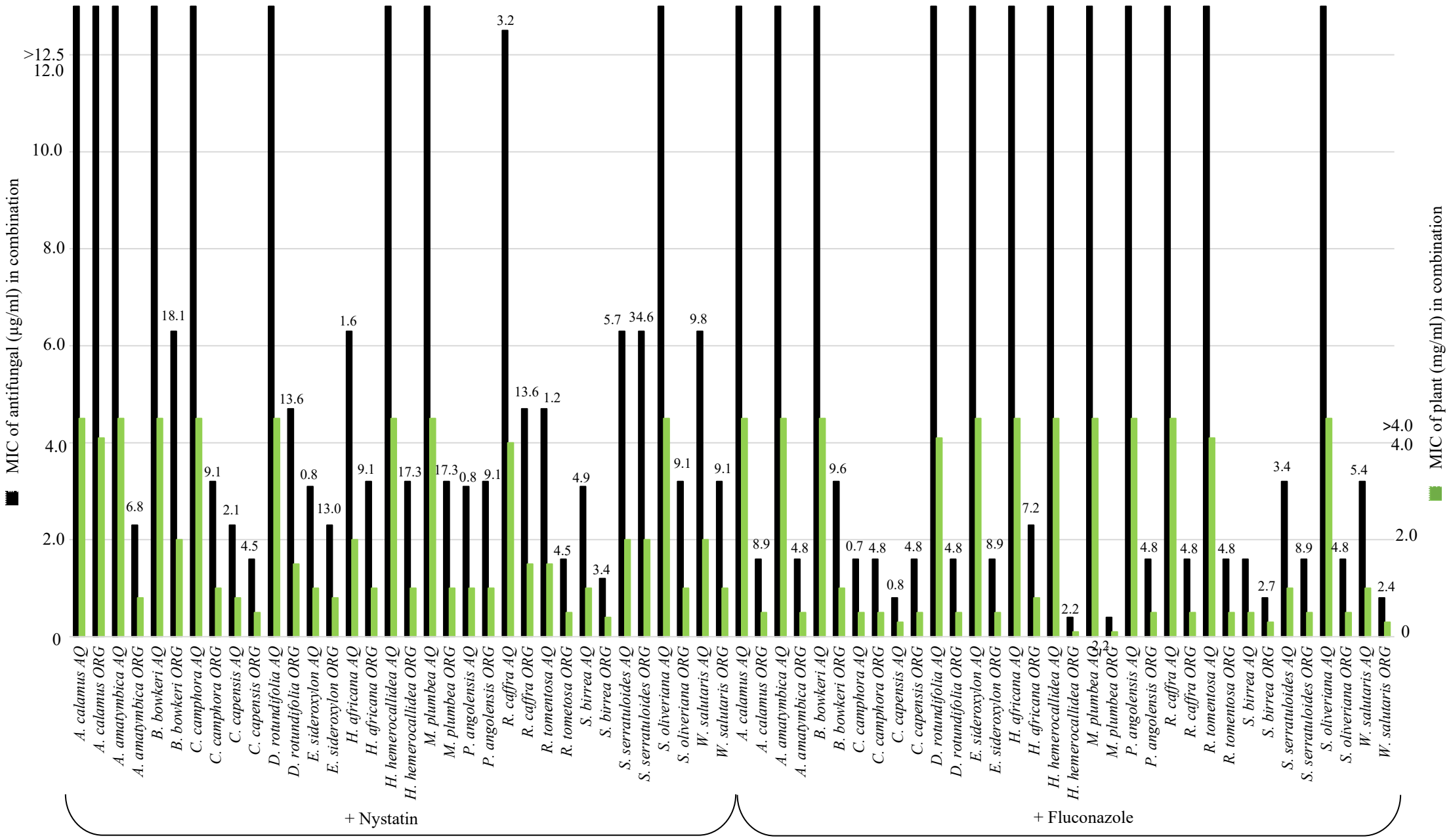

Fig.19. Interactive profiles of antifungals with plant extracts when tested against the *Candida albicans.*

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of nystatin (0.1 mg/ml) 5.210 µg/ml; culture control >8.0 mg/ml.

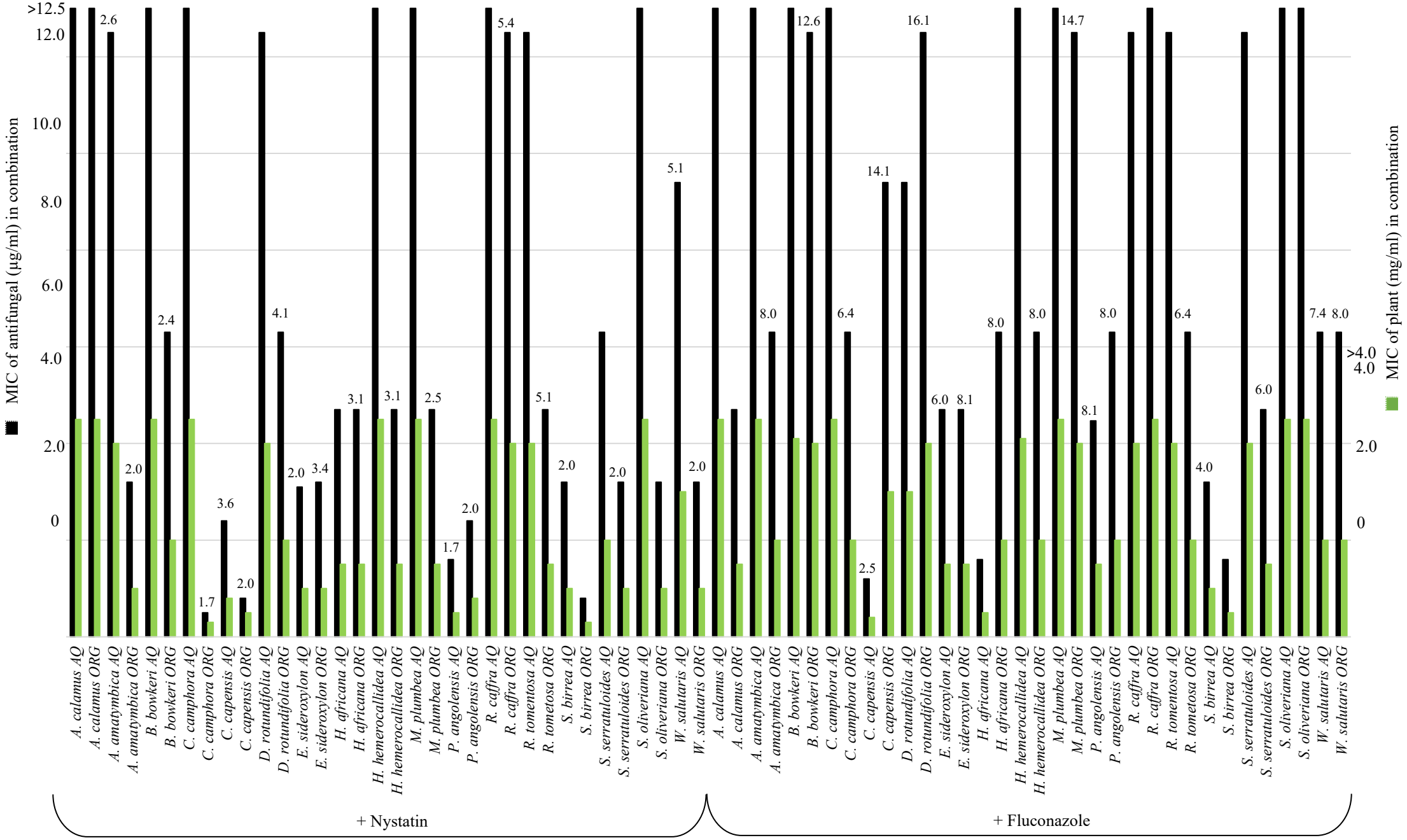

Fig.20. Interactive profiles of antifungals with plant extracts when tested against the *Candida glabrata*.

*Values indicated above columns = ΣFIC; where no value indicated above column = ΣFIC could not be determined; for brevity, all ΣFIC values have been rounded to one decimal place; AQ = Aqueous extract; ORG = Organic extract; Negative control of acetone in water >8.0 mg/ml; positive control of nystatin (0.1 mg/ml) 6.250 µg/ml; culture control >8.0 mg/ml.

Hubsch et al. (2014) studied combinations of other South African medicinal plants with antifungals, nystatin and amphotericin B, where mostly non-interactive profiles were noted against *C. albicans* and *Cryptococcus neoformans*, however 13 antagonistic interactions were identified, with one synergistic interaction exhibited by the *Lippia javanica* in combination with nystatin tested against *C. albicans* (ΣFIC 0.5). Kumar et al. (2015), investigated the isolated compounds from *A. calamus* and found that the asarone compounds were synergistic with antifungals, amphotericin B, clotrimazole and fluconazole, against *C. albicans* and *C. tropicalis*. It was found that there was substantial synergy with this compound when combined with antifungals, where MIC values for the combinations were reduced by eight times, with further inhibition of biofilms, which is advantageous, particularly in overcoming antimicrobial resistance (Kumar et al., 2015). The antagonism for *A. calamus* with fluconazole found in the current study, compared to the synergy observed by Kumar et al. (2015), further demonstrates the effect of variability of chemical constituencies of medicinal plants that could impact on the interactive profiles with conventional medicines. Kumar et al. (2015) has reported that drug resistance to existing conventional antifungal agents is rapidly increasing, posing many challenges to the effective treatment of *Candidal* infections. With the lack of synergistic plant: antifungal interactions identified in this current study, dual medicinal use with plants investigated in this study against *Candidal* pathogens should not be proposed as a novel strategy to reduce antimicrobial resistance toward antifungals.

3.2. Toxicity studies (BSLA)

Plant samples that demonstrated synergism with either azithromycin or ciprofloxacin were tested for toxicity in the BSLA, to determine if synergism was because of general toxicity or alternatively increased antimicrobial potency of the combination. Toxicity was first determined for the independent samples (Supplementary Table S4) and then in combination (Table 5). Bussman et al. (2011) classified toxicity for BSLA mortality rates of greater than 50%. Ciprofloxacin was the only individual antibiotic demonstrating toxicity (50.83% mortality ± 29.15 after 48 h) (Supplementary Table S4). When tested individually, no aqueous extracts were found to be toxic, however, three organic extracts, exhibited toxic effects in the BSLA, namely: *M. plumbea* (79.50% mortality ± 15.25 after 48 h); *W. salutaris* (93.80% mortality ± 7.08 after 48 h), and *S. oliveriana* (51.41% mortality ± 18.05 after 48 h) showing the least toxicity of the three (Supplementary Table S4).

When evaluating the toxicity of the combinations, only two combinations were found to exhibit mortality rates above 50%, namely *S. oliveriana*: azithromycin (organic extract - 54.51% mortality ±6.17 after 48 h) and more so *W. salutaris*: azithromycin (organic extract - 100.00% mortality ±0.00 after 48 h), but the aqueous extract combination with azithromycin showing no toxicity (0% mortality after 24 and 48 h).

Furthermore, when reflecting on individual versus combination toxicity, it was interesting to note that the substantial toxicity of the individual organic extract of *M. plumbea* was found to be non-toxic when combined with azithromycin (9.09% mortality ± 15.75 after 48 h). The aqueous extract of *M. plumbea* was non-toxic when tested individually (Supplementary Table S4) and in combination with azithromycin (Table 5). Therefore, the synergistic interaction noted for the *M. plumbea*; azithromycin combinations (organic extract ΣFIC 0.26; aqueous extract ΣFIC 0.32 against *S. aureus*) can be confirmed as a true reflection of antimicrobial synergism, rather than being attributed to toxicity. Contrastingly, the organic extract of *S. oliveriana*, although showing negligible toxicity when tested individually (51.41% mortality ± 18.05 after 48 h), was found to increase slightly (54.51% mortality ± 6.17 after 48 h) when combined with azithromycin. Limited information exists specifically on the toxicity of *S. oliveriana*, which is one of the most frequently purchased medicinal plants from Faraday *muthi* market, for multiple uses (SANBI, 2021).

Another noteworthy finding for *W. salutaris*, the plant found to be most toxic when tested individually (organic extract - 70.47% mortality ± 34.23 after 24 h; 93.80% mortality ± 7.08 after 48 h), was that it was found to increase in toxicity when in combination with azithromycin (100.0% mortality ± 0.0 after 48 h), although the onset of toxicity within 24 h for *W. salutaris* in combination was far less after 24 h (40.98% mortality ± 22.52), as opposed to when tested individually after 24 h (70.47% mortality ± 34.23). The toxicity analysis still confirms that the synergistic antimicrobial interaction (ΣFIC 0.34) observed may be resultant of toxicity rather than true antimicrobial synergism. Furthermore, *W. salutaris* was the only plant found to exhibit toxicity within 24 h of exposure when tested individually (70.47% mortality ± 34.23) (Supplementary Table S4). The toxicity of the organic extract of *W. salutaris* was also identified by Soyingbe et al. (2018), however, not in the BLSA, but rather against human breast adenocarcinoma cells.

The lack of toxicity for most other synergistic plant-antibiotic combinations evaluated in this study demonstrates the true increased antimicrobial activity against the tested pathogens rather than synergism reflective of toxicity. However, it is still advised for further toxicological analysis of these plants individually and in combination with antimicrobials, particularly

cytotoxicity against human cell lines. Okaiyeto and Oguntibeju (2021) reviewed various adverse effects reported on African herbal medicines, where it was stated that these plants, when prepared for commercial trade at informal *muthi* markets, may contain contaminants, impurities, and be subject to harmful adulterations.

Table 5

Average mortality (%) rates yielded in the brine-shrimp lethality assay of the synergistic combinations between antimicrobials and plant extracts.

Sample		**Average mortality (%) ± S.D. after 24 h**	**Average mortality (%) ± S.D. after 48 h**
Azithromycin + Organic extracts	*Acorus calamus*	0.00 ± 0.00	5.25 ± 4.61
	Alepidia amatymbica	0.00 ± 0.00	0.00 ± 0.00
	Bauhinia bowkeri	0.00 ± 0.00	0.00 ± 0.00
	Cinnamomun camphora	0.00 ± 0.00	0.00 ± 0.00
	Cunonia capensis	0.00 ± 0.00	6.67 ± 11.55
	Dombeya rotundifolia	0.00 ± 0.00	17.65 ± 30.57
	Eucalyptus sideroxylon	0.00 ± 0.00	0.85 ± 1.48
	Hydnora africana	0.00 ± 0.00	6.67 ± 11.55
	Hypoxis hemerocallidea	1.52 ± 2.62	6.06 ± 10.50
	Merwilla plumbea	0.00 ± 0.00	9.09 ± 15.75
	Pterocarpus angolensis	0.00 ± 0.00	5.11 ± 4.65
	Rauvolfia caffra	0.00 ± 0.00	1.96 ± 3.40
	Rhoicissus tomentosa	0.00 ± 0.00	0.00 ± 0.00
	Sclerocarya birrea	0.00 ± 0.00	0.00 ± 0.00
	Synaptolepis oliveriana	6.27 ± 5.69	**54.51 ± 6.17**
	Senecio serratuloides	0.00 ± 0.00	0.00 ± 0.00
	Warburgia salutaris	40.98 ± 22.52	**100.00 ± 0.00**
Azithromycin + Aqueous extracts	*Bauhinia bowkeri*	0.00 ± 0.00	5.56 ± 9.62
	Merwilla plumbea	8.63 ± 10.28	32.66 ± 12.32
	Pterocarpus angolensis	0.00 ± 0.00	0.00 ± 0.00
	Rhoicissus tomentosa	0.00 ± 0.00	0.00 ± 0.00
	Senecio serratuloides	0.00 ± 0.00	1.85 ± 3.21
	Warburgia salutaris	0.00 ± 0.00	0.00 ± 0.00
Ciprofloxacin + Organic extracts	*Cunonia capensis*	0.00 ± 0.00	0.00 ± 0.00
Ciprofloxacin + Aqueous extracts	*Rhoicissus tomentosa*	0.00 ± 0.00	0.00 ± 0.00
	Rauvolfia caffra	0.00 ± 0.00	0.00 ± 0.00
	Cunonia capensis	0.00 ± 0.00	0.00 ± 0.00

*S.D. = standard deviation; Individual samples prepared at concentration of 4 mg/ml resulting in concentration of each component when combined to be 2 mg/ml resulting in testing concentration within wells of the micro-titre plate for the combination of 1 mg/ml; Bold = toxicity observed. Negative control consisted of 32 g/l salt water and the positive control consisted of 1.6 mg/ml potassium dichromate.

It was also reported that several plants themselves, contain toxic bio-active compounds which could lead to cellular toxicity and cell mutation. Pertinent to the current study, Okaiyeto and Oguntibeju (2021) also highlights the concern for patients with co-morbidities and those making use of medicinal plants with conventional medicines, since there have been several studies that have demonstrated these herbal remedies either to be toxic, contaminated or to alter the functioning of the liver required for metabolism of these treatments (Iroezindu et al., 2013; Van Vuuren et al., 2014; Omage et al., 2017).

4. Conclusion

Of the antibiotic: plant extract combinations tested against the ESKAPE pathogens, 6.63% were synergistic and 47.45% were antagonistic. Synergy was only prevalent when azithromycin and ciprofloxacin was combined with the plant extracts. Interestingly the organic extracts of all 17 plants tested were synergistic in combination with azithromycin when tested against *S. aureus*, providing potential alternative sources of new antimicrobial formulations aimed at *S. aureus* infections, particularly for the treatment of respiratory tract and skin infections. Furthermore, when these 17 combinations were tested for toxicity, the only two plants exhibiting any toxicity with azithromycin was *S. oliveriana* and *W. salutaris.* The synergistic combinations with ciprofloxacin, however, demonstrated no toxicity in the BSLA. The lack of toxicity observed for most of the synergistic combinations evaluated with azithromycin and ciprofloxacin, demonstrates that synergism for these combinations is a true reflection of increased antimicrobial potency, rather than toxicity.

No synergy was observed for the antifungal-plant extract combinations tested against *C. albicans* and *C. glabrata*, but a concerning 61.91% antagonism was documented.

The notably high number of overall antagonistic interactions observed in this study warrants concern when considering the impact on antimicrobial treatments of patients indicating concurrent use of traditional and conventional medicines. To achieve the safe integration of traditional medicine it is necessary for validated antagonistic combinations to be cautioned against by healthcare professionals, whereas validated synergistic combinations could provide a worthy source of new antimicrobials for future formulation research and development. Future studies should also focus on determining if chemical variability with frequently purchased medicinal plants from the Faraday *muthi* markets, could alter the antagonistic profile when combined with antimicrobials.

Declaration of interest

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. Full data sets are available upon request.

CRedIT author statement

ZB: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization, Project administration, Funding acquisition. **GK:** Plant identification, Writing – review and editing, Visualization. **SvV:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition.

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Table S1
Conventional antimicrobials to be tested in combination with medicinal plant samples (SAMF, 2022; EMGuidance 2024).

Antimicrobial	Class of antimicrobial	Clinical uses	Spectrum of activity
Amoxicillin	Beta-lactam antibiotic	Respiratory tract infections (upper and lower): ■ Tonsillitis, sinusitis, pharyngitis, bronchitis, bronchopneumonia. Gastrointestinal infections: ■ Gastroenteritis, typhoid fever, salmonella infection, peptic ulcers. Urinary tract infections: ■ Cystitis, urethritis, pyelonephritis, bacteruria. Others: ■ Skin infections, acute otitis media, sepsis, meningitis, sexually transmitted infections, dental abscesses.	Gram-positive and certain Gram-negative micro-organisms (enterococci, haemophilus, and listeria).
Azithromycin	Macrolide antibiotic	Respiratory tract infections ■ Bronchitis, sinusitis, pharyngitis, tonsilitis, community-acquired pneumonia. Others: • Skin infections, soft tissue infections, otitis media, uncomplicated sexually transmitted infections.	Gram-positive and Gram-negative aerobic cocci (excluding *enterococci* and Methicillin-resistant *Staphylococcus* spp.); some Gram-negative bacilli
Ciprofloxacin	Fluoroquinolone	Respiratory tract infections: ■ Tonsillitis, sinusitis, pharyngitis, bronchitis, bronchopneumonia. Urinary tract infections: ■ Cystitis, urethritis, pyelonephritis, bacteruria. Gastrointestinal infections: ■ Infective diarrhoea. Others: ■ Skin and soft tissue infections, bone infections, sexually transmitted infections.	Gram-positive cocci and many Gram-negative bacterial species.
Doxycycline	Tetracycline antibiotic	Respiratory tract infections (upper and lower): ■ Sinusitis, pharyngitis, pneumonia, and chronic bronchitis. Urinary tract infections: ■ Non-specific urethritis Others: ■ Skin infections and soft tissue infections, conjunctivitis, malaria prophylaxis, sexually transmitted infections.	Gram-positive and gram-negative aerobic and anaerobic pathogens.
Nystatin	Polyene antifungal	Candidal infections of the oral cavity, skin, and vaginal cavity.	Limited to yeasts, specifically Candida sp.
Fluconazole	Azole antifungal	Cryptococcal meningitis, systemic candidiasis, oropharyngeal, oesophageal candidiasis, vaginal candidiasis, skin yeast infections.	Limited to yeasts and some clinical activity against endemic fungi.

Table S2

Conventional antimicrobial MIC values (µg/ml) when tested individually against ESKAPE pathogens and yeasts, respectively.

	MIC values of individual antimicrobials against respective pathogens						
Antibiotic (0.01 mg/ml)	**Bacteria**						
	***E. faecium* (ATCC 27270)**	***S. aureus* (ATCC 29212)**	***K. pneumoniae* (ATCC 13883)**	***A. baumannii* (ATCC 19606)**	***P. aeruginosa* (ATCC 27853)**	***E. cloacae* (ATCC 13460)**	**Breakpoints (EUCAST-CLSI)**
Amoxicillin (purity ≥ 95%)	0.039	0.078	0.039	0.039	1.800	0.313	0.001 – 8
Azithromycin (purity ≥ 98%)	1.880	1.880	0.390	0.640	0.040	2.500	0.01 – 4
Ciprofloxacin (purity ≥ 98%)	0.039	0.078	0.039	0.039	1.800	0.313	0.001 – 4
Doxycycline (purity ≥ 93.5%)	1.880	1.880	1.880	0.240	1.880	1.880	0.1 - 4
Antifungal (0.1 mg/ml)	**Yeasts**						
	***C. albicans* (ATCC 10231)**	***C. glabrata* (ATCC 90030)**					
Nystatin (purity ≥ 98%)	5.210	6.250					0.125 – 8
Fluconazole (purity ≥ 98%)	3.130	1.560					0.001 – ≤ 16

*All values All conventional antimicrobials, when tested individually, against respective ESKAPE pathogens and yeasts, were within EUCAST-CLSI 2021 breakpoints. Shaded area= no relevant data necessary.

Table S3

Plant extract MIC values (mg/ml) when tested individually against ESKAPE pathogens.

Individual plant sample (32 mg/ml)	MIC values (mg/ml) of individual plant extracts against respective ESKAPE pathogens											
	***E. faecium* (ATCC 27270)**		***S. aureus* (ATCC 29212)**		***K. pneumoniae* (ATCC 13883)**		***A. baumannii* (ATCC 19606)**		***P. aeruginosa* (ATCC 27853)**		***E. cloacae* (ATCC 13460)**	
	Aqueous extract	Organic extract	Aqueous extract	Organic extract	Aqueous extract	Organic extract	Aqueous extract	Organic extract	Aqueous extract	Organic extract	Aqueous extract	Organic extract
Acorus calamus	>8.0	6.0	>8.0	4.0	>8.0	4.0	>8.0	1.0	>8.0	4.0	>8.0	3.0
Alepidia amatymbica	>8.0	4.0	2.0	2.0	3.0	>8.0	2.7	0.5	8.0	>8.0	>8.0	>8.0
Bauhinia bowkeri	>8.0	8.0	8.0	>8.0	8.0	3.0	>8.0	2.0	>8.0	>8.0	>8.0	>8.0
Cinnamomum camphora	4.0	2.0	>8.0	3.0	4.0	1.0	4.0	2.0	4.0	3.0	>8.0	1.5
Cunonia capensis	4.0	4.0	1.0	3.0	1.5	4.0	4.0	1.5	2.0	2.0	2.0	3.0
Dombeya rotundifolia	4.0	4.0	4.0	3.0	>8.0	8.0	>8.0	1.3	8.0	3.0	>8.0	1.5
Eucalyptus sideroxylon	2.0	4.0	4.0	4.0	4.0	1.5	3.0	1.0	6.0	1.0	2.0	1.0
Hydnora africana	>8.0	4.0	3.0	2.0	>8.0	>8.0	>8.0	1.0	>8.0	3.0	>8.0	1.5
Hypoxis hemerocallidea	>8.0	3.3	>8.0	4.0	3.3	>8.0	3.3	4.0	2.7	6.0	2.0	>8.0
Merwilla plumbea	>8.0	1.5	3.0	4.0	>8.0	>8.0	>8.0	2.7	>8.0	>8.0	>8.0	>8.0
Pterocarpus angolensis	4.0	4.0	3.0	4.0	8.0	8.0	>8.0	1.0	4.0	3.0	>8.0	2.0
Rauvolfia caffra	4.0	6.0	>8.0	8.0	>8.0	>8.0	8.0	1.0	8.0	2.0	>8.0	2.0
Rhoicissus tomentosa	>8.0	4.0	8.0	4.0	4.0	8.0	8.0	1.0	4.0	2.0	8.0	1.0
Sclerocarya birrea	4.0	2.0	2.0	2.0	4.0	1.0	6.0	0.5	4.0	2.0	1.0	0.8
Senecio serratuloides	4.0	3.3	5.3	3.0	2.0	2.0	8.0	3.3	4.0	3.3	3.0	3.0
Synaptolepis oliveriana	>8.0	>8.0	>8.0	4.0	8.0	2.0	8.0	1.0	>8.0	4.0	>8.0	>8.0
Warburgia salutaris	4.0	4.0	8.0	2.0	6.0	2.0	3.0	1.0	4.0	3.0	>8.0	1.5

*Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

Table S3
Plant extract MIC values (mg/ml) when tested individually against yeasts.

Individual plant sample (32 mg/ml)	**MIC values (mg/ml) of individual plant samples against yeasts**			
	***C. albicans* (ATCC 10231)**		***C. glabrata* (ATCC 90030)**	
	Aqueous extract	Organic extract	Aqueous extract	Organic extract
Acorus calamus	>8.0	0.3	>8.0	>8.0
Alepidia amatymbica	>8.0	0.5	>8.0	3.0
Bauhinia bowkeri	2.0	0.5	8.0	8.0
Cinnamomum camphora	>8.0	0.5	>8.0	1.5
Cunonia capensis	2.0	0.5	1.0	2.0
Dombeya rotundifolia	>8.0	0.5	>8.0	3.0
Eucalyptus sideroxylon	>8.0	0.3	3.0	1.5
Hydnora africana	>8.0	0.5	>8.0	3.0
Hypoxis hemerocallidea	>8.0	0.3	>8.0	3.0
Merwilla plumbea	>8.0	0.3	>8.0	4.0
Pterocarpus angolensis	>8.0	0.5	1.5	2.0
Rauvolfia caffra	>8.0	0.5	>8.0	6.0
Rhoicissus tomentosa	>8.0	0.5	>8.0	1.5
Sclerocarya birrea	2.0	0.3	>8.0	3.0
Senecio serratuloides	1.0	0.5	3.0	>8.0
Synaptolepis oliveriana	>8.0	0.5	>8.0	>8.0
Warburgia salutaris	1.0	0.5	4.0	3.0

*Negative control of acetone in water >8.0 mg/ml; positive control of ciprofloxacin (0.01 mg/ml) 0.039 – 1.800 µg/ml; culture control >8.0 mg/ml.

Table S4

Average mortality (%) rates in the BSLA of individual antimicrobials and plant samples proving synergistic in various combinations.

Sample		Average mortality (%) ± S.D. after 24 h	Average mortality (%) ± S.D. after 48 h
Antibiotics (1 mg/ml)	Azithromycin	1.39 ± 3.40	4.02 ± 4.71
	Ciprofloxacin	13.50 ± 21.61	**50.83 ± 29.15**
Organic extracts (1 mg/ml)	*Acorus calamus*	14.04 ± 18.12	38.93 ± 41.80
	Alepidia amatymbica	20.34 ± 24.62	42.63 ± 37.44
	Cinnamomun camphora	8.77 ± 9.72	24.15 ± 26.53
	Cunonia capensis	18.26 ± 22.80	**59.94 ± 8.85**
	Dombeya rotundifolia	10.79 ± 11.90	28.54 ± 32.44
	Eucalyptus sideroxylon	21.51 ± 24.34	43.71 ± 43.12
	Hydnora africana	11.70 ± 15.52	28.25 ± 31.05
	Hypoxis hemerocallidea	25.06 ± 8.73	35.71 ± 39.53
	Merwilla plumbea	49.32 ± 29.26	**79.50 ± 15.25**
	Pterocarpus angolensis	12.35 ± 14.87	34.57 ± 38.93
	Rauvolfia caffra	11.02 ± 14.15	37.48 ± 26.55
	Rhoicissus tomentosa	14.41 ± 17.77	32.95 ± 37.69
	Sclerocarya birrea	14.50 ± 17.29	39.67 ± 43.72
	Senecio serratuloides	17.42 ± 18.43	39.44 ± 39.03
	Synaptolepis oliveriana	11.36 ± 5.71	**51.41 ± 18.05**
	Warburgia salutaris	**70.47 ± 34.23**	**93.80 ± 7.08**
Aqueous extracts (1 mg/ml)	*Bauhinia bowkeri*	0.85 ± 1.48	11.77 ± 1.05
	Cunonia capensis	0.00 ± 0.00	0.00 ± 0.00
	Merwilla plumbea	1.99 ± 2.30	25.49 ± 11.69
	Pterocarpus angolensis	0.00 ± 0.00	5.22 ± 6.75
	Rauvolfia caffra	0.00 ± 0.00	0.00 ± 0.00
	Rhoicissus tomentosa	0.00 ± 0.00	0.00 ± 0.00
	Senecio serratuloides	0.00 ± 0.00	9.17 ± 10.16
	Warburgia salutaris	0.00 ± 0.00	5.77 ± 6.61

*Individual samples prepared at concentration of 2 mg/ml resulting in testing concentration within wells of 1 mg/ml; Bold = toxicity observed. Negative control consisted of 32 g/l salt water and the positive control consisted of 1.6 mg/ml potassium dichromate.

References for Supplementary data

EMGuidance, 2024. Available at: https://emguidance.com/discover (Accessed: 15 March 2024).

SAMF (South African Medicines Formulary), 2022. Division of Clinical Pharmacology, Faculty of Health Sciences, University of Cape Town. Published by Health and Medical Publishing Group for the South African Medical Association. Cape Town, South Africa.

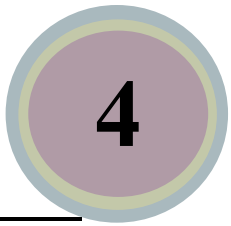
3.2. Chapter summary

Of the combinations tested, approximately half were antagonistic in nature, raising the concern for ineffective infection treatment outcomes and increased antimicrobial resistance amongst the Johannesburg patient population. However, further *in vivo* studies are required to evaluate if this identified *in vitro* antagonism could be clinically translated.

Combinations of plant extracts with azithromycin and ciprofloxacin demonstrated the most synergism, with most of these found to be non-toxic in the BSLA. These findings provide an indication of combinations worth noting for future research endeavours.

Chapter 4

Interactive antimicrobial and toxicity profiles of KwaZulu-Natal traditional medicinal plants with conventional antimicrobials



4.1. Chapter introduction

Chapter 4 sought to achieve objectives set out in phase 3 "a" to "e" of this study, specific to the KwaZulu-Natal sourced medicinal plant samples. Similarly to the Faraday *muthi* market in Johannesburg, the Warwick *muthi* market in Durban, KwaZulu-Natal (KZN), ensures availability of locally harvested plant material to Durban city centre.

In Chapter 2, it was revealed that 94.1% of patients at the King Edward Hospital in Durban, KwaZulu Natal, used traditional medicine alongside their prescribed conventional medicines or in the past. Interactive combination studies are therefore of particular relevance in this province of South Africa, given the heavy reliance on the traditional healthcare modality.

In this final section of phase 3 (*Publication 3*) of the study, the evaluation of the *in vitro* antimicrobial interactions and toxicity profiles of commonly traded traditional medicinal plants (Table 4.1) from the Warwick *muthi* market in KZN, in combination with various conventional antimicrobials has been presented.

Table 4.1. Traditional medicinal plant material purchased from the Warwick *muthi* market in Durban, KwaZulu-Natal.

Scientific plant name	Plant sample image	Scientific plant name	Plant sample image	Scientific plant name	Plant sample image
Acorus calamus L.		*Hydnora africana* Thunb.		*Sansevieria hyacinthoides* (L.) Druce	
Artemisia afra Jacq. ex Willd.		*Jatropha zeyheri* Sond.		*Senecio oxyriifolius* DC.	
Berchemia discolor (Klotzsch) Hemsl.		*Lippia javanica* (Burm.f.) Spreng		*Senecio serratuloides* DC.	
Callilepis laureola DC.		*Rapanea melanophloeos* (L.) Mez		*Strychnos henningsii* Gilg	
Elaeodendron transvaalense (Burtt Davy) R.H.Archer		*Rauvolfia caffra* Sond.		*Warburgia salutaris* (G.Bertol.) Chiov.	
Gunnera perpensa L.		*Rhoicissus tridentata* (L.f.) Wild & R.B.Drumm.			

*Plant identification and verification by Dr. Gugulethu Khumalo. Images by Prof. Sandy van Vuuren.

P.3

Publication 3

Booth, Z., Essack, S.Y., van Vuuren, S.F., 2025. Conventional antimicrobial and medicinal plants from a traditional medicine market in South Africa: An interactive antimicrobial and toxicity study. *Antibiotics*, 14(5): 512-530. https://doi.org/10.3390/antibiotics14050512

*Proof of submission to a journal is provided in Appendix B. A graphical abstract reflecting this publication has been presented in Figure 4.1.

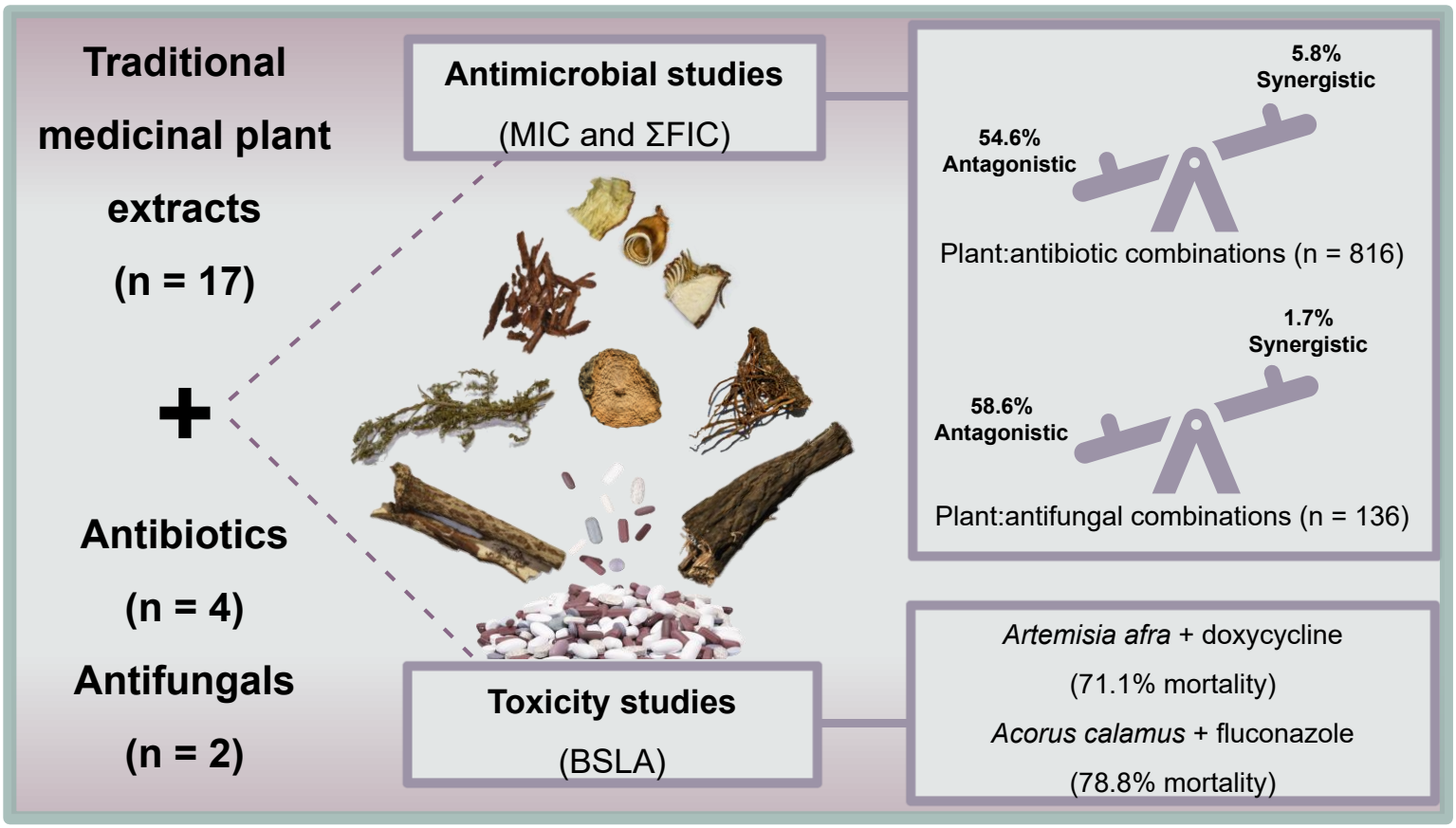


Figure 4.1. A graphical abstract for *Publication 3*.

Conventional Antimicrobial and Medicinal Plants from a Traditional Medicine Market in South Africa: An Interactive Antimicrobial and Toxicity Study

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Abstract: Background: The World Health Organization (WHO) has proposed the use of integrative medicine to achieve extended healthcare coverage in developing countries facing high morbidity. Traditional remedies are frequently employed to prevent and treat infections among South Africans; however, the ways in which they interact with conventional antimicrobials are largely unknown. Therefore, this study aimed to explore the interactions between commonly traded medicinal plants at a traditional medicine market in KwaZulu-Natal (KZN), South Africa, and conventional antibiotics and antifungals. Methods: To determine the interactive antimicrobial profiles for plant/conventional antimicrobial combinations, minimum inhibitory concentration (MIC) assays were performed against ESKAPE pathogens and the yeasts *Candida albicans* and *Candida glabrata*. Calculated fractional inhibitory concentration (ΣFIC) values were used to identify synergism or antagonism, with synergistic interactions further tested *in vitro* for toxicity. Results: A total of 952 combinations were tested, of which 5.8% and 54.6% of the plant/antibiotic combinations were synergistic and antagonistic, respectively; additionally, 1.7% and 58.6% of the plant/antifungal combinations showed synergism or antagonism, respectively. The most toxic plant/antibiotic combination was *Artemisia afra* with doxycycline (71.1% mortality). The most toxic plant/antifungal combination was *Acorus calamus* with fluconazole (78.8% mortality). Conclusions: When medicinal plants acquired from a traditional medicine market in South Africa are used in combination with conventional antibiotics and antifungals, more than half of the combinations exhibit antagonism, which is concerning.

Keywords: antibiotics; antifungals; traditional medicinal plants; antimicrobial combinations; interactions; synergistic; antagonistic; toxicity

1. Introduction

An estimated 60–80% of South Africans make use of traditional medicines sourced from various traditional medicine markets [1–3]. With the country's diverse array of indigenous plants, exposure to a wide range of traditional medicinal plants is highly prevalent among the population, particularly for the treatment of infectious conditions [4]. Given the widespread use of medicinal plants in South Africa and the substantial burden of infections [5], there is a significant likelihood of combined traditional and conventional antimicrobial use.

Consequently, it is crucial to investigate the interactions of these combinations. Synergistic antimicrobial interactions could prove beneficial as alternatives to addressing resistant infections. The potentiation of antimicrobial efficacy could be harnessed without having to increase dosing, which subsequently reduces toxicity. Of concern, however, is the potential for antagonism. Specifically, the findings from a recent study on antimicrobial interactions between traditional medicinal plants purchased from an informal Johannesburg (JHB) traditional medicine market (Faraday market) and conventional antimicrobials, revealing that most combinations were antagonistic [6].

This study aimed to investigate whether this pattern of antagonism extends to traditional medicinal plants with varied geographic distributions in South Africa. Specifically, we evaluated the *in vitro* antimicrobial interactions and toxicity profiles of commonly used traditional medicinal plants from KwaZulu-Natal (KZN), traded at the Warwick market, when used in combination with conventional antimicrobials against selected bacterial and fungal species.

2. Results

2.1. Individual Antimicrobial Activity of Plants

This study focused specifically on combinations, but, to evaluate interactions, baseline minimum inhibitory concentration (MIC) values for individual samples needed to be established. All antimicrobials were found to be aligned with the EUCAST [7]-CLSI [8] breakpoint ranges (Supplementary Tables S1 and S2), and the individual plant extract activities against the ESKAPE pathogens (*Enterococcus faecium* ATCC 27270, *Staphylococcus aureus* ATCC 25923; *Klebsiella pneumoniae* ATCC 13883, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853, and *Enterobacter cloacae* NCTC 13406; and two yeasts (*Candida albicans* ATCC 10231 and *Candida glabrata* ATCC 90030) are summarized in Supplementary Tables S1 and S2, respectively. An evaluation of the individual plant samples for antimicrobial activity established that *Elaeodendron transvaalense* (organic extract) was

the most active amongst the plant samples when tested against *A. baumannii*, with an MIC of 0.4 mg/mL (Supplementary Table S1).

2.2. Plant Extracts in Combination with Commercial Antimicrobials

A total of 952 combinations of plant (aqueous and organic) extracts with conventional antimicrobials were tested against the six ESKAPE pathogens and the two yeasts (Supplementary Figures S1–S19). When the extracts of the 17 plants (50 µL of 32 mg/mL extract) were combined with antimicrobials (50 µL of 0.01 mg/mL antibiotic, or 50 µL of 0.1 mg/mL antifungal) and tested against their respective pathogens, many combinations lacked definitive interactive ΣFIC classifications, since the MIC endpoints could not be reached (i.e., MIC > 4 mg/mL for plant extracts or MIC >1.25 µg/mL for antibiotics and >12.5 µg/mL for antifungal agents). The interactive summary of the 308 plant/antibiotic combinations with definitive interactive values, determined using the sum of the fractional inhibitory concentration (ΣFIC) calculation (Supplementary Figures S1–S17), is presented in Figure 1.

Furthermore, the interactive classification overview of the 58 plant/antifungal combinations with definitive ΣFIC values (Supplementary Figures S18 and S19) is presented in Figure 2.

Most plant/antimicrobial combinations demonstrated antagonism, which is concerning. Regarding the treatment of infectious conditions, antagonistic interactions pose a threat to the efficacy of antimicrobial therapies. Reduced antimicrobial potency further promotes the development of antimicrobial resistance among pathogens, rendering the existing antimicrobial agents ineffective [9].

Some interactions were synergistic (5.8% of plant/antibiotic combinations; 1.7% of plant/antifungal combinations), and these interactions could lead to more effective therapeutic outcomes that could prevent antimicrobial resistance. As additive and non-interactive profiles have little therapeutic impact, the combinations demonstrating antagonism (reduced antimicrobial efficacy) and synergy (enhanced antimicrobial efficacy) are only further described to emphasize their potential therapeutic impact on antimicrobial outcomes.

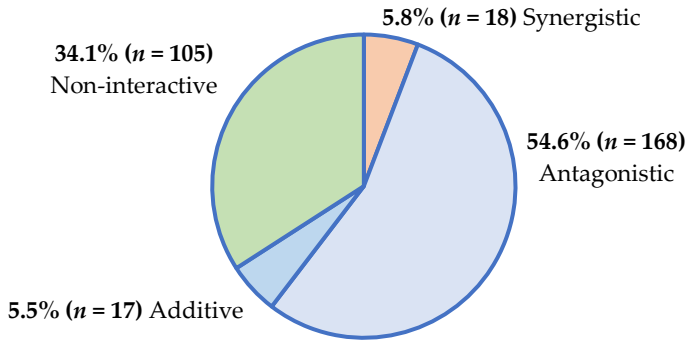

Figure 1. Interactive antimicrobial profiles of plant/antibiotic combinations (n = 308) against ESKAPE pathogens.

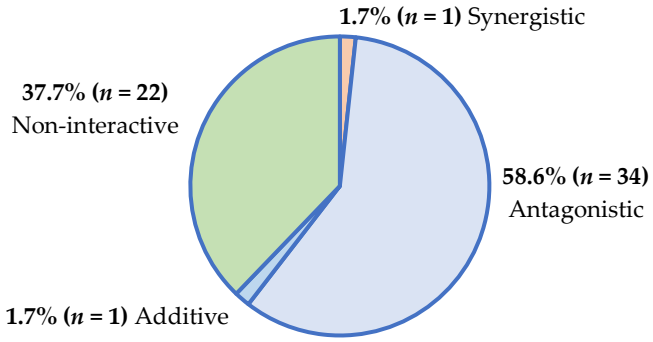

Figure 2. Interactive antimicrobial profiles of plant/antifungal combinations (n = 58) against yeasts.

2.2.1. Plant Combinations with Amoxicillin

Among the plant extract combinations with amoxicillin that have a definitive ΣFIC (n = 182), no synergistic interactions were identified, with 26.4% of the combinations being antagonistic (n = 48). Plant combinations with amoxicillin were most antagonistic against *A. baumannii*, where 16 of the 17 tested plants were antagonistic in combination (Table 1). The most prominent antagonistic interaction was between *S. serratuloides* (aqueous extract) and amoxicillin, yielding a ΣFIC of 135.49 against *K. pneumoniae*. No synergistic interactions were exhibited for further reporting.

Table 1. Interactive antagonistic plant/amoxicillin combinations.

Plant	Pathogen	MIC [1]	MIC [2]	ΣFIC
Organic extract antagonism				
Artemisia afra	*E. faecium*	0.16	0.50	17.09
Berchemia discolor		0.16	0.50	17.09
Elaeodendron transvaalense		0.16	0.50	17.25
Gunnera perpensa		0.16	0.50	17.09
Hydnora africana		0.16	0.50	17.09
Jatropha zeyheri		0.16	0.50	17.09
Lippia javanica		0.16	0.50	17.09
Rapanea melanophloeos		0.16	0.50	17.09
Rauvolfia caffra		0.16	0.50	17.09
Rhoicissus tridentata		0.16	0.50	17.01
Sansevieria hyacinthoides		0.47	1.50	50.76
Senecio serratuloides		0.16	0.50	17.09
Strychnos henningsii		0.31	1.00	33.89
Warburgia salutaris		0.16	0.50	17.09
Artemisia afra	*S. aureus*	0.63	2.00	34.53
Berchemia discolor		1.25	4.00	68.62
Elaeodendron transvaalense		0.63	1.00	36.28
Rapanea melanophloeos		0.63	2.00	34.97
Sansevieria hyacinthoides		1.25	4.00	67.96
Senecio oxyriifolius		1.25	4.00	68.62
Senecio serratuloides		0.31	1.00	17.16
Strychnos henningsii		1.25	4.00	67.96
Warburgia salutaris		0.31	1.00	17.35
Artemisia afra	*A. baumannii*	0.31	1.00	34.98
Berchemia discolor		0.31	1.00	34.45
Callilepis laureola		0.31	1.00	34.16
Elaeodendron transvaalense		0.31	1.00	36.79
Gunnera perpensa		0.63	2.00	70.07
Hydnora africana		0.63	2.00	68.75
Jatropha zeyheri	*A. baumannii*	0.63	2.00	68.75
Lippia javanica		0.47	1.50	51.50
Rapanea melanophloeos		0.31	1.00	34.65
Rauvolfia caffra		0.63	2.00	68.42
Rhoicissus tridentata		0.63	2.00	68.75
Sansevieria hyacinthoides		0.63	2.00	68.75
Senecio oxyriifolius		0.63	2.00	68.75
Senecio serratuloides		0.63	2.00	70.07
Strychnos henningsii		0.63	2.00	68.75
Warburgia salutaris		0.63	2.00	68.42
Gunnera perpensa	*P. aeruginosa*	0.63	2.00	4.63
Artemisia afra	*E. cloacae*	1.25	4.00	19.40
Senecio serratuloides		0.47	1.50	7.48
Aqueous extract antagonism				
Acorus calamus	*K. pneumoniae*	1.25	4.00	134.60
Sansevieria hyacinthoides		0.94	3.00	101.00
Senecio serratuloides		1.25	4.00	135.49
Hydnora africana	*A. baumannii*	0.47	1.50	50.76
Rauvolfia caffra		0.47	1.50	50.76
Senecio serratuloides		0.31	1.00	33.86

[1] MIC for plant extract (mg/mL). [2] MIC for amoxicillin (µg/mL).

2.2.2. Plant Combinations with Azithromycin

Azithromycin is not typically effective against many ESKAPE pathogens; however, it is a broad-spectrum antibiotic that is frequently prescribed, and unnecessarily overused, in conventional healthcare settings [10]. Among the plant extract combinations with azithromycin

that had a definitive ΣFIC (n = 77), 11.7% were synergistic (n = 9) and 54.6% were antagonistic (n = 42) (Table 2). The greatest antagonism was noted for plant/azithromycin combinations when tested against *P. aeruginosa*, where 16 of the 17 combinations were antagonistic. Synergism was observed only against the Gram-positive pathogens, *S. aureus* and *E. cloacae*, where *J. zeyherii* (organic extract) with azithromycin against *S. aureus* had the greatest synergistic effect (ΣFIC of 0.22).

Table 2. Interactive antagonistic plant/azithromycin combinations.

Plant	Pathogen	MIC [1]	MIC [2]	ΣFIC
Organic extract antagonism				
Artemisia afra	*E. faecium*	1.25	4.00	4.11
Hydnora africana		1.25	4.00	4.11
Senecio serratuloides		1.25	4.00	4.11
Warburgia salutaris		1.25	4.00	4.11
Artemisia afra	*S. aureus*	1.25	4.00	4.54
Berchemia discolor	*K. pneumoniae*	0.47	1.50	5.79
Elaeodendron transvaalense		0.31	1.00	4.24
Warburgia salutaris		0.47	1.50	5.38
Artemisia afra	*A. baumannii*	0.63	2.00	6.73
Elaeodendron transvaalense		0.31	1.00	5.18
Hydnora africana		1.25	4.00	10.83
Jatropha zeyherii		0.47	1.50	4.06
Lippia javanica		1.25	4.00	10.83
Rauvolfia caffra		0.63	2.00	5.08
Senecio oxyriifolius		0.47	1.50	4.10
Strychnos henningsii		1.25	4.00	10.83
Artemisia afra	*P. aeruginosa*	0.16	0.50	16.73
Berchemia discolor		0.31	1.00	34.13
Callilepis laureola		0.63	2.00	66.28
Elaeodendron transvaalense		0.24	0.75	25.94
Gunnera perpensa		0.31	1.00	34.39
Hydnora africana		0.12	0.38	12.80
Jatropha zeyherii		0.16	0.50	16.83
Lippia javanica		0.24	0.75	25.12
Rapanea melanophloeos		0.16	0.50	16.80
Rauvolfia caffra		0.24	0.75	25.00
Rhoicissus tridentata		0.47	1.50	49.99
Sansevieria hyacinthoides		0.31	1.00	33.14
Senecio oxyriifolius		0.63	2.00	65.95
Senecio serratuloides		0.16	0.50	16.80
Strychnos henningsii		0.47	1.50	49.74
Warburgia salutaris		0.47	1.50	49.84
Aqueous extract antagonism				
Callilepis laureola	*A. baumannii*	0.94	3.00	6.65
Artemisia afra	*P. aeruginosa*	0.63	2.00	66.28
Berchemia discolor		0.16	0.50	16.48
Elaeodendron transvaalense		0.16	0.50	16.73
Gunnera perpensa		0.16	0.50	16.48
Lippia javanica		1.25	4.00	131.91
Rapanea melanophloeos		0.31	1.00	33.07
Rauvolfia caffra		1.25	4.00	131.91
Senecio serratuloides		0.16	0.50	16.52
Strychnos henningsii		1.25	4.00	131.90
Organic extract synergism				
Callilepis laureola	*S. aureus*	0.08	0.25	0.26
Elaeodendron transvaalense		0.08	0.25	0.50
Hydnora africana		0.16	0.50	0.50
Jatropha zeyherii		0.08	0.25	0.22
Senecio serratuloides		0.16	0.50	0.50

Plant	Pathogen	MIC [1]	MIC [2]	ΣFIC
Berchemia discolor	*E. cloacae*	0.08	0.25	0.53
Elaeodendron transvaalense		0.08	0.25	0.33
Aqueous extract synergism				
Berchemia discolor	*E. cloacae*	0.12	0.38	0.49
Gunnera perpensa		0.16	0.50	0.36

[1] MIC for plant extract (mg/mL). [2] MIC for azithromycin (µg/mL).

2.2.3. Plant Combinations with Ciprofloxacin

Among the plant extract combinations with ciprofloxacin with a definitive ΣFIC (n = 77), 2.6% were synergistic (n = 2) and 64.9% were antagonistic (n = 50) (Table 3). The greatest antagonism was evident when combinations were tested against *S. aureus* and *A. baumannii*, where 14 and 15, respectively, of the 17 plant sample combinations were antagonistic.

Only two synergistic interactions were noted for the plant/ciprofloxacin combinations; however, overall, the most synergistic interaction for this study was identified between *Strychnos henningsii* and ciprofloxacin against *P. aeruginosa* (ΣFIC of 0.07). Conversely, the most notable antagonistic interaction was found for *S. serratuloides*/ciprofloxacin against *A. baumannii* (ΣFIC of 139.87).

Table 3. Interactive antagonistic plant/ciprofloxacin combinations.

Plant	Pathogen	MIC [1]	MIC [2]	ΣFIC
Organic extract antagonism				
Jatropha zeyheri	*E. faecium*	0.94	3.00	102.01
Lippia javanica		1.25	4.00	135.93
Strychnos henningsii		0.31	1.00	33.86
Warburgia salutaris		1.25	4.00	135.93
Acorus calamus	*S. aureus*	1.25	4.00	5.25
Artemisia afra		0.94	3.00	51.83
Berchemia discolor		1.25	4.00	68.62
Callilepis laureola		1.25	4.00	68.62
Elaeodendron transvaalense		1.25	4.00	72.56
Gunnera perpensis		1.25	4.00	70.31
Jatropha zeyheri		1.25	4.00	68.06
Lippia javanica		0.94	3.00	51.50
Rapanea melanophloeos		0.94	1.00	52.48
Sansevieria hyacinthoides		1.25	4.00	67.96
Senecio oxyriifolius		1.25	4.00	68.62
Senecio serratuloides		1.25	4.00	68.62
Strychnos henningsii		1.25	4.00	67.96
Warburgia salutaris		1.25	4.00	69.41
Elaeodendron transvaalense	*K. pneumoniae*	0.12	0.38	13.02
Lippia javanica		0.16	0.50	17.01
Rapanea melanophloeos		0.08	0.25	8.47
Rauvolfia caffra		0.08	0.25	8.47
Senecio serratuloides		1.25	4.00	135.27
Warburgia salutaris		1.25	4.00	135.49
Artemisia afra	*A. baumannii*	0.63	2.00	70.07
Berchemia discolor		1.25	4.00	137.76
Callilepis laureola		0.31	1.00	34.16
Elaeodendron transvaalense		0.31	1.00	36.79
Hydnora africana		1.25	4.00	137.24
Jatropha zeyheri		0.31	1.00	34.32

Plant	Pathogen	MIC [1]	MIC [2]	ΣFIC
Lippia javanica		0.47	1.50	51.50
Rapanea melanophloeos		0.63	2.00	69.41
Rauvolfia caffra		0.16	0.50	17.17
Rhoicissus tridentata		0.63	2.00	68.75
Sansevieria hyacinthoides	*A. baumannii*	0.31	1.00	34.32
Senecio oxyriifolius		0.31	1.00	34.32
Senecio serratuloides		1.25	4.00	139.87
Strychnos henningsii		0.63	2.00	68.75
Warburgia salutaris		1.25	4.00	136.58
Artemisia afra	*P. aeruginosa*	1.25	44.00	5.54
Aqueous extract antagonism				
Sansevieria hyacinthoides	*E. faecium*	0.63	2.00	67.31
Lippia javanica		0.039	0.13	4.35
Rapanea melanophloeos		0.12	0.38	12.75
Rauvolfia caffra	*K. pneumoniae*	0.06	0.19	6.45
Senecio serratuloides		0.16	0.50	17.03
Strychnos henningsii		0.06	0.19	6.44
Callilepis laureola		1.25	4.00	135.27
Rauvolfia caffra	*A. baumannii*	0.63	2.00	50.76
Senecio serratuloides		1.25	4.00	135.40
Berchemia discolor	*E. cloacae*	0.47	1.50	7.28
Organic extract synergism				
Callilepis laureola	*E. cloacae*	0.03	0.90	0.43
Strychnos henningsii	*P. aeruginosa*	0.02	0.06	0.07

[1] MIC for plant extract (mg/mL). [2] MIC for ciprofloxacin (µg/mL).

2.2.4. Plant Combinations with Doxycycline

Among the plant/doxycycline combinations with definitive ΣFIC values (n = 91), 7.7% were synergistic (n = 7) and 30.8% were antagonistic (n = 28) (Table 4). The most notable synergism was between *R. melanophloeos*/doxycycline against *E. cloacae* (ΣFIC of 0.30). Four plant organic extracts (*H. africana*, *J. zeyheri*, *S. hyacinthoides*, and *S. serratuloides*) with doxycycline against *A. baumannii*, were most antagonistic (ΣFIC of 24.50).

Table 4. Interactive antagonistic and synergistic plant/doxycycline combinations.

Plant	Pathogen	MIC [1]	MIC [2]	ΣFIC
Organic extract antagonism				
Lippia javanica	*E. faecium*	1.25	4.00	4.11
Artemisia afra		1.25	4.00	4.54
Hydnora africana		1.25	4.00	4.11
Lippia javanica		1.25	4.00	4.11
Senecio oxyriifolius	*S. aureus*	1.25	4.00	4.11
Senecio serratuloides		1.25	4.00	4.11
Warburgia salutaris		1.25	4.00	4.89
Artemisia afra		0.63	2.00	13.56
Elaeodendron transvaalense		0.16	0.50	4.30
Gunnera perpensa		0.63	2.00	13.56
Hydnora africana		1.25	4.00	24.50
Jatropha zeyheri	*A. baumannii*	1.25	4.00	24.50
Sansevieria hyacinthoides		1.25	4.00	24.50
Senecio oxyriifolius		1.25	4.00	24.50
Senecio serratuloides		0.47	1.50	7.44
Callilepis laureola		1.25	4.00	4.11
Elaeodendron transvaalense		0.63	2.00	4.56
Jatropha seyheri	*P. aeruginosa*	1.25	4.00	5.42
Rhoicissus tridentata		1.25	4.00	4.76

Plant	Pathogen	MIC [1]	MIC [2]	ΣFIC
Callilepis laureola	*E. cloacae*	1.25	4.00	4.11
Lippia javanica		1.25	4.00	4.11
Rauvolfia caffra		1.25	4.00	4.37
Aqueous extract antagonism				
Gunnera perpensa	*K. pneumoniae*	1.25	4.00	13.29
Callilepis laureola	*A. baumannii*	0.94	3.00	16.91
Rauvolfia caffra		0.47	1.50	8.45
Artemisia afra	*P. aeruginosa*	1.25	4.00	4.11
Berchemia discolor	*E. cloacae*	1.25	4.00	5.42
Elaeodendron transvaalense		1.25	4.00	5.42
Organic extract synergism				
Artemisia afra	*E. faecium*	0.16	0.50	0.50
Rapanea melanophloeos		0.12	0.38	0.39
Rapanea melanophloeos	*K. pneumoniae*	0.12	0.38	0.50
Berchemia discolor	*P. aeruginosa*	0.06	0.19	0.38
Rapanea melanophloeos	*E. cloacae*	0.08	0.25	0.30
Aqueous extract synergism				
Gunnera perpensa	*E. cloacae*	0.16	0.50	0.43
Rapanea melanophloeos		0.16	0.50	0.45

[1] MIC for plant extract (mg/mL). [2] MIC for doxycycline (μg/mL).

2.2.5. Plant Combinations with Nystatin

Among the plant extract combinations with nystatin that had a definitive ΣFIC value (n = 25), no synergy was observed, and a considerable 60.0% antagonism was noted against the yeasts *C. albicans* and *C. glabrata* (Table 5). The combination of *H. africana* (organic extract) and nystatin against *C. albicans* was the most antagonistic (ΣFIC of 19.67) among the antifungal combinations. A greater frequency of antagonism was noted with the organic extracts (*C. albicans* where n = 8) than with the aqueous extracts (*C. albicans* where n = 1) for the plant/nystatin combinations. However, both the organic and aqueous extracts in combination with nystatin had the same frequency of antagonistic effects on *C. glabrata* (n = 3). No synergy was observed for conventional antifungal agents.

Table 5. Interactive antagonistic plant/nystatin combinations.

Plant	Pathogen	MIC [1]	MIC [2]	ΣFIC
Organic extract antagonism				
Berchemia discolor	*C. albicans*	3.13	1.00	4.92
Hydnora africana		12.50	4.00	19.67
Jatropha zeyheri		6.25	2.00	4.33
Lippia javanica		4.69	1.50	7.38
Rapanea melanophloeos		4.69	1.50	4.28
Rauvolfia caffra		4.69	1.50	7.38
Rhoicissus tridentata		4.69	1.50	7.38
Strychnos henningsii		6.25	2.00	5.71
Callilepis laureola	*C. glabrata*	12.50	4.00	11.90
Gunnera perpensa		1.56	0.50	7.91
Lippia javanica		12.50	4.00	13.22
Aqueous extract antagonism				
Strychnos henningsii	*C. albicans*	12.50	4.00	5.23
Lippia javanica	*C. glabrata*	12.50	4.00	4.70
Senecio hyacinthoides		12.50	4.00	4.70
Strychnos henningsii		12.50	4.00	4.70

[1] MIC for plant extract (mg/mL). [2] MIC for nystatin (μg/mL).

2.2.6. Plant Combinations with Fluconazole

For the plant/fluconazole combinations with a definitive ΣFIC value (n = 33) against *C. albicans* and *C. glabrata*, only 3.0% were synergistic (n = 1), and 57.6% were antagonistic (n = 19) (Table 6). The only synergistic interaction was identified for the combination of *A. calamus* (organic extract) and fluconazole, with *C. albicans*, yielding a ΣFIC value of 0.34. The most antagonistic combination was exhibited when *R. melanophloeos* (organic extract) was combined with fluconazole and *R. tridentata* (organic extract) with fluconazole, both of which were tested against *C. glabrata*, yielding a ΣFIC of 16.08.

Table 6. Interactive antagonistic and synergistic plant/fluconazole combinations.

Plant	Pathogen	MIC [1]	MIC [2]	ΣFIC
Organic extract antagonism				
Gunnera perpensa	*C. albicans*	6.25	2.00	6.76
Jatropha zeyheri		6.25	2.00	5.39
Lippia javanica		3.13	1.00	5.45
Rhoicissus tridentata		6.25	2.00	10.89
Sansevieria hyacinthoides		6.25	2.00	10.89
Senecio serratuloides		6.25	2.00	6.76
Strychnos henningsii		6.25	2.00	6.76
Warburgia salutaris		6.25	2.00	10.89
Berchemia discolor	*C. glabrata*	6.25	2.00	9.41
Elaeodendron transvaalense		12.50	4.00	14.70
Hydnora africana		9.38	3.00	10.00
Lippia javanica		6.25	2.00	9.41
Rapanea melanophloeos		12.50	4.00	16.08
Rhoicissus tridentata		12.50	4.00	16.08
Senecio oxyriifolius		12.50	4.00	12.64
Senecio serratuloides		4.69	1.50	4.74
Aqueous extract antagonism				
Rapanea melanophloeos	*C. glabrata*	6.25	2.00	4.07
Senecio oxyriifolius		12.50	4.00	4.70
Senecio serratuloides		12.50	4.00	4.70
Organic extract synergism				
Acorus calamus	*C. albicans*	0.39	0.13	0.34

[1] MIC for plant extract (mg/mL). [2] MIC for fluconazole (µg/mL).

2.3. Toxicity Studies

The relevant samples were tested for toxicity individually via the brine shrimp lethality assay (BSLA) (Supplementary Table S3). To determine whether synergism was reflective of toxicity or the true increased antimicrobial potency of a combination, only plant samples that demonstrated synergistic interactions with conventional antimicrobials were tested for toxicity (Table 7).

Table 7. Toxicity of plant/antimicrobial combinations in the BSLA.

Combination	Plant sample	Mortality (%) ± S.D. [1] at 24 h	Mortality (%) ± S.D [1]. at 48 h
Azithromycin + organic extracts	*Acorus calamus*	27.48 ± 8.67	27.48 ± 8.67
	Callilepis laureola	4.76 ± 8.25	4.76 ± 8.25
	Elaeodendron transvaalense	0.00 ± 0.00	11.76 ± 8.92
	Hydnora africana	0.00 ± 0.00	0.00 ± 0.00
Azithromycin + organic extracts	*Jatropha zeyheri*	9.10 ± 5.48	15.62 ± 6.59
	Rapanea melanophloeos	0.00 ± 0.00	0.00 ± 0.00
Azithromycin + aqueous extracts	*Berchemia discolor*	0.00 ± 0.00	0.00 ± 0.00
	Gunnera perpensa	0.00 ± 0.00	52.88 ± 8.55
	Rapanea melanophloeos	0.00 ± 0.00	0.00 ± 0.00
Ciprofloxacin + organic extracts	*Callilepis laureola*	0.00 ± 0.00	9.90 ± 13.43
	Strychnos henningsii	0.00 ± 0.00	8.50 ± 6.82
Doxycycline + organic extracts	*Artemisia afra*	0.00 ± 0.00	**71.10 ± 11.51**
	Rapanea melanophloeos	0.00 ± 0.00	**51.33 ± 25.03**
	Rauvolfia caffra	0.45 ± 1.10	2.05 ± 3.41
Doxycycline + aqueous extracts	*Berchemia discolor*	1.03 ± 1.63	15.74 ± 19.32
	Gunnera perpensa	0.00 ± 0.00	**57.45 ± 27.08**
Fluconazole + organic extracts	*Acorus calamus*	0.00 ± 0.00	**78.79 ± 28.49**

[1] standard deviation; bold text shows toxicity. Negative control consisted of saltwater (32 g/L) – 0.00 ± 0.00% ± S.D. mortality; positive control consisting of potassium dichromate (1.6 mg/mL) - 100.00 ± 0.00% ± S.D. mortality.

Among the individual plant extracts studied, only the *A. calamus* and *E. transvaalense* organic extracts were found to be toxic, with average mortality rates of 100.0% and 55.8%, respectively (Supplementary Table S3).

Among the 17 synergistic combinations evaluated for toxicity (Table 7), only four (*A. afra* organic extract with doxycycline 71.1%; *R. melanophloeos* organic extract with doxycycline 51.3%; *G. perpensa* aqueous extract with doxycycline 57.5%; and *A. calamus* organic extract with fluconazole 78.8%) were toxic after only 48 h of exposure.

The independent plant samples that were toxic had reduced mortality rates when combined, except for the *A. calamus* (organic extract)/fluconazole combination, where considerable toxicity was still evident (78.8% average mortality after 48 h). This combination was also the only combination with an antifungal agent that was found to have synergistic antimicrobial effects. When the *A. calamus* (organic extract)/azithromycin combination was tested, the toxicity was reduced to 27.5% for the combination.

3. Discussion

Combinations of various antimicrobials with plant extracts demonstrating pertinent synergistic and antagonistic profiles were evaluated in this study. They may present enhanced or reduced antimicrobial activity in terms of the therapeutic impact.

Amoxicillin belongs to the penicillin family and is commonly used for the treatment of bacterial infections of the respiratory tract, skin, and oral cavity [10–13]. *Staphylococcus aureus* poses a serious health threat, as the pathogen has intrinsic virulence, the ability to cause a diverse array of life-threatening infections, and the capacity to adapt to different environmental conditions [14,15]. Furthermore, a literature review was conducted [9] in which a surge in the resistance of *S. aureus* toward amoxicillin was frequently reported in the literature. The identification of new, more effective antimicrobial options is therefore paramount, and synergistic plant: antimicrobial combinations, could serve as alternative avenues for investigation. Conversely, antagonistic plant/antimicrobial combinations could further exacerbate the health threat posed by resistant Staphylococcal infections.

Lippia javanica, A. afra, R. melanophloeos, R. caffra and *S. serratuloides*, which are traditionally used for the treatment of respiratory tract infections [16–18] (Supplementary Table S4), all exhibited antagonism in combination with amoxicillin, particularly against three prominent causative pathogens of respiratory tract infections (*A. baumannii, S. aureus* and *K. pneumoniae*).

Antibacterial activity of *A. afra* (organic extract) was investigated [19] and it was reported that *A. afra* was highly active against *S. aureus*, and active even against methicillin-resistant *S. aureus*. With amoxicillin commonly employed to treat respiratory tract infections in conventional healthcare settings [10–13], it is likely that the combination of amoxicillin with medicinal plants is used traditionally for similar respiratory complaints. Considering the antagonism identified in the current study, a combination of this nature should not be used.

Amoxicillin is also frequently used for the treatment of skin infections in conventional health settings [10–13,20]. *Berchemia discolor, G. perpensa*, *H. africana*, *J. zeyheri*, *L. javanica*, *R. caffra*, *S. serratuloides*, *S. henningsii* and *W. salutaris* are all traditionally used for the treatment of skin infections, particularly in wound treatment [16–18] (Supplementary Table S4). Hence, with the prevalence of antagonism between amoxicillin and these medicinal plants is cause for concern, particularly against the pathogens *S. aureus*, *P. aeruginosa*, *A. baumannii, E. cloacae* and *E. faecium*, which are known for their potential to infect wounds [10]. Although *S. aureus* is a natural skin commensal, when the skin is perforated by injury, the bacteria can

become pathogenic and result in soft skin tissue infections [20]. *Staphylococcus aureus* has become resistant to many ß-lactam antibiotics, including amoxicillin [15,19] and it emphasizes that the rapid development of multidrug-resistant *S. aureus* has resulted in difficulties in achieving effective treatment [20]. Therefore, the antagonism noted in the present study warrants concern in relation to the efficacy of amoxicillin in the treatment of infections caused by *S. aureus*.

Azithromycin is a macrolide antibiotic used to treat respiratory tract infections (pneumonia and sinusitis), in addition to gastro-intestinal and skin infections [10–13]. Azithromycin, exhibits known activity against both *P. aeruginosa* and *S. aureus* [10–13]. Furthermore, *S. serratuloides* is traditionally used for respiratory tract infections and was found to be synergistic with azithromycin against *S. aureus* (ΣFIC of 0.50). This combination is promising for treating *S. aureus* sinusitis in the future, particularly since this combination was further found to be non-toxic (Table 7).

Conversely, antagonism between 16 of the 17 plant samples tested with azithromycin against *P. aeruginosa* is worrisome, since azithromycin is clinically employed for the treatment of *P. aeruginosa* infections [10–13].

Azithromycin is further used to treat various gastrointestinal complaints and associated infections, such as traveler's diarrhoea [10–13]. Both *C. laureola* and *E. transvaalense* are used traditionally for gastro-intestinal infections [16–18] (Supplementary Table S4). Given that *S. aureus* is causative of food poisoning, and that azithromycin is a potential conventional treatment [10–13], these synergistic interactions between traditional and conventional medicines should be investigated further. Generally, aqueous extracts exhibit poor antimicrobial activity. Interestingly, however, in this study, aqueous extracts of *G. perpensa* (ΣFIC of 0.36) and *B. discolor* (ΣFIC of 0.49) combined with azithromycin and tested against *E. cloacae* were synergistic, providing further impetus for further exploration. These synergistic combinations offer an even more favorable approach for treating resistant skin infections, since combinations were also found to be non-toxic in the present study.

A further potential combination is *J. zeyheri* with azithromycin for the treatment of skin infections [16–18] (Supplementary Table S4), since synergism (ΣFIC of 0.22) against *S. aureus* was observed, along with a lack of toxicity in the BSLA (Table 7). Azithromycin is often prescribed in conventional healthcare settings [10–13], and *J. zeyheri* [16–18] (Supplementary Table S4) is used traditionally for the treatment of skin infections. *Staphylococcus aureus* commonly causes skin infections, with ailments such as boils, abscesses, cellulitis, atopic dermatitis, impetigo, folliculitis, and secondary wound infections [10]. Hence, this synergistic

interaction should be explored further to determine its clinical relevance, particularly since *S. aureus* has the potential to rapidly develop resistance toward conventional antibiotic treatments, often resulting in resistance to multiple antibiotics (multidrug-resistant *S. aureus*) [20].

Ciprofloxacin is a broad-spectrum antibiotic from a group of antibiotics classified as fluoroquinolones [10–13]. Ciprofloxacin is used in the clinical setting to treat uncomplicated urinary tract infections and respiratory tract infections, such as pneumonia, as well as skin, bone, eye and ear infections [10–13].

Strychnos henningsii is traditionally used for the treatment of gynecological, respiratory, oral cavity, skin, and gastro-intestinal infections [16–18] (Supplementary Table S4). *Pseudomonas aeruginosa* is a Gram-negative pathogen that causes bloodstream and respiratory tract infections, as well as infections of other parts of the body following surgery (nosocomial infections) [10–13]. The organic extract of *S. henningsii* with ciprofloxacin demonstrated the most noteworthy synergistic interaction when tested against *P. aeruginosa* (ΣFIC of 0.07). Antibiotic resistance is prominent in Pseudomonal infections [10–13], so the identified synergistic interaction could be explored as a mechanism to overcome this resistance, particularly since the combination is non-toxic (Table 7) and ciprofloxacin is an antibiotic that is used extensively in treating *P. aeruginosa* infections [10–13].

Doxycycline is a tetracycline antibiotic [11]. It is used for the treatment of respiratory, skin and dental infections [10–13]. Doxycycline is often employed in conventional treatments for skin infections [10–13]. With *B. discolor* being traditionally used for skin infections [16–18] (Supplementary Table S4), there is an alignment between traditional and conventional treatments, which could be of clinical relevance.

Nystatin is an antimicrobial used to treat fungal or yeast infections of the skin and oral cavity [10–13]. *Candida* spp. are well known for causing topical yeast infections, particularly in immunocompromised patients [10]. *Hydnora africana* is used traditionally to treat skin infections [16–18] (Supplementary Table S4). Hence, the antagonism documented in this study is a cause for concern in the treatment efficacy of conventional nystatin. For skin infections caused by both *C. albicans* and *C. glabrata*, there is concern should a patient make use of *H. africana* simultaneously with nystatin.

Fluconazole is an antifungal agent used to treat infections of the skin, genitalia, and oral cavity [10–13]. It is also employed for the treatment of systemic Candidal infections [10–13]. The only synergistic antifungal interaction was with *A. calamus* and fluconazole against *C. albicans*. This combination is even more promising considering the lack of toxicity in the

BSLA (Table 7). *Acorus calamus* has been studied for its antimicrobial, anthelmintic, antidiarrheal, antioxidant, anti-ulcer, and analgesic activities, among other properties [21,22]. Moderate antifungal activity of *A. calamus* extracts against *C. albicans* has been reported [22–24]. However, there are few studies on the combination of *A. calamus* with conventional antifungals. A study that explored their combined use was conducted, where *A. calamus* compounds were tested against *C. albicans* in combination with the antifungal's amphotericin B, clotrimazole and fluconazole and synergy was predominantly observed [23].

A similar study [6] investigated the interactions between conventional antimicrobials (amoxicillin, azithromycin, ciprofloxacin, doxycycline, nystatin and fluconazole) and traditional medicinal plants purchased from a Johannesburg (JHB) traditional medicine market (Faraday market) in Gauteng, South Africa [6]. A total of 816 plant/antibiotic combinations were tested against the ESKAPE pathogens, and synergistic (6.6%) and antagonistic (47.5%) effects were observed. Interactive profiles documented for JHB plant/antibiotic combinations [6] are similar to those obtained in the current study on KZN plant/antibiotic combinations (5.8% synergy; 54.6% antagonism). Furthermore, the greatest synergy was observed in both studies when JHB or KZN plants were combined with azithromycin and tested against the ESKAPE pathogens. Five common medicinal plants were tested in both the previous JHB study and the current KZN study, including *A. calamus*, *H. africana*, *R. caffra*, *S. serratuloides* and *W. salutaris*. Among these, the only shared interactive outcome was evident with *H. africana*/azithromycin combinations against *S. aureus*, where synergy was observed despite *H. africana* being purchased from alternate sources (JHB ΣFIC of 0.5; KZN ΣFIC of 0.5).

The pathogens with the greatest antagonism were *P. aeruginosa* and *A. baumannii*. This is concerning, since these two pathogens pose challenges to existing antimicrobial therapies because of resistance mechanisms [10–13]. Infections caused by *A. baumannii* are increasing, and these infections are resistant to conventional antibiotics because of their ability to develop biofilms [24].

Interactive findings for the plant/antifungal combinations used in the present study were similar to those previously studied [6], where the interactions were predominantly antagonistic (JHB 61.9%; KZN 58.6%). However, the synergy in the KZN study for plants with antifungal combinations (1.7%) was greater than that previously reported [6] for JHB plant combinations with antifungal agents, where no synergy was observed. Limited evidence of synergistic interactions with the antifungals that are clinically used against pathogenic fungi has been highlighted [25]. The findings from this study, therefore, offer valuable insights into the

interactive nature of antifungals with medicinal plants, which are rarely studied or documented in literature.

The strong antagonism evident in the current study requires further investigation as a potential cause of rapidly increasing resistance patterns toward conventional antifungal treatments. This is a serious public health threat, particularly in low- to middle-income countries, such as South Africa, where immunocompromising factors exist [25]. Furthermore, reports [26–28] have shown that candidiasis is still strongly associated with HIV infection. The antagonism evident in the present study between the plant/antifungal combinations against two common *Candida* species could be a compounding factor in antifungal resistance development, increasing the health threat posed by *Candida* opportunistic infections among HIV-infected patients.

When both South African studies were compared, a similar number of plant species were antagonistic with nystatin (n = 11) and fluconazole (n = 13) against both *C. albicans* and *C. glabrata*, indicating that the pattern of antagonism remained constant. This pattern should be further evaluated against other *Candida* strains to substantiate the commonality of the antagonism, although *C. albicans* remains the most common causative pathogen for fungal infections [29]. Non-albicans fungal infections are increasing and include *C. tropicalis*, *C. glabrata, C. parapsilosis*, and *C. krusei* [30–32]. Consequently, identifying antifungal therapies with broad-spectrum activity is vital.

The limited range of effective conventional antifungal agents used to treat fungal infections exacerbates the health threat they pose, even more so because antimicrobial resistance has now extended its reach [29–32]. The findings of the present study underscore that combination therapy is not a feasible option for identifying alternative, more effective treatment options for Candidal infections.

4. Materials and Methods

4.1. Plant Material Preparation

The plant samples (n = 24) were purchased from commercial traditional medicine traders at the Warwick traditional medicine market in Durban, KZN (South Africa), in February 2022. Appropriate assistance was garnered to facilitate effective communication with traders in the isiZulu local language. The purchases were made in accordance with those products most frequently sold to individuals presenting symptoms of an infectious disease. Services were garnered from a qualified botanist, with extensive knowledge of the plant material traded at the Warwick informal market and who was in good standing with the traders for communication

purposes. Botanical identification of the plant material purchased for analysis was based on the physical properties (appearance and sensory) of the plant material. Seven plant samples that could not be botanically verified were removed from the study, and seventeen medicinal plant samples (Supplementary Table S4) remained for *in vitro* combination antimicrobial testing.

The raw plant materials (leaves/stems/roots/bulbs) were dried and macerated to prepare both aqueous and organic extracts. The organic extracts were prepared by ensuring plant material immersion (20 g of plant material per 250 mL of solvent) in a 1:1 mixture of dichloromethane and methanol for 24 h at 37°C. Aqueous extracts were obtained by soaking the plant material in sterile water under the same conditions. The resulting filtrates were either evaporated, through exposure to fume hood conditions, to remove solvents (organic extracts) or frozen at −70°C and lyophilized via a Virtus freeze dryer (aqueous extracts). To ensure sterility, aqueous extracts were exposed to ultraviolet light. Both types of extracts were prepared at a concentration of 32 mg/mL for antimicrobial testing; sterile water was used for the aqueous extracts, and acetone was used for the organic extracts.

4.2. Interactive Antimicrobial Analysis

The ESKAPE (*Enterococcus faecium* ATCC 27270, *Staphylococcus aureus* ATCC 25923; *Klebsiella pneumoniae* ATCC 13883, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853, and *Enterobacter cloacae* NCTC 13406) pathogens and two yeasts (*Candida albicans* ATCC 10231 and *Candida glabrata* ATCC 90030) (American Type Culture Collection purchased from Davies Diagnostics (Randburg, South Africa)) were cultured in optimal growth media, Tryptone Soya broth (TSB) (Sigma–Aldrich, Johannesburg, South Africa), at 37°C for 24 h and 48 h at 37°C for the bacteria and yeasts, respectively. Pathogens were selected due to their ability to cause prominently occurring infectious diseases that are considered by the World Health Organization (WHO) to be "critical or high priority" on their global pathogen list because of their significant impact on morbidity and mortality rates [33].

The conventional antimicrobials, amoxicillin (purity ≥ 95%), azithromycin (purity ≥ 98%), ciprofloxacin (purity ≥ 98%), doxycycline (purity ≥ 93.5%), nystatin (purity ≥ 98%) and fluconazole (purity ≥ 98%) (Sigma–Aldrich, Johannesburg, South Africa), which are regularly used in the public healthcare sector against the selected pathogens, were prepared accordingly for analysis [12].

The antimicrobial activities of the plant samples (100 µL) and conventional antimicrobials (100 µL) were determined, after which the plant/antimicrobial combinations (50 µL of each agent of the combination) were assessed via the minimum inhibitory concentration (MIC) assay

and CLSI [8]-approved transfer techniques. The positive control of ciprofloxacin 0.01 mg/mL or nystatin 0.1 mg/mL and negative (32 mg/mL of acetone in water) controls, due to spectrum of activity, along with culture control (TSB), were included. After the serial doubling dilution method was completed, the 0.5 McFarlands turbidity standard was added, and the plates were tightly sealed and incubated accordingly (bacteria were incubated at 37°C for 24 h and yeasts were incubated at 37°C for 48 h). The *p*-iodonitrotetrazolium violet (0.4 mg/mL) (Sigma–Aldrich, Johannesburg, South Africa) indicator was added to all the wells at 40 µL and left until the culture control turned purple/pink in the presence of microbial growth, with MIC readings taken thereafter at the lowest concentration to inhibit microbial growth (the first well had no pink-purple coloring from the indicator) [34]. All individual samples and combinations were tested in duplicate or triplicate when needed.

4.3. Fractional Inhibitory Concentration Index

The sum of the fractional inhibitory concentration index (ΣFIC) was calculated according to [31], using the equation $\Sigma FIC = FIC^{(i)} + FIC^{(ii)}$, where (a) represents the plant sample and (b) the conventional antimicrobial sample:

$$FIC^{(i)} = \frac{\text{MIC (a) in combination with (b)}}{\text{MIC (a) independently}}$$

$$FIC^{(ii)} = \frac{\text{MIC (b) in combination with (a)}}{\text{MIC (b) independently}}$$

The interactions with definitive ΣFIC values (MIC endpoints reached) were classified as synergistic ($\Sigma FIC \leq 0.5$), additive ($\Sigma FIC > 0.5–1.0$), noninteractive ($\Sigma FIC > 1.0$ and ≤ 4.0) or antagonistic ($\Sigma FIC > 4.0$) [34]. Since combinations demonstrating synergy or antagonism are highly relevant to antimicrobial treatment outcomes, these two classifications were the focus of this study [6].

4.4. Toxicity Studies

Only synergistic antimicrobial combinations were tested for toxicity to determine whether the observed synergism was due to enhanced antimicrobial activity or was simply reflective of toxicity. Tropic Marine® Sea Salt (32 g) was dissolved in 500 mL of distilled water to prepare artificial saltwater for the addition of dried brine shrimp (*Artemia franciscana*) larvae (0.5 g) (Ocean Nutrition™, Cape Town, South Africa). The incubation conditions included constant light (LED bulb, 220–240 V), rotary pump aeration (Kiho), and maintenance at 25°C for 24–48 h [35].

After hatching, 400 µL of saltwater containing live brine shrimp was added to each well of a 48-well sterile microtiter plate. Subsequently, 400 µL of the sample (individual plant extracts, antimicrobials, or their combinations) was prepared at 2 mg/mL in sterile water (aqueous extracts) or 2% *v/v* dimethyl sulfoxide (DMSO) (organic extracts) and added to the wells in triplicate, resulting in a final test concentration of 1 mg/mL [35–36]. The negative control consisted of 32 g/L saltwater, while the positive control included 1.6 mg/mL potassium dichromate (Sigma–Aldrich, Johannesburg, South Africa).

Dead brine shrimp were excluded from the mortality calculations by observing the plates under a light microscope (Leica DMi1, 40× magnification) immediately after sample addition (time 0). Mortality assessments were further conducted after 24 h and 48 h. Following the final observation, 50 µL of glacial acetic acid (100% *v/v*; Saarchem, Johannesburg, South Africa) was added to each well to determine the final mortality. Samples causing greater than 50% mortality were classified as toxic [32,33].

5. Conclusions

Synergistic (5.8% plant/antibiotic; 1.7% plant/antifungal) and non-toxic combinations require further *in vivo* interactive validation. Only then can combination therapy be promoted in a clinical setting; alternatively, combinations can be transitioned into formulation studies. The three concerning toxicity results for plant/antimicrobial combinations require further dose–response analysis to determine toxic doses. The considerable antagonism of 54.6% for plant/antibiotic combinations and 58.6% for plant/antifungal combinations found in this study raises concerns for patient safety if considered for use clinically. Furthermore, a similar prevalence of antagonism for plant/antimicrobial combinations was reported in our previous study with JHB plants. This demonstrates a wider distribution of antagonism, which potentially translates into adverse clinical effects and treatment failure among patients receiving both traditional and conventional antimicrobial therapies [6]. While these plant materials are being sold in an informal market, where there is no South African Health Products Regulatory Authority (SAHPRA) regulation of these product sales in South Africa, attention should be given to stricter guidelines for use in future. Consultation between traders and authoritative bodies could be implemented to advise against concurrent use with prescription drugs.

The findings from this study, therefore, serve to inform future research, aiming to discern the potential risks posed by antagonistic interactions. Furthermore, this study sheds light on possible beneficial synergistic interactions, offering valuable insights into later formulation studies aimed at addressing the ongoing antimicrobial resistance crisis.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1. Additional files in the form of tables (Tables S1–S4) and figures (Figures S1– S19) can be found in SM file. Table S1: The MIC values of plant extracts (mg/ml) and conventional antibiotics (µg/ml) when tested individually against ESKAPE pathogens; Table S2: MIC values for plant extracts (mg/ml) and conventional antifungals when tested individually against yeasts; Table S3: Average mortality (%) rates in the BSLA of individual plant samples proving synergistic in various combinations; Table S4: Medicinal plants procured from the Warwick traditional medicine market in Durban, KZN. Figure S1*: Acorus calamus* combined with antibiotics against ESKAPE pathogens; Figure S2: *Artemisia afra* combined with antibiotics against ESKAPE pathogens; Figure S3: *Berchemia discolor* combined with antibiotics against ESKAPE pathogens; Figure S4: *Callilepis laureola* combined with antibiotics against ESKAPE pathogens; Figure S5: *Elaeodendron transvaalense* combined with antibiotics against ESKAPE pathogens; Figure S6: *Gunnera perpensa* combined with antibiotics against ESKAPE pathogens; Figure S7: *Hydnora africana* combined with antibiotics against ESKAPE pathogens; Figure S8: *Jatropha zeyheri* combined with antibiotics against ESKAPE pathogens; Figure S9: *Lippia javanica* combined with antibiotics against ESKAPE pathogens; Figure S10: *Rapanea melanophloeos* combined with antibiotics against ESKAPE pathogens; Figure S11: *Rauvolfia caffra* combined with antibiotics against ESKAPE pathogens; Figure S12: *Rhoicissus tridentata* combined with antibiotics against ESKAPE pathogens; Figure S13: *Sansevieria hyacinthoides* combined with antibiotics against ESKAPE pathogens; Figure S14: *Senecio oxyriifolius* combined with antibiotics against ESKAPE pathogens; Figure S15: *Senecio serratuloides* combined with antibiotics against ESKAPE pathogens; Figure S16: *Strychnos henningsii* combined with antibiotics against ESKAPE pathogens; Figure S17: *Warburgia salutaris* combined with antibiotics against ESKAPE pathogens; Figure S18: Plant extracts and conventional antifungals against *Candida albicans*; Figure S19: Plant extracts and conventional antifungals against *Candida glabrata*.

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Abbreviations

The following abbreviations are used in this manuscript:

MIC	Minimum Inhibitory Concentration
ΣFIC	Sum of the Fractional Inhibitory Concentration
KZN	KwaZulu-Natal
JHB	Johannesburg
SAHPRA	South African Health Products Regulatory Authority

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Supplementary Materials

Table S1. The MIC values of plant extracts (mg/ml) and conventional antibiotics (μg/ml) when tested individually against ESKAPE pathogens.

Plant	*E. faecium* ATCC 27270		*S. aureus* ATCC 25923		*K. pneumoniae* ATCC 13883		*A. baumannii* ATCC 19606		*P. aeruginosa* ATCC 27853		*E. cloacae* NCTC 13406	
	AE	OE	AE	OE	AE	OE	AE	OE	AE	OE	AE	OE
Acorus calamus	>8.0	>8.0	>8.0	4.0	>8.0	>8.0	>8.0	4.0	8.0	>8.0	>8.0	>8.0
Artemisia afra	>8.0	4.0	>8.0	3.0	>8.0	>8.0	>8.0	1.0	4.0	2.0	>8.0	2.0
Berchemia discolor	>8.0	4.0	>8.0	4.0	>8.0	2.7	>8.0	1.7	8.0	1.0	2.0	0.8
Callilepis laureola	>8.0	>8.0	>8.0	4.0	>8.0	>8.0	8.0	2.7	>8.0	4.0	>8.0	4.0
Elaeodendron transvaalense	>8.0	2.0	>8.0	1.0	>8.0	1.5	>8.0	0.4	2.0	0.8	2.0	1.7
Gunnera purpensa	>8.0	4.0	>8.0	1.8	0.5	2.7	8.0	1.0	8.0	0.8	8.0	1.3
Hydnora africana	>8.0	4.0	>8.0	4.0	>8.0	>8.0	8.0	2.0	>8.0	1.7	>8.0	2.7
Jatropha zeyheri	>8.0	4.0	>8.0	7.0	>8.0	>8.0	>8.0	2.0	>8.0	2.0	>8.0	>8.0
Lippia javanica	>8.0	4.0	>8.0	4.0	8.0	8.0	>8.0	2.0	8.0	2.7	>8.0	4.0
Rapanea melanophloeos	>8.0	4.0	>8.0	2.0	8.0	2.0	>8.0	1.3	5.3	1.7	4.0	2.0
Rauvolfia caffra	>8.0	4.0	>8.0	>8.0	6.0	5.3	8.0	2.7	8.0	4.0	8.0	3.3
Rhoicissus tridentata	>8.0	8.0	>8.0	>8.0	>8.0	>8.0	>8.0	2.0	>8.0	2.7	8.0	>8.0
Sansevieria hyacinthoides	>8.0	8.0	>8.0	8.0	>8.0	>8.0	>8.0	2.0	>8.0	4.0	>8.0	>8.0
Senecio oxyriifolius	>8.0	>8.0	>8.0	4.0	8.0	>8.0	>8.0	2.0	>8.0	8.0	>8.0	>8.0
Senecio serratuloides	>8.0	4.0	>8.0	4.0	6.0	8.0	6.7	1.0	5.3	1.7	>8.0	1.7
Strychnos henningsii	>8.0	6.0	>8.0	8.0	8.0	>8.0	>8.0	2.0	8.0	4.0	8.0	8.0
Warburgia salutaris	>8.0	4.0	>8.0	2.5	>8.0	6.0	>8.0	2.7	>8.0	3.3	>8.0	2.0
Antibiotic												
Amoxicillin	0.04		0.08		0.04		0.04		1.80		0.31	
Azithromycin	1.88		1.88		0.39		0.64		0.04		2.50	
Ciprofloxacin	0.04		0.08		0.04		0.04		1.80		0.31	
Doxycycline	1.88		1.88		1.88		0.24		1.88		1.88	

(AE) aqueous extract. (OE) organic extract. EUCAST [7]-CLSI [8] breakpoints: amoxicillin 0.001 – 8.00 μg/ml; azithromycin 0.01 – 4.0 μg/ml; ciprofloxacin 0.001 – 4.0 μg/ml; doxycycline 0.1 – 4.0 μg/ml. Negative control of acetone in water >8.0 mg/ml. Positive control of ciprofloxacin (0.01 mg/ml) 0.04 – 1.8 μg/ml. Culture control >8.0 mg/ml.

Table S2. MIC values for plant extracts (mg/ml) and conventional antifungals when tested individually against yeasts.

Plant	*C. albicans* ATCC 10231		*C. glabrata* ATCC 90030	
	AE	OE	AE	OE
Acorus calamus	>8.0	3.0	>8.0	6.0
Artemisia afra	>8.0	2.0	>8.0	8.0
Berchemia discolor	>8.0	1.0	>8.0	6.0
Callilepis laureola	>8.0	1.5	>8.0	>8.0
Elaeodendron transvaalense	8.0	2.0	>8.0	3.0
Gunnera purpensa	8.0	1.0	8.0	1.0
Hydnora africana	>8.0	3.0	>8.0	3.0
Jatropha zeyheri	>8.0	1.0	>8.0	1.0
Lippia javanica	8.0	2.0	8.0	2.0
Rapanea melanophloeos	8.0	1.0	8.0	2.0
Rauvolfia caffra	8.0	1.0	8.0	8.0
Rhoicissus tridentata	8.0	2.0	8.0	>8.0
Sansevieria hyacinthoides	>8.0	8.0	>8.0	4.0
Senecio oxyriifolius	>8.0	1.0	>8.0	2.0
Senecio serratuloides	>8.0	2.0	>8.0	2.0
Strychnos henningsii	>8.0	1.0	>8.0	1.0
Warburgia salutaris	>8.0	8.0	>8.0	8.0
Antifungal				
Fluconazole	3.13		1.56	
Nystatin	5.21		6.25	

(AE) aqueous extract. (OE) organic extract. EUCAST [7]-CLSI [8] breakpoints: fluconazole 0.001 - ≤ 16.00 μg/ml; nystatin 0.13 – 8.00 μg/ml. Negative control of acetone in water >8.0 mg/ml. Positive control of nystatin (0.1 mg/ml) 5.21 – 6.25 μg/ml. Culture control >8.0 mg/ml.

Table S3. Average mortality (%) rates in the BSLA of individual plant samples proving synergistic in various combinations.

Extract type	Plant	Average mortality (%) ± S.D. at 24 h	Average mortality (%) ± S.D. at 48 h
Organic extracts	*Acorus calamus*	**100.00 ± 0.00**	**100.00 ± 0.00**
	Artemisia afra	36.11 ± 55.49	45.37 ± 47.82
	Callilepis laureola	0.00 ± 0.00	8.50 ± 9.15
	Elaeodendron transvaalense	**75.38 ± 3.85**	**55.75 ± 3.35**
	Hydnora africana	0.00 ± 0.00	0.00 ± 0.00
	Jatropha zeyheri	7.83 ± 0.61	30.37 ± 10.32
	Rapanea melanophloeos	0.00 ± 0.00	9.38 ± 4.88
	Rauvolfia caffra	0.00 ± 0.00	3.46 ± 3.58
	Senecio serratuloides	0.00 ± 0.00	2.56 ± 4.44
	Strychnos henningsii	0.00 ± 0.00	0.00 ± 0.00
Aqueous extracts	*Berchemia discolor*	0.00 ± 0.00	2.22 ± 3.85
	Gunnera perpensa	0.00 ± 0.00	1.15 ± 1.99
	Rapanea melanophloeos	0.00 ± 0.00	0.00 ± 0.00

Bold values - toxicity observed. Negative control of salt water (32 g/l) (average mortality of 0.0 ± 0.00%). Positive control of potassium dichromate (1.6 mg/ml) (average mortality of 100.0 ± 0.00%).

Table S4. Medicinal plants procured from the Warwick traditional medicine market in Durban, KZN.

Plant species collected	Vernacular and common name	Voucher number	Plant part	Traditional uses for the medicinal plant
Acorus calamus L.	*Ikalimuzi* (Z); flag root, sweet flag (E); *makkalmoes* (A)	ZB306	Roots	Digestive complaints, such as dysentry [34-36].
Artemisia afra Jacq. ex Willd.	*Umhlonyane* (Z); African wormwood (E); *wildeals* (A)	ZB315	Leaves and stems	Respiratory tract infections, gastrointestinal complaints and intestinal worms [34-36].
Berchemia discolor (Klotzsch) Hemsl.	*Uvuka* (Z); brown ivory, bird plum, mountain date, wild almond (Eng); *bruin-ivoor, voëlpruim* (A)	ZB320	Bark	Wounds and other skin infections [34-36].
Callilepis laureola DC.	*Impila* (Z); ox-eye daisy (E)	ZB310	Bark	Gastrointestinal complaints [34-36].
Elaeodendron transvaalense (Burtt Davy) R.H.Archer	*Ingwavuma* (Z); bushveld saffron (E); *bosveld-saffraan, lepelhout* (A)	ZB319	Bark	Gastrointestinal complaints and fever, sexually transmitted infections [34-36].
Gunnera perpensa L.	*Ugobho* (Z); river pumpkin (E); *rivierpampoen* (A)	ZB309	Roots	Urinary tract and skin infections [34-36].
Hydnora africana Thunb.	*Umavumbuka* (Z) jackal food (E); *jakkalskos, bobbejaankos* (A)	ZB308	Bark	Gastrointestinal complaints, skin infections, urinary tract and bladder infections [34-36].
Jatropha zeyheri Sond.	*Ugodide* (Z); *verfbol* (A)	ZB312	Bark	Skin ailments and wounds, sexually transmitted infections, urinary tract infections and eye infections [34-36].
Lippia javanica (Burm.f.) Spreng	*Umsuzwane* (Z); fever tea; fever tree, lemon bush (E); *lemoenbossie* (A)	ZB316	Bark and stems	Respiratory and gastrointestinal infections, skin infections, wounds, malaria [34-36].
Rapanea melanophloeos (L.) Mez	*Umaphipha* (Z); Cape Beech (E); *boekenhout, beukenhout* (A)	ZB321	Bark	Respiratory complaints and stomach conditions [34-36].
Rauvolfia caffra Sond.	*UmHlambamanzi, umKhadluvungu* (Z); quinine tree (E); *kinaboom* (A); *umJelo* (X)	ZB322	Bark	Wounds and other skin infections, gastrointestinal complaints and respiratory infections [34-36].
Rhoicissus tridentata (L.f.) Wild & R.B.Drumm.	*Isinwazi* (Z); bitter grape, Bushman's grape, common forest grape (E); *bitterdruif, bobbejaantou, droog-my-keel* (A)	ZB313	Bark	Gynaecological conditions, gastrointestinal complaints, urinary tract and bladder infections [34-36].
Sansevieria hyacinthoides (L.) Druce	*Isikholokotho* (Z); mother-in-law's tongue (E*); skoonma-se-tong, wildeatel, aambeiwortel* (A)	ZB317	Roots	Intestinal parasites, fever, oral cavity infections and ear infections [34-36].
Senecio oxyriifolius DC.	*Idumbe* (Z); false nasturtium (E); *Kappertjieblaar* (A)	ZB323	Roots	Fever associated with bacterial infections [34-36].

Table S4 continued. Medicinal plants procured from the Warwick traditional medicine market in Durban, KZN.

Plant species collected	Vernacular and common name	Voucher number	Plant part	Traditional uses for the medicinal plant
Senecio serratuloides DC.	*Unsukumbili* (Z); two-day cure (E)	ZB307	Leaves and stems	Respiratory infections, skin infections and wounds, sexually transmitted infections [34-36].
Strychnos henningsii Gilg	*Umqalothi* (Z); red bitterberry, coffee hard pear, hard pear (E); *rooibitterbessie, koffiehardepeer, hardepeer* (A)	ZB318	Bark	Gynaecological complaints, wounds, respiratory tract and oral infections, gastrointestinal infections and parasitic infestation [34-36].
Warburgia salutaris (G.Bertol.) Chiov.	*Isibhaha* (Z); pepperbark tree (E)	ZB311	Bark	Respiratory tract infections, malaria, gastrointestinal complaints, sexually transmitted infections, skin infections [34-36].

A = Afrikaans; E = English; X = Xhosa; Z = Zulu. Plants were purchased from traders by Prof. S.F van Vuuren - PhD and Z. Booth - Master of Pharmacy (Department of Pharmacy and Pharmacology, University of the Witwatersrand, South Africa). Plant identification was conducted by Dr. G Khumalo – PhD (Queen Mary University of London, William Harvey Research Institute

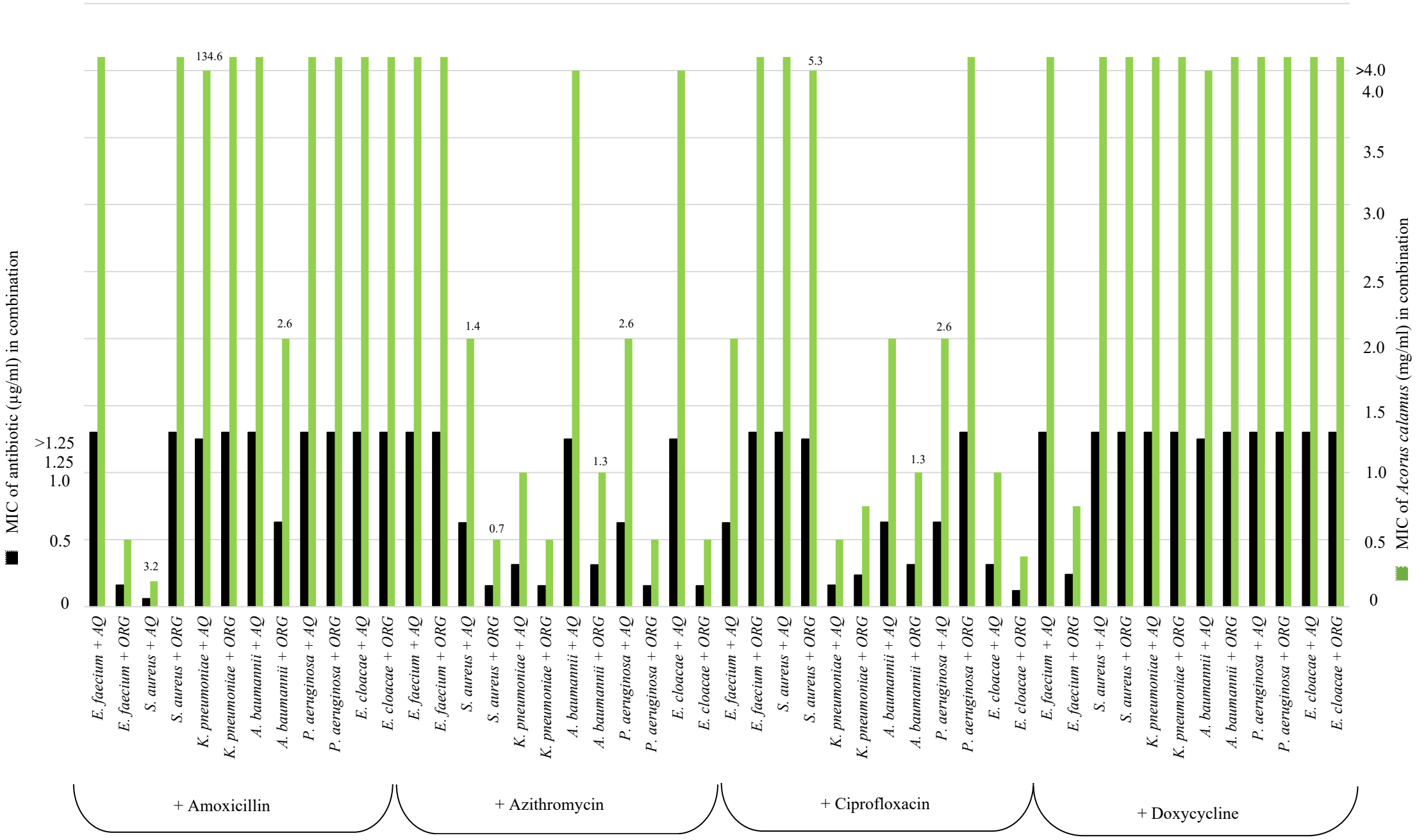


Figure S1. *Acorus calamus* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

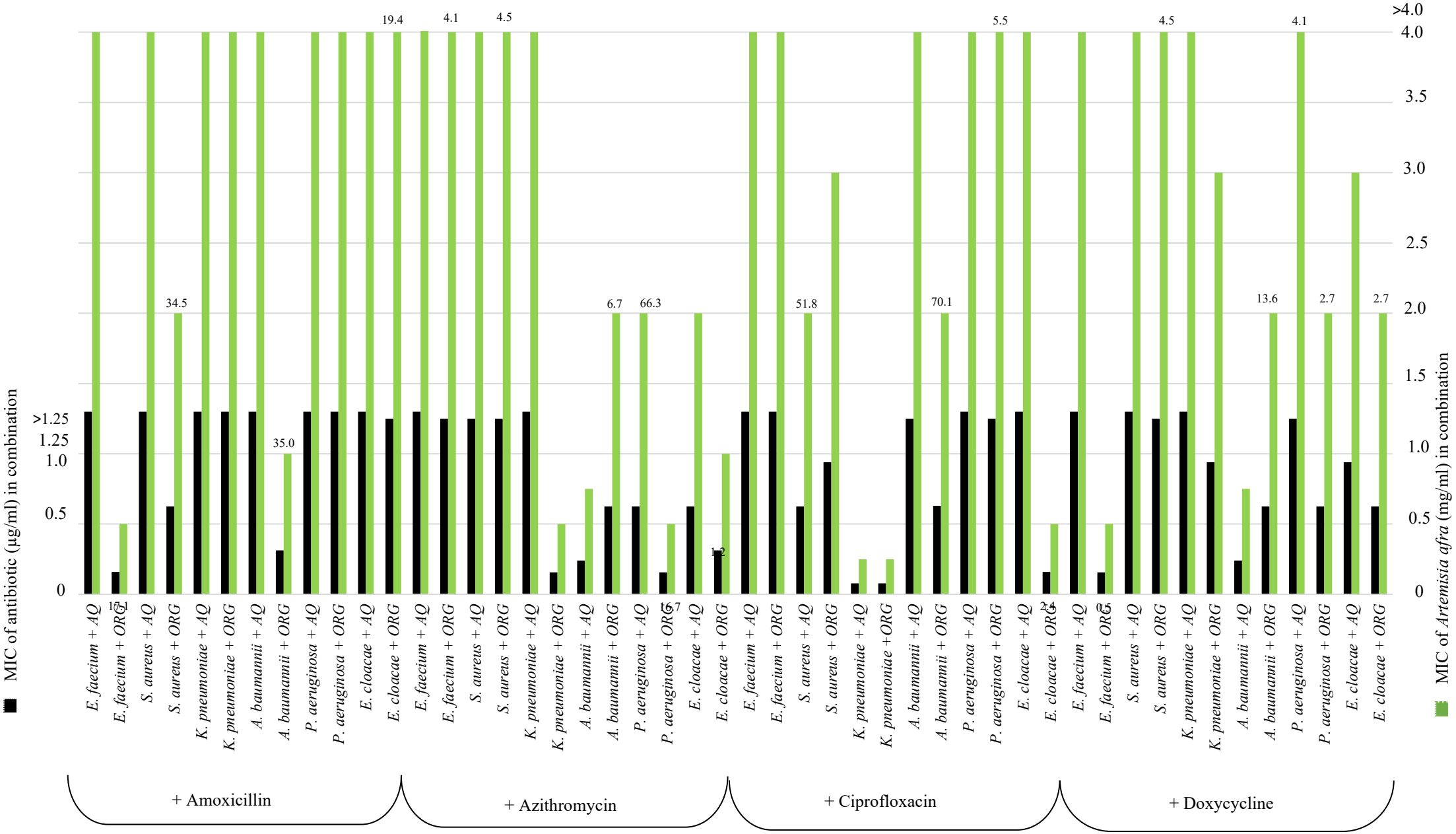


Figure S2. *Artemisia afra* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

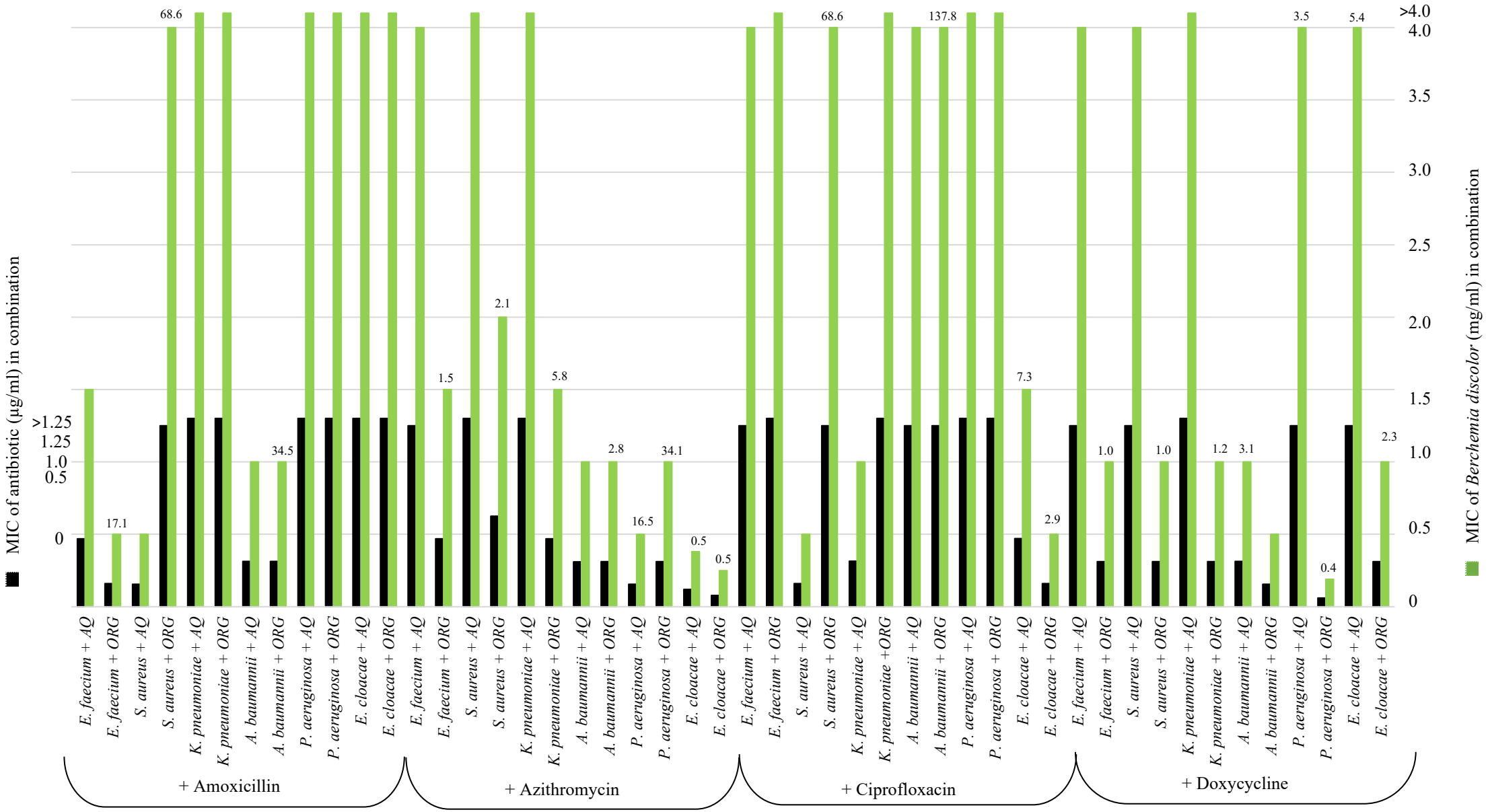


Figure S3. *Berchemia discolor* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

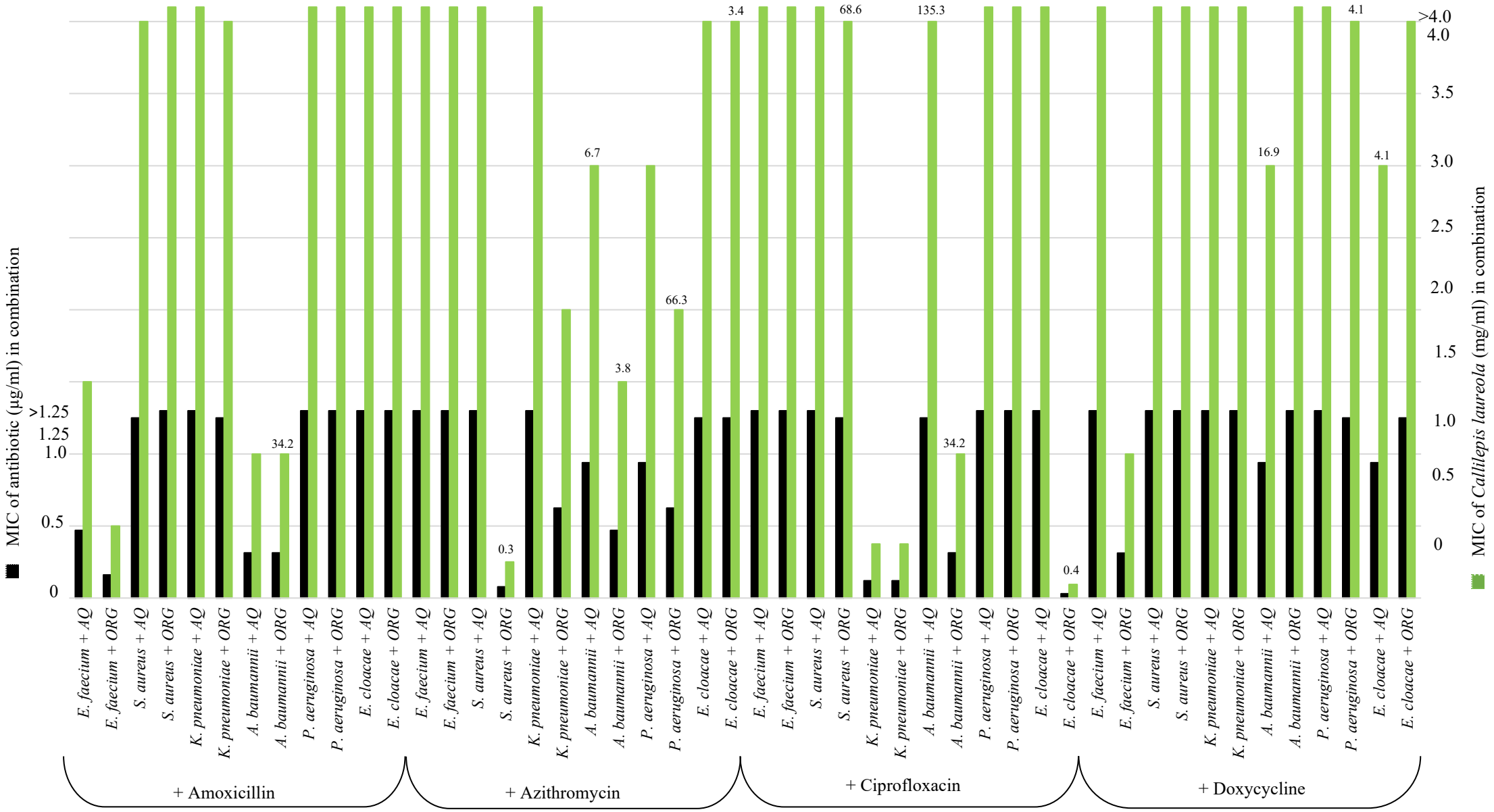


Figure S4. *Callilepis laureola* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

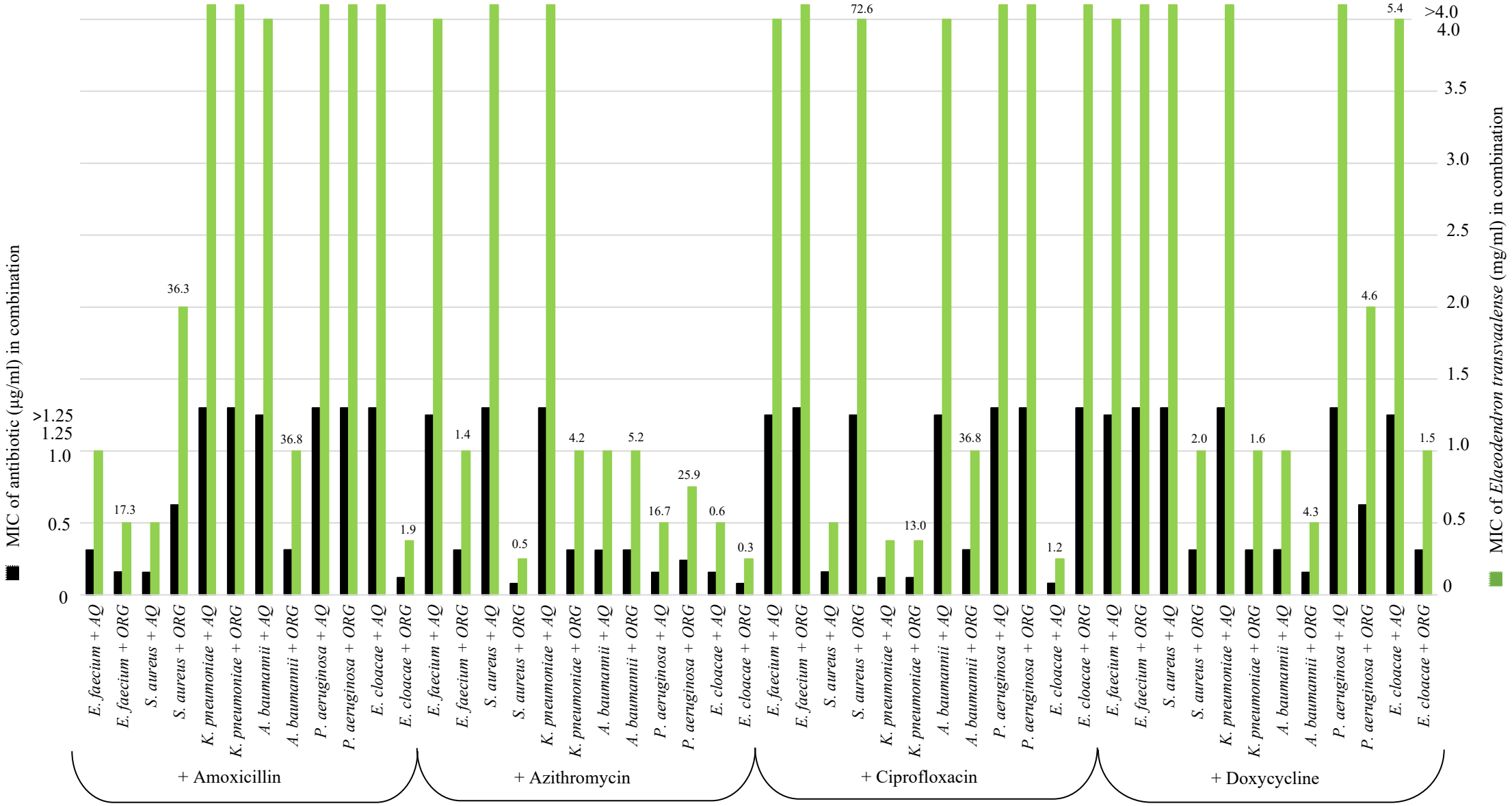


Figure S5. *Elaeodendron transvaalense* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

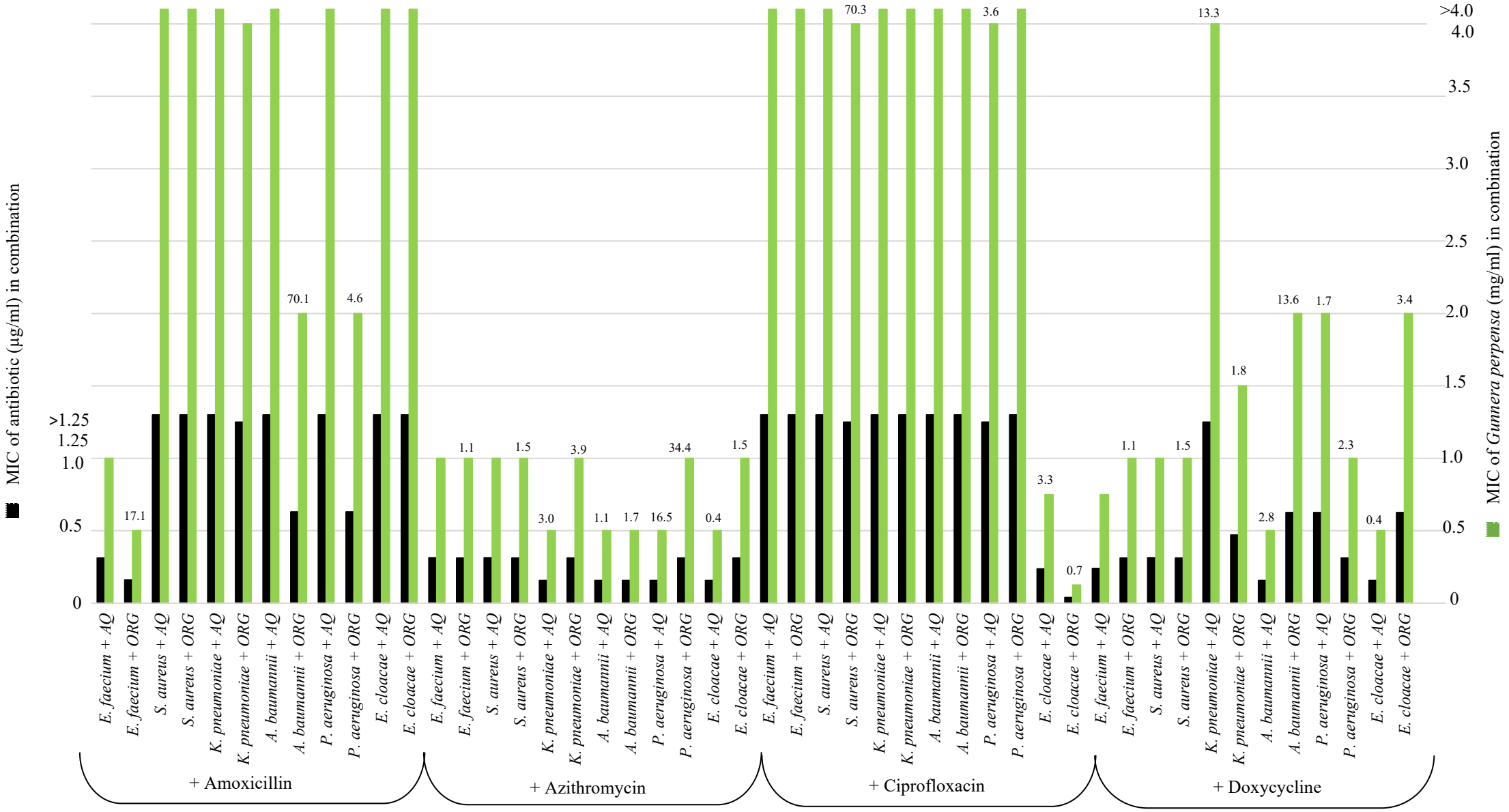


Figure S6. *Gunnera perpensa* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

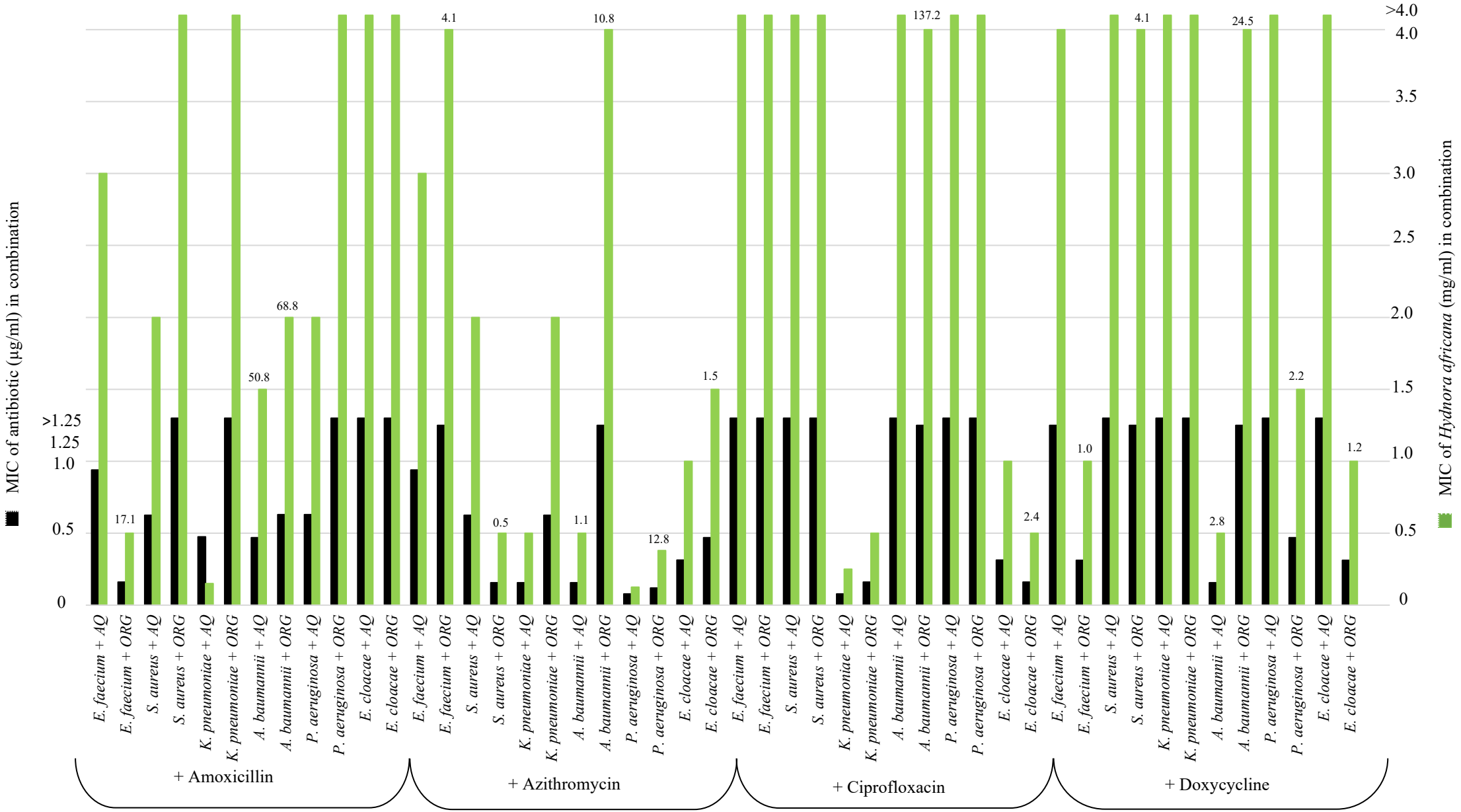


Figure S7. *Hydnora africana* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

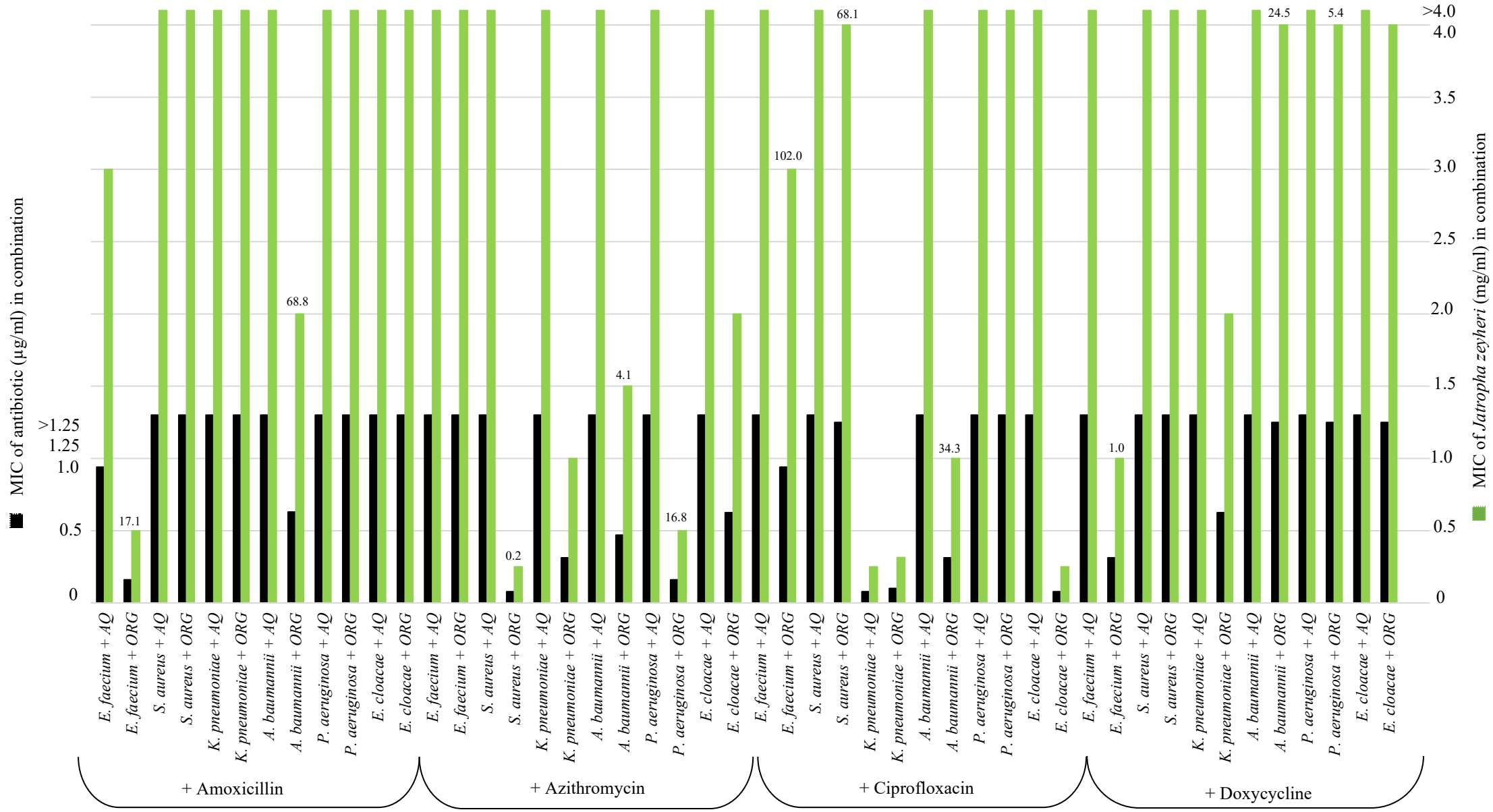


Figure S8. *Jatropha zeyheri* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

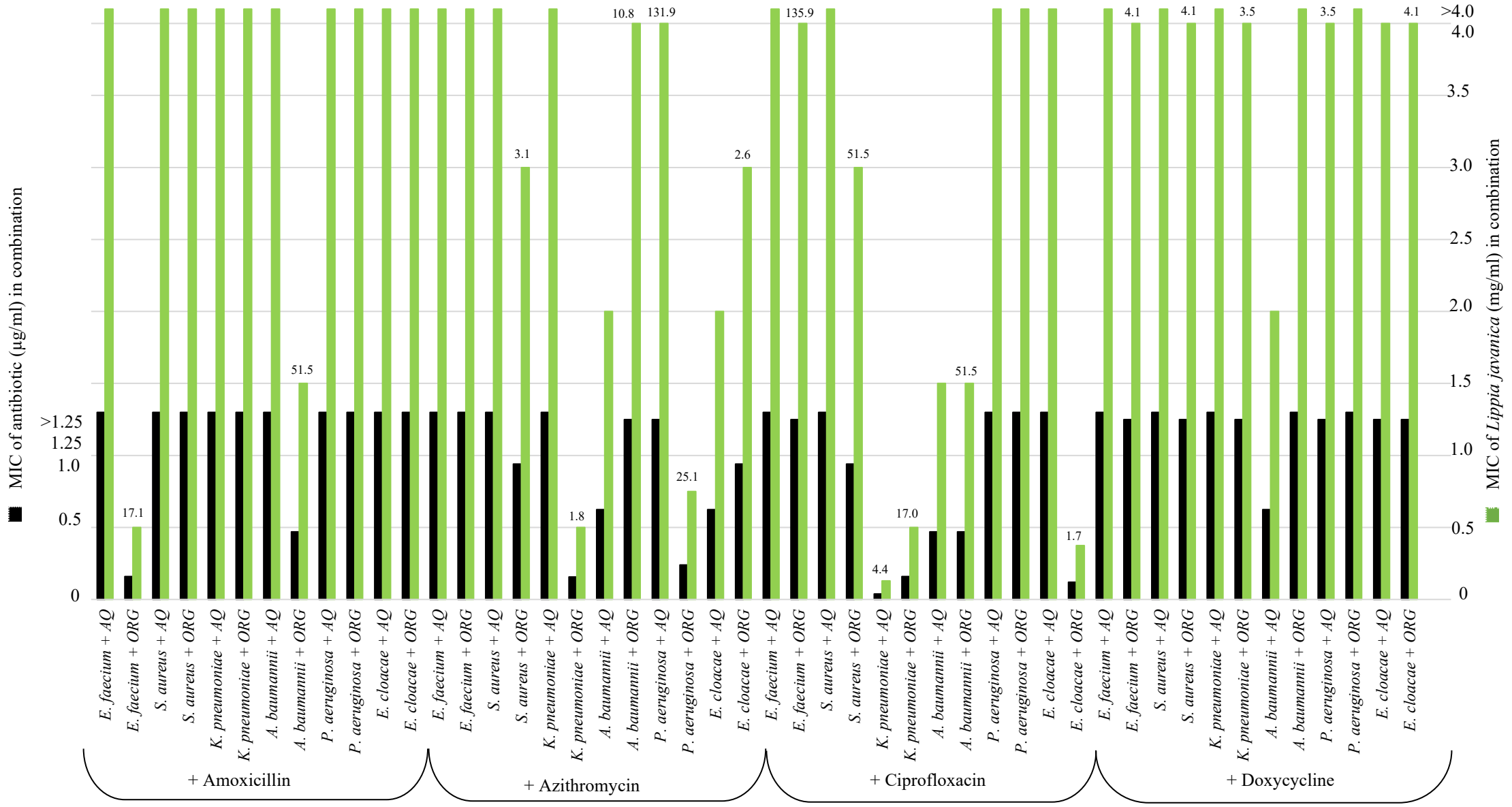


Figure S9. *Lippia javanica* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

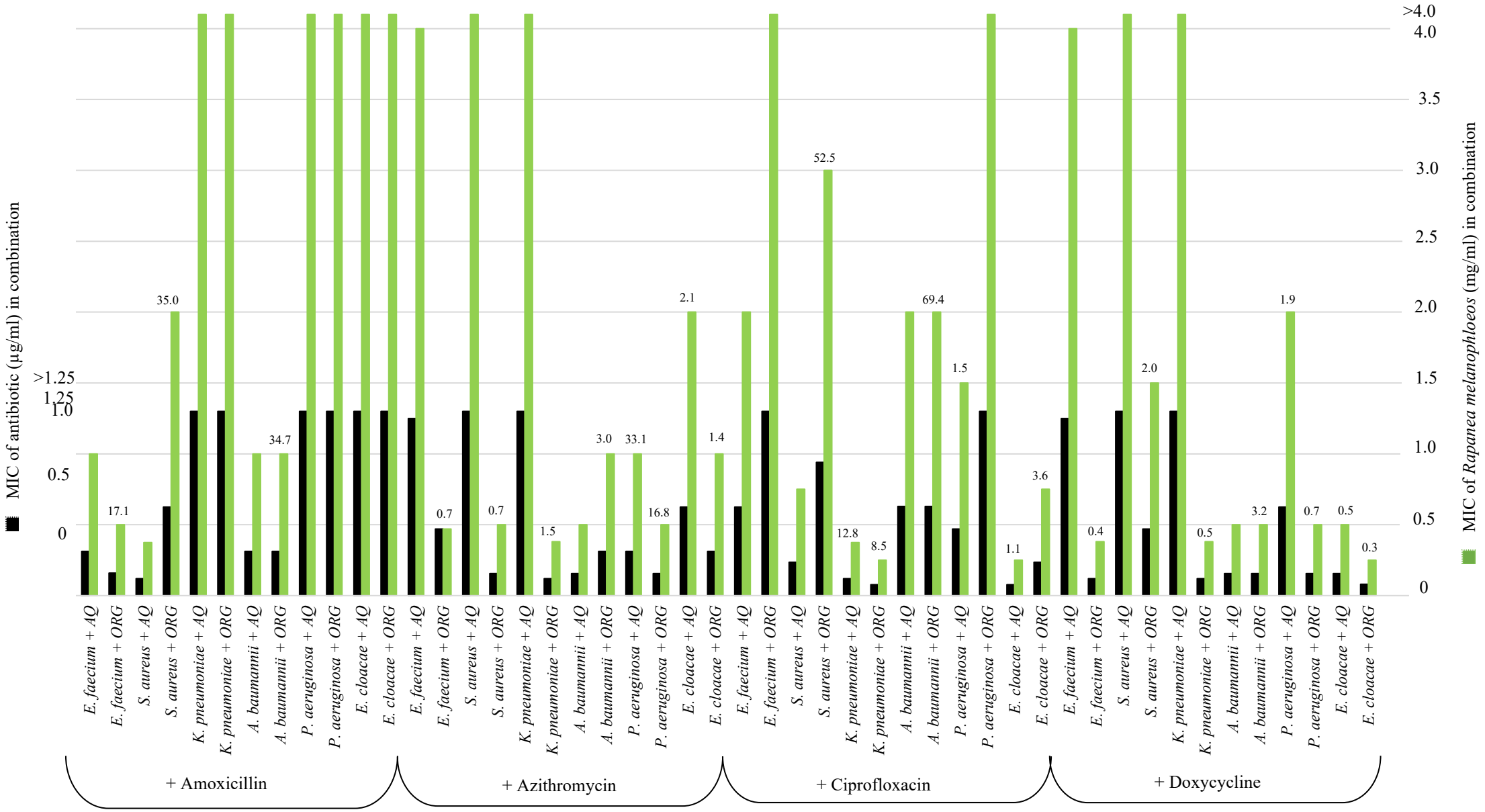


Figure S10. *Rapanea melanophloeos* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

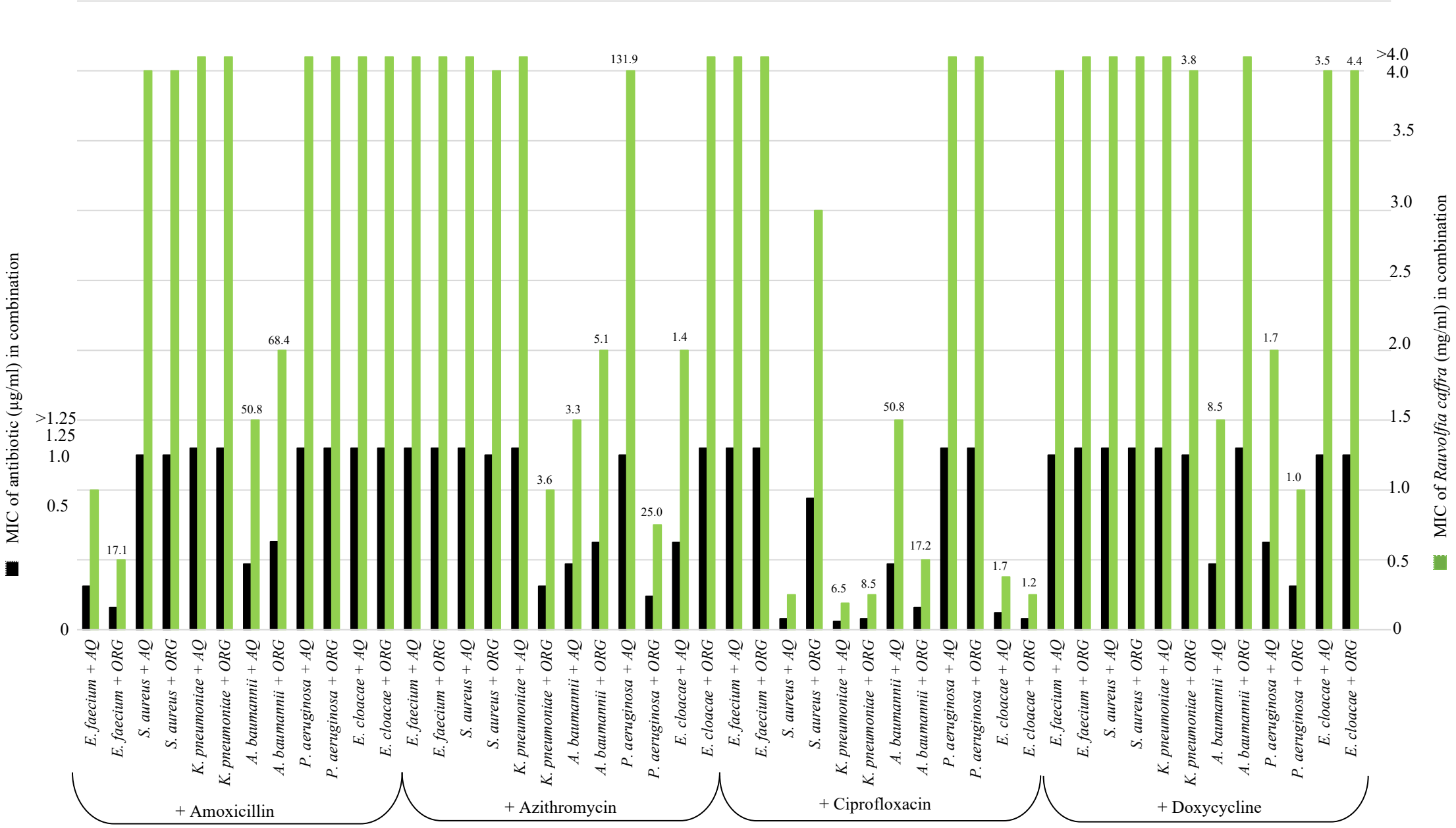


Figure S11. *Rauvolfia caffra* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

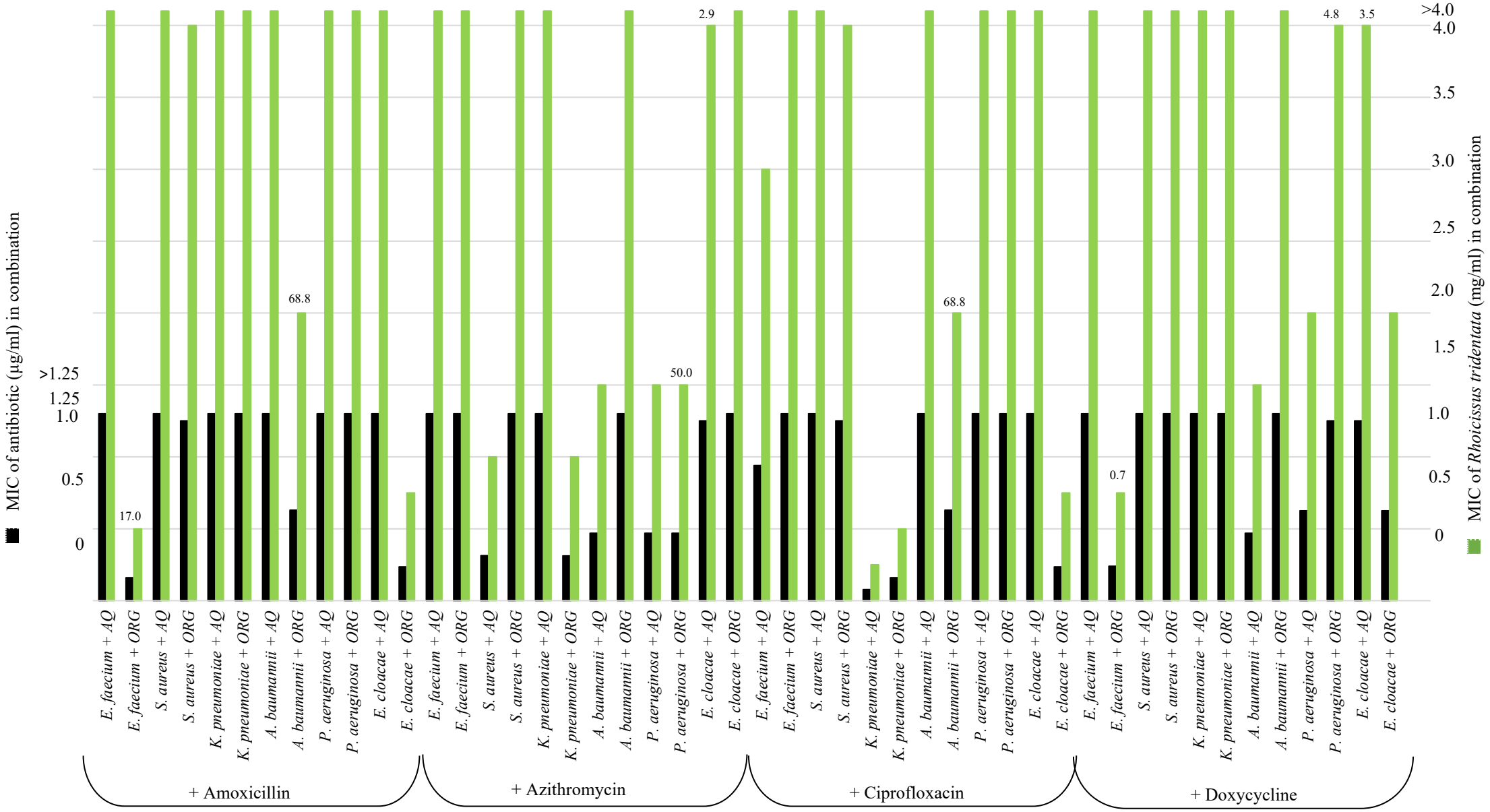


Figure S12. *Rhoicissus tridentata* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

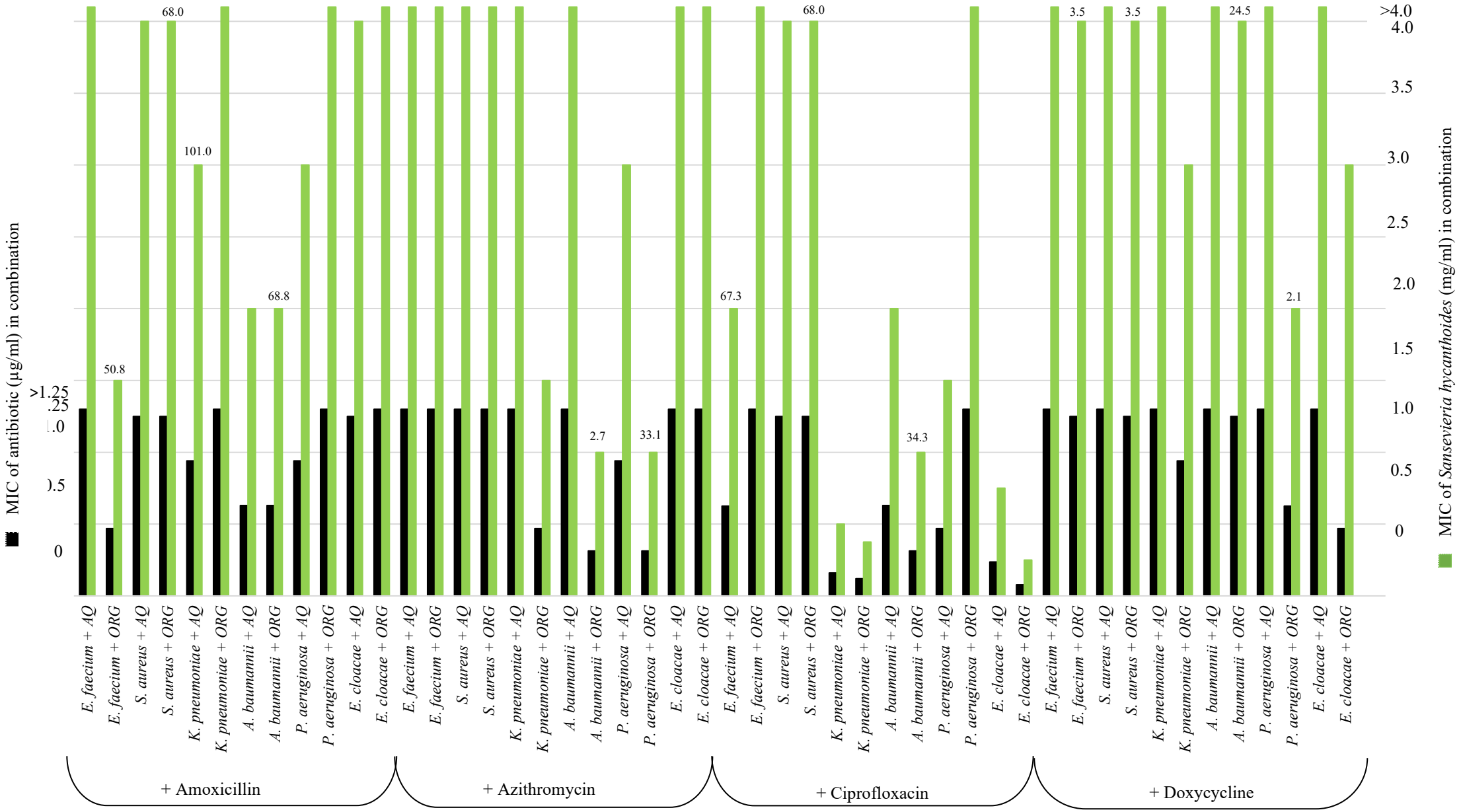


Figure S13. *Sansevieria hyacinthoides* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

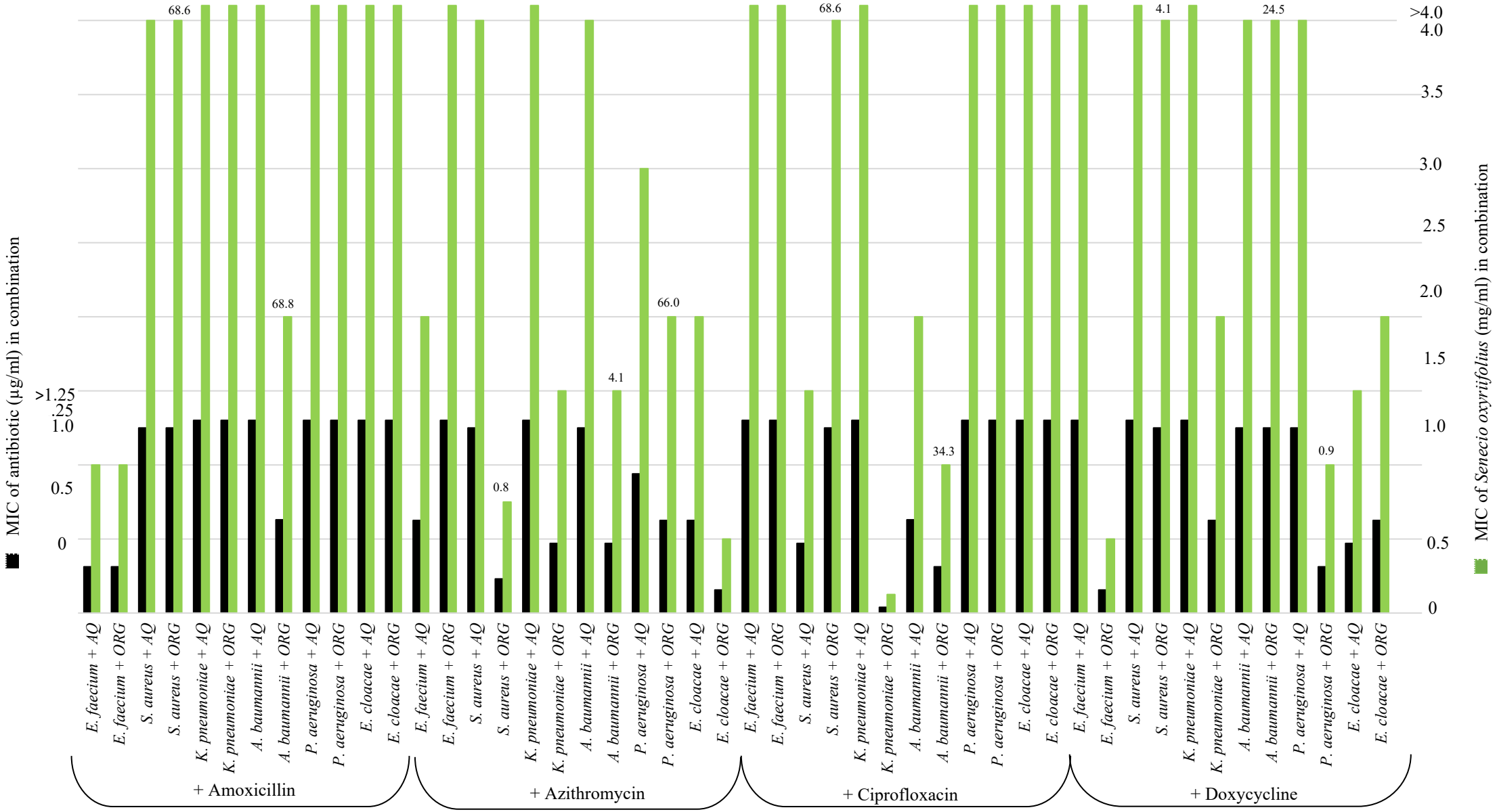


Figure S14. *Senecio oxyriifolius* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

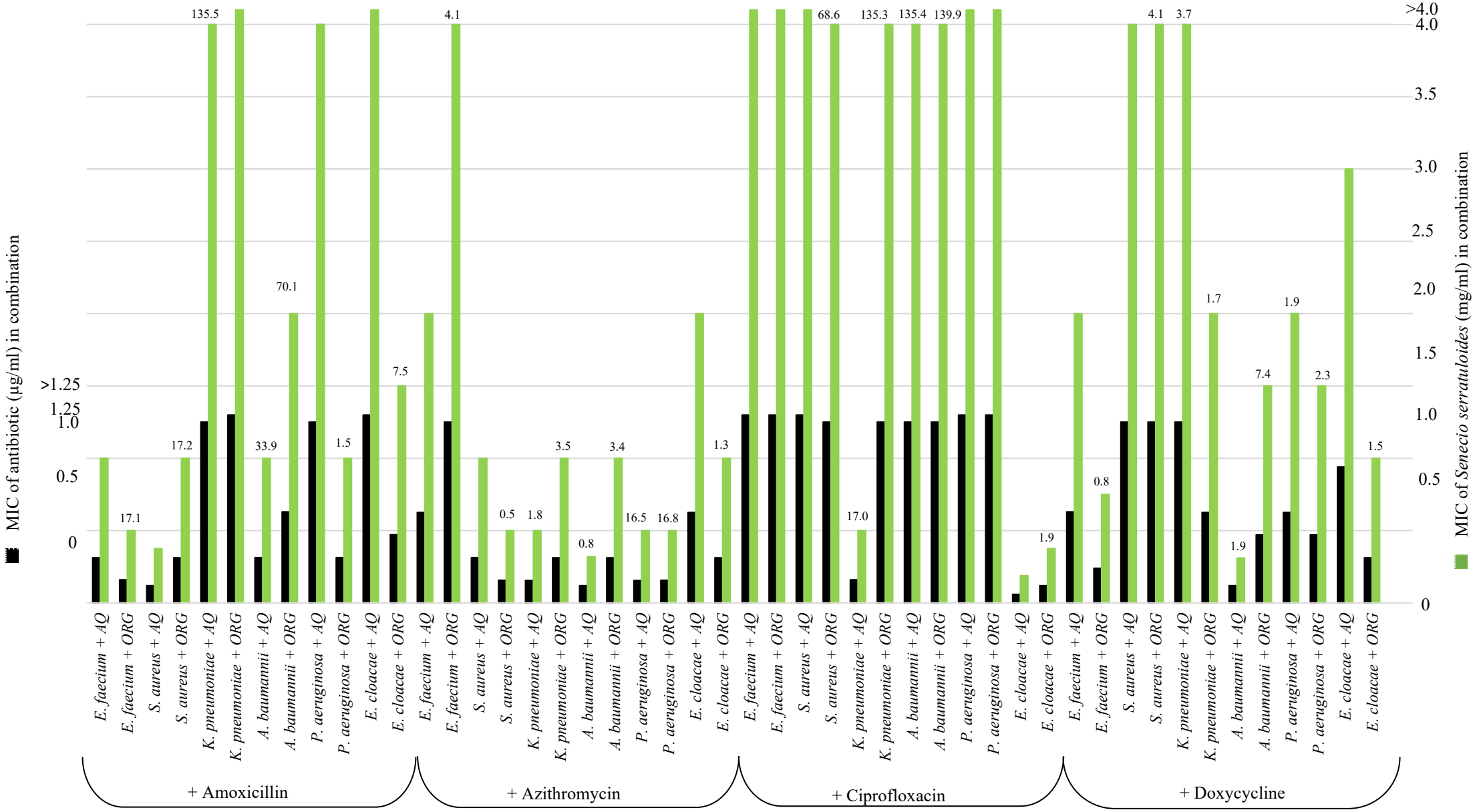


Figure S15. *Senecio serratuloides* combined with antibiotics against ESKAPE pathogens

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

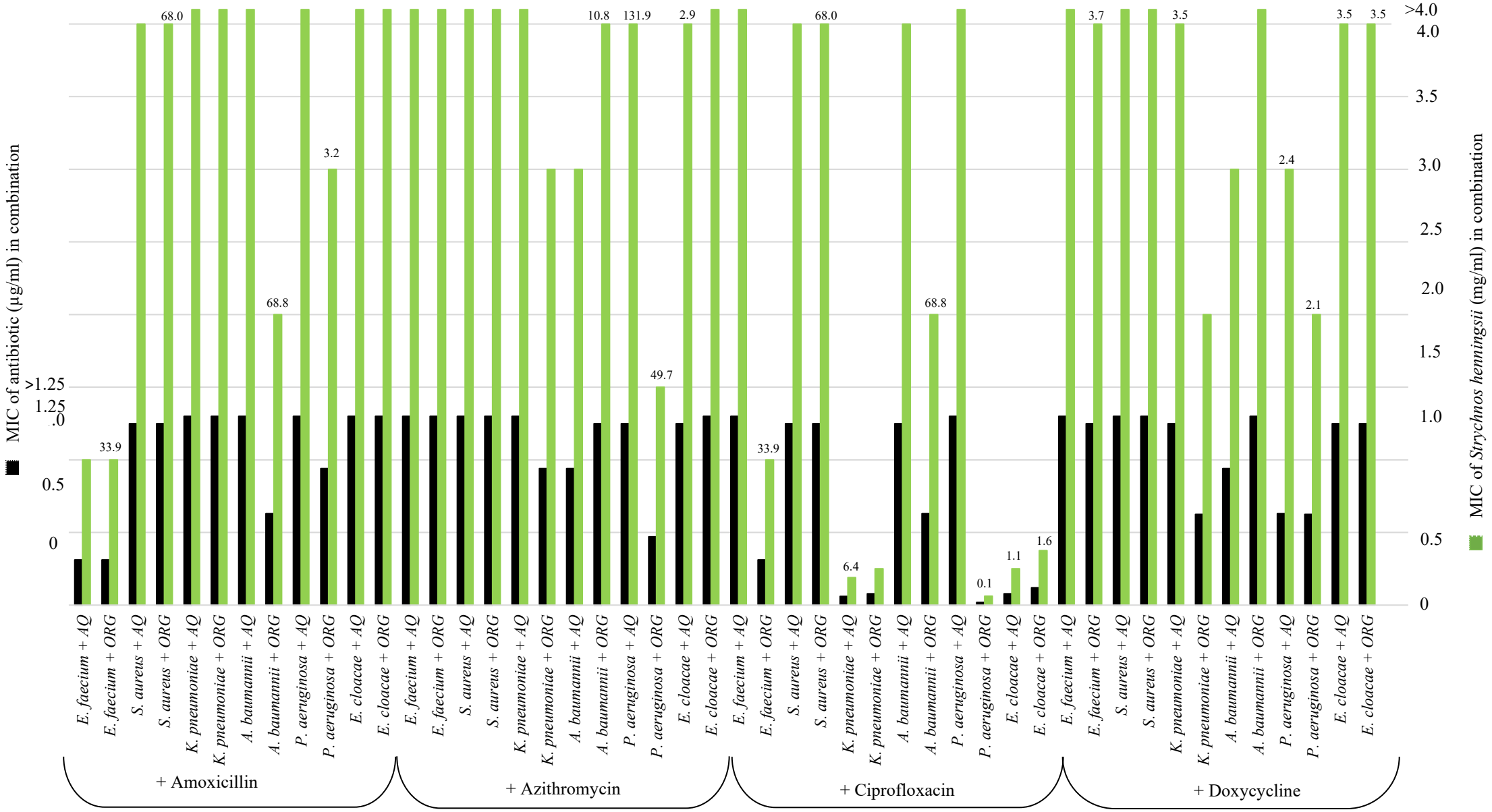


Figure S16. *Strychnos henningsii* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

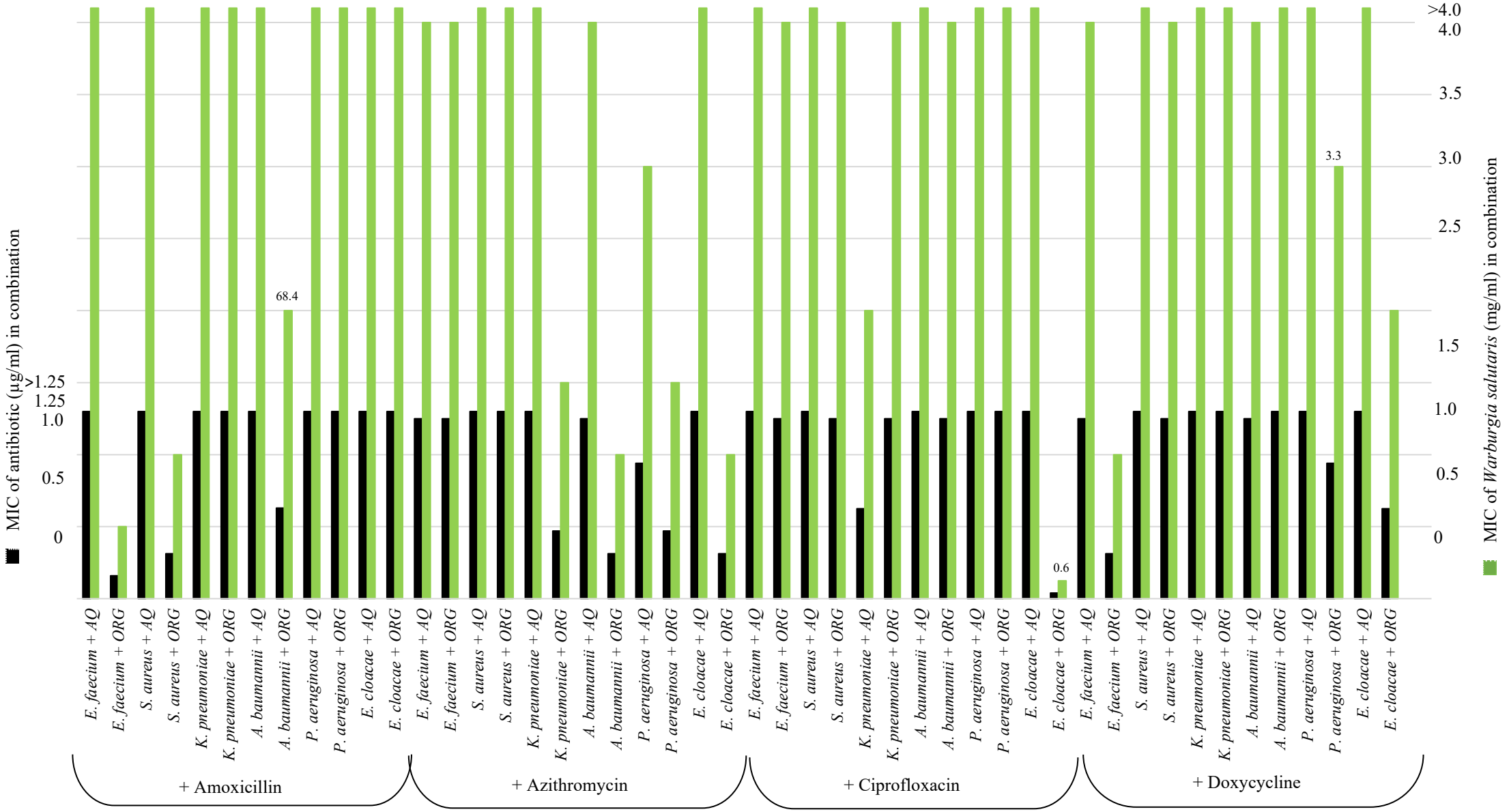


Figure S17. *Warburgia salutaris* combined with antibiotics against ESKAPE pathogens.

Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as negative control (MIC >8.0 mg/ml). Ciprofloxacin (0.01 mg.ml) as positive control (MIC 0.039 – 1.800 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

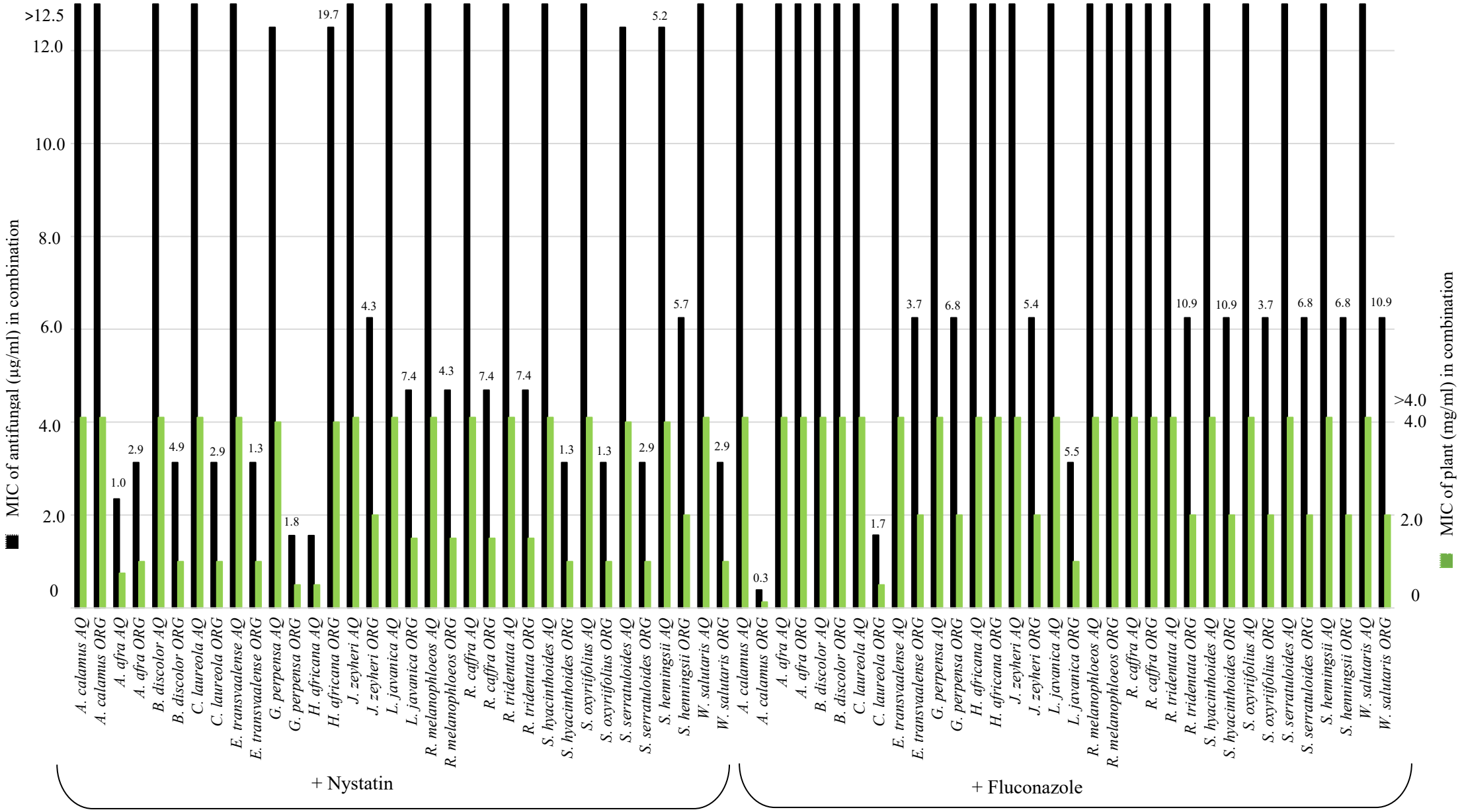


Figure S18. Plant extracts and conventional antifungals against *Candida albicans*.

Individual MIC values in tables as mg/ml. Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as the negative control (MIC >8.0 mg/ml). Nystatin (0.1 mg/ml) as the positive control (MIC 5.210 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

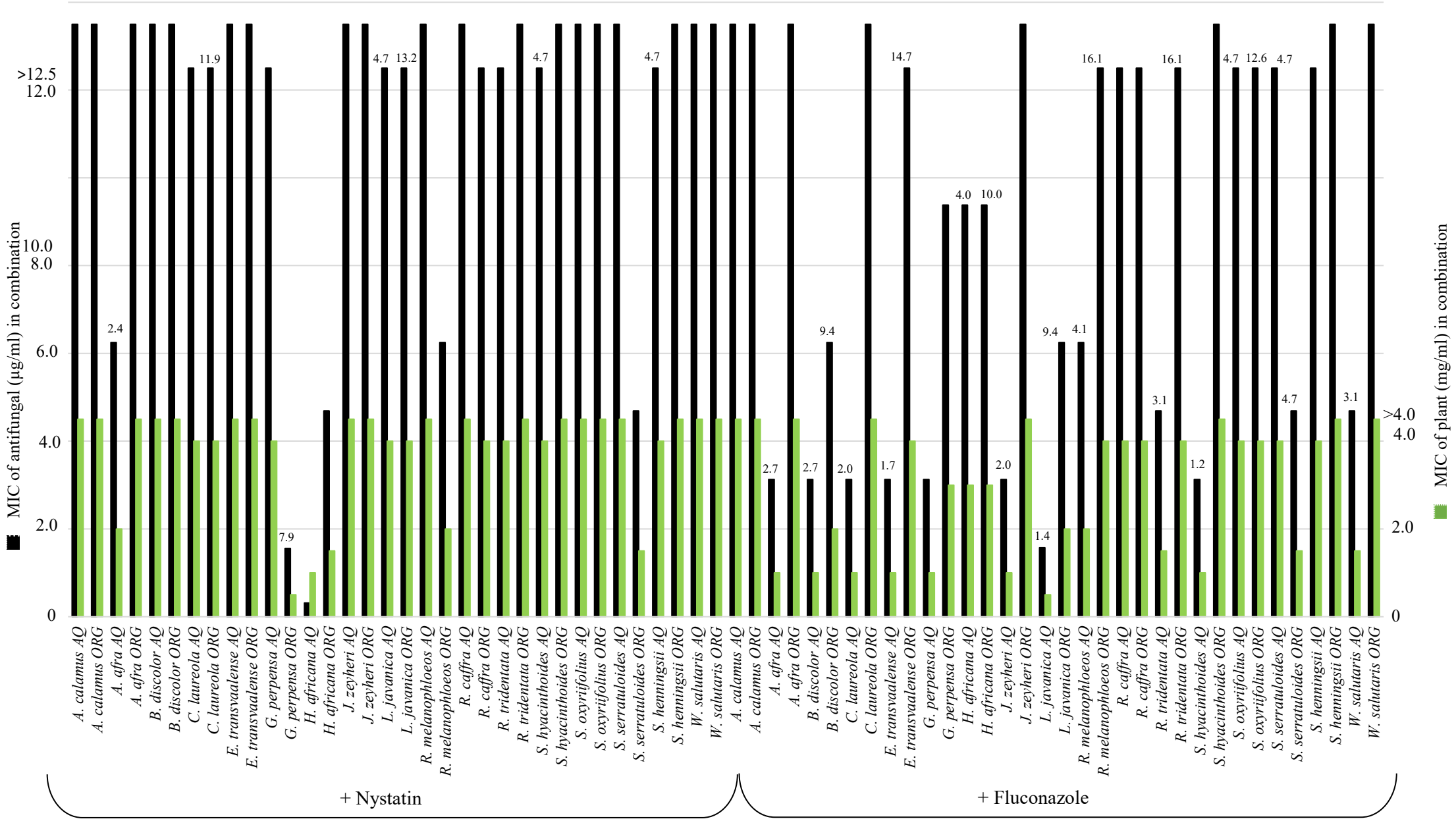


Figure S19. Plant extracts and conventional antifungals against *Candida glabrata.*

Individual MIC values in tables as mg/ml. Values indicated above columns ΣFIC could be determined. No value indicated above column ΣFIC could not be determined. (*AQ*) aqueous extract. (*ORG*) organic extract. Acetone in water (32.0 mg/ml) as the negative control (MIC >8.0 mg/ml). Nystatin (0.1 mg/ml) as the positive control (MIC 5.210 µg/ml). Culture in TSB as the culture control (MIC >8.0 mg/ml).

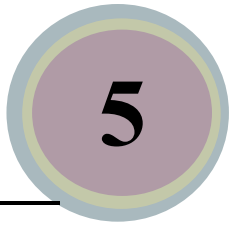
4.2. Chapter summary

Of the combinations tested, just over half were antagonistic in nature, with a slightly higher antagonism for plant: conventional antifungal combinations as opposed to the plant: conventional antibiotic combinations. Similarly, as documented in Chapter 3 for Johannesburg, this antagonism warrants concerns related to infection treatment failure and the contribution to antimicrobial resistance. Further *in vivo* studies are, however, necessary to confirm clinical translation.

Synergistic (5.8% plant:antibiotic; 1.7% plant:antifungal) and non-toxic combinations require further *in vivo* interactive validation. Only then can combination therapy be promoted in a clinical setting, or combinations be transitioned into formulation studies.

Chapter 5

Thesis summary and conclusion



5.1. Study overview

A three-phased approach was followed to achieve the aim of this study. Phase 1 served to document existing literature on antimicrobial synergistic or antagonistic combinations between (a) African natural products and conventional antimicrobials; and (b) medicinal plant extracts and conventional antimicrobials, thereby emphasising the need for further investigations, either to drive new antimicrobial development (synergism) or draw attention to harmful interactions to be cautioned against in clinical practice (antagonism), in a systematic framework.

Key findings from phase 1 of the study:

- The rise in antimicrobial resistance in Africa deems it important to identify new antimicrobials or alternative avenues to enhance antimicrobial activity of existing agents.
- Interactive profiles of combination therapies are poorly investigated, however, natural product combinations with conventional antimicrobials is arguably a potential avenue of research to assist in discovering new medicines that prove effective against resistant micro-organisms.
- Synergistic interactions are more frequently reported on, compared to antagonistic interactions. The underreporting of antagonism could potentially be due to preferential publication of positive results or lack of incentive to study harmful interactions.
- The lack of patient disclosure of natural products for infectious disease treatment, as well as the enquiry prior to prescribing or dispensing in modern healthcare facilities, has been noted.
- Africa, with its rich diversity in flora, offers the ideal opportunity for researchers to identify and develop innovative products and advantageous combination therapies to treat infections.
- Studies focusing on medicinal plant combinations with conventional antimicrobials are severely lacking.

- *In vitro* combination studies were found to focus on essential oil and isolated plant compounds with conventional antimicrobials, indicating a clear gap in research pertaining to plant extract combinations with conventional antimicrobials.
    - Further investigations are urgently needed, for foundational work aimed at establishing the safety of integrative medicine practices, since antagonism, although infrequently reported, still presented in findings and any antagonism should justify concern of safety.
    - Furthermore, toxicity studies were lacking in many studies.

Phase 2 aimed at investigating the prevalence of use of a combination of traditional and conventional medicines, amongst patients at two large public hospitals in South Africa.

Key findings from phase 2 of the study:

- The majority (94.1%) of participants indicated past and/or current use of traditional medicine.
- A greater reliance on traditional medicine was observed amongst KwaZulu-Natal (94.1%) participants compared to the study in Johannesburg (76.8%).
- A third of the study sample (31.6%) indicated concurrent use with conventional medicines.
- Participants indicated use of traditional medicine for preventing and treating of chronic diseases (60.3%), more so than for infection treatment (26.4%).
- Medicinal plants (88.3%) were prevalently used, with *Artemisia afra* Jacq. ex Willd. (African wormwood), *Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall. ("African potato"), *Aloe ferox* Mill. (bitter Aloe), *Lippia javanica* (Burm.f.) Spreng (fever tea), *Gunnera perpensa* L. (river pumpkin) and *Acorus calamus* L. (sweet-flag) frequently indicated.

Phase 3 set out to determine the *in vitro* interactive profiles of conventional antimicrobials in combination with the most popular traditional, medicinal plants for infectious diseases, purchased from major *muthi* markets in South Africa. Chapters 3 and 4 presented results for Johannesburg and KwaZulu-Natal sourced medicinal plant extracts, respectively, in combination with conventional antimicrobials.

Key findings for Chapter 3 (JHB) included:

- Against the ESKAPE pathogens, antimicrobial antagonism was more prevalent (47.5%) than synergy (6.63%) for plant: antibiotic combinations.
- Against the two yeasts tested, no synergy was observed, but 61.9% antagonism was noted for plant: antifungal combinations.
- All 17 medicinal plants were found to be synergistic when combined with azithromycin and tested against *S. aureus*.
- The highest synergistic interaction was observed between *H. hemerocallidea* and azithromycin when tested against *S. aureus* (ΣFIC 0.06), with no toxicity evident in the BLSA (6.1% mortality).
- The most antagonistic combination was noted for *E. sideroxylon* with amoxicillin when tested against *K. pneumoniae* (ΣFIC 138.1).
- In the toxicity analysis, the most toxic combination was found when azithromycin and *S. oliveriana* was combined (54.5% mortality), and of most concern when azithromycin and *W. salutaris* were combined, since a 100% mortality rate was observed.

Key findings from Chapter 4 (KZN) included:

- Just over half of the plant: antibiotic combinations tested against the ESKAPE pathogens were found to be antagonistic (54.6%).
- Only 5.8% synergy was observed for plant: antibiotic combinations when tested against the ESKAPE pathogens.
- The highest synergistic interaction was observed between the organic extract of *S. henningsii* and ciprofloxacin against *P. aeruginosa* (ΣFIC of 0.07).
- Five of the medicinal plants (*C. lauerola*, *E. transvaalense*, *H. africana*, *J. zeyheri* and *S. serratuloides*) were found to be synergistic with azithromycin against *S. aureus*.
- The most antagonistic combination, was noted for *S. serratuloides* (aqueous extract) and amoxicillin, yielding a ΣFIC of 135.49 against *K. pneumoniae*.
- When evaluating the plant: antifungal interactive antimicrobial profiles, yet again, just over half of the combinations were antagonistic (58.6%) when tested against *C. albicans* and *C. glabrata*.

- Only one synergistic plant: antifungal combination was observed (ΣFIC 0.34), when *A. calamus* was combined with the antifungal, fluconazole, and tested against *C. albicans*.
- The only synergistic combination identified, was also the only toxic plant: antifungal combination (78.8% mortality). This indicates that the enhanced antimicrobial activity of the combination in the antimicrobial analysis of the combination was not due to synergy, but rather due to toxic effects of the combination.

5.2. Study limitations

Limited scientific investigation of interactive profiles was a major limitation to phase 1 of the study, where inclusion parameters for the reviews needed to be extended further than 10 years. This, however, also served to reinforce the need for research of this nature to be conducted. Furthermore, the underreporting of antagonism could potentially be due to preferential publication of positive results or lack of incentive to study harmful interactions.

The lack of established integrative medicine practices in the country also resulted in survey participation hesitancy, where indigenous knowledge remains garded. Participants were sceptical when approached with questions based on their traditional medicine knowledge, with many questions pertaining to the reason for enquiry for this information. When questions requesting details to be specified in writing by the participants, and particularly with "*Question 20: Do you know the names of the traditional medicines that you have used in the past or are using now?*", responses were infrequent and poorly attended.

In phase 2 of the study, the questionnaire design could have been improved by including additional questions and refining the wording of certain items. For example, a question on the frequency of traditional medicine product use could have helped determine whether participants use traditional medicines daily or have only used it once in their lifetime.

A further study limitation is related to the methodological approach adopted with the selection of the medicinal plant samples in phase 3 of the study. Although those plants, indicated as most frequently purchased for infectious conditions through consultation with the market traders, this is not an exhaustive list of plants used traditionally from the survey participation. Furthermore, the host of common names for medicinal plants in South Africa, proved

challenging when having to scientifically validate plant samples purchased according to their trading classification provided at the time of purchase.

A further research limitation, for practical translation considerations, is that although baseline synergistic *in vitro* findings may be useful in the identification of an active pharmaceutical ingredient during formulation and commercialisation of new antimicrobials, it does not truly represent the practical doses during traditional methods of administration.

In addition, sharing of findings scientifically, due to the infancy of the research field of integrative medicine, proved challenging. This challenge has also been recognised and acknowledged by Ng et al. (2024) and Raja et al. (2024). These challenges not only obstruct the advancement of integrative medicine but also reinforce negative attitudes and biases within the healthcare and research communities. Addressing these issues requires a comprehensive strategy focused on enhancing research literacy and promoting open science practices.

5.3. Future recommendations

Synergistic and antagonistic antimicrobial interactions need to be tested on pathogens other than the ESKAPE pathogens and two yeasts (*C. albicans* and *C. glabrata*). Furthermore, combinations need to be tested on clinical and resistant strains, as this would provide valuable clinical impact, particularly for the synergistic interactions identified. The synergistic pattern between all 17 JHB organic extracts with azithromycin identified in this study against *S. aureus* needs to further be explored at different ratios other than than the 1:1 combination studied here. Furthermore, the plant: conventional antimicrobial interactions on biofilms and quorum sensing could be explored further to further determine activity against pathogen resistance to antimicrobial therapy. Betoni et al. (2006) found that protein synthesis inhibitors like aminoglycosides and macrolides tend to exhibit a stronger synergistic interaction when combined with medicinal plant extracts, whereas nucleic acid synthesis inhibitors tend to show weaker synergism. In the current study, the macrolide antibiotic, azithromycin was found to show the highest synergistic potential in combination with medicinal plant extracts in both the JHB (n = 45) and KZN (n = 9) study (Publication 2 and 3). Macrolides are protein synthesis inhibitors, which aligns with the synergism of macrolides observed with the plant extracts from this study. Further studies should thus be undertaken to determine if interactions between

medicinal plants and conventional antimicrobials vary based on the class of the conventional antimicrobial as suggested by Betoni et al. (2006). Furthermore, an indepth phytochemical analysis of plants should be established to determine a favourable chemotype for optimum combination. Additional studies to include further detail of promising examples, such as investigating the synergism between Johannesburg medicinal plants and azithromycin further, dose-response analysis, and further toxicity studies is recommended. Further synergistic combinations (such as *Hypoxis hemerocallidea* with azithromycin) should be prioritized for *in vivo* studies, and pharmacokinetic assessments are needed before these combinations can be considered for human use, since this current study focused on *in vitro* studies, and one cannot compare *in vitro* findings directly to clinical outcomes for application in the South African healthcare context.

Much of the literature reviewed in Chapter 1 lacked toxicity analysis. In the current study, the BSLA was used to determine toxicity. The BSLA is a basic, inexpensive, rapid screening assay, and it is recommended that synergistic combinations be further evaluated for cytotoxicity using human cell-based assays, such as the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay.

Mechanism of action studies for combinations are lacking in the current study as well when examining the literature, presenting further avenues for investigation, particularly for synergistic combinations.

Further *in vivo* studies are recommended for synergistic interactions identified in this study. Furthermore, antagonistic interactions studied *in vivo* would bolster claims for these combinations to be cautioned against in clinical practice. *In vitro* studies do not fully represent the complex biological effects that medicinal plants exhibit because of their highly complex chemical constituency (van Vuuren, 2007) and this emphasises the need for supporting *in vivo* studies. Despite this, *in vitro* studies are necessary to provide screening as the foundational framework to build upon, with the aim of formulary development for TCAM: conventional drug interactions (Mayoka and Cheuka, 2023).

Healthcare professionals in South Africa tend to only be aware of a few natural product interactions such as St. John’s wort and grapefruit juice with conventional drugs, which are

also generally recorded in accessible formularies. However, there is limited knowledge about the interactions between traditional medicinal plants and conventional therapies, which is concerning given that this current study has highlighted that many South Africans still regularly make use of traditional remedies and may utilise both forms of healthcare concurrently. To address this gap, undergraduate medical and pharmacy programs should include coursework on potential interactions between traditional and conventional medicines, extending beyond the few commonly addressed herbal preparations (Alehegn et al., 2022).

Public awareness is also crucial, as many people assume natural products are inherently safe and free from adverse effects. This misconception often leads patients to omit mentioning their use of natural products or traditional medicines during consultations with healthcare providers (Ioannides, 2002), further increasing the risk of harmful interactions. To mitigate this issue, healthcare professionals should routinely be involved in raising awareness and actively inquiring about and documenting the use of natural products and traditional medicines as part of patient history-taking, treating it with the same importance as questions about other medications or allergies. This practice needs to be instilled within healthcare professionals within the undergraduate program (Bahall and Legall, 2017; Lubago et al., 2022; Lam Ung et al., 2023). Furthermore, it is important from these findings for a phased policy rollout to include: (1) mandatory TCAM disclosure in clinical histories, (2) community workshops to educate traditional healers on AMR risks, and (3) inclusion of TCAM interactions in South Africa's National Antimicrobial Surveillance Framework.

The provision of high-quality patient care and public health interventions aimed at preventing infectious diseases in southern Africa is hindered by fragmented healthcare systems, ineffective referral pathways, and the lack of coordination in managing patients with chronic infectious diseases across different levels of care. However, advancements in diagnostics, treatments, and the use of strategic data from national laboratory record linkages present valuable opportunities to reverse current trends in infectious disease burdens. A key recent achievement in combating antimicrobial resistance (AMR) in the private healthcare sector has been the South African Antibiotic Stewardship Programme. To replicate this success in the public sector, it is crucial to leverage the combined expertise of professional societies and committed specialists to advocate for AMR initiatives at the national level and to strengthen Antimicrobial Stewardship Programmes (ASPs) within public healthcare settings. Establishing targeted AMS training

programmes for public sector healthcare workers is highly recommended (Govender and Reubenson, 2018).

5.4. Final conclusion

This study provides a strong foundation for understanding both the potential beneficial and harmful effects of integrative traditional: conventional antimicrobial therapies in the South African context. Traditional medicinal plant use for infections in South Africa remains a prevalent practice, and with the high frequency of antagonism documented in this current study, along with the few toxic combinations identified, combination antimicrobial therapies should be cautioned against amongst patients. Findings from this study serve as an agent for informing future *in vivo* studies, facilitating clinical translation, or alternatively, aiding in raising awareness of potential risks posed by dual use antagonism. Additionally, it offers valuable insights that contribute to advancing South Africa's healthcare system toward the goal of universal healthcare, in alignment with the World Health Organisation's mandate prioritising integrative medicine. Currently, South Africa only has two statutory councils mandated to regulating TCAM through practitioner regulating, namely the Allied Health Professions Council of South Africa, and the Interim Traditional Health Practitioners Council of South Africn (Mullinder, 2023). However, more stringent regulation and policy development is necessary.

By furthering our understanding of the contextual framework for integrative medicine implementation, we can foster well-informed holistic medicine research, develop essential skills, and facilitate the integration of scientifically validated and evidence-based integrative medicine therapies into a more holistic healthcare system in South Africa.

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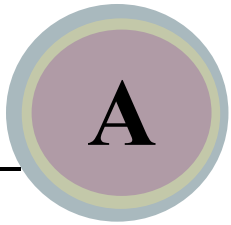
Appendices



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Appendix B – Agreements to make use of publications in thesis / proof of submission to the journal

B

P.1

Publication 1

Chapter 14
The Combined Use of African Natural Products and Conventional Antimicrobials: An Alternative Tool Against Antimicrobial Resistance

Z. Booth and S. F. van Vuuren

14.1 Introduction

14.1.1 Natural Product Classification

Natural products are groups of non-synthetic molecules exhibiting biological activity and hence often used for their medicinal properties. Many products feature under this broad classification of "natural products". Traditional medicines, herbal or supplementary products, are often derived from medicinal plants in various forms (essential oils, extracts, tinctures, teas and isolated phytochemicals), along with commonly consumed foodstuff (most often culinary herbs, spices, seeds, fruit and bee products), containing medicinal compounds (Cragg and Newman 2013). These natural products used for medicinal purposes are often referred to as traditional, complementary and/or alternative medicines (TCAMs). The TCAM framework describes all medicines or healing practices that fall outside the standard biomedical healthcare models (James et al. 2018).

Traditional medicine comprises of local herbal medicines or products, indigenous healthcare practices and local or imported complementary and alternative medicine products and practices (James et al. 2018). Traditional medicine is defined as "the use of theories, beliefs, and native experiences from different cultures, in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (WHO 2019). Complementary and alternative medicines both refer to a wide range of healthcare products and techniques that are not part of a country's traditional medicine framework and are not fully incorporated into the mainstream (conventional) healthcare system (WHO 2019). The

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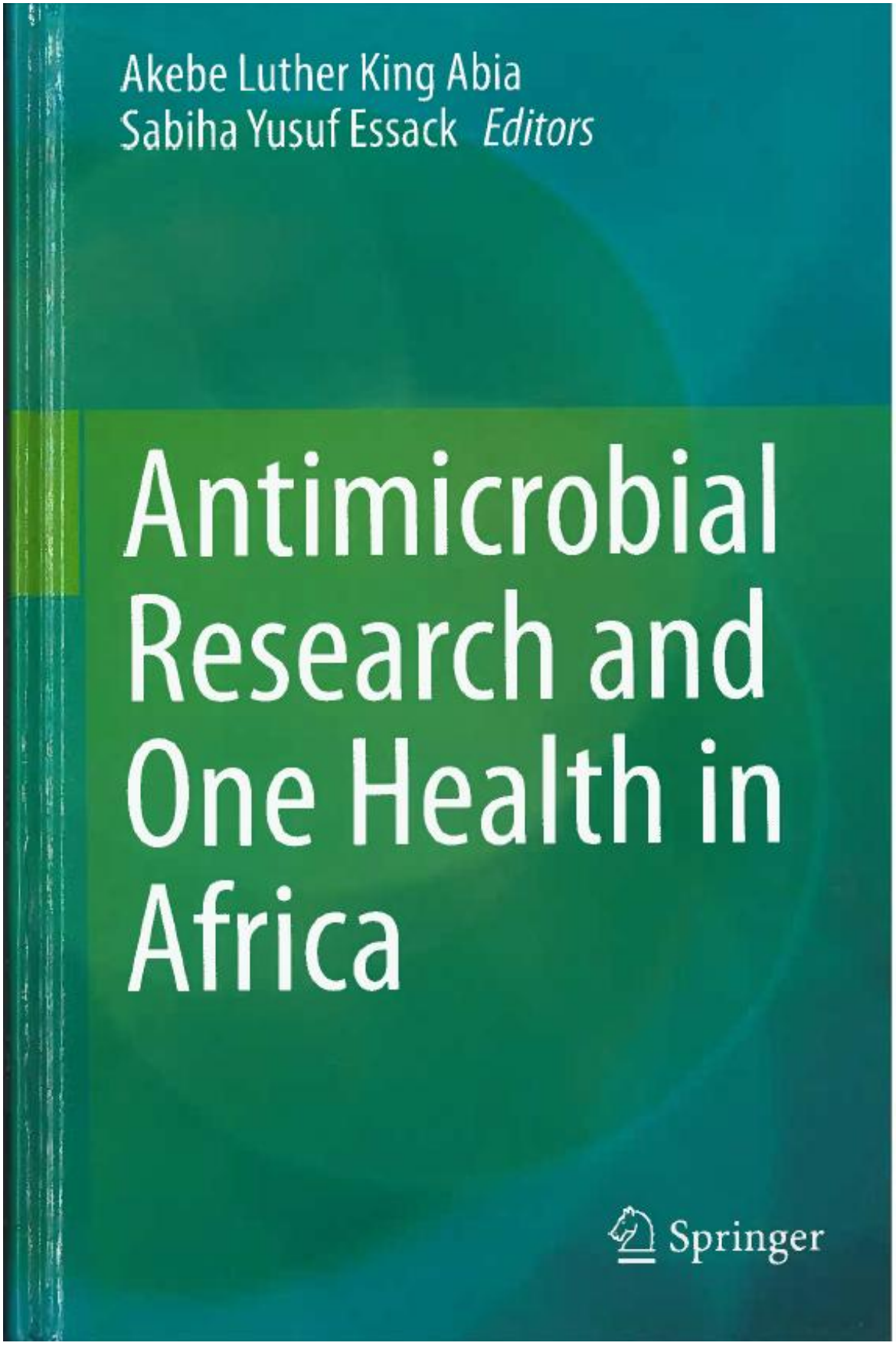
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Toxicity and antimicrobial interactions with conventional antimicrobials of commonly traded medicinal plants from Faraday *muthi* market (Johannesburg, South Africa)

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ABSTRACT

In South Africa, medicinal plants are frequently used to treat infections, with the Faraday *muthi* market in Johannesburg ensuring accessibility to these medicines. This study aimed to determine the *in vitro* interactive antimicrobial and toxicity profiles of commonly traded traditional medicinal plants at the Faraday *muthi* market, with a range of conventional antimicrobials. Minimum inhibitory concentration (MIC) assays were conducted to determine the *in vitro* interactive profiles and tested against the ESKAPE pathogens (*Enterococcus faecium* ATCC 27270, *Staphylococcus aureus* ATCC 29212, *Klebsiella pneumoniae* ATCC 13883, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloacae* ATCC 13460) and yeasts, *Candida albicans* ATCC 10231 and *Candida glabrata* ATCC 90030. The fractional inhibitory concentration index (ΣFIC) was determined to classify interactions as synergistic, additive, non-interactive or antagonistic. The brine-shrimp lethality assay (BSLA) was conducted to determine the toxicity of synergistic interactions. A total of 816 plant: antibiotic combinations were tested against the ESKAPE pathogens, with 392 combinations exhibiting a definitive ΣFIC. Synergy (6.63 %), antagonism (47.45 %), additive (8.67 %) and non-interactive (37.24 %) profiles were observed. Medicinal plants were most widely synergistic with azithromycin against *S. aureus*. *Hypoxis hemerocallidea* with azithromycin against *S. aureus* (ΣFIC 0.06) was the most synergistic, with no toxicity evident in the BLSA (6.06 % mortality). The most antagonistic interaction was with *Eucalyptus sideroxylon* and amoxicillin against *K. pneumoniae* (ΣFIC 138.12). Furthermore, a total of 136 plant: antifungal combinations were tested, where a definitive ΣFIC was obtained for 84 of the combinations. No synergy was observed, but 61.91 % of the combinations were antagonistic. The most antagonistic plant: antifungal combination was observed with *Senecio serratuloides* and nystatin against *C. albicans* (ΣFIC 34.6). The vast antagonism against the ESKAPE pathogens (47.49 %) and yeasts (61.90 %) is cause for concern, particularly with antimicrobial resistance development. The most toxic combinations in the BSLA were when azithromycin was combined with *Synaptolepis oliveriana* (54.50 % average mortality after 48 h) and with *Warburgia salutaris* (100 % average mortality after 48 h). The synergy and antagonism observed in this study warrants further investigation for clinical translation, to determine potentially beneficial or harmful combinations where applicable.

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1. Introduction

In South Africa, cultural heritage is intricately linked to the traditional use of medicinal plants. A previous study by Booth et al. (2024), found that 78.6 % and 94.1 % of patients surveyed at a public hospital in Johannesburg and Durban (South Africa), respectively used traditional medicine either with currently prescribed conventional medicines or used traditional medicine in the past.

With the high prevalence of medicinal plant use amongst patients visiting conventional hospitals, along with the high infectious disease burden in South Africa, interactions between medicinal plants and conventional antimicrobials are a concern. Patient safety and optimal health outcomes are a priority. Interactions could either increase (synergy) or decrease (antagonism) the potency of conventional antimicrobials (Zhang and Lui, 2020).

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Article

Conventional Antimicrobial and Medicinal Plants from a Traditional Medicine Market in South Africa: An Interactive Antimicrobial and Toxicity Study

Zelna Booth [1,*], Sabiha Essack [2] and Sandy van Vuuren [1]

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[2] Antimicrobial Research Unit, University of KwaZulu-Natal, Durban 4000, South Africa; essacks@ukzn.ac.za
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Abstract: Background: The World Health Organization (WHO) has proposed the use of integrative medicine to achieve extended healthcare coverage in developing countries facing high morbidity. Traditional remedies are frequently employed to prevent and treat infections among South Africans; however, the ways in which they interact with conventional antimicrobials are largely unknown. Therefore, this study aimed to explore the interactions between commonly traded medicinal plants at a traditional medicine market in KwaZulu-Natal (KZN), South Africa, and conventional antibiotics and antifungals. Methods: To determine the interactive antimicrobial profiles for plant/conventional antimicrobial combinations, minimum inhibitory concentration (MIC) assays were performed against ESKAPE pathogens and the yeasts *Candida albicans* and *Candida glabrata*. Calculated fractional inhibitory concentration (ΣFIC) values were used to identify synergism or antagonism, with synergistic interactions further tested in vitro for toxicity. Results: A total of 952 combinations were tested, of which 5.8% and 54.6% of the plant/antibiotic combinations were synergistic and antagonistic, respectively; additionally, 1.7% and 58.6% of the plant/antifungal combinations showed synergism or antagonism, respectively. The most toxic plant/antibiotic combination was *Artemisia afra* with doxycycline (71.1% mortality). The most toxic plant/antifungal combination was *Acorus calamus* with fluconazole (78.8% mortality). Conclusions: When medicinal plants acquired from a traditional medicine market in South Africa are used in combination with conventional antibiotics and antifungals, more than half of the combinations exhibit antagonism, which is concerning.

Keywords: antibiotics; antifungals; traditional medicinal plants; antimicrobial combinations; interactions; synergistic; antagonistic; toxicity

check for updates

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1. Introduction

An estimated 60–80% of South Africans make use of traditional medicines sourced from various traditional medicine markets [1–3]. With the country's diverse array of indigenous plants, exposure to a wide range of traditional medicinal plants is highly prevalent among the population, particularly for the treatment of infectious conditions [4]. Given the widespread use of medicinal plants in South Africa and the substantial burden of infections [5], there is a significant likelihood of combined traditional and conventional antimicrobial use.

Consequently, it is crucial to investigate the interactions of these combinations. Synergistic antimicrobial interactions could prove beneficial as alternatives to addressing resistant

Appendix C – Research study ethics and site approvals

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Ms Z Booth

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M190534

NAME: (Principal Investigator)	Ms Z Booth
DEPARTMENT:	School of Therapeutic Sciences Department of Pharmacy and Pharmacology Medical School University
PROJECT TITLE:	*Traditional medicine use in two public South African hospitals: prevalence and interactive antimicrobial and toxicity studies for combinations with conventional antimicrobials* *Change of study title noted on 2020/08/03*
DATE CONSIDERED:	2019/05/31
DECISION:	Approved unconditionally
CONDITIONS:	This approval applies only to the study sites listed in Annex 1 attached Further sites may be added on presentation of evidence of management approval to the HREC (Med)
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Professor S van Vuuren
APPROVED BY:	Dr CB Penny, Chairperson HREC (Medical)
DATE OF APPROVAL:	2019/08/12

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **May** and therefore reports and re-certification will be due in the month of **May** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

18 – 01 – 2022
Date

Annex 1

Protocol No. M190534 **Ms Z Booth**

Study sites approved under this Clearance Certificate

Study site	Date of HREC (Med) Approval
Charlotte Maxeke Johannesburg Academic Hospital	2019/08/12
King Edward VIII Hospital, Durban, KZN	2022/01/18

Dr CB Penny
Chair: HREC (Medical)
University of the Witwatersrand, Johannesburg

Date: 2022/01/18

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Innovation)

TO: Ms Z Booth
School: Therapeutic Sciences
Department: Pharmacy and Pharmacology
Division: Pharmaceutical Micribiology
Medical School
University
E-mail: Zelna.Booth@wits.ac.za

CC: Supervisor: Professor S van Vuuren <Sandy.vanVuuren@wits.ac.za>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 14/03/2022

REF: *R14/49*

PROTOCOL NO: W-CBP-220314-01 (This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)

PROJECT TITLE: *Traditional medicine use in two public South African hospitals: prevalence and interactive antimicrobial and toxicity studies for combinations with conventional antimicrobials*

Please find attached the Ethics Waiver Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Innovation)

14/03/2022

Ref: W-CBP-220314-01

TO WHOM IT MAY CONCERN

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical)

Investigator: Ms Z Booth
Student No. (if appropriate): 300896
Staff No. (if appropriate): A0026806

Supervisor: Professor S van Vuuren

School: Therapeutic Sciences
Department: Pharmacy and Pharmacology
Pharmaceutical Micribiology
Medical School
University

Project title: *Traditional medicine use in two public South African hospitals: prevalence and interactive antimicrobial and toxicity studies for combinations with conventional antimicrobials*

Reason: In vitro laboratory study
No human participants will be involved in the study

CB Penny

Dr CB Penny
Chairperson: Human Research Ethics Committee (Medical)

Research Office Secretariat:
Third Floor, Phillip Tobias Building, corner of St Andrews and York Roads, Parktown, Johannesburg 2193
Postal address: Private Bag 3, Wits 2050
Tel Nos: +27 (0)11 717 1234/1252/2656/2700
Office E-mail: HREC-Medical.ResearchOffice@wits.ac.za
Website:
https://www.wits.ac.za/research/researcher-support/research-ethics/ethics-committees/

Proposal Details: GP_201908_005

GAUTENG HEALTH RESEARCH COMMITTEE

APPLICATION DETAILS

TITLE OF RESEARCH PROJECT

"A Survey: Determining the prevalence of traditional medicine use in a tertiary academic hospital", a part of the PhD study entitled "Traditional medicine use in two public South African hospitals: Prevalence and interactive antimicrobial and toxicity studies for combinations with conventional antimicrobials".

TYPE OF STUDY

Academic

STATUS OF APPLICATION

Approved

STATUS OF PROJECT

On-Going

PROPOSAL SUBMISSION DATE

2019/08/06

You will find a list of all comments made on the selected research application. The list below displays comments visible to both the Applicant and Research Committee

COMMENTS

Comment	Comment Date	Comment By

PRIMARY INVESTIGATOR OF THE PROJECT/PROPOSAL

Title	Name	Surname	Role	Institution	E-Mail	Telephone No.	Mobile No.	CV/Resume
MRS	Zelna	Booth	Principal Investigator		zelna.booth@wits.ac.za	0117172912	0829212688	Download CV (/Researcher/Download/44784)

GAUTENG PROVINCE

HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Ms. N. Mzila
Office of the Clinical Director
Email: Nolwazi.Mzila@gauteng.gov.za
Tell: (011): 488-4812
31st July 2019

Dear Ms. Z. Booth

STUDY TITLE: A Survey: Determining the Prevalence of Traditional Medicine Use in a Tertiary Academic Hospital (Charlotte Maxeke Johannesburg Academic Hospital).

Permission to review patient file for conduction of the above mentioned study is provisional approved. Your study can only commence once Ethics approval is obtained. Please forward a copy of your Ethics Clearance Certificate as soon as the study is approved by the Ethics Committee for the CEO's office to give you the final approval to conduct the study.

Supported / ~~Not supported~~

Dr. M.I. Mofokeng
Clinical Director
DATE: 1/08/2019

Approved / not approved

Ms. B. Bogoshi
Chief Executive Officer
DATE: 01-08-2019

KWAZULU-NATAL PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

KING EDWARD VIII HOSPITAL
CLINICAL MANAGER
Corner of Sydney and Rick Turner Roads, Umbilo, Durban
Private Bag x02, Congella 4013
Tel: 031 360 3854 Fax: 031 206 1457 Email: E-mail: KE8.MedicalManagerSecretary@kznhealth.gov.za
www.kznhealth.gov.za

Ref: KE 2/7/12/ (01/2021
Enq: Mr. W.P. Kubheka
Research Programming

12 January 2022

University of the Witwatersrand
Johannesburg

Dear Ms. Z Booth

HREC REFERENCE NO: M190534
Protocol: "A SURVEY: DETERMINING THE PREVALENCE OF TRADITIONAL MEDICINE HOSPITAL."

Your request to conduct research at King Edward VIII Hospital has been approved.

Please ensure the following:

- That King Edward VIII Hospital receives full acknowledgment in the study on all publications and reports and also kindly present a copy of the publication or report on completion.

Before commencement:

* Discuss your research project with our relevant Clinical Head/Assistant Nursing Manager
* Sign an indemnity form at Room8, CEO's Complex, Admin. Block.

The Management of King Edward VIII Hospital reserves the right to terminate the permission for the study should circumstances so dictate.

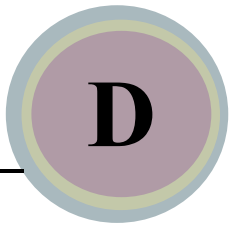
Yours faithfully

SUPPORTED/NOT SUPPORTED

DR. V. KALALA
CLINICAL MANAGER

13/01/2022
DATE

Appendix D – Questionnaire used in survey study



QUANTITATIVE RESEARCH QUESTIONNAIRE, FOR A SURVEY UNDERTAKEN AT PHARMACY WAITING AREAS IN A PUBLIC HOSPITAL IN SOUTH AFRICA.

Date: _____Questionnaire Number: _____

Age: _____

Where were you born? _____

Where do you live now? _____

Please circle your answer to each of the questions below.

SECTION 1 - DEMOGRAPHIC INFORMATION

1. **Gender:**
   a. Male.
   b. Female.
   c. Other.

2. **Ethnic group:**
   a. Black.
   b. White.
   c. Indian.
   d. Coloured.
   e. Chinese.
   f. Other:

   _____

3. **Employment:**
    a. Unemployed.
    b. Employed.
    c. Self-employed.
    d. Student.

4. **Education:**
    a. None.
    b. Primary (Grade R to Grade7).
    c. Secondary (Grade 8 to Grade 12).
    d. Tertiary (University degree/certificate, college diploma/certificate).

5. **Cultural practice:**
    a. Christian.
    b. Muslim.
    c. Hinduism.
    d. Judaism.
    e. African traditional beliefs/practices.
    f. None.
    g. Other (please explain): _____

6. **Do you have any chronic health problems (chronic = a disease that is not an infection caused by germs; but a disease that you have had for a long period of time)?**
    a. Yes.
    b. No.

    If yes, please give the chronic health problem: _____

7. **Why have you come to the hospital pharmacy today?**
    a. I saw the doctor and have a prescription for medicine to treat a new sickness.
    b. I need to fetch more of my medicine for a sickness I have had for a long time.
    c. I have questions about my medicine that I am using.
    d. Other (please explain): _____

SECTION 2 - TRADITIONAL MEDICINE USE INFORMATION

Traditional medicine is the natural health practice, knowledge and belief to treating or preventing sickness. It includes using medicines made from plants, herbs and animal products.

8. What do you understand by traditional medicines (more than one can be chosen)?

a. Traditional medicine is made from plants and herbs.
b. Traditional medicine is made from animals, like animal blood or bones.
c. Traditional medicine can also be food or drinks (eg: milk powder).
d. Traditional medicine involves spiritual healing and prayers.

9. Have you used traditional medicines before?

a. Yes.
b. No.

10. Are you using any traditional medicines now?

a. Yes.
b. No.

11. If you answered "Yes" to question 9 or question 10, do you use traditional medicine together with medicine you get from the doctor, clinic, pharmacy or hospital?

a. Yes.
b. No.

12. Are traditional medicines your first choice for healthcare before going to the doctor, clinic, pharmacy or hospital?

a. Yes.
b. No.

13. What do you use traditional medicines for (more than one can be chosen)?

a. To not get disease.
b. To not get infections from germs.
c. To treat a disease that you have just recently found out about (Please give disease name):

   _____

d. To treat a disease that you have had for a long time (Please give disease name):

   _____

e. To treat an infection from germs that you have just gotten sick from (Please give place of infection in the body – example: on the skin):

   _____

14. Where did you first learn about traditional medicines?

a. Family or friends.
b. A traditional healer.
c. A community leader.
d. Media (radio, newspaper, magazines, books).
e. A pharmacist.
f. Other healthcare worker (example: nurses or doctors).

15. Why would you use traditional medicines instead of medicine from the doctor, clinic, pharmacy or hospital (more than one can be chosen)?

a. Traditional medicine is cheaper than getting medicine at the doctor, clinic, pharmacy or hospital.
b. Traditional medicine is easy to get where I live.
c. I think traditional medicine is safer than medicines from the doctor, clinic, pharmacy or hospital.
d. Medicine from the doctor, clinic, pharmacy or hospital did not help me.
e. Other: _____

16. How do you know if traditional medicines work (more than one can be chosen)?

a. I was told that traditional medicine works well by a friend or family member.
b. I believe traditional medicine works well because it is a part of my culture.
c. I got better when I used traditional medicine.
d. I am trying traditional medicine myself to see if it works.
e. Other: _____

17. How do you use the traditional medicines (more than one can be chosen)?

a. Drinking (swallowing).
b. Mouthwash (not swallowing).
c. Rectal.
d. Breathing it in.
e. Bathing in or with it.
f. Drops in the ear.
g. Drops in the eye.
h. On the skin.
i. Other (please explain): _____

18. Have you had any bad things happen to you from using traditional medicine?

a. Yes.
b. No.

If yes, please explain (example – skin rash): _____

19. What type of traditional medicines do you use most of the time?

a. Plants or herbs.

   Please give part of plant used (example - root): _____
b. Animal products (example: animal blood).
c. Both plants/herbs and animal products.
d. Other (please explain): _____

20. Do you know the names of the traditional medicines that you have used in the past or are using now?

a. Yes.
b. No.

If yes, please give the name that you know it by: _____

21. Where do you get your traditional medicine from (more than one can be chosen)?

a. The pharmacy.
b. A health shop.
c. A traditional healer.
d. A street market (please give name of market): _____
e. Other (please give details): _____

22. Did your doctor, nurse, dentist or pharmacist at the hospital ever ask you if you use traditional medicine?

a. Yes.
b. No.

23. Do you think that traditional medicine can affect how medicine from the doctor, clinic, pharmacy or hospital works in your body?

a. Yes.
b. No.

Thank you for helping us with this research study. It will help us in understanding traditional medicine use in patients visiting the hospital pharmacies.

Appendix E – Patient information sheet

E

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

DEPARTMENT OF PHARMACY AND PHARMACOLOGY

PARTICIPANT INFORMATION SHEET FOR A SURVEY UNDERTAKEN AT PHARMACY WAITING AREAS IN A PUBLIC HOSPITAL IN SOUTH AFRICA.

Name of Principle Investigator: Zelna Booth

Name of Organization: Department of Pharmacy and Pharmacology

School of Therapeutic Sciences

Faculty of Health Sciences

University of the Witwatersrand, Johannesburg

Name of Project: "A Survey: Determining traditional medicine use in a tertiary academic hospital", a part of the PhD study entitled "Traditional medicine use in two public South African hospitals: Prevalence and interactive antimicrobial and toxicity studies for combinations with conventional antimicrobials".

Study site: Charlotte Maxeke Johannesburg Academic Hospital.

Dear Sir or Madam,

This is an invitation to participate in a study conducted by researchers at the University of Witwatersrand. The purpose of the research is to determine the prevalence of traditional medicine use in a large public hospital in South Africa.

I am Zelna Booth, a postgraduate student and academic staff member at the University of the Witwatersrand, in the Department of Pharmacy and Pharmacology. I am conducting research on the prevalence of traditional medicine use in the public hospital pharmacies. I am completing a survey under the supervision of Professor Sandy van Vuuren (University of the Witwatersrand) and Professor Sabiha Essack (University of Kwa-Zulu Natal).

I am going to give you information about the content of this survey and invite you to be a part of this research. I would appreciate it if you could give 25 minutes of your time, while you are waiting to receive your medication in the pharmacy. You are more than welcome to refuse to take part in this survey. If you do not feel that the time of approach is appropriate, I could arrange for conducting of the survey at an alternate, more suitable time and place. Before you decide to participate in the study, you are more than welcome to consult with anyone regarding your involvement in the study, should that make you feel more comfortable in participating.

If the participant information sheet, consent form or questionnaire contains anything that is not clearly understood, please feel free to ask me to go through the information with you. Should you have any further questions later, you are more than welcome to ask me or anyone else.

Purpose of the research

The use of traditional medicine is said to be high amongst Africans and it is said to be the first health care choice taken by many patients for health-related problems. I want to verify if the statement is true as it is important to know whether patients use traditional medicines. The healthcare professionals such as pharmacists, doctors and nurses will need to know about the traditional medicine use especially when used with medicines that are given to you at the hospital that could possibly lead to problems. We believe you can help us to understand better if patients use traditional medicines by answering our research questions. In addition, your participation will assist healthcare professionals to identify the need for traditional medicine use to be questioned during history taking practices.

Type of research intervention

This research will involve your participation by answering 23 questions about your use of traditional medicine in a self-administered questionnaire.

Participant selection

You are being invited to take part in this research because you have visited the hospital pharmacy today and I am focusing on patients who are waiting for their medication in the hospital pharmacy waiting areas. Your participation in this research is entirely voluntary. It is your choice whether to participate or not. If you choose not to participate, all the services you receive from the hospital and the pharmacy will continue and nothing will change. You may change your mind at any stage throughout the process and can stop participating at any time, even if you have originally consented.

Procedures

A questionnaire consisting of 23 questions will be handed out to you, along with a pen, whilst you are sitting within the waiting area of the hospital pharmacy. The questionnaire can be read through and answered at your own pace and there will be no interaction with the researcher, unless requested. The researcher will be available at all times should you have any questions or require any clarity. If you feel that certain questions or information to be discussed requires privacy, please inform the researcher, who will then arrange for a private consultation area for maintenance of confidentiality. No one else but the researcher, along with two research assistants, will be disseminating and collecting the completed questionnaires. The information recorded is confidential, and no one else except for the researcher, research assistants and supervisors stipulated previously. It is highly likely that the information may be published, however, any personal details that could directly link you to the information will remain confidential at all times.

Duration

Data will be collected over one to three month/s in total. During that time, patients interested in participating in the study for about 25 minutes will be provided with a questionnaire to complete whilst awaiting their medication to be dispensed in the pharmacy. I will not need any follow ups with you, as the information provided through answering the questionnaire will provide sufficient information.

Risks

I am asking you to share information with me regarding your traditional medicine use, which may be considered confidential information. Please be aware that you do not have to answer any question or take part in the survey if you don't wish to do so. You do not have to give us any reason for not responding to any question, or for refusing to take part in the study.

Reimbursements

You will not be provided any incentive or direct benefit to take part in the research. However, we would highly appreciate your time to participate in the study. No costs will be incurred by any participant in the study.

Confidentiality

The survey being done in the pharmacy may draw attention and if you participate you may be asked questions by other people in the pharmacy. I will not be sharing information about you to anyone outside of the research team. The information that I collect from this research project will be kept confidential. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is, and the coding system will be password secured on a laptop. It will not be shared with or given to anyone except to the research team.

Sharing the results

The information from the study will be presented to relevant healthcare professionals at the hospital, with the intention of treating if any adverse effects have resulted due to combination traditional and western medicines being taken. The information that you share with us will be used for the purposes of research that will be published, but it will not include your personal information. Should you wish to be informed of the outcomes from this study, please feel free to contact the principle investigator, Mrs. Zelna Booth via the email address provided below.

Right to refuse or withdraw

You do not have to take part in this research if you do not wish to do so, and choosing to participate will not affect your visit to the hospital in any way. You may stop participating in the study at any time that you wish without your visit to the hospital being affected. I will give you an opportunity at the end of completing the questionnaire to review your remarks, and you can ask to modify or remove portions.

Who to contact

If you have any questions, you can ask the researchers or supervisors now or later. If you wish to ask questions later, you may contact any of the following persons:

- Zelna Booth (Zelna.booth@wits.ac.za/ 011 717 2912)
- Prof Sandy van Vuuren (sandy.vanvuuren@wits.ac.za/ 011 717 2157)
- Prof. Sabiha Essack (essacks@ukzn@ac.za/ 031 260 7785)
- This proposal has been reviewed and approved by the Wits Human Research Ethics Committee (Medical), which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find out more about the committee or you have any concern over the way the study is being conducted, please contact the Chairperson of this Committee who is Dr. Clement Penny, who may be contacted on the telephone number 011 717 2301, or by e-mail on Clement.Penny@wits.ac.za. The telephone numbers for the Committee secretariat are 011 717 2700/1234 and the e-mail addresses are Zanele.Ndlovu@wits.ac.za and Rhulani.Mukansi@wits.ac.za.

Thank you for reading the participant information sheet. Date: January 2020



UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

DEPARTMENT OF PHARMACY AND PHARMACOLOGY

PARTICIPANT INFORMATION SHEET FOR A SURVEY UNDERTAKEN AT PHARMACY WAITING AREAS IN A PUBLIC HOSPITAL IN SOUTH AFRICA.

Name of Principle Investigator: Zelna Booth

Name of Organization: Department of Pharmacy and Pharmacology

School of Therapeutic Sciences

Faculty of Health Sciences

University of the Witwatersrand, Johannesburg

Name of Project: "A Survey: Determining traditional medicine use in a tertiary academic hospital", a part of the PhD study entitled "Traditional medicine use in two public South African hospitals: Prevalence and interactive antimicrobial and toxicity studies for combinations with conventional antimicrobials".

Study site: King Edward VIII Hospital.

Dear Sir or Madam,

This is an invitation to participate in a study conducted by researchers at the University of Witwatersrand. The purpose of the research is to determine the prevalence of traditional medicine use in a large public hospital in South Africa.

I am Zelna Booth, a postgraduate student and academic staff member at the University of the Witwatersrand, in the Department of Pharmacy and Pharmacology. I am conducting research on the prevalence of traditional medicine use in the public hospital pharmacies. I am completing a survey under the supervision of Professor Sandy van Vuuren (University of the Witwatersrand) and Professor Sabiha Essack (University of Kwa-Zulu Natal).

I am going to give you information about the content of this survey and invite you to be a part of this research. I would appreciate it if you could give 25 minutes of your time, while you are waiting to receive your medication in the pharmacy. You are more than welcome to refuse to take part in this survey. If you do not feel that the time of approach is appropriate, I could arrange for conducting of the survey at an alternate, more suitable time and place. Before you decide to participate in the study, you are more than welcome to consult with anyone regarding your involvement in the study, should that make you feel more comfortable in participating.

If the participant information sheet, consent form or questionnaire contains anything that is not clearly understood, please feel free to ask me to go through the information with you. Should you have any further questions later, you are more than welcome to ask me or anyone else.

Purpose of the research

The use of traditional medicine is said to be high amongst Africans and it is said to be the first health care choice taken by many patients for health-related problems. I want to verify if the statement is true as it is important to know whether patients use traditional medicines. The healthcare professionals such as pharmacists, doctors and nurses will need to know about the traditional medicine use especially when used with medicines that are given to you at the hospital that could possibly lead to problems. We believe you can help us to understand better if patients use traditional medicines by answering our research questions. In addition, your participation will assist healthcare professionals to identify the need for traditional medicine use to be questioned during history taking practices.

Type of research intervention

This research will involve your participation by answering 23 questions about your use of traditional medicine in a self-administered questionnaire.

Participant selection

You are being invited to take part in this research because you have visited the hospital pharmacy today and I am focusing on patients who are waiting for their medication in the hospital pharmacy waiting areas. Your participation in this research is entirely voluntary. It is your choice whether to participate or not. If you choose not to participate, all the services you receive from the hospital and the pharmacy will continue and nothing will change. You may change your mind at any stage throughout the process and can stop participating at any time, even if you have originally consented.

Procedures

A questionnaire consisting of 23 questions will be handed out to you, along with a pen, whilst you are sitting within the waiting area of the hospital pharmacy. The questionnaire can be read through and answered at your own pace and there will be no interaction with the researcher, unless requested. The researcher will be available at all times should you have any questions or require any clarity. If you feel that certain questions or information to be discussed requires privacy, please inform the researcher, who will then arrange for a private consultation area for maintenance of confidentiality. No one else but the researcher, along with two research assistants, will be disseminating and collecting the completed questionnaires. The information recorded is confidential, and no one else except for the researcher, research assistants and supervisors stipulated previously. It is highly likely that the information may be published, however, any personal details that could directly link you to the information will remain confidential at all times.

Duration

Data will be collected over one to three month/s in total. During that time, patients interested in participating in the study for about 25 minutes will be provided with a questionnaire to complete whilst awaiting their medication to be dispensed in the pharmacy. I will not need any follow ups with you, as the information provided through answering the questionnaire will provide sufficient information.

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or take part in the survey if you don't wish to do so. You do not have to give us any reason for not responding to any question, or for refusing to take part in the study.

Reimbursements

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Confidentiality

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Sharing the results

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Right to refuse or withdraw

You do not have to take part in this research if you do not wish to do so, and choosing to participate will not affect your visit to the hospital in any way. You may stop participating in the study at any time that you wish without your visit to the hospital being affected. I will give you an opportunity at the end of completing the questionnaire to review your remarks, and you can ask to modify or remove portions.

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- Prof Sandy van Vuuren (sandy.vanvuuren@wits.ac.za/ 011 717 2157)
- Prof. Sabiha Essack (essacks@ukzn@ac.za/ 031 260 7785)
- This proposal has been reviewed and approved by the Wits Human Research Ethics Committee (Medical), which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find out more about the committee or you have any concern over the way the study is being conducted, please contact the Chairperson of this Committee who is Dr. Clement Penny, who may be contacted on the telephone number 011 717 2301, or by e-mail on Clement.Penny@wits.ac.za. The telephone numbers for the Committee secretariat are 011 717 2700/1234 and the e-mail addresses are Zanele.Ndlovu@wits.ac.za and Rhulani.Mukansi@wits.ac.za.

Thank you for reading the participant information sheet. Date: January 2020

F

Appendix F – Plagiarism report

Zelna Booth Thesis

ORIGINALITY REPORT

13%	5%	12%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Z. Booth, S. F. van Vuuren. "Chapter 14 The Combined Use of African Natural Products and Conventional Antimicrobials: An Alternative Tool Against Antimicrobial Resistance", Springer Science and Business Media LLC, 2023 Publication	4%
2	www.pure.ed.ac.uk Internet Source	3%
3	Hübsch, Zelna. "Antimicrobial Efficacy and Toxicity Profiles of Conventional Antimicrobial Agents in Combination with Commercially Relevant Southern African Medicinal Plants", University of the Witwatersrand, Johannesburg (South Africa), 2025 Publication	1%
	Submitted to University of Hull	
4	Student Paper	1%
5	hdl.handle.net Internet Source	1%
6	Nciki, Sibongile. "Validating the Traditional Use of Medicinal Plants in Maputaland to Treat Skin Diseases", University of the Witwatersrand, Johannesburg (South Africa), 2025 Publication	<1%
7	Submitted to South African Pharmacy Council Student Paper	<1%
8	Keiichi Hiramatsu, Longzhu Cui, Makoto Kuroda, Teruyo Ito. "The emergence and evolution of methicillin-resistant Staphylococcus aureus", Trends in Microbiology, 2001 Publication	<1%
9	Julia Zabielska, Agnieszka Tyfa, Alina Kunicka-Styczyńska. "Methods for eradication of the biofilms formed by opportunistic pathogens using novel techniques – A review", Acta Universitatis Lodziensis. Folia Biologica et Oecologica, 2016 Publication	<1%

10	Mahumane, Gillian Dumsile. "Antimicrobial Activity and Chemical Analysis of Eucalyptus radiata Leaf Essential Oil", University of the Witwatersrand, Johannesburg (South Africa), 2025 Publication	<1 %
11	de Abreu, Ana Cristina Ralha. "Reversing Antibacterial Resistance of Staphylococcus Aureus: The Use of Phytochemicals as Antibiotic-Adjuvants and Resistance-Modifying Agents Towards More Effective Therapies", Universidade do Porto (Portugal), 2022 Publication	<1 %
12	everyspec.com Internet Source	<1 %
13	Suliman, Sajida. "Antimicrobial Interactions of Artemisia afra Used in African Traditional Medicine", University of the Witwatersrand, Johannesburg (South Africa), 2025 Publication	<1 %
14	www.ncbi.nlm.nih.gov Internet Source	<1 %
15	Submitted to University of Witwatersrand Student Paper	<1 %
16	Submitted to Universidad San Francisco de Quito Student Paper	<1 %
17	Miriam Falkenberg, Eliana Nakano, Leonardo Zambotti-Villela, Gabriele A. Zatelli et al. "Bioactive compounds against neglected diseases isolated from macroalgae: a review", Journal of Applied Phycology, 2018 Publication	<1 %
18	Zelna Booth, Gugulethu Khumalo, Sandy Van Vuuren. "Toxicity and antimicrobial interactions with conventional antimicrobials of commonly traded medicinal plants from Faraday muthi market (Johannesburg, South Africa)", South African Journal of Botany, 2024 Publication	<1 %
19	Joan Camunas-Soler, Xiao-Qing Dai, Yan Hang, Austin Bautista et al. "Patch-Seq Links Single-Cell Transcriptomes to Human Islet Dysfunction in Diabetes", Cell Metabolism, 2020 Publication	<1 %

20	Nebyu Daniel Amaha, Samson Goitom Mebrahtu, Nuru Abdu. "Saponins and their synergistic antibacterial activity with traditional antibiotics against Staphylococcus aureus and Escherichia coli: Review", Qeios Ltd, 2022 Publication	<1%
21	academicjournals.org Internet Source	<1%
22	vital.seals.ac.za:8080 Internet Source	<1%

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Exclude bibliography	On		