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In Vitro Antimicrobial Activity of *Conyza bonariensis* and *Tribulus terrestris* Growing in Tanzania

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Abstract

Objective: The aim of this study was to evaluate antimicrobial activity of *Conyza bonariensis* and *Tribulus terrestris* growing in Tanzania.

Background statement: The improvement of antimicrobial agents has protected and improved human life from fatal diseases since their discovery. The increase in microbial drug resistance demands for new effective drugs of which medicinal plants are a promising source.

Methods: Minimum inhibitory concentration (MIC) in 96-well micro dilution was used to determine antimicrobial activity. The method involved loading of 50 µL of Sabouraud's dextrose broth in each well. Then, 50 µL of plant extract fetched from 100 mg/mL stock solution to form 100 µL. Thereafter, 50 µL of the mixture from the first rows was repeatedly down the columns until the last row where 50 µL were discarded. Subsequently, 50 µl of microbial suspensions was added in each well and incubated at 37°C for 24 hours. In each well, 20 µL of 0.02% Para-iodonitrotetrazolium chloride dye (INT) was added to micro plates in order to distinguish between the wells with live microbes from those with dead microbes.

Results: All extracts demonstrated antimicrobial activity to tested bacterial and fungal strains. Seven extracts namely *C. bonariensis* leaf chloroform, *C. bonariensis* leaf ethyl acetate, *C. bonariensis* stem chloroform, *C. bonariensis* stem ethyl acetate, *C. bonariensis* root ethyl acetate, *T. terrestris* leaf ethyl acetate and *T. terrestris* root ethyl acetate inhibited *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Salmonella typhi* and *Cryptococcus neoformans* at 0.78125 mg/mL. Plant extracts demonstrated synergistic effect of their chemical components against the tested microbes. The strongest bactericidal and fungicidal of combined extracts was exhibited on *E. coli* and *C. neoformans* respectively. The extracts from *C. albicans* and *T. terrestris* revealed antimicrobial activity against seven tested microbes.

Conclusion: It was concluded that *C. bonariensis* and *T. terrestris* can be considered as possible sources of antimicrobial drug leads upon further phytochemical investigations.

Keywords: Antimicrobial; *Conyza bonariensis*; *Tribulus terrestris*; Extract; MIC; Synergistic effect; Ethno-medical information

Introduction

Humans have used antibiotics to manage bacterial and fungal infections for the last seven decades [1]. Infectious diseases killed 3.25 million of children worldwide in the year 2013 [2] and bacterial infections accounted for 17.8% global human death [3]. The situation is worse in developing countries that may have been attributed by poor medication and little or non-affordability of proper antimicrobial drug. The prevalence of infectious diseases is high despite the availability of conventional antimicrobial drugs mainly because some microbes have acquired resistance to the present drugs [4].

In these circumstances, the search for effective, safe and affordable antimicrobial drugs is an appealing need. Ethno-medical information has gained popularity as one of reliable sources for drug templates [4]. It is estimated that about 25% of conventional drugs originate from medicinal plants [5].

It is further reported that about 56% of people in Kilimanjaro region use traditional medicines in their primary health care [6]. Tanzania is estimated to have about 1,000 medicinal plant species [7]. Despite the fact that the country is blessed with high diversity of medicinal plants, only few of them have been evaluated for their antimicrobial activity.

Conyza bonariensis is an annual herb present all over the world except in Antarctica [8,9]. It is used for treatment of ailment such as sore throat, ringworm, chicken pox, bleeding from injuries, toothache diarrhea and constipation in Pakistan and China [10,11]. In Tanzania, *C. bonariensis* is used for treatment of HIV/AIDS opportunistic infections [12]. *Tribulus terrestris* which is commonly known as spine/puncher vine is distributed in tropical, subtropical and warm temperate areas

[13]. Leaves of the *T. terrestris* are used traditionally to treat gonorrhea, inflammation, leprosy, skin diseases, ulitis, and general body weakness at Gujarat region in India [14]. Traditionally, this herb is used to enhance libido, stimulate spermatogenesis, vermifuge and medicine to skin diseases [12,15].

Fungal and bacterial infections continue to cost the wellbeing of humans because, some pathogenic bacteria and fungi have acquired adaptive mechanisms to present antibacterial and antifungal drugs respectively [16]. Furthermore, the problem continues to cost peoples' life because of insufficient efforts to search for new and effective antimicrobial drugs. Although currently medicinal plants have been regarded as promising source of antimicrobial drugs, most of them have not been scientifically evaluated to be considered in drug production [17].

In searching for new antimicrobial drugs, *in vitro* evaluation of unstudied medicinal plants is essential at initial stages. That is why this study reports the antifungal and antibacterial activity on *C. bonariensis* and *T. terrestris* growing in Tanzania on four Gram-negative bacteria which are *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, one Gram-positive Methelin-resistant bacterium *Staphylococcus aureus* as well as two fungal strains namely *Cryptococcus neoformans* and *Candida albicans*.

Materials and Methods

Acquisition of materials

Distilled water was collected from Arusha Technical College while seawater from Indian Ocean along the Dar es Salaam coast. Para-iodonitrotetrazolium chloride dye (INT) and dimethyl sulfoxide (DMSO) were purchased from Sigma and Alrich, St Louis, USA. Ketoconazole was purchased from S Kant Healthcare LTD, Gujarat, India and Ciprofloxacin tablets were bought from Micro Lab LTD, India. Chloroform, ethyl acetate and methanol were purchased from Avantor performance materials in India. Both nutrient (agar and broth), Sabouraud's dextrose (agar and broth) were purchased from Hi Media Laboratories Pvt Ltd (Mumbai-India). Selection of microorganisms for testing the potency of medicinal plants depended on the availability during the study on their pathogenic representativeness as suggested by Cos et al. [18]. The test microorganisms were two fungal strains namely *Cryptococcus neoformans* (clinical isolate) and *Candida albicans* (ATCC 90028), one Gram-positive bacterium *Staphylococcus aureus* (ATCC29213) and four Gram-negative bacteria namely *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC 29953), *Salmonella typhi* (ATCC 6539) and *Salmonella typhimurium* (ATCC 14023) were obtained from the Department of Microbiology, Muhimbili University of Health and Allied Sciences (MUHAS).

Sample collection

Leaves, stem bark and roots of *C. bonariensis* and *T. terrestris* were collected from Nambala, Themi and Kaloleni areas in Arusha. Plant species were identified by Mr. Mboya from Tropical Pesticides Research Institute (TPRI) and voucher specimens AMB501 and AMB502 were are assigned for *C. bonariensis* and *T. terrestris* respectively. The leaves, stem barks and roots of *C. bonariensis* and *T. terrestris* were harvested while observing the sustainability of the plants.

Extraction process

The collected plant materials were washed thoroughly with tape water, dried in shade and pulverized with electrical grinder to form fine powders to increase surface area for extraction. From each of the plant material, 350 g of the powder was sequentially macerated twice by being completely immersed in 99.8% chloroform, 99.5% ethyl acetate and 99.5% methanol solvents for 48 hours at room temperature. All extracts were stored in the refrigerator at -20°C until the time of conducting bioassay experiments.

Antimicrobial assays

Minimum inhibitory concentration (MIC) of the extracts against bacteria and fungi was determined through micro dilution method using 96-well plates as proposed by Eloff [19] with minor modifications. Firstly, 100 mg of each plant extract was dissolved in 1 ml of DMSO to form 100 mg/mL stock solutions. In testing extracts' synergistic effect, 50 mg of one plant extract and the same amount of the other extract were mixed in a 1:1 ratio and then dissolved into 1 ml of DMSO to form 100 mg/mL stock solutions.

A 50 µL of Sabouraud's dextrose broth was loaded into sterilized 96-well microtitre plates followed by addition of 50 µl of 100 mg/mL extract in first well of each row to make a total volume of 100 µl in each of the first-row wells. The plant extracts and Sabouraud's dextrose broth were thoroughly mixed in the first rows. After that, 50 µl of the mixture from the first rows was shifted to the second rows and shifting continued down the columns until the last row where 50 µl from each well was discarded.

Next, 50 µl of microbial suspensions (0.5 MacFarland) were added in each well and incubated at 37°C for 24 hours followed by 20 µl of 0.02% p-iodonitrotetrazolium (INT) chloride dye and incubated for 1-hour at 37°C. Ketoconazole (100 µg/mL) and Ciprofloxacin were used as positive control for fungal and bacterial tests respectively. Dimethyl sulfoxide (DMSO) and the row which contained only broth were used as growth control representing negative control.

The change in the color of INT was observed where formation of pink indicated live microbes and persistence of the color of INT meant the microbes were dead. Even though 100% DMSO prohibits *C. albicans* growth, it has no effect in determining MIC because INT only causes color change to living organisms [20]. The lowest concentration of the extracts that killed or did not allow the survival of microbes was described as Minimum Inhibitory Concentration (MIC) that is the lowest effective dose against the tested organism.

Results

Results emanated from antimicrobial evaluation of *Conyza bonariensis* and *Tribulus terrestris* extracts are summarized in **Table 1**. The extracts were tested against four Gram-negative and one Gram-positive bacteria and two fungal strains and all the microbes are pathogenic to human being. The minimum inhibitory concentration (MIC) shows the activity of the extract against the microbes as presented in **Tables 1 and 2**. Eighty percent of the tested bacteria and 50% of the tested fungi were inhibited by the extracts at the MIC value of 0.78125 mg/mL.

Table 1 Antimicrobial activities of *C. bonariensis* and *T. terrestris* (leaf, stem and root bark).

Plant Extracts	Minimum Inhibition Concentration (mg/mL)							
	E. coli	*S. typhimurium*	*S. aureus*	*S. typhi*	*P. aeruginosa*	*C. albicans*	*C. neoformans*	S.E
CLC	6.25	6.25	0.78125	3.125	6.25	3.125	3.125	0.8125
CLE	1.5625	3.125	1.5625	6.25	0.78125	1.5625	3.125	0.697
CLM	3.125	1.5625	3.125	6.25	1.5625	12.5	3.125	1.4637
CSC	6.25	3.125	6.25	1.5625	3.125	12.5	0.78125	1.5609
CSE	6.25	0.78125	6.25	6.25	3.125	12.5	6.25	1.3623
CSM	3.125	3.125	6.25	12.5	3.125	6.25	1.5625	1.394
CRC	1.5625	6.25	1.5625	3.125	3.125	6.25	1.5625	0.7944
CRE	6.25	1.5625	0.78125	0.78125	1.5625	1.5625	3.125	0.7319
CRM	6.25	3.125	1.5625	1.5625	3.125	12.5	3.125	1.4637
TLC	3.125	12.5	6.25	12.5	1.5625	12.5	6.25	1.7717
TLE	3.125	6.25	3.125	0.78125	3.125	6.25	1.5625	0.797
TLM	12.5	12.5	6.25	12.5	6.25	6.25	12.5	1.2627
TSC	6.25	25	6.25	1.5625	3.125	6.25	1.5625	3.0849
TSE	6.25	6.25	3.125	3.125	1.5625	6.25	6.25	0.7624
TSM	3.125	12.5	6.25	6.25	6.25	6.25	3.125	1.1811
TRC	12.5	12.5	6.25	3.125	3.125	6.25	12.5	1.6504
TRE	6.25	3.125	6.25	6.25	0.78125	12.5	12.5	1.6592
TRM	3.125	6.25	1.5625	3.125	6.25	25	12.5	3.101
Cipr	0.78125	0.390625	0.78125	0.39062	0.390625	N/A	N/A	--
Keto		N/A	N/A	N/A	N/A	0.78125	0.390625	--

Key: CLC-*C. bonariensis* leaf chloroform, CLE-*C. bonariensis* leaf ethyl acetate, CLM-*C. bonariensis* leaf methanol, CSC-*C. bonariensis* stem chloroform, CSE-*C. bonariensis* stem ethyl acetate, CSM-*C. bonariensis* stem methanol, CRC-*C. bonariensis* root chloroform, CBRE-*C. bonariensis* root ethyl acetate, CRM-*C. bonariensis* root methanol, TLC-*T. terrestris* leaf chloroform, TLE-*T. terrestris* leaf ethyl acetate, TLM-*T. terrestris* leaf methanol, TSC-*T. terrestris* stem chloroform, TSE-*T. terrestris* stem ethyl acetate, TSM-*T. terrestris* stem methanol, Keto-Ketoconazole, Cipr-Ciprofloxacin, S.E-Standard error.

According to Rios and Recio [21], plant extracts that are suggested in the drug discovery initiatives are the ones with MIC values less than 1 mg/mL. In this study, *C. bonariensis* leaf chloroform (CLC), *C. bonariensis* leaf ethyl acetate (CLE), *C. bonariensis* stem chloroform (CSC), *C. bonariensis* stem ethyl acetate (CSE), *C. bonariensis* root ethyl acetate (CRE), *T. terrestris* leaf ethyl acetate (TLE) and *T. terrestris* root ethyl acetate (TRE) met that criterion. They exhibited MIC value of 0.78125 mg/mL against *S. aureus*, *P. aeruginosa*, *C. neoformans*, *S. typhimurium* and *S. typhi* (**Table 1**).

Table 2 Antimicrobial activities of mixed extracts from *C. bonariensis* and *T. terrestris* (leaf and root extracts).

Combined Plant Extracts	Minimum inhibition concentration (mg/mL)							
	E. coli	*S. typhimurium*	*S. aureus*	*S. typhi*	*P. aeruginosa*	*C. albicans*	*C. neoformans*	S. E
CLCCLE	0.390625	3.125	1.5625	0.78125	1.5625	1.5625	1.5625	0.3238

CLCCLM	1.5625	1.5625	6.25	1.5625	3.125	3.125	0.78125	0.697
CLCCRC	0.78125	6.25	1.5625	6.25	1.5625	6.25	3.125	0.9448
CLCCRE	0.390625	1.5625	0.7812	3.125	3.125	3.125	1.5625	0.4429
CLCCRM	1.5625	3.125	3.125	1.5625	6.25	1.5625	3.125	0.6313
CLECRC	12.5	6.25	3.125	6.25	0.78125	0.78125	1.5625	0.16057
CLECLM	3.125	6.25	12.5	1.5625	3.125	3.125	1.5625	1.464
CLECRE	3.125	0.78125	3.125	3.125	1.5625	1.5625	6.25	0.6789
CLECRM	0.78125	1.5625	1.5625	1.5625	3.125	3.125	1.5625	0.3348
CLMCRC	3.125	1.5625	3.125	0.78125	6.25	1.5625	1.5625	0.697
CLMCRE	6.25	1.5625	0.78125	0.78125	1.5625	6.25	1.5625	0.9315
CLMCRM	6.25	3.125	1.5625	12.5	6.25	1.5625	6.25	1.441
CLCTLC	0.78125	6.25	1.5625	0.39062	3.125	3.125	3.125	0.7466
CLCTLE	1.5625	6.25	6.25	1.5625	6.25	6.25	1.5625	0.947
CLCTLM	3.125	3.125	0.7812	1.5625	6.25	1.5625	1.5625	0.697
CLETLC	6.25	3.125	1.5625	0.78125	1.5625	0.78125	0.78125	0.757
CLETLE	0.7812	6.25	1.5625	3.125	0.78125	12.5	3.125	1.585
CLETLM	1.5625	0.78125	3.125	1.5625	0.78125	6.25	3.125	0.7319
CLMTLC	6.25	0.78125	3.125	3.125	1.5625	3.125	1.5625	0.6789
CLMTLE	1.5625	1.5625	6.25	1.5625	6.25	1.5625	3.125	0.8352
CLMTLM	1.5625	6.25	1.5625	6.25	1.5625	6.25	1.5625	0.947
CLCTRC	3.125	1.5625	3.125	1.5625	1.5625	6.25	3.125	0.6313
CLCTRE	3.125	3.125	0.7812	1.5625	3.125	1.5625	1.5625	0.3702
CLCTRM	0.78125	1.5625	3.125	3.125	1.5625	6.25	0.78125	0.7319
CLETRC	0.7812	1.5625	1.5625	0.78125	6.25	1.5625	3.125	0.7319
CLETRE	1.5625	6.25	1.5625	1.5625	1.5625	6.25	6.25	0.947
CLETRM	6.25	3.125	0.7812	6.25	1.5625	1.5625	0.78125	0.9135
CLMTRC	3.125	0.78125	1.5625	6.25	6.25	1.5625	1.5625	0.8764
CLMTRE	0.78125	0.78125	3.125	3.125	3.125	6.25	0.78125	0.757
CLMTRM	1.5625	6.25	0.7812	1.5625	1.5625	6.25	6.25	0.992
TLCTLE	0.78125	0.78125	3.125	6.25	3.125	3.125	6.25	0.8475
TLCTLM	1.5625	1.5625	3.125	3.125	1.5625	12.5	3.25	1.4794
TLETLM	1.5625	3.125	6.25	1.5625	1.5625	6.25	6.25	0.8929
TLETRC	0.78125	1.5625	1.5625	1.5625	3.125	1.5625	3.125	0.3348
TLETRE	1.5625	3.125	3.125	0.78125	0.78125	1.5625	1.5625	0.3702
TLETRM	0.78125	3.125	0.7812	1.5625	1.5625	3.125	3.125	0.4126
TRCTRE	0.78125	0.78125	1.5625	1.5625	0.78125	6.25	1.5625	0.7403
TRETRM	1.5625	1.5625	3.125	3.125	1.5625	1.5625	3.125	0.3157
TLMTRC	3.125	1.5625	6.25	3.125	3.125	1.5625	6.25	0.7403
TLMTRE	1.5625	3.125	3.125	6.25	1.5625	3.125	3.125	0.5906

TLMTRM	3.125	6.25	3.125	3.125	1.5625	6.25	3.125	0.6696
Cipro	0.78125	0.390625	0.78125	0.39062	0.39063	N/A	N/A	
Keto	N/A	N/A	N/A	N/A	N/A	0.78125	0.390625	

Key: CLCCLE-*C. bonariensis* leaf chloroform and *C. bonariensis* leaf ethyl acetate, CLCCLM-*C. bonariensis* leaf chloroform and *C. bonariensis* leaf ethyl methanol, CLCCRC-*C. bonariensis* leaf chloroform and *C. bonariensis* root chloroform, CLCCRE-*C. bonariensis* leaf chloroform and *C. bonariensis* root ethyl acetate, CLCCRM-*C. bonariensis* leaf chloroform and *C. bonariensis* root methanol, CLECRC-*C. bonariensis* leaf ethyl acetate and *C. bonariensis* root chloroform, CLECLM-*C. bonariensis* leaf ethyl acetate and *C. bonariensis* leaf methanol, CLECRE-*C. bonariensis* leaf ethyl acetate and *C. bonariensis* root ethyl acetate, CLECRM-*C. bonariensis* leaf ethyl acetate and *C. bonariensis* root methanol, CLMCRC-*C. bonariensis* leaf methanol and *C. bonariensis* root chloroform, CLMCRE-*C. bonariensis* leaf methanol and *C. bonariensis* root ethyl acetate, CLMCRM-*C. bonariensis* leaf methanol and *C. bonariensis* root methanol, CLCTLC-*C. bonariensis* leaf chloroform and *T. terrestris* leaf chloroform, CLCTLE-*C. bonariensis* leaf chloroform and *T. terrestris* leaf ethyl acetate, CLCTLM-*C. bonariensis* leaf chloroform and *T. terrestris* leaf methanol, CLETLC-*C. bonariensis* leaf ethyl acetate and *T. terrestris* leaf chloroform, CLETLE-*C. bonariensis* leaf ethyl acetate and *T. terrestris* leaf ethyl acetate, CLETLM-*C. bonariensis* leaf ethyl acetate and *T. terrestris* leaf methanol, CLMTLC-*C. bonariensis* leaf methanol and *T. terrestris* leaf chloroform, CLMTLE-*C. bonariensis* leaf methanol and *T. terrestris* leaf ethyl acetate, CLMTLM-*C. bonariensis* leaf methanol and *T. terrestris* leaf methanol, CLCTRC-*C. bonariensis* leaf chloroform and *T. terrestris* root chloroform, CLCTRE-*C. bonariensis* leaf chloroform and *T. terrestris* root ethyl acetate, CLCTRM-*C. bonariensis* leaf chloroform and *T. terrestris* root methanol, CLETRC-*C. bonariensis* leaf ethyl acetate and *T. terrestris* root chloroform, CLETRE-*C. bonariensis* leaf ethyl acetate and *T. terrestris* root ethyl acetate, CLETRM-*C. bonariensis* leaf ethyl acetate and *T. terrestris* root methanol, CLMTRC-*C. bonariensis* leaf methanol and *T. terrestris* root chloroform, CLMTRE-*C. bonariensis* leaf methanol and *T. terrestris* root ethyl acetate, CLMTRM-*C. bonariensis* leaf methanol and *T. terrestris* root methanol, TLCTLE-*T. terrestris* leaf chloroform and *T. terrestris* leaf ethyl acetate, TLCTLM-*T. terrestris* leaf chloroform and *T. terrestris* leaf methanol, TLETLM-*T. terrestris* leaf ethyl acetate and *T. terrestris* leaf methanol, TLETRC-*T. terrestris* leaf ethyl acetate and *T. terrestris* root chloroform, TLETRE-*T. terrestris* leaf ethyl acetate and *T. terrestris* root ethyl acetate, TLETRM-*T. terrestris* leaf ethyl acetate and *T. terrestris* root methanol, TRCTRE-*T. terrestris* root chloroform and *T. terrestris* root ethyl acetate, TRETRM-*T. terrestris* root ethyl acetate and *T. terrestris* root methanol, TLMTRC-*T. terrestris* leaf methanol and *T. terrestris* root chloroform, TLMTRE-*T. terrestris* leaf methanol and *T. terrestris* root ethyl acetate, TLMTRM-*T. terrestris* leaf methanol and *T. terrestris* root methanol, S.E-Standard error.

Apparently, *C. albicans* and *E. coli* were the least susceptible to the tested extracts in which none of the extracts exhibited their growth at MIC value of 0.78125 mg/mL. Two extracts, namely CLE and CRE inhibited *C. albicans* at MIC value 1.5625 mg/mL while CLE and *C. bonariensis* root chloroform (CRC) inhibited *E. coli* at the same concentration.

It was noted that, 75% of extracts with MIC values below 1 mg/mL emanated from *C. bonariensis* extracts and 25% from *T. terrestris* extracts. This means *C. bonariensis* is preferably a more promising source for antimicrobial agents than *T. terrestris*. The lethality of phytochemicals contained in extracts was specific to the tested pathogens. The type of solvent used in extraction appeared to influence antimicrobial activity of the extracts. In a total of seven 0.78125 mg/mL MIC value, five of them were ethyl acetate extracts, two chloroform extracts and none methanol extracts.

Some phytochemicals demonstrate more pharmacokinetic when in combination with other relevant compounds than when in isolation [5].

In light of that, the synergistic effect of phytochemicals from different plant extracts were examined. The findings of the investigation demonstrated the presence of antimicrobial synergistic effect in the combined extracts which was evidenced by increasing antimicrobial efficacy of extracts. Microbial susceptibility to phytochemicals in combined extracts was determined to be species specific. *Escherichia coli* that was the most resistant bacterium in uncombined extracts became the most susceptible to mixed extracts. This was attributed by the observation that 32% of extracts' combinations were effective against *E. coli* at MIC values of 0.78125 mg/mL and 0.390625 mg/mL. The strongest synergistic effect came from CLC (6.25 mg/mL)-CLE (1.5625 mg/mL) and CLC (6.25 mg/mL)-CRE (6.25 mg/mL) combinations which inhibited the growth of *E. coli* at MIC values of 0.390625 mg/mL. Combinations CLC (06.25 mg/mL)-CRC (1.5625 mg/mL); CLE (1.5625 mg/mL)-CRM (6.25 mg/mL); CLE (1.5625 mg/mL)-CRM (6.25 mg/mL); CLC (6.25 mg/mL)-TLC (3.125 mg/mL); CLE (1.5625 mg/mL)-TLE (3.125 mg/mL); CLC (6.25 mg/mL)-TRM (3.125 mg/mL) and CLE (1.5625 mg/mL)-TRC (12.5 mg/mL) inhibited the growth of *E. coli* at MIC value 0.7825 mg/mL. Other combinations which inhibited the growth of *E. coli* at 0.7825 mg/mL MIC value to attest antimicrobial synergism of their phytochemicals were CLM (3.125 mg/mL)-TRE (6.25 mg/mL); TLC (3.125 mg/mL)-TLE (3.125 mg/mL); TLE (3.125 mg/mL)-TRC (12.5 mg/mL); TLE (3.125 mg/mL)-TRM (3.125 mg/mL); and TRC (12.5 mg/mL)-TRE (6.25 mg/mL).

Extract combinations of CLE (3.125 mg/mL)-CRE (1.5625 mg/mL); CLE (3.125 mg/mL)-TLM (12.5 mg/mL); CLM (1.5625 mg/mL)-TLC (12.5 mg/mL); CLM (1.5625 mg/mL)-TRC (12.5 mg/mL); CLM (1.5625 mg/mL)-TRE (3.125 mg/mL); TLC (12.5 mg/mL)-TLE (6.25 mg/mL) and TRC (12.5 mg/mL)-TRE (3.125 mg/mL) had strong antibacterial activity of 0.7825 mg/mL values against *S. typhimurium*.

Seven percent of the 41 extract combinations namely CLE (1.5625 mg/mL)-TRM (1.5625 mg/mL); CLM (3.125 mg/mL)-TRM (1.5625 mg/mL) and TLE (3.125 mg/mL)-TRM (1.5625 mg/mL) demonstrated antibacterial synergistic effect against *S. aureus* which was revealed lowered MIC values to 0.78125 mg/m. For the case of *S. typhi*, 4 out of 41 extract combinations had lower MIC values than before they were mixed. These combinations were (6.25 mg/mL)-CRC (3.125 mg/mL); CLE (6.25 mg/mL)-TLC (6.25 mg/mL) and CLE (6.25 mg/mL)-TRC (6.25 mg/mL) to 0.78125 mg/mL. However, extract combination CLC (3.125 mg/mL)-TLC (6.25 mg/mL) produced the strongest synergistic effect against *S. typhi* that lowed the MIC values to 0.390625 mg/mL. Extracts did not show remarkable synergistic effect against *Pseudomonas aeruginosa* bacterium.

The fungus *C. neoformans* was more susceptible to phytochemical synergistic effect than *C. albicans*. The extracts' combinations CLE (1.5625 mg/mL)-CRC (6.25 mg/mL) and CLE (6.25 mg/mL)-TLE (6.25 mg/mL) showed strong antifungal activity to *C. albicans* at MIC value of 0.78125 mg/mL while

combinations CLC (3.125 mg/mL) CLM (3.125 mg/mL); CLE (3.125 mg/mL)-TLC (6.25 mg/mL); CLC (3.125 mg/mL)-TRM (12.5 mg/mL); CLE (3.125 mg/mL)-TRM (12.5 mg/mL); and CLM (3.125 mg/mL)-TRM (12.5 mg/mL) had strong antifungal activity to *C. neoformans* at MIC value of 0.78125 mg/mL.

Discussion

Evaluation of traditional medicine has been a reliable approach in drug discovery process [8]. However, the medicinal efficacy of some traditionally used medicinal plants have not been validated. In light of that fact, this study is reporting for the first time the antibacterial and antifungal activity of *C. bonariensis* and *T. terrestris* growing in Arusha region of Tanzania. According to Eloff [19], extracts with MIC values 0.05-0.5 mg/mL, 0.6-1.5 mg/mL and above 1.5 mg/mL represented strong, moderate and weak antimicrobial activity respectively. According to Rios and Recio [21], plant extracts which should be considered in drug discovery enterprises are the ones with MIC values less than 1 mg/mL. Extracts which displayed antifungal and antibacterial activity with MIC values 0.78125 and 0.39065 mg/mL were qualified for further examination as foundations of drug leads.

In this aspect, *C. bonariensis* leaf chloroform, *C. bonariensis* stem ethyl acetate, *C. bonariensis* root ethyl acetate, *C. bonariensis* leaf ethyl acetate, *T terrestris* leaf ethyl acetate and *T. terrestris* root ethyl acetate demonstrated antibacterial against *S. aureus*, *S. typhimurium*, *S. typhi* and *P. aeruginosa* with MIC of 0.78125 mg/mL are possible antibiotic templates for the diseases caused by these bacteria. Similarly, *C. bonariensis* stem chloroform extract inhibited *C. neoformans* with MIC of 0.78125 mg/mL qualify to be a possible antifungal drug lead for treatment of *C. neoformans* caused infections.

According to Uzun et al., [22] antimicrobial synergistic effect of phytochemicals occurs when different extracts are combined. Phytochemicals in *C. bonariensis* extracts produced synergistic effect against tested bacteria when combined with synthetic antibiotics [23]. The findings showed that *E. coli* was selectively more prone to combined extracts than rest of tested bacteria. The bacteria was resistant to all extracts at MIC values below 1 mg/mL. However, the bacterium was sensitive to about 32% of extracts' combinations. The fungus, *C. albicans* which initially was not inhibited by extracts before they were mixed, became susceptible to CBLE-CBRC and CBLE-TTLC extract combinations at MIC value of 0.78125 mg/mL. The effect of antimicrobial synergism of phytochemicals suggest that formulations of antimicrobial drugs should involve antimicrobial agents from different sources and extracted from different solvents. For better extraction, the type of solvent used, method of extraction and specifics during collection of materials have effects on results of antimicrobial activity of the extracts and should be valued [21].

The antimicrobial activity of *C. bonariensis* has been investigated in different parts of the world. *Conyza bonariensis* which is used to manage HIV/AIDS opportunistic infections in Tanzania and Uganda [24,25] possess antimicrobial activity. In Pakistan, the methanol and ethyl acetate extracts failed to inhibit the growth of *C. albicans*, *E. coli*, *S. aureus* and *S. typhimurium* at the MIC of 20 mg/mL [26]. However, the same study revealed that methanolic and ethyl acetate extracts from *C. bonariensis* inhibited *P. aeruginosa* and *Staphylococcus epidermidis* at 13.3 mg/mL and 16 mg/mL respectively [26]. Another study in Yemen discovered the antibacterial activity of *C. bonariensis* ethanol extracts against *S. aureus*, *E. coli* and *S. typhimurium* bacteria whereas *S. aureus* was mostly inhibited by extracts like in this study [11]. Furthermore, essential oil from Kenyan *C. bonariensis* exhibited antibacterial activity against two Gram-negative bacterial strains namely *E. coli* and *S. typhi* [27]. They found that, *S. typhi* was also more vulnerable than *E. coli* to *C. bonariensis* extracts. Although not very much studied, the antimicrobial activity of *T. terrestris* have been reported [15]. According to Hashim et al. [15], *T. terrestris* contain high amount of spirostanol saponins which inhibited fungal growth of *C. albicans* and *C. neoformans*. The antimicrobial activity of *T. terrestris* extracts are also in line with reported antifungal and antibacterial activity in Iran [28]. However, unlike the findings from this study, the Pakistani *T. terrestris* showed no antibacterial activity against *P. aeruginosa* bacterium [29]. The ethno-medical uses of *C. bonariensis* and *T. terrestris* in management of bacterial and fungal infections are endorsed by the findings of this study. The medicinal value of *T. terrestris* is highly associated with its high content of saponins which its composition varies due to geographical locations [15].

Conclusion

The extracts from *C. bonariensis* and *T. terrestris* revealed antifungal activity to two fungal strains namely *C. neoformans* and *C. albicans*. The extracts from the same plants also revealed antibacterial activities against four tested Gram-negative bacteria namely *E. coli*, *S. typhimurium*, *S. typhi* and *P. aeruginosa* and against one Methicillin- resistant Gram-positive bacterium namely *S. aureus*.

This study has publicized the antifungal and antibacterial activity of *C. bonariensis* and *T. terrestris* growing in Tanzania. It has further revealed the influence of phytochemicals' synergistic effect in management of bacterial and fungal infections.

Conflict of Interest

Authors declare that there is no competing interests exist.

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