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ORIGINAL ARTICLE

Chemical profiling and antimicrobial potential of crude extract andessential oil from *Conyza bonariensis* leaves

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ABSTRACT

Background and objectives: Microbial infections have made significant contributions to the global health burden, particularly in developing countries with lagging economies. Challenges arising from the evolution of antimicrobial-resistant (AMR) bacteria have led to the search for novel antimicrobial agents, especially those derived from medicinal plants. Therefore, this study aimed to assess the antimicrobial activity of ethyl acetate crude extracts and essential oils from C. bonariensis collected from the Mbeya region of Tanzania.

Methods: Initial experimental activities involved the preparation of crude extracts and essential oil, which were then, tested for antimicrobial activity against selected test organisms (Staphylococcus aureus (ATCC29213), Bacillus subtilis (ATCC6051), Escherichia coli (ATCC8736), Salmonella typhi (ATCC6539), and Candida albicans (DSM1665)) by using the disc diffusion method. On the other hand, the chemical composition profiling of ethyl acetate and essential oils was performed by gas chromatography-mass spectrometry (GC-MS).

Results: The crude extracts and essential oil of C. bonariensis leaves from Tanzania demonstrated to have antibacterial and antifungal activity. The minimum inhibitory concentration (MIC) of ethyl acetate leaf crude extract was 25 mg/mL > MIC > 12.5 mg/mL for tested microorganisms, with the exception of C. albicans, where the MIC was 50 mg/mL > MIC > 25 mg/mL. Similarly, for all tested microorganisms, the MIC of leaves essential oils was 1:1 > MIC > 1:2, except S. typhi, where the MIC was 2:1 > MIC > 1:1. On the other hand, 2,4-di-tert-butyl phenol in leaves crude extracts had the highest percentage composition of peak area (41%); whereas, in essential oil, 2,4-Di-tert-butyl phenol had the highest percentage composition (38.8%).

Conclusions: The present findings have demonstrated antimicrobial potential of ethyl acetate crude extract and essential oil of C. bonariensis leaves from Tanzania, and validate the wide use of the plant by local communities, particularly the local community of Mbeya region in Tanzania.

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Keywords Conyza bonariensis, Antimicrobial Potential, Essential Oil

INTRODUCTION

Microbial infections have a significant contribution to the health burden worldwide, particularly in developing countries dueto lagging economies. [1]The microbial infection burden is further exacerbated by the emergence of antimicrobial resistant microbes. [1], [2] It has been estimated that about 700,000 people die each year as a result of antimicrobial resistance (AMR) around the world. [3] In Sub-Saharan Africa (SSA), it was found that 73.4% of deaths caused by microbial infections were linked to antibiotic resistance of infectious agents. [3] Specifically, in Tanzania, about 25% to 50% of the mortality caused by microbial infections was associated with antimicrobial resistance. Furthermore, antibiotic resistance threatens not just the country's mortality rate but also its economic stability, particularly in developing nations like Tanzania.[4] Moreover, within clinical settings in Tanzania, the prevalence of multidrug-resistant bacteria ranges from 25% to 50%. [1], [4], [5] These challenges caused by the evolution of antimicrobial resistant bacteria entail searching for novel antimicrobial agents, particularly from medicinal plants.

Medicinal plants are plants with therapeutic properties and have beneficial pharmacological effects on the human or animal body.[6] Medicinal plants are a good source of bioactive substances such as flavonoids, tannins, curcumin, and resveratrol, which have antibacterial, anti-diabetic, anti-ulcer, and antioxidant properties. [7] For example, *Plantago major* from Spain. [8] Has been reported to have antimicrobial activities. *Aloe vera* which is from China, has been reported to have anti-diabetic effects [9] and *Momordica charantia* which is from Turkey has antimicrobial and antioxidant activities. [10] Toassess the antibacterial activity of medicinal plants leaves crude extracts and essential oil have been found to be more appropriate.

Essential oils are aromatic, volatile liquids obtained from plant material through steam distillation and named after the plant from which they are derived. [11] Essential oils extracted from various medicinal plants have been shown to contain high levels of bioactive metabolites that have antimicrobial activity.[12] Essential oils have been widely used in the treatment of infectious diseases like skin diseases, respiratory diseases, and intestinaldisorders. For example, essential oil from *Abies balsamea* is used for the treatment of skin infections. [13] Also, essential oils from *Lavandula angustifolia* and *Mentha piperita* medicinal plants have been used in treatment of respiratory disorders. [14]

One empirical investigation was conducted in Tanzania to assess the antibacterial efficiency of *C. bonariensis*, and the results were promising. [15] The inhibitory concentration of ethyl crude extracts of *C. bonariensis* leaves collected in Tanzania was around 1.6, 1.6, 6.3 and 1.7 mg/mL against *E. coli*, *S. aureus*, *S. typhi*, and *C. albicans*, respectively.[15] However, essential oil from C. bo*nariensis was* not tested for antibacterial activity. Also, it is widely understood that the composition of secondary metabolites in medicinal plants can be influenced by variety of circumstances, including temperature, water, and soil properties.[16] This suggests a wide-ranging surveillance study to assess the antimicrobial activity of crude extracts and essential oils of *C. bonariensis* collected from other places including Mbeya region of Tanzania. The aim of the present study was to determine the antimicrobial activity of crude extracts and essential oil from C. *bonariensis* leaves and to profile chemical composition of *C. bonariensis* leaves crude extracts and essential oil.

MATERIALS AND METHODS

Collection of plant materials

Plant samples were collected from Mbeya region of Tanzania. Purposive random sampling was used to select healthy leaves between November and December 2021 for extraction of leaves crude extracts and essential oil from *C. bonariensis*. For each subsequent sampling, a sample voucher was included for identification and was provided with voucher number FMM4139 after identification by a plant taxonomist at Botany Department, University of Dar Es Salaam.

Tested microorganisms

Five test microorganisms used were Gram-positive bacteria *Staphylococcus aureus* (ATCC29213), *Bacillus subtilis* (ATCC6051) Gram-negativebacteria E. coli (ATCC8736), *Salmonella typhi* (ATCC 6539) and the yeast *Candida albicans* (DSM1665), all obtained from the microbial strains library of the Department of Molecular Biology and Biotechnology (MBB), University of Dar Es Salaam. Bacteria and fungi were grown in nutrient agar and potato dextrose agar and incubated at 37 ˚C and 30˚C for 24 hours and 48 hours respectively. Bacterial and fungal colonies were suspended in normal saline, which were then compared with 0.5 McFarland standards (prepared by adding 0.05 mL of 1.175% $BaCl_2$ to 9.95 mL of 1% H_2SO_4). Suspensions of tested organisms were kept in the test tubes at 4 ˚C for a maximum of overnight. [17]

Leavescrude extractsandessential oil preparation

Leaves crude extracts preparation

Leaves were kept in the shade for 21 days to dry. The dried leaves were ground into a fine powder using a grinder (Panasonic AC MX-AC300-H 550-Watt Mixer Grinder). The ground powder was soaked in 99.55% ethyl acetate and left for 72 hours so as to extract the bioactive constituents. The mixture was filtered using Whatman No.1 filter paper (pore size 11 μm) and the filtrate was then concentrated on the rotary evaporator (Moddel No RE-501) at 40˚C and 100 mbar pressure. The obtained crude extract was stored at 4˚Cfor further use. [6, 16]

Essential oil preparation

Fresh leaves were cleaned in tap water and placed in a round-bottomed flask. An electric burner was used to set up a steam distillation setup, which was followed by heating the fresh leaves to 100 ˚C. [16], [18] The essential oil was gathered in the collector after 45 minutes of boiling and followed by the addition of an equivalent volume of dichloromethane to the harvest. The lower layer was removed and concentrated using a rotary evaporator at 40 ˚C and 100 mbar pressure. The oil was collected and stored in dark glass tubes at 4 ˚C for later use. [12], [16]

Screening for antimicrobial activity and Minimum inhibitory concentration

Screening for antimicrobial activities

Sterile 5 mm discs, which were prepared from Whatman No. 1 filter paper (pore size of 11 μm), were soaked with 20μL of various concentrations (200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL) of C. bonariensis leaves crude extract. For C. bonariensis essential oils 20 μL of 1:1 with Dimethyl sulfoxide (DMSO) was used to soak discs. Discs were placed at 4^{o}C in the refrigerator for 30 minutes to allow proper soaking before transferring to Petri dishes. Well-soaked discs were placed on Petri dishes, which were initially inoculated with test organism suspension by using sterile cotton swabs. Then, Petri dishes were incubated at 37˚C and 30˚C for 24 hours and 48 hours for bacteria and yeast, respectively. Ten percent of dimethyl sulfoxide (DMSO) was used as negative control; whereas, Chloramphenicol (0.25mg/ml) and Fulconazole (0.25mg/ml) were used as positive control for Bacteria and Fungi, respectively. The experiment was performed in triplicate then, zones of growth inhibition were measured after 24 hours of incubation for bacteria, and after 48 hours of incubation for fungi. [19]

Minimum inhibitory concentration

For test organisms that were inhibited by extracts and/or essential oils, the minimum inhibitory concentration (MIC) was determined. The MIC was carried out using the disc diffusion method, as described before. [19] Each of the twofold serial dilutions of crude extracts and essential oil was tested for growth inhibition of test organisms. The discs were prepared, the test organisms were inoculated into Petri dishes, and the incubation period was as indicated in the section on screening for antimicrobial activities

Chemical analysis of Crude extract and essential oils

Chemicalanalysis was performedby Gas Chromatography-Mass Spectrometryrecorded in GCMS-2010 Shimadzu instrument operating in Electron Ionization (EI) mode (MS) at 70ev, and Flame Ionization Detector (FID) for GC. A Restek-5MS column (30mx 0.25mmx 0.25μm) was used. The oven temperature program was 90^{o}C to 280 ^{o}C and held at 90 ^{o}C for two minutes. The temperature was increased to 280 ^{o}C for 10 minutes (hold time) at a rate of 15 ^{o}C per minute. The injection temperature was 250^{o}C with a split injection mode. The flow rate of carrier gas helium was 1.21ml min-1. The ion source temperature and interface temperature in MS were 230 ^{o}C and 300 ^{o}C, respectively. The identification of chemicals in theextracts was donebythescan method, which involves theuse of Mass Spectral Library&

Search Software (NIST 11). The quantification of phytochemicals in the extracts was done using the Peak Integration method (area normalization), whereby the ion allowance was 20%, the target ion and other five quantization ions were used in the quantitative analysis. 10 μL of the sample was dissolved in dichloromethane to make 1mL and injected in GC da Silva et al., 2021, The results were reported as percentage compositions derived from peak area of all scanned compounds in the extracts.[20], [21]

DATA ANALYSIS

InR software, descriptive and inferential statistics were used to examine the data (R core team). For descriptive statistics, bar graphs were used to summarize the statistics; for inferential statistics, two-way analysis of variance (ANOVA) was employed to test whether therewas a difference between the means of the zone of inhibition index of test among tested microorganisms. Following ANOVA, Tukey HSD was performed for pair wisecomparison, with a significant difference set at P<0.05.

RESULTS

Antimicrobial activity of C. bonariensis leaves crude extract and essential oil

The antimicrobial activity of essential oil and crude extract from *Conyza bonariensis* leaves was investigated using the disc diffusion method, as explained in subsection 2.2. Zones of inhibition against test microorganisms are depicted in Figure 1-2.

Activity indices, which were derived from zones of inhibition values, are presented in Table 1. The activity index for essential oil ranged from 0.22 to 0.47 against S. typhi and B. subtilis, respectively, and the crude extract activity index ranged from 0.35 to 0.4 against S. aureus and B. subtilis, respectively. Generally, crude extract had slightly elevated (P>0.05) antimicrobial activity as compared to essential oils (Table 1).

Table 1. Antimicrobial activity of extracts from *Conyza bonariensis* leaves presented as activity index and standard error of mean

Testorganism	Positivecontrol	Essential oil	Leaves crude extract
	Mean values of activity indices ± standard deviation		
Escherichia coli	$1.00±0.00^{b,1}$	$0.31±0.04^{a,2}$	$0.38±0.03^{b,2}$
Candidaalbicans	$1.00±0.00^{b,1}$	$0.44±0.04^{b,2}$	$0.37±0.03^{b,2}$
Staphylococcus aureus	$1.00±0.00^{b,1}$	$0.38±0.06^{a,2}$	$0.35±0.03^{b,2}$
Salmonella typhi	$1.00±0.00^{b,1}$	$0.22±0.31^{a,1}$	$0.38±0.03^{b,1}$
Bacillussubstilis,	$1.00±0.00^{b,1}$	$0.47±0.06^{b,2}$	$0.40±0.01^{b,2}$

Column that bear different superscript later are significant different (P<0.05), and row that bear different superscript number are significant different (P<0.05); positive control was chloramphenicol and fluconazole for bacteria and fungus, respectively. Concentrations

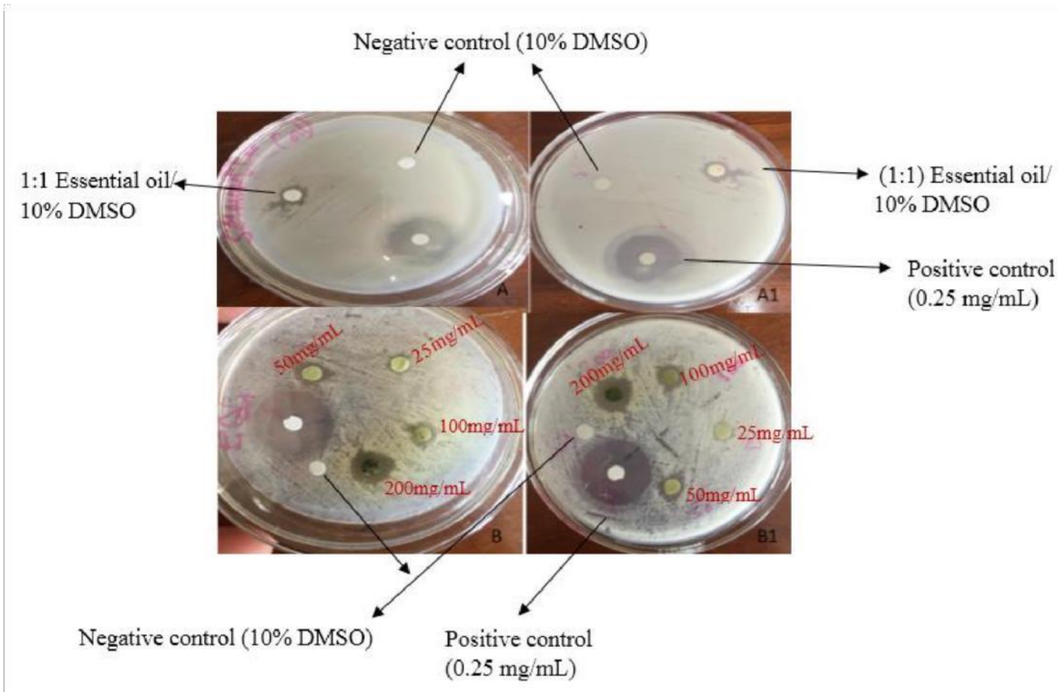

Figure 1 Representative images of zone of inhibition for crude extracts and essential oils from C. bonariensis leaves. A and A1 are front and back views of inhibition zone for C. bonariensis essential oil against S.typhi; B and B1 are front and back views of inhibition zone for C. bonariensis leaves crude extract against E. coli.

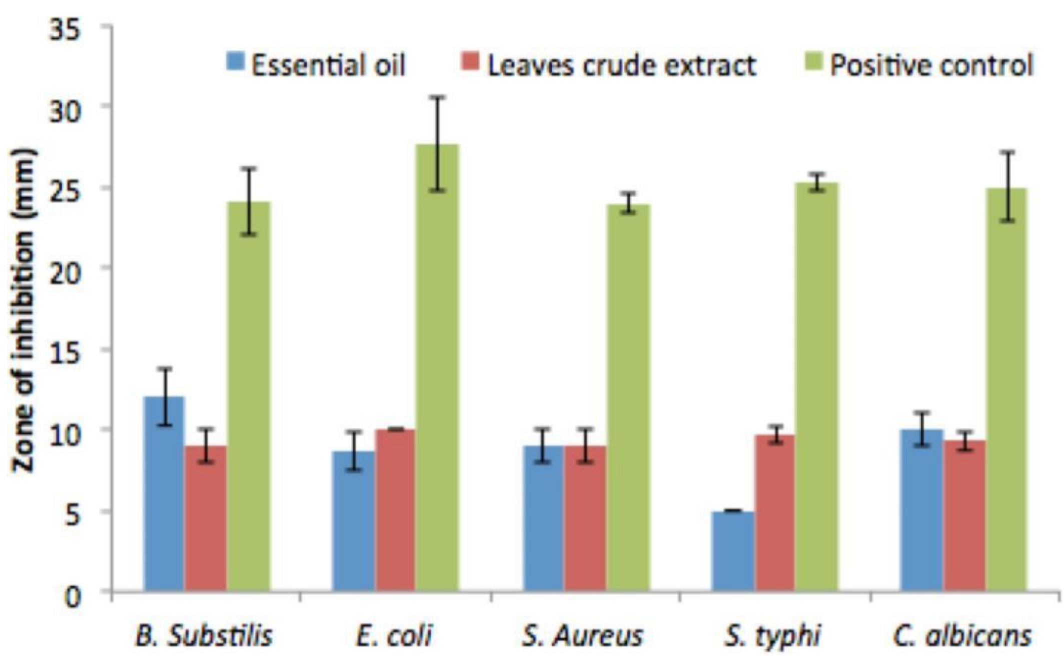

Figure 2 A bar graph representing zone of inhibition (mm) of ethyl acetate crude extracts and essential oils from C. bonariensis leave against test organisms; error bars represent standard deviation, Positive control were chloramphenicol and fluconazole (0.25 mg/mL) for bacteria and fungi, respectively.

of positive control and leaves crude extract were 0.25 mg/mL and 100mg/mL, respectively. Essential oil concentration was 1:1μL dilution with 10% DMSO

The minimum inhibitory concentration of C. bonariensis leaves crude extract and essential oil

The MIC of crude extract and essential oil was evaluated for each of the tested microorganisms as demonstrated in Table 2. For leavecrude extract, the highest MIC was 25 mg/mL >MIC> 12.5 mg/mL, for four tested microorganisms, and the lowest MIC was 50 mg/mL >MIC> 25 mg/mL for C. albicans. Furthermore, for essential oil higher MIC was 1:1>MIC> 1:2 (means that essential oil was diluted with equal volume (20 μL) of 10% DMSO) for all microorganisms and the lowest MIC was 2:1>MIC> 1:1 (means 20 μL of essential oil was diluted with 10 μL volume of 10% DMSO) for S. typhi.

Table 2. Minimum inhibitory concentration of crude extracts in mg/mL and essential oil v/v (μl) extracted from *Conyza bonariensis*.

Extracts	**Minimum inhibitory concentration of the crude extracts on selected microorganisms**				
	S. aureus	B. substilis	E. coli	S. typhi	C. albicans
Leaves crude	25>MIC>12.5	25>MIC>12.5	25>MIC>12.5	25>MIC>12.5	50>MIC>25
Essential oil	1:1>MIC> 1:2	1:1>MIC> 1:2	1:1>MIC> 1:2	2:1>MIC> 1:1	1:1>MIC> 1:2

For essential oils, the ratio 1:1 means that essential oil was diluted with equal volume (20 μL) of 10% dimethyl sulfoxide (DMSO), and therefore the essential oil was diluted to half of original concentration. Whereas, for ratio 1:2, means that the essential oil was diluted to one third of original concentration.

Chemical composition of C. bonariensis leaves crude extract

Phytochemical analysis of the crude extract from *C. bonariensis* leaves detected different compounds based on peak area, retention time, and peak area and peak height. As illustrated in Figure 3 below, alkene and phenol predominated in crude extract from *C. bonariensis* leaves, especially 2,4-Di-tert-butylphenol (41.6%), Phytol (11.9%), 1-Nonadecene (8.7%), (-)-Spathulenol (6.6%), 1-Heptadecenol (6.4%), n-Tetracosanol-1 (6.4%), and Caryophyllene oxide (3.3%), as illustrated in figure 3 below. It is very interesting that most of the compounds detected in the present study have been demonstrated to have various health benefits as shown in Table 3.

Table 3. Biologically active chemical compounds of leaves crude extract from *Conyza bonariensis*

Name of compound	% Composition (Peak area)	R. Time	Chemical formula	Target Peak	P. Area	P. Height	Activity
2,4-Di-tert-butylphenol	41.6231	10.439	$C_{14}H_{22}O$	191.15	86349	49914	Antioxidant and antifungal [22]
(-)-Spathulenol	6.57777	11.244	$C_{15}H_{24}O$	119.15	17506	7888	Antibacterial Activity [22]
Caryophyllene oxide	3.26053	11.306	$C_{15}H_{24}O$	79.05	6422	3910	Antimicrobial [23]
1-Heptadecene	6.4443	13.537	$C_{17}H_{34}$	57.1	16961	7728	Antifungal [24]
Phytol, acetate	15.01263	14.013	$C_{22}H_{42}O_2$	68.1	33252	18003	Antioxidant [25]Anti-inflammatory [22]
1-Nonadecene	8.72339	15.533	$C_{19}H_{38}$	57.1	19951	10461	Antifungal [26]
Phytol	11.94556	16.66	$C_{20}H4_{0}O$	71.1	31524	14325	Ant nociceptive [27] and Antioxidant [28], anti-inflammatory and [29] anti-allergic
n-Tetracosanol-1	6.41266	17.355	$C_{24}H_{50}O$	97.1	14111	7690	Antibacterial [30]

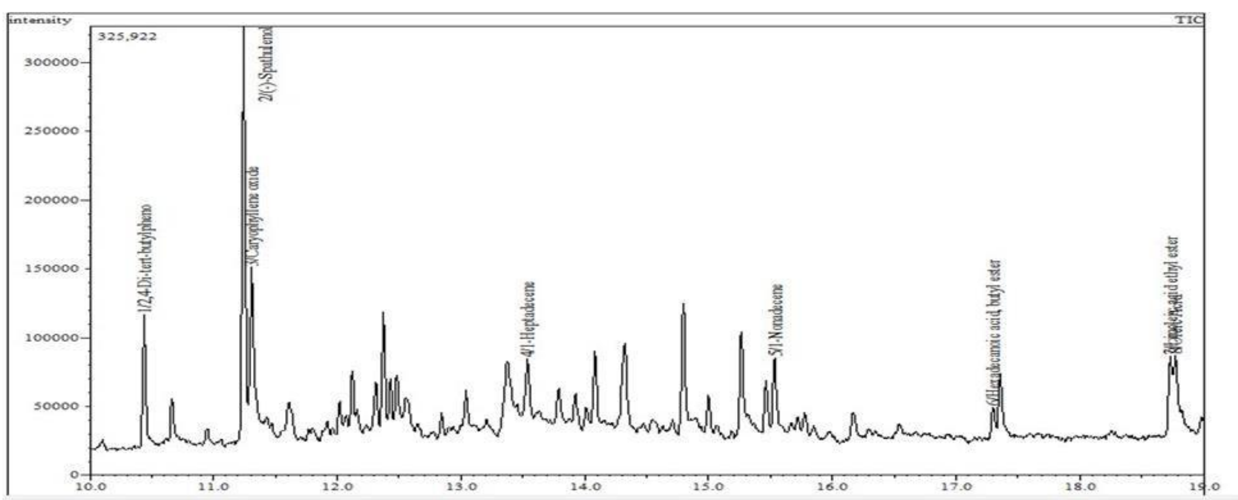

Figure 3: Chromatogram of *Conyza bonariensis* leaves crude extract

were 2,4-di-tertbutylphenol (38.8%), (-)-spathulenol (25.5%), 1-heptadecene (6.4%), n-tetracosanol-1 (6.4%), and caryophyllene oxide (8.7%) dominating, and 1-Nonadecene (6.4%), 1-Heptadecene (5.4%), Hexadecanoic acid, butyl ester (5.3%), Linoleic acid ethyl ester (5.6%) and Oleic Acid (4.2%) were relatively small . Also, most of the compounds detected from essential oil in the present study have been demonstrated to have various health benefits as demonstrated in Table4.

Table 4. Biologically active chemical compounds of essential oil extracted from Conyza bonariensis Leaves

Name of compound	% composition (P.area)	R. Time	Chemical formula	Target Peak	P. Area	P. Height	Activity
2,4-Di-tert-butylpheno	38.8342	10.44	$C_{14}H_{22}O$	191.15	58675	33411	Antioxidant and antifungal [31], [26]
(-)-Spathulenol	25.51055	11.243	$C_{15}H_{24}O$	119.15	49539	21948	Antibacterial Activity [32]
Caryophyllene oxide	8.73482	11.308	$C_{15}H_{24}O$	79.1	12611	7515	Antimicrobial activities [33]
1-Heptadecene	5.40129	13.537	$C_{17}H_{34}$	57.1	11883	4647	Antifungal [34]
1-Nonadecene	6.40902	15.533	$C_{19}H_{38}$	57.1	11729	5514	Antioxidant and antimicrobial [35], [36]
Hexadecanoic acid, butyl ester	5.33853	17.3	$C_{17}H_{32}O_2$	56.1	9466	4593	Antioxidant [37]
Linoleic acid ethyl ester	5.64189	18.733	$C_{20}H_{36}O$	67.1	10399	4854	Anti-inflammatory [38]
Oleic Acid	4.12971	18.771	$C_{18}H_{34}O_2$	55.1	8537	3553	Antioxidant activity [38]

DISCUSSION

Antimicrobial substances are plentiful in medicinal plants and several research have been undertaken to investigate the antimicrobial activity of plant parts such as seeds, flowers, stems, androots. [39], [40] Toassess antibacterial activity frommedicinalplants, leaves crude extracts and essential oil have been found to be more appropriate because they posses a higher content of secondary metabolites than other form. [12], [18]

C. bonariensis is an annual or short-lived perennial plant. The members of genus Conyza are most difficult weeds to control. [41] Conyza bonariensis crude extracts and essential oils have been reported to have chemical constituents such as saponins, tannins, flavonids, steroids, glycosides, diterpenoids andtripernoids (Gutierrez et al.,2020), which haveantioxidant, antimicrobial activities, ant-inflammatory activities. In Tanzania, C. bonariensis has traditionally been used to cure ailments like bacterial infections, fungal infections, and wound healing among various populations. [15] The Safwa tribe in Tanzania's Mbeya region traditionally uses C. bonariensis leaves to cure microbiological illnesses, notably fungal infections.

There is limited empirical evidence on C. bonariensis found in Tanzania, for example before the present findings, one study reported on C. bonariensis obtained from one geographical region of Tanzania (Arusha and Kilimanjaro regions, Northern part of Tanzania). [15] Furthermore, essential oil from C. bonariensis found in Tanzania was not yet evaluated, and it is widely understood that the composition of secondary metabolites in medicinal plants can be influenced by variety of circumstances, including temperature, water, and soil properties. [16] Suggesting crude extracts and essential oils of C. bonariensis from different geographic regions may have difference antimicrobial performance.

In present study, crude extracts of Conyza bonariensis leaves had high antimicrobial activities against some of selected microorganisms. The highest MICs was found to be 25mg/mL>MIC>12.5mg/mL against all tested bacteria (E.coli, S.aureus and S.typhi) and lowest MIC was 50mg/mL>MIC>25mg/m against C. albicans. On other hand essential oil extractedfrom C. bonariensis leaves also hadantimicrobialactivities against tested microorganisms, whereby the MIC ranged from 1:1>MIC> l:2 for four tested microorganisms with the exception of S. typhi where the MIC range was 2:1>MIC> 1:1. Variability in antimicrobial activities to different microorganisms may be explained differences in biochemical composition of cell walls and other inherent factors of a particular microorganisms. [42], [43] On the other hand, overall antimicrobial activity exhibited by crude extracts was slightly higher (P>0.05) than essential oil, these findings are challenging to compare since the two formulations (crude extracts and essential oil) have different units of measurement.

Previous studies on ethyl acetate crude extracts of C. bonariensis from Yemen and Tanzania reported higher antimicrobial activities compared to the findings of the present study. [44], [15] For example, in the study that was conducted in Tanzania the MIC for E.coli, S.aureus, and S.typhi were 1.56 mg/mL, 6.25 mg/mL and 0.78 mg/mL, respectively. This observation may be due to a difference in geographical location from which C. bonariensis grows. [39] Also, the influence of slight genetic variability among tested microorganisms cannot be ignored.[45]

In addition, in previous studies, leaves crude extracts and essential oils from medicinal plants were also reported to haveantimicrobial activities. [42] Study of antimicrobial activities of leaves crude extracts from various plants, such as Cichorium endivia leaves from Egypt using methanol. [46] C. bonariensis leaves from Arusha Tanzania using ethyl acetate. [15] Crude extracts from five medicinal plants found in Saudi Arabia using ethanol extract. [21] show high antimicrobial activities compared to the current study. In contrast, Psidium guajava leaves from the USA using (hexane, methanol, ethanol, and water) had shown low activity compared to the present findings. [47] The variation in antibacterial activity could be due to the crude extracts being from various plant species and the solvent utilized being different as well as variances in tested microorganisms and geographical conditions. [48], [49], [50]

Furthermore, chemical analysis by gas chromatography mass spectrometry (GC-MS) showed that there were antimicrobial compounds in both leaves crude extracts and essential oil of C. bonariensis, such as 2,4-Di-tert-butylphenol, (-)-Spathulenol, Caryophyllene oxide, and 1-Heptadecene.[51], [52] In leavecrudeextracts, Phytol, acetate, 1-Nonadecene, Phytol, n-Tetracosanol-1 were found. In contrast Hexadecanoic acid, butyl ester, linoleicacid ethyl ester, and Oleic Acid were observed in essential oil.[52],[27], [31] The difference in antimicrobial activities of crude extracts and essential oil may be due to differences in chemical composition as shown in Table 3 and4.

CONCLUSIONS

Based on in vitro antimicrobial activities and compounds screened by GC-MS, this study revealed that leaves extract and essential oils from C. bonariensis have antimicrobial properties. This study paves the way forward in the discovery of novel broad-spectrum drugs that can help to solve antimicrobial challenges, and chemical analysis provides directions for further in vivo research. Further studies should be done to isolate pure compounds from active fungi metabolites responsible for the shown activities.

DATA AVAILABILITY

All data presented in this article are available with the authors.

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No external fund was received

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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