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Comparative Antibacterial and Antifungal Efficacy of Selected Tanzania Medicinal Plants

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Authors' contributions

This work was carried out in collaboration between both authors. Authors BAN and MC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author BAN managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To evaluate the antimicrobial activity of *Crassocephalum vitellinum* (Benth), *Euphorbia hirta* (L), *Cupressus lusitanica* (Mill), *Euphorbia inaquilatera* (Sond), *Ajuga remota* (Benth) and *Lantana camara* (linn) extracts against six Gram negative bacterial strains and two fungal strains.
Study Design: *In vitro* antibacterial and antifungal assay was done using micro dilution method.
Place and Duration of Study: This study was conducted at Nelson Mandela African Institution of Science and Technology, Arusha-Tanzania, between December 2014 and June 2015.
Methodology: Plant materials were grinded and soaked in solvents which later removed by using vacuum rotary evaporator to obtain crude extracts. *Escherichia coli* ATCC 11775, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* ATCC 6539, *Klebsiella oxytoca, Salmonella kisarawe* and two strains of fungi; *Cryptococcus neoformans* and *Candida albicans* were tested on the prepared crude extracts. The micro dilution method using 96 wells plates was used to determine minimum inhibition concentration (MIC). After 24 hours of incubation, 20 µL of 0 .02% *p*ara–iodo nitrotetrazolium (INT) chloride dye prepared in distilled water

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was added to each well then incubated for 1 hour and 30 minutes at 37℃.
Results: Thirty seven extracts from 6 plant species investigated exhibited antimicrobial activity against tested fungi (*C. neoformans* and *C. albicans*) and Gram negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa, S. typhi*, *K. oxytoca and S. kisarawe*). *C. vitellinum* leaf methanolic extract, *E. inaequilatera* methanolic extract, *A. remota* leaf and stem methanolic extracts exhibited the highest antibacterial activity against *K. oxytoca*, *P. aeruginosa, S. typhi* and *K. pneumoniae e* with a MIC value of 0.78 mg/mL. *L. camara* leaf chloroform, ethyl acetate extracts and *A. remota* leaf chloroform demonstrated high activity against *S. typhi*, *K. pneumoniae e* and *C. neoformans* with MIC value of 0.78 mg/mL. The highest antibacterial activity against *E. coli* was exhibited by *E. hirta* methanolic, *E. inaquilatera* chloroform and ethyl acetate extracts with MIC value of 1.56 mg/mL.
Conclusion: Results showed that *Crassocephalum vitellinuum* (leaves and stem), *Lantana camara (*leaves, whole flowers and petals*), Cupressus lusitanica* (leaves, seed cover and seeds*), Euphorbia hirta and Euphorbia inaquilatera* possess antimicrobial activities against Gram negative bacterial strains and fungal strains.

Keywords: Antimicrobial activity; medicinal plant extracts; Tanzania.

1. INTRODUCTION

The increasing incidence of bacterial infections from clinical and non-clinical isolates are becoming resistant to most of the antimicrobial drugs currently available and has severely threatened the therapeutic choices and is becoming a global health problem [1]. Among the causative agents of infectious diseases include Gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa Salmonella typhi*, *Klebsiella oxytoca and Salmonella kisarawe* [2]. Fungi such as *Cryptococcus neoformans* and *Candida albicans* have been mentioned as among the causative agents of nosocomial bloodstream infections [3,4]. The mortality rate caused by *C. albicans* is rapidly increasing globally despite of the new drugs being developed [5]. In developing countries particularly sub-Saharan countries the acquired bloodstream infections due to fungi and bacteria are increasing rapidly [6]. For instance, in Tanzania the mortality rate of 34.9% to children is due to blood stream infections including the Gram negative bacteria and *Candida albicans* [7]. These strains have developed multi drug resistance against most of the available drugs [8]. The rising level of microorganism resistance to antibiotics necessitates search for the new alternative drugs [9] with high potency against resistant microbial strains [10]. Validation of ethnomedical data provides one of the ways of developing new drug templates. It is in this vein that *Crassocephalum vitellinum*, *Euphorbia hirta*, *Cupressus lusitanica*, *Euphorbia inaquilatera*, *Ajuga remota* and *Lantana camara* extracts were evaluated against Gram negative bacteria and fungi species in this study.

Euphorbia inaequilatera is used in the treatment of stomach microbial infections for children in Northern Tanzania particularly in Arumeru district. *Crassocephalum vitellinum* is used by many communities in Tanzania for treatment of Gonorrhea and the urinary tract infection as one of the common diseases caused by *E. coli* pathogens [11]. In Kenya, local practitioners have been using *C. vitellinum* in treating malaria and mouth diseases [12]. In Democratic Republic of Congo *C. vitellinum* leaves have been used in treatment of swollen legs [13], mastitis and fever which are caused by virulent strains of *E. coli* and *K. pneumoniae* [14]. *Euphorbia hirta* is also used for management of diarrhea, amoebic dysentery, asthma, bronchitis in Nigeria [15]. In Mauritius, the *E. hirta* is used for treating respiratory tract infections [16]. In Australia the folk use of *E. hirta* is covering a wide range of ailments such as asthma, coughs, diarrhea and dysentery [17]. Similarly in East and Central Africa, the *E. hirta* is used for treating oral thrush, sores, boils skin and wound infections [18]. Subsequently the *E. hirta* has long history as an antispasmodic, depurative, febrifuge, purgative and vermifuge by most of East African communities [19]. *Ajuga remota* on the other hand has been reported in Central part of Kenya for treatment of malaria, ear, nose and throat infections [20]. Rheumatism, whooping cough, hemorrhoids, skin diseases and styptic problems have been managed by the essential oil of *Cupressus lusitanica* leaves [21]. Several cases of respiratory tract infections like tuberculosis have been treated with plant leaves of *Lantana*

camara [22]. The extracts of *Lantana camara leaves* have been exploited as fungicides, nematicides, regulating high blood pressure, treating malaria, tumors, swellings and asthma [23]. This paper is therefore reporting the antimicrobial activity of *Crassocephalum vitellinum*, *Euphorbia hirta*, *Cupressus lusitanica*, *Euphorbia inaquilatera*, *Ajuga remota* and *Lantana camara* extracts against *Escherichia coli* ATCC 11775, *Klebsiella pneumoniae* ATCC13 883, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* ATCC 6539, *Klebsiella oxytoca*, *Salmonella kisarawe, Cryptococcus neoformans* and *Candida albicans*.

2. MATERIALS AND METHODS

2.1 Plant Materials

Plants materials were collected between December 2014 and February 2015 in Nambala and Akeri villages of Northern part of Tanzania. Plants were identified by Mr. E. Mboya, a plant taxonomist from Tropical Pesticides Research Institute (TPRI), and voucher specimens were deposited at the Herbarium located in TPRI, Arusha, Tanzania.

2.2 Extraction

The plant materials were air dried at 25℃ under shade and pulverized using a laboratory grinder. The fine powders were sieved through 0.5 mm sieve to obtain the powder form then separately soaked in methanol, ethyl acetate and chloroform for 48 hours at room temperature. The extracts were filtered and concentrated using rotary evaporator at 50℃ and 100 mbar. Further preparation was done according to Gebre-Mariam et al. [24]. The extracts were kept at 4℃ until they were analysed for antimicrobial activities.

2.3 Testing for Antibacterial Activity

2.3.1 Test organisms

Six strains of Gram negative bacteria (*Escherichia coli* ATCC 11775, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* ATCC 6539, *Klebsiella oxytoca and Salmonella kisarawe*) and two fungal strains (*Candida albicans* and *Cryptococcus neoformans*) were obtained from the Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences (MUHAS).

2.4 Antibacterial Assay

The antimicrobial activities of the plant samples were tested using sterile 96-well micro plates. The micro dilution method was used to determine minimum inhibition concentration (MIC) as described by Eloff [25]. One hundred milligrams of the test sample were weighed by using analytical balance and dissolved in 1 mL of Dimethyl sulphoxide (DMSO) to make up a concentration of 100 mg/mL. It has however reported in [26] that 100% DMSO diminished the growth of *C*. *Albicans*. This fact does not influence the present study as the minimum inhibition concentration was taken for the cell that had not expressed colour change after the iodonitrotetrazolium spray indicating the absence of the viable fungal cell. The sterilized microplates were first preloaded with 50 µL of the broth media followed by 50 µL of the plant extract sample which was early prepared in the concentration of 100 mg/mL which make a total of 100 µL in the wells of the first row. Gentamicin (50 µL) was used as a positive control, the broth was added as media for bacteria to grow and the DMSO also was added as negative control. After introducing 50 µL of extracts and controls (positive, negative and broth) in the first row of micro plates then serial dilution based on the concentration downward were followed as there was thorough mixing of 50 µL then after mixing the sample of 50 µL was drawn from each well downward until the last well. The last 50 µL from the last well at the bottom were discarded. Thereafter a 50 µL of 0.5 McFarland Standard turbidity made from the one day old cultured bacteria suspension [27] was added to all wells to make up a total of 100 mL. The incubation procedures were followed whereby the adjustment of 37℃ for 24 hours was done. After the maximum time of 24 hours the 0.02% *para* – iodo nitrotetrazolium (INT) chloride dye were made and 20 µL of each INT was added to each well then incubated for 1hour and 30 minutes at 37℃. The dye simply is for indicating whether bacteria are alive or dead whereby the bacteria growth was indicated by pink colour. The minimum inhibition concentration (MIC) was considered as the lowest concentration which shows no bacteria growth.

3. RESULTS AND DISCUSSION

Thirty seven extracts from six medicinal plants used for the management of microbial infections in Tanzania were evaluated for antibacterial and

antifungal activities using 96 wells micro dilution method. Plant extracts exhibited antimicrobial activities at different degrees of potency as presented in Tables 1 and 2. The results revealed minimum inhibition concentration (MIC) range of 0.78 – 6.25 mg/mL against *S*. *typhi*, *P*. *aeruginosa* and *K. pneumoniae*; 1.56 – 6.25 mg/mL against *K*. *oxytoca*; 3.12 – 12.5 mg/mL against *S*. *kisarawe*; 1.56 – 12.5 mg/mL against *E*. *coli*; 0.78 – 25.0 mg/mL against *C*. *neoformans* and *C*. *albicans*. Specifically, 10 extracts (27%) from 6 plant species were found to exhibit MIC values of 0.78 mg/mL which is the lowest concentration tested. Thirty five percent, 19% and 11% of all tested extracts exhibited MIC values of 1.56, 3.12 and 6.25 mg/mL respectively while 8% of extracts had MIC values of 12.5 mg/mL and 25.0 mg/mL.

The *Ajuga remota* leaf chloroform, ethyl acetate and methanolic extracts exhibited MIC value range of 0.78 – 12.5 mg/mL. However, *Ajuga remota* leaf chloroform and ethyl acetate extracts exhibited the highest activity against *C. neoformans* while *Ajuga remota* leaf methanolic extract had the highest activity against *S*. *typhi* both with MIC value of 0.78 mg/mL (Table 1). The *E. coli, K. oxytoca, K. pneumoniae* and *S. kisarawe* were sensitive on *A. remota* leaf extracts with MIC value range of 3.12 mg/mL -6.25 mg/mL. *Ajuga remota* leaf chloroform extract was 4 times active than *A. remota* stem chloroform extract against *C*. *neuforman.* The trend was revised against *S*. *typhi* in which stem chloroform extract was 4 times active than stem leaf extract. The antibacterial evaluation of *A. remota* growing in Kenya was reported to exhibit antibacterial activity against *S. aureus, B. cereus* and *E. coli* with MIC values of 7.8, 15.6 and 31.25 mg/mL respectively [28]. The *A. remota* leaf chloroform, ethyl acetate and methanolic extracts were less susceptible to *Candida albicans* with MIC range of 6.25 – 12.5 mg/mL. They were however more susceptible to *C. neoformans* with MIC range of 0.78 – 6.25 mg/mL, Previous antifungal and antibacterial activity evaluation of the aerial part of *A. remota* growing in Kenya revealed that petroleum ether, dichoromethane and methanolic extracts possess antifungal properties [29]. The significant antifungal and antibacterial activity of crude extracts of *A. remota* could possibly due to the presence of secondary metabolites such as flavonoids, tannin and sterols in the leaf which could be beneficial remedies for various infectious diseases caused by these pathogens [30].

The antimicrobial evaluation of *Euphorbia hirta* extracts revealed that–methanolic extract had high activity against *S*. *typhi* with MIC value of 0.78 mg/mL. It also inhibited the growth of *K. pneumoniae* and *E.coli* with MIC value of 1.56 mg/mL. The *E*. *hirta* chloroform extract exhibited antibacterial activity against *S. kisarawe, E. coli, P. aeruginosa* and *K. pneumoniae* with MIC range of 3.12 - 6.25 mg/mL.It was however observed that *C. albicans* was resistant to *E. hirta* methanolic extract (12.5 mg/mL) compared to ethyl acetate and chloroform extracts (6.26 mg/mL). Previous studies on *E. hirta* extracts against different strains of Gram negative bacteria and fungus demonstrated antimicrobial potency of the extracts. For instance, in Malaysia, the *Euphorbia hirta* was tested against *Escherchia coli, Salmonella typhi, Klebsiella pneumonia* using disc diffusion method and revealed to exhibit antibacterial activity with MIC value of 3.13, 100.00 and 100.00mg/mL respectively [31]. Similarly, in Nigeria the agar diffusion method was employed to evaluate *E. hirta* ethanolic extract against *E. coli, S. aureus,* and *P. aeruginosa* and found the exhibit MIC of 58.09, 22.55, 57.64 mg/mL respectively [32]. The report of anthroquinones, tannins, alkaloids saponins and flavonoids that possess antimicrobial activity from *E*. *hirta* growing in Nigeria and Kenya [33] [34,35] suggest the presence of antimicrobial secondary metabolites from *E*. *hirta* growing in Tanzania. The *Lantana camara* leaf chloroform, ethyl acetate and methanolic extracts exhibited antimicrobial activity with the MIC range of 0.78 – 6.25 mg/mL, 0.78 – 12.5 mg/mL and 3.12 – 12.5 mg/mL respectively (Table 1). *S*. *typhi* and *C. neoformans* were susceptible to *L*. *camara* leaf chloroform extract with MIC value of 0.78 mg/mL. The *L*. *camara* leaf ethyl acetate extract demonstrated high activity against *K. pneumoniae* with MIC value of 0.78 mg/mL. Antimicrobial activity of leaf aqueous extract of *L. camara* growing in India indicated that the extracts inhibited the growth of *S. typhi*, *B. subtilis* and *P. vulgaris* with zones of inhibition of 24 mm 22 mm and 28 mm respectively [36]. Similarly in Nigeria, the essential oil from *L. camara* leaves inhibited the growth of *B. subtilis* and *P. mirabilis* at 1000 ppm and the growth of *C. albicans, B*. *aureus, S. typhi*, *P*. *aeruginosa* at MIC 10000 ppm in disc well diffusion method [37]. It has also reported that *L*. *camara* leaf methanolic extract growing in Kenya inhibited the growth of of *E. coli* at 62.5 mg/mL using agar-well diffusion method [38].

Table 1. Antimicrobial activity of *Ajuga remota, Euphorbia hirta* and *Lantana camara* extracts

Extracts	Minimum inhibition concentration (mg/mL)							
	S. kisarawe	***E. coli***	***S. typhi***	***K. oxytoca***	***P. aeruginosa***	***K. pneumonia***	***C. neoformans***	***C. albicans***
AJLC	12.5	6.25	3.12	3.12	6.25	3.12	0.78	12.5
AJLEA	6.25	6.25	3.12	6.25	6.25	3.12	0.78	12.5
AJLM	12.5	3.15	0.78	6.25	6.25	12.5	6.25	6.25
AJSC	6.25	6.25	0.78	3.12	6.25	3.12	3.12	12.5
AJSEA	12.5	12.5	3.12	3.12	6.25	6.25	6.25	12.5
AJSM	12.5	12.5	1.56	3.12	12.5	6.25	3.12	25.0
AJRC	12.5	3.12	1.56	6.25	6.25	6.2	6.25	6.25
AJREA	12.5	12.5	3.12	6.25	6.25	3.12	6.25	12.5
AJRM	12.5	12.5	1.56	6.25	6.25	6.25	6.25	12.5
EHC	6.25	6.25	3.12	3.12	6.25	6.25	3.12	6.25
EHEA	3.12	12.5	1.56	6.25	3.12	6.25	6.25	6.25
EHM	3.12	1.56	0.78	3.12	3.12	1.56	6.25	12.5
LCLC	6.25	3.15	0.78	3.12	3.12	1.56	0.78	1.56
LCLEA	12.5	6.25	3.12	3.12	6.25	0.78	1.56	1.56
LCLM	12.5	6.25	3.12	3.12	3.12	3.12	3.12	12.5
LCWFC	6.25	3.15	3.12	6.25	3.12	3.12	3.12	12.5
LCWFEA	3.12	6.25	3.12	1.56	3.12	3.12	12.5	12.5
LCWFM	12.5	12.5	6.25	6.25	6.25	12.5	6.25	>25
LCPC	3.12	12.5	1.56	3.12	6.25	3.12	6.25	3.12
LCPEA	6.25	12.5	1.56	3.12	6.25	1.56	6.25	12.5
LCPM	6.25	1.56	3.15	6.25	6.25	12.5	6.25	12.5
GENT	3.12	3.15	3.12	0.78	0.19	0.78	NA	NA
FLUC	NA	NA	NA	NA	NA	NA	6.25	

Key: GENT = GENTAMYCIN; FLUC = FLUCONAZOLE

ARLC = Ajuga remota leaf chloroform, ARLEA = Ajuga remota leaf ethyl acetate, ARLM = Ajuga remota leaf methanol, ARSC = Ajuga remota stem chloroform, ARSEA = Ajuga remota stem ethyl acetate, ARSM = Ajuga remota stem methanol, ARRC = Ajuga remota roots chloroform, ARREA = Ajuga remota root ethyl acetate, ARRM = Ajuga remota roots methanol. EHC = Euphorbia hirta chloroform, EHEA = Euphorbia hirta ethyl acetate, EHM = Euphorbia hirta methanol. LCLEA = Lantana camara leaf ethyl acetate, LCLM = Lantana camara leaf methanol, LCWFC = Lantana camara whole flower chloroform, LWFEA = Lantana camara whole flower ethyl acetate, LWFM = Lantana camara whole flower methanol. LCPC = Lantana camara petal chloroform, LCPEA = Lantana camara petal ethyl acetate, LCPM = Lantana camara petal methanol. MIC = Minimum Inhibition Concentration

Table 2. Antimicrobial activity of *Crassocephalum vitellinum, Euphorbia inaequilatera* and *Cupressus lusitanica* extracts

Extracts	Minimum inhibition concentration (mg/mL)							
	S. kisarawe	***E. coli***	***S. typhi***	***K. oxytoca***	***P. aeruginosa***	***K. pneumonia***	***C. neoforman***	***C. albicans***
CVLC	6.25	3.12	1.56	1.56	12.5	3.12	1.56	3.12
CVLEA	6.25	6.25	3.12	3.12	1.56	1.56	1.56	1.56
CVLM	3.12	6.25	3.12	0.78	0.78	0.78	3.12	3.12
CVSC	3.12	6.25	1.56	3.12	3.12	6.25	0.78	0.78
CVSM	12.5	3.12	3.12	6.25	6.25	3.12	6.25	6.25
EIC	6.25	1.56	1.56	1.56	3.12	3.12	3.12	1.56
EIEA	12.5	1.56	3.12	6.25	6.25	3.12	1.56	3.12
EIM	3.12	3.12	0.78	6.25	6.25	3.12	3.12	3.12
CLLC	6.25	6.25	6.25	6.25	6.25	6.25	6.25	12.5
CLLE	12.5	6.25	3.12	3.12	6.25	1.56	6.25	12.5
CLLM	6.25	3.15	1.56	1.56	1.56	1.56	12.5	25.0
CLSCC	12.5	12.5	6.25	6.25	6.25	6.25	25.0	25.0
CLSCE	6.25	12.5	6.25	6.25	6.25	12.5	12.5	6.25
CLSCM	3.12	12.5	3.12	6.25	25	12.5	12.5	25.0
CLSC	6.25	12.5	>25	6.25	25	12.5	25	6.25
CLSM	6.25	6.25	12.5	6.25	6.25	12.5	6.25	6.25
GENT	3.12	3.15	3.12	0.78	0.19	0.78	NA	NA
FLUC	NA	NA	NA	NA	NA	NA	6.25	

Key: GENT= GENTAMYCIN; FLUC= FLUCONAZOLE

CVLC=Crassocephalum vitellinum leaves Chloroform, CVLEA= Crassocephalum vitellinum leaves Ethyl acetate,CVLM= Crassocephalum vitellinum leaves Methanol CVSC= Crassocephalum vitellinum stem Chloroform, SVSM= Crassocephalum vitellinum stem Methanol. LCLC=Lantana camara leaves Chloroform, EIC=Euphorbia inaquilatera Chloroform, EIEA =Euphorbia inaquilatera Ethyl acetate,EIM=Euphorbia inaquilatera Methanol, CLLC=Cupressus lusitanica leaf chloroform, CLLE = Cupressus lusitanica leaf ethyl acetate, CLLM = Cupressus lusitanica leaf methanol, CLSCC = Cupressus lusitanica seed cover Chloroform, CLSCEA = Cupressus lusitanica seed cover ethyl acetate, CLSCM= Cupressus lusitanica seed cover methanol, CLSEC = Cupressus lusitanica seed chloroform, CLSM = Cupressus lusitanica seed methanol, MIC=Minimum Inhibition Concentration in milligrams per mills

As it has been indicated above and supported by available literatures [39-42] that significant efforts has been focused towards unveiling medicinal potential of *L*. *camara* leaf extracts and very little is known so far about the activity of the petals and flowers of this plant and rare literatures are available. The present study revealed that flower and petal extracts exhibited antimicrobial activity with MIC range of 1.56 – 12.5 mg/mL. It was striking to observe that *L*. *camara* flower ethyl acetate was more potent against *K*. *oxytoca* with MIC value of 1.56 mg/mL compared to the rest of plant extracts evaluated in this study (Table 1). Interestingly, *L*. *camara* petal methanolic extract inhibited the growth of *E*. *coli* with MIC of 1.56 mg/mL. Of extracts evaluated in this study this was the second extract which demonstrated such activity against *E*. *coli* (Table 1). *Lantana camara* petal ethyl acetate was two times more active than *L*. *camara* whole flower ethyl acetate against *S. typhi* and *K. pneumoniae*. The related findings have been reported the activity of the flower of ethyl acetate, chloroform and acetone on aqueous extracts against *E. coli, P. aeruginosa* and *B. subtilis* with zones of inhibition value of 10-21mm [43]. It has been found that the presence of high antibacterial and antifungal activity of *L. camara* leaves and flowers is due to the presence of bioactive compounds such as lantadenes, alkaloids, theveside, anthocyanins, triterpenoids and phenolics [44].

Similarly, *Crassocephalus vitellinum* leaf Ethyl acetate, chloroform and methanollic extracts, showed antimicrobial activity against *K. oxytoca, P. aeruginosa, K. pneumoniae* e, *C. neuformans, C. albicans*, *S. typhi* and *K. oxytoca* with a MIC value of 0.78 mg/mL - 1.56 mg/mL (Table 2). However, the current study showed interested results on *Crassocephalus vitellinum* stem chloroform that possessing strong activity with MIC value of 0.78 mg/mL against *C. neoformans*and *C. albicans* (Table 2). Previous study conducted in Tanzania by Moshi evidenced the antimicrobial activity of *C. vitellinum* aerial parts ethanolic and ethyl acetate extracts (3.125 mg/mL) against *S. typhi* and *E. coli* [45]. Interestingly, the current study through different soaking solvents found the *C. vitellinum* leaf possessing higher antibacterial and antifungal activity (0.78 mg/mL - 1.56 mg/mL) for more than five different strains of Gram negative bacteria (Table 2) compared to results reported by Moshi [44].

Euphorbia inaequilatera methanolic, ethyl acetate and chloroform extracts revealed antibacterial activity with MIC value of 0.78 mg/mL and 1.56 mg/mL against *S. typhi, E. coli* and *K. oxytoca* (Table 2). It was interesting to observe that only *Euphorbia inaequilatera* chloroform and ethyl acetate inhibited the growth of *E*. *coli*, *Candida albicans* and *Cryptococcus neoformans* with MIC of 1.56 mg/mL. The antifungal activity of *E. inaequilatera* suggests the presence of antifungal templates that be developed into another generation of antifungal agents and thus replacing azoles and echnocandins [45] that have been shown to face resistance against fungal species. This herb has been used by Tanzania rural communities for the treatment of microbial infections in children [46,47]. To the best of our knowledge, this is the first report of antimicrobial activity of *E. inaequilatera*.

The antimicrobial evaluation of *Cupressus lusitanica* leaf and seed cover demonstrated antibacterial and antifungal activity with MIC range of 1.56 – 12.5 mg/mL and 6.25 – 25.0 mg/mL respectively. Only *Klebsiella oxytoca, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were susceptible on *Cupressus lusitanica* leaf methanol and ethyl acetate with MIC value of 1.56 mg/mL. Previous study on *C*. *lusitanica* growing in Cameroon revealed that its essential oil had antibacterial activity against *E. coli, K. pneumoniae , P. aeruginosa* and *S. typhi* with inhibition zones of 11 – 18 mm [48]. It has also reported that the essential oil from *C*. *lusitanica* growing in Brazil inhibited the growth of endophytic fungi (Xylaria and Guignardia sp) [49]. Phytochemical report on *C*. *lusitanica* growing in Cameroon indicated the presence of monoterpenes, sesquiterpenes and diterpenes with antifungal properties [50]. Since this plant is used for the management of cough and skin infections [51], the present results validates its ethnomedical use.

4. CONCLUSION

The crude extracts of *C. vitellinuum* (leaves and stem), *L. camara* (leaves, whole flowers and petals), *C. lusitanica* (leaves, seed cover and seeds), *E. hirta and E. inaquilatera* possess antimicrobial activities against Gram negative bacterial strains and fungal strains. Thus the present results, suggests the presence of potential antimicrobial drug leads. Therefore, further study should be taken to isolate the bioactive compounds present in *E. inaquilatera* and *C. vitellinuum* that could significantly contribute in drug discovery.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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