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Evaluation of Antibacterial Activity of Five Selected Medicinal Plants in Tanzania against Gram Negative Bacteria

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Authors' contributions

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

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ABSTRACT

Aims: To evaluate antibacterial activity from five selected medicinal plants namely *Embelia schimperi*, *Maerua decumbens*, *Ocimum gratissimum, Conyza floribunda* and *Plectranthus barbatus* used for the management of bacterial infections in Tanzania.
Study Design: *In vitro* antibacterial activity was carried out by using 96 well microplates method.
Place and Duration of Study: The samples were collected in three region of Tanzania namely Kilimanjaro, Arusha and Dodoma. Extraction and antibacterial assay was conducted at School of Life Sciences and Bioengineering, Nelson Mandela African Institution of Science and Technology, Tanzania, between February and June 2015.
Methodology: Minimum Inhibitory Concentration (MIC) of plants extracts against the tested Gram negative bacteria was determined by using 96 well microdilution methods.
Results: Plant extracts exhibited antibacterial activity with MIC range of 1.56 mg/mL to >25 mg/mL.

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About 36% (8) of extracts, out of 22 extracts demonstrated antibacterial activity with MIC of 1.56 mg/mL against *K. oxytoca*, *P. aeruginosa*, *P. mirabilis*, *E. coli* and *S. typhii*. The inter-species activity comparison indicated that antibacterial activity of the evaluated plant species are in order of *Conzya floribunda* > *Plectranthus barbatus* > *Maerua decumbens* > *Embelia schimperi* and *Ocimum gratissimum*. The *Conyza floribunda* extracts exhibited a narrow range antibacterial activity (MIC of 1.56 to 6.25 mg/mL) compared to the rest of plant species in this study.
Conclusion: The highest inhibitory effects exhibited by *C. floribunda* root chloroform, *O. gratissimum* leaf methanolic and *O. gratissimum* flower ethyl acetate extracts against at least two bacteria strains validates the traditional uses of these plants for the management of infections caused by Gram negative bacteria.

Keywords: Gram negative; medicinal plants; antibacteial; Embelia schimperi; Conyza floribunda Ocimum gratissium.

1. INTRODUCTION

The world has been facing a big challenge of antimicrobial resistance (AMR) that affects the efforts under taken to prevent and control infectious diseases caused by bacteria, fungi and virus [1]. The problem of antibacterial resistance particularly the Gram-negative bacteria has been witnessed over the years [2]. Gram-negative bacteria which are responsible for infectious diseases such as pneumonia, bloodstream infections, wound or surgical site infections and meningitis in healthcare settings have developed resistance to the multiple available drugs [3,4]. Gram-negative bacteria such as *Acinetobacter* spp, *Pseudomonas aeruginosa*, and Enterobacteriaceae have been reported to be worrisome due to production of extended-spectrum β-lactamase [5] which is responsible in the cleavage of β-lactam ring of the penicillins [6]. Both intrinsic and extrinsic factors have been mounting the problem of antibiotics resistance in Gram-negative bacteria [7,8]. A strategy that has been preferred by the pharmaceutical companies in addressing challenges posed by antibacterial resistance has been to extend the shelf life of antibiotics by development of synthetic analogues with better activity than the parent drugs [8,9]. However with the portfolio of chemotherapeutics currently available, it has been acknowledged that researchers are getting close to the end game in terms of parent structure alterations. A call has therefore been made for the development of new classes of drugs that work on different target sites to those in current use [10]. It is in this vein that five medicinal plants namely *Embelia schimperi* , *Maerua decumbens*, *Ocimum gratissimum, Conyza floribunda* and *Plectranthus barbatus* used for the management of bacterial infections in Tanzania were evaluated against Gram-negative strains.

2. MATERIALS AND METHODS

2.1 Collection of Plants Materials

The plant materials were collected from different parts of Tanzania, *Embelia schimperi* (leaves, fruits and stem bark), *Ocimum gratissimum* (leaves and flowers) and *Plectranthus barbatus* (stem) were collected from Kilimanjaro region. *Maerua decumbens* (leaves) was collected from Dodoma region and *Conzya floribunda* (leaves and stem bark) was collected from Ngateu village, Arusha region. The plant species were identified by Mr. Emmanuel Mboya a senior botanist from National Herbarium, Tropical Pesticides Research Institute. The plant specimens for *Embelia schimperi*, *Maerua decumbens*, *Ocimum gratissimum, Conyza floribunda* and *Plectranthus barbatus* coded ES-EG12, MD-EG13, OG-EG14, CF-EG15 and PB-EG16 respectively are kept at Nelson Mandela African Institution of Science and Technology.

2.2 Solvent and Reagent, Media

Chloroform and ethyl acetate were purchased from Loba Chemie Pvt Ltd, Mumbai, INDIA). Methanol (absolute) was bought from Fluka Chemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands). Dimethyl sulfoxide (DMSO) was purchased from RFCL Limited, Hayana, India. Nutrient broth and Nutrient agar were bought from HIMEDIA®, INDIA. Iodonitrotetrazolium chloride was purchased from SIGMA (Sigma Aldrich, St Louis, USA) and Gentamycin was supplied by Lincoln pharmaceutical ltd (Guj, India).

2.3 Preparation of Plant Extracts

The collected plant materials were washed with tap running water to remove debris and dried

under room temperature. Dried plant materials were pulverized into fine powdered by using an electric grinding machine, the pulverized plant materials (250 g per plant part) were sequentially extracted using chloroform, ethyl acetate and methanol for 48 hours repeated twice. The extracts were then dried under vacuum using rotary evaporator and stored at -20℃ until the time for testing.

2.4 Source of Bacteria Strains

Gram-negative bacteria namely *Salmonella kisarawe* (clinical isolate), *Klebsiella oxytoca* (clinical isolate), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 29953), *Proteus mirabilis* (NCTC 1075), *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (NCTC 8385) were obtained from the Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences (MUHAS).

2.5 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) for all twenty two plant extracts were evaluated according to method described by [11] with minor modification employing 96 well micro plates. For each plate 50 µL of nutrient broth were placed to each well followed by 50 µL of plant extracts (100 mg/mL) which were added to the first row only. This made each well of the first row to have a total volume of 100 µL. Some columns in 96 well plates were set as positive and negative control in which Getamycin were loaded as positive control and Dimethyl Sulfoxide as negative control. Starting from the first row serial dilution was conducted downward to the columns, the final volumes (50 µL) drawn from the last row were casted off. 0.5 Mac Farhland standard turbidity was prepared then 50 µL of microbes were added to each well of the plate. Subsequently the plates were incubated for 24 hrs at 37C. The minimum Inhibitory Concentration (MIC) was determined by adding 20 µL (2 mg/mL) of 0.02% p-iodonitrotetrazolium chloride (INT) and incubated at 37℃ for 30 minutes. INT was used as an indicator for bacteria growth; bacteria metabolize it and changed into pink. The wells which had no change in color after the addition of INT indicated no growth and they were taken as Minimum Inhibitory Concentration (MIC).

3. RESULTS AND DISCUSSION

The minimum inhibitory concentration (MIC) of *Embelia schimperi, Ocimum gratissimum Plectranthus barbatus*, *Maerua decumbens* and *Conzya floribunda* extracts were evaluated against seven Gram-negative bacteria and results are summarized in Tables 1 and 2. The antibacterial activities of these plants were found to vary between plant species and plant parts. The MIC range against different indicator bacteria was 1.56 – 6.25 mg/mL for *Conzya floribunda*, 1.56 to >25 mg/mL for *Embelia schimperi* and *Ocimum gratissimum*, 1.56 – 25 mg/mL for *Maerua decumbens and* 3.12 – 12.5 mg/mL for *Plectranthus barbatus*. Thus the order of antibacterial activities of plant species was thus in the order of *Conzya floribunda> Plectranthus barbatus > Maerua decumbens > Embelia schimperi* and *Ocimum gratissimum*.

Table 1. Antibacterial activity of *E. schimperi* fruit, leaf and stem extracts

Plant extracts	Minimum Inhibitory concentration MIC (mg/mL)						
	P. mirabilis	***K. pneumoniae***	***P. aeruginosa***	***S. typhii***	***K. oxytoca***	***E. coli***	***S. kisarawe***
ESFC	6.25	25.0	6.25	3.12	3.12	3.12	6.25
ESFE	12.5	>25.0	1.56	6.25	NT	NT	6.25
ESFM	3.12	6.25	1.56	3.12	6.25	12.5	3.12
ESSC	6.25	25.0	6.25	3.12	6.25	6.25	6.25
ESSE	12.5	25.0	12.5	6.25	25.0	25.0	12.5
ESSM	12.5	6.25	3.12	6.25	25.0	6.25	3.12
ESLC	25.0	25.0	12.5	3.12	25.0	12.5	12.5
ESLE	25.0	6.25	12.5	3.12	25.0	25.0	12.5
ESLM	3.12	6.25	6.25	3.12	12.5	6.25	3.12
GC	0.015625	0.03125	0.015625	0.0625	0.03125	0.0156	0.0625

Key: ESFC- E. schimperi fruits chloroform, ESFE- E. schimperi fruits ethyl acetate, ESFM- E. schimperi fruits methanol, ESSC- E. schimperi stem chloroform, ESSE- E. schimperi stem ethyl acetate, ESSM- E. schimperi stem methanol, ESLC- E. schimperi leaves chloroform, ESLE- E. schimperi leaves ethyl acetate, ESLM- E. schimperi leaves methanol, GC-Gentamycin and NT-Not tested

Table 2. Antibacterial activities of *O. gratissimum* (flower and leaf), *C. floribunda (root,* stem and leaf), *M. decumbens* (leaf extracts) *and P. barbatus* (stem)

Plant extracts	Minimum Inhibitory concentration MIC (mg/mL)						
	P. mirabilis	***K. pneumoniae***	***P. aeruginosa***	***S. typhii***	***K. oxytoca***	***E. coli***	***S. kisarawe***
OGLE	6.25	12.5	>25.0	12.5	6.25	6.25	12.5
OGLM	12.5	25.0	6.25	1.56	12.5	12.5	12.5
OGFC	6.25	12.5	3.12	6.25	12.5	6.25	25.0
OGFE	6.25	6.25	1.56	3.12	1.56	1.56	3.12
OGFM	3.12	6.25	NT	6.25	1.56	3.25	3.12
CFRC	6.25	6.25	6.25	1.56	1.562	3.12	6.25
CFSE	6.25	6.25	3.12	6.25	3.12	6.25	6.25
CFLM	3.12	6.25	NT	3.12	1.56	3.25	6.25
MDLC	6.25	6.25	25.0	3.12	25.0	25	12.5
MDLE	1.56	3.12	25.0	25	25.0	12.5	12.5
PBSC	12.5	12.5	12.5	12.5	12.5	12.5	12.5
PBSE	12.5	12.5	3.12	3.12	3.12	12.5	12.5
PBSM	12.5	12.5	6.25	3.12	3.12	12.5	12.5
GC	0.015625	0.03125	0.015625	0.0625	0.03125	0.015625	0.0625

Key: OGLE- O. gratissimum leaves ethyl acetate; OGLM- O. gratissimum leaves methanol, OGFC- O. gratissimum flower chloroform, OGFE- O. gratissimum flower ethyl acetate, OGFM- O. gratissimum flower methanol, CFRC- C. floribunda roots chloroform, CFSE- C. floribunda stem ethyl acetate, CFLM- C. floribunda leaves methanol, MDLC- M. decumbens leaves chloroform, MDLE- M. decumbens leaves ethyl acetate, NT-Not tested and GC-Gentamycin

The *E*. *schimperi* fruit ethyl acetate and methanolic extracts exhibited the higher antibacterial activity against *P. aeruginosa* (MIC values of 1.56 mg/mL) compared to chloroform, stem and leaf extracts as depicted in Table 1. *Embelia schimperi* fruit ethyl acetate and methanolic extracts had however lower antibacterial activity against other tested bacterial strains suggesting the presence of secondary metabolites with relative selectivity against *P*. *aeruginosa*. The *P*. *aeruginosa* has been reported as one of troublesome Gram-negative multiple drugs resistance bacteria. It is known as common opportunistic pathogen that is responsible for hospital-acquired infections as well as in community, it causes serious problems such as pneumonias, urinary tract infections, wound or surgical site infections and bloodstream infections [12]. The highest susceptibilty shown by *P*. *aeruginosa* towards fruits extract suggesting that the fruit might contain secondary metabolites that can be used in management of infections caused by *P*. *aeruginosa*. Also it validates the tradition uses of *E. schimperi* in treating chest infections, as a disfectant and in cleaning the wounds [13]. Previous reported antibacterial studies on *E*. *schimperi* fruits, twigs and leaf extracts growing in Ethiopia demonstrated antibacterial activity against Gram-positive bacteria; *Bacillus cereus*, *Listeria monocytogenes*, and *Streptococcus pyogenes* [14] collaborating the reported results. Methyleneoxy bridged - oleanane type pentacyclic triterpenoids with antibacterial activity against *Escherichia coli*, *Pseudomonas putida*, *Bacillus subtilis* were reported from the *E*. *schimperi* stem bark in Kenya [15] which suggests that antibacterial activity against Gram-negative bacteria reported in this paper might due to the presence of oleanane triterpenes. Another antibacterial investigation conducted by disc diffusion using pure compounds and crude extracts from *E. schimperi* stem ethyl acetate grown in Kenya demostrated that the crude extract was inactive while 2,5-dihydroxy-3-methyl-1,4-benzoquinone showed significant activities against Gram-negative *Salmonella* spp., *Proteus* spp., *P. aeruginosa, K. pneumoniae,* and Gram-positive *E. coli, Shigella dysentriae* and *Staphylococcus aureus*. The most sensitive microorganism was established to be *P. aeruginosa* [13]. Howeover another antibacterial investigation from Kenya conducted by using Embelin from *E.schimperi* fruit ethyl acetate extract and Embelin synthetic derivative indicated they were inactive against *P*. *aeruginosa and E. coli.* [16]. Futhermore it has been reported earlier that Gram negative bacteria such as *E*. *coli* is resistant against Embelin [17].

The *O.gratissimum* flower ethyl acetate extract demonstrated high activity with MIC values of 1.56 mg/mL against *P. aeruginosa, K. oxytoca* and *E. coli* compared to the other tested *O.gratissimum* extracts. The *O. gratissimum* flower methanolic and *O. gratissimum* leaf methanolic extracts significantly inhibited the growth of *K. oxytoca* and *S*. *typhi* respectively with MIC value of 1.56 mg/mL (Table 2). It was therefore deduced that *O*. *gratissimum* flower extract had high antibacterial activity against

Gram-negative bacteria as compared to the leaf extracts of the same plant. Since *O. gratissimum* flower ethyl acetate extract demonstrated high activity against the three bacterial strains, it therefore evident that compounds contained might have a wider spectrum of antibacterial activity. Previous reported antibacterial studies on *O. gratissimum* leaves growing in Kenya, Brazil, Nigeria and Benin indicated that they possess antibacterial activity against both Gram-positive and Gram-negative bacteria. Leaves were established to be rich in essentil oil (EO) which the principal compound was eugenol and is responsible for the displayed antibacterial activity [18-25]. The chemical composition of the essential oil was however found to depend of the growth stage of plant [25]. In the current investigation, it has been establishment that *O. gratissimum* flower extracts possess much higher antibacterial activity compared to leaf extracts suggesting the potential source of antibacterial compounds in the *O. gratissimum* flowers. This finding is in collaborating with previously study conducted by [25] which found that flowers from *O. gratissimum* growing in Benin exhibited antimicrobial activity compare to other plant parts.

The C*. floribunda* extracts demonstrated a narrow MIC range of 1.56 – 6.25 mg/mL against Gram-negative bacteria compared to other plant species (Table 2). It was also vivid that the *C. floribunda* root chloroform extract was the most active which exhibited antibacterial activity against *S*. *typhi* and *K*. *oxytoca* at 1.56 mg/mL. *Conzya floribunda* leaf methanolic and *Maerua decumbens* selectively displayed high antibacterial activity with MIC value of 1.56 mg/mL against *K*. *oxytoca* and *P*. *mirabilis* respectively (Table 2). The antibacterial selectivity of these plant extracts entails the presence of secondary metabolites with selectivity against Gram-negative bacteria. Unlike other plant species, *P. barbatus* displayed a relatively wide MIC range of 3.12 to 12.5 mg/mL. The *P. barbatus* stem ethyl acetate and methanolic extracts demonstrated MIC values of 3.12 mg/mL against *S. typhii* and *K. oxytoca*. Additionally, *P. barbatus* exhibited MIC value of 3.12 mg/mL against *P*. *aeruginosa*. Previous antibacterial investigation of *C. floribunda* extracts growing in Kenya established that dichloromethane and methanol extracts inhibit both Gram-negative bacteria and Gram- positive bacteria [26]. These findings corroborate with the present findings, despite the geographical separation of the plants. The antibacterial results emanated the present and reported studies indicate that the plant could be a useful remedy for some of the disease conditions caused by the tested bacteria. It was further established in [26] that *C. floribunda* extracts had antifungal activity against *Candida albicans*, *Trichophyton mentagrophytes* and *Microsporum gypsium*. The antibacterial principles from *C*. *floribunda* were reported in [26] to be (24S)-ethylcholesta-5, 22E, 25-dien-3-O-β-glucoside and cyasterone while 3-oxofriedooleanane and betullinic acid exhibited antifungal activities. Results emanated from this study which focused on C*. floribunda* growing in Tanzania and the reported data for the same plant growing in Kenya [26] indicate that the plant could be a useful remedy for disease conditions caused by the tested bacteria and fungi.

Despite the fact that *Maerua decumbens* has been utilized in Tanzania for the management of bacterial diseases, the antibacterial activity of *M*. *decumbens* extracts is reported for the first time in this paper. However, previous studies have reported the use of its root to clean water turbidity and to eliminate heterotrophic bacteria [27]. The *M. decumbens* leaf extracts have also been established to exhibit antifungal activity and the phytochemical analysis revealed the presence of flavonoids and cardiac glycoside**s** [28].Flavonoids and cardiac glycosides have also been reported from *Maerua angolensis* and extracts from this plant were found to exhibit antibacterial activity against Gram positive and Gram negative bacteria with MIC range of 6.25 mg/mL to 25 mg/mL [29]. Furthermore an antibacterial investigation of *P. barbatus* growing in Kenya was found to inhibit Gram negative bacteria [30]. Likewise, the essential oils from *P. barbatus* growing in Brazil have been reported to exhibit antibacterial activity against *E*. *coli*, *Proteus vulgaris*, *Bacillus cereus, Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus aureus* (multiresistant) [31]. The chemical profile of *P. barbatus* essential oil was found to containα-pinene, β-phellandrene, (Z)-β-ocimene, manol, and abietadiene as main constituents [32]. The labdane forskolin reported from *P*. *barbatus* has been established to exhibit a range of pharmacological properties and could explain many of the diverse medicinal uses of *P*. *barbatus* [33].

4. CONCLUSION

The extracts of *Embelia schimperi*, *Ocimum gratissimum Plectranthus barbatus*, *Maerua decumbens* and *Conzya floribunda* showed

varies degree of antibacterial activity against Gram negative bacterial .The study revealed that the extracts C*. floribunda* had a narrow MIC range of 1.56 – 6.25 mg/mL against all tested bacteria strains compared to other plant species. The C*. floribunda* root chloroform, *O. gratissimum* leaf methanolic and *O. gratissimum* flower ethyl extract were able to inhibit atleast two bacteria strain with MIC 1.56 mg/mL. This suggests that they have a wider antibacterial spectrum and they can be used for treating more than one infections caused by different Gram-negative bacteria.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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