

## In Vitro Antibacterial Activity of Selected Tanzania Medicinal Plants

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### Abstract

**Objective:** To evaluate antibacterial activity from four selected medicinal plants namely *Mystroxydon aethiopicum*, *Lonchocarpus capassa*, *Albizia anthelmentica* and *Myrica salicifolia* used for management of bacterial infection in Tanzania.

**Methods:** Minimum Inhibitory Concentration (MIC) of plants extracts against the tested bacterial species was determined by using 96 wells microdilution method. In this method, 50  $\mu$ L of nutrient broth were loaded in each well followed by 50  $\mu$ L of extract (100 mg/mL) to make a final volume of 100  $\mu$ L. Subsequently 50  $\mu$ L were transferred from first rows of each well to the second rows and the process was repeated down the columns to the last wells from which 50  $\mu$ L were discarded. Thereafter, 50  $\mu$ L of the selected bacterial suspension were added to each well thus making a final volume of 100  $\mu$ L. The lowest concentration which showed no bacterial growth was considered as MIC.

**Results:** It was revealed that *L. capassa* leaf ethyl acetate extract exhibited antibacterial activity against *Salmonella kisarawe* and *Salmonella typhi* with MIC values of 0.39 and 0.781 mg/mL respectively. Likewise, *L. capassa* root bark ethyl acetate extracts inhibited growth of *S. typhi* and *E. coli* with MIC values of 0.39 and 0.781 mg/mL respectively. The *M. aethiopicum* leaf and root bark chloroform extracts displayed antibacterial activity against *S. kisarawe* and *S. typhi* respectively with MIC value of 0.781 mg/mL. The *M. salicifolia* stem bark ethyl acetate exhibited antibacterial activity against *P. aeruginosa* with MIC value of 0.39 mg/mL whereas the methanolic stem and root bark of the same plant inhibited the growth of *Proteus mirabilis* and *Klebsiella pneumoniae* with MIC value of 0.781 mg/mL.

**Conclusion:** It was concluded that *M. aethiopicum*, *L. capassa*, *A. anthelmentica* and *M. salicifolia* are potential source of antibacterial agents. Further studies to establish structures of antibacterial and evaluate active ingredients are recommended.

**Keywords:** Antibacterial; *Mystroxydon aethiopicum*; *Lonchocarpus capassa*; *Albizia anthelmentica*; *Myrica salicifolia*

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### Introduction

Bacterial infections are responsible for about 17.8% of all human deaths world-wide, amounting to almost 10 million deaths per year [1]. Despite the fact that conventional drugs have been used as remedy for human infectious diseases globally, there are some limitations which include, among others, antibiotic resistance, toxicity, inhibitive costs and allergic reactions in some patients

[2]. In view of such limitations, search for alternative drugs for the management of infectious diseases is desirable. Screening for bioactive compounds from medicinal plants in counteracting some limitations facing conventional drugs offers a promising approach [3]. Indeed, medicinal plants have been established to contain phytochemicals such as flavonoids, tannins, terpenoids and alkaloids which are responsible for antibacterial activities, and are potential antimicrobial agents [4].

Tanzania is endowed with more than 10,000 widely distributed plant species of which about 1,000 are considered to be medicinal [5]. Some of the plants commonly used for the treatment of human infectious diseases include *Mystroxydon aethiopicum* (Celastraceae), *Lonchocarpus capassa* (Papilionoideae), *Albizia anthelmentica* (Mimosaceae) and *Myrica salicifolia* (Myricaceae). The *M. aethiopicum* which is also known as the spike thorn, is widely distributed in highlands of Arusha and Kilimanjaro regions, where it is locally known as “*Oldonyanangu*” in Maasai language. It is reportedly used by many ethnic groups for treatment of dysentery [6].

The *L. capassa* is usually found in deciduous woodlands and wooded grasslands especially along water courses, is locally known as “*Mvale*” in Swahili language. In Tanzania, the plant is commonly found in Arusha, Tabora, Dodoma, Kondo, Morogoro and Iringa [7]. Fine powder prepared from stem bark of this plant is reportedly used by the Nyamwezi people for management of wounds by mixing and taking the same with porridge [8].

Likewise, *A. anthelmentica* which is a thorny/spiny, deciduous, multi-stemmed tree is commonly found in deciduous or evergreen bush lands and scrubland, especially along seasonal rivers and on termite-mound clump thickets. It is medium canopy and grows to an average height of 8 meters [9]. The plant is native in Botswana, Kenya, Namibia, Somalia, South Africa, Swaziland, Tanzania and Uganda. It is reportedly used by pastoralists throughout East Africa for treatment of helminthic infections of livestock [10].

Regarding *M. salicifolia*, is a shrub with average height of about one meter and may occasionally grow to a tree of up to 20 m and is usually aromatic and resinous [11]. The plant is mostly found in temperate, subtropical and tropical-montane regions of the world [11]. Traditionally, root extracts of *M. salicifolia* is consumed by Maasai warriors in Kenya to prime themselves for battle. They believed that consumption of these extracts would produce feelings of detachment from the environment, invincibility, irritability, aggressiveness, overreaction to extraneous sounds and tendency to keep a posture for a long time [12].

In view of the foregoing information on the contribution of medicinal plants for management of infectious diseases, the efficacy of limited number of medicinal plants has been scientifically validated [13]. This study therefore reports the antibacterial activity of four medicinal plants namely *M. aethiopicum*, *L. capassa*, *A. anthelmentica* and *M. salicifolia* against seven Gram negative bacteria. This include: *Pseudomonas aeruginosa* (ATCC 29953), *Klebsiella oxytoca* (clinical isolate), *Proteus mirabilis* (NCTC 1075), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella kisarawe* (clinical isolate), *Salmonella typhi* (NCTC 8385) and *Escherichia coli* (ATCC 25922).

## Materials and Methods

### Acquisition of material

Methanol and chloroform were purchased from Avantor Performance Materials Limited, Gujarat, India. Dimethyl sulphoxide (DMSO) and ethyl acetate were bought from RFCL Limited, Haryana, India. Nutrient broth and Nutrient agar were

supplied by HIMEDIA Laboratories Pvt. Limited, Mumbai, India. Bacterial strains namely *Pseudomonas aeruginosa* (ATCC 29953), *Klebsiella oxytoca* (clinical isolate), *Proteus mirabilis* (NCTC 1075), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella kisarawe* (clinical isolate), *Salmonella typhi* (NCTC 8385) and *Escherichia coli* (ATCC 25922) were obtained from the department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences (MUHAS) in Tanzania. Gentamycin and iodinitrotetrazolium chloride were supplied by Mediatech Incorporation, Manassas, USA and SIGMA® (Sigma- Aldrich®, St. Louis, USA) respectively.

### Preparation of plant extracts and extraction

The plant materials were collected from different parts of Arusha region in Tanzania. Leaves, stems and roots of *L. capassa* and *A. anthelmentica* were collected from Esilalei village in Monduli district, Tanzania while the same plant parts of *M. aethiopicum* and *M. salicifolia* were collected from Imbibya and Engalaoni villages respectively, both in Arusha rural district, Tanzania. Plant species were identified by Mr. Gabriel Laizer, a botanist from Tropical Pesticide Research Institute (TPRI) and voucher specimens coded MA-1, LC-2, AA-3 and MS-4 for *M. aethiopicum*, *L. capassa*, *A. anthelmentica* and *M. salicifolia* respectively are kept at Nelson Mandela African Institution of Science and Technology (NM-AIST). Plant materials were air dried under the shade and pulverized into fine particles using electric blender. Pulverized materials (250 g, per plant part) were successively macerated in chloroform, ethyl acetate and methanol for 48 hours. The respective extracts were filtered through Whatman No. 1 filter paper on a plug of glass wool in a glass column and solvents were evaporated through the vacuum using a rotary evaporator and stored in a deep freezer at -20°C.

### Determination of antibacterial activity

Minimum inhibitory concentrations (MICs) were determined by microdilution method using 96-well plates according to procedure reported by Ellof [14]. Plates were first preloaded with 50 µL of the nutrient broth media in each well followed by addition of 50 µL of 100 mg/mL extract (prepared in DMSO) into the first wells of each row so as to make a total volume of 100 µL in each of the first row wells. The contents were thoroughly mixed and 50 µL of the same were drawn from each of the first row wells and put into the next row wells. The process was repeated down the columns to the last wells at the bottom from which 50 µL were discarded. Thereafter, 50 µL of the selected bacterial suspension (0.5 Mac Farland standard turbidity) were added to each well thus making a final volume of 100 µL per well. Gentamycin sulphate was used in two rows of each plate to serve as standard positive control drugs against the test bacteria while DMSO was used as negative control. Likewise, nutrient broth was used to monitor bacterial growth. The plates were then incubated at 37°C for 24 hrs. MICs for each extract, were determined by adding 20 µL of 0.02% *p*-iodinitrotetrazolium (INT) chloride dye in each well followed by incubation at 32°C for 1 hr. Bacterial growth was indicated by change of colour to pink. The lowest concentration which showed no bacterial growth was considered as MIC.

## Results

The antibacterial activity of *M. aethiopicum* *L. capassa*, *A. anthelmintica* and *M. salicifolia* extracts were evaluated against seven Gram-negative bacterial species. The findings presented as minimum inhibition concentrations (MIC) indicated that plant extracts possess varying antibacterial potencies as summarized in **Tables 1 and 2**. Out of seven Gram negative bacteria tested, only three bacteria namely *Salmonella kisarawe*, *Salmonella typhi* and *Pseudomonas aeruginosa* were inhibited by plant extracts at MIC value of 0.39 mg/mL. Seventy one percent of tested bacterial were susceptible to plant extracts at MIC value of 0.781 mg/mL. According to Rios and Recio [15], plant extracts that are recommended in the drug discovery initiatives are those with MIC values less than 1 mg/mL, and thus plant extracts that exhibited MIC values of 0.39 and 0.781 mg/mL are potential source of drug templates.

*Mystroxydon aethiopicum* leaf chloroform (MALC) and stem bark methanolic (MASM) exhibited antibacterial activity against all tested bacteria with narrow MIC range of 0.781-3.125 mg/mL and 1.562-3.125 mg/mL respectively suggesting that the two extracts are more active than other plant parts of *M. aethiopicum*. However, the highest antibacterial activity was exhibited by MALC and *M. aethiopicum* root bark (MARC) extracts which displayed MIC value of 0.781 mg/mL against *S. kisarawe* and *S. typhi* respectively.

As regards *L. capassa*, the leaf extracts had high antibacterial activity which is evidenced by the narrow MIC range of 0.39–6.25 mg/mL followed by root extracts with MIC range of 0.39-12.5 mg/mL and stem extracts were the least with MIC range of 3.125-25.0 mg/mL. However, the highest antibacterial activity was displayed by leaf ethyl acetate (LCLE) and root bark ethyl acetate (LCRE) which the former extract had MIC values of 0.39 and 0.781 mg/mL against *S. kisarawe* and *S. typhi* respectively whereas the latter had MIC values of 0.39 and 0.781 mg/mL against *S. typhi* and *E. coli* respectively.

Antibacterial investigation of *M. salicifolia* revealed that, the stem bark ethyl acetate (MSSE) extract exhibited the lowest MIC value of 0.39 mg/mL against *P. aeruginosa*. Apparently, stem bark methanolic (MSSM) and root bark methanolic (MSRM) extracts displayed MIC value of 0.781 mg/mL against *P. mirabilis* and *K. pneumoniae*. Unlike *L. capassa* leaf extracts, which was comparatively more active than other parts of the same plant, *M. salicifolia* leaf extracts exhibited antibacterial activity with MIC range of 1.562-6.25 mg/mL.

*Albizia anthelmintica* extracts displayed the least antibacterial activity as compared with the rest of plant extracts tested. None of its extracts exhibited antibacterial activity with MIC value below 3.125 mg/mL. Comparatively, *Albizia anthelmintica* leaf ethyl acetate (AALE) was the most active; it exhibited antibacterial activity against *K. oxytoca*, *K. pneumoniae* and *S. kisarawe* with MIC value of 3.125 mg/mL. *Albizia anthelmintica* leaf chloroform

**Table 1** Antibacterial activity of *M. aethiopicum* and *L. capassa* (leaf, stem and root bark).

Plant Extracts	Minimum inhibitory concentrations (MICs) in mg/ml						
	<i>P. aeruginosa</i>	<i>K. oxytoca</i>	<i>P. mirabilis</i>	<i>K. pneumoniae</i>	<i>S. kisarawe</i>	<i>S. typhi</i>	<i>E. coli</i>
MALC	1.562	3.125	3.125	1.562	0.781	3.125	3.125
MALE	6.25	1.562	6.25	6.25	3.125	3.125	6.25
MALM	12.5	1.562	6.25	6.25	3.125	3.125	12.5
MASC	3.125	12.5	6.25	3.125	1.562	3.125	6.25
MASE	12.5	12.5	3.125	12.5	6.25	1.562	3.125
MASM	3.125	3.125	1.562	3.125	3.125	1.562	1.562
MARC	12.5	6.25	12.5	3.125	6.25	0.781	1.562
MARE	12.5	12.5	25	25	25	3.125	3.125
MARM	1.562	25	25	3.125	25	1.562	1.562
LCLC	3.125	1.562	6.25	3.125	1.562	1.562	3.125
LCLE	3.125	3.125	6.25	6.25	0.39	0.781	3.125
LCLM	6.25	3.125	3.125	3.125	3.125	1.562	3.125
LCSC	12.5	12.5	12.5	6.25	12.5	6.25	12.5
LCSE	6.25	25	25	12.5	3.125	3.125	12.5
LCSM	25	12.5	12.5	12.5	12.5	6.25	12.5
LCRC	12.5	12.5	12.5	6.25	12.5	6.25	12.5
LCRE	3.125	12.5	6.25	6.25	3.125	0.39	0.781
LCRM	12.5	12.5	6.25	6.25	6.25	12.5	12.5
Gentamycin	0.0078125	0.015625	0.125	0.0625	0.03125	0.0625	0.0625

**Key:** MALC=*M. aethiopicum* leaf chloroform extract, MALE=*M. aethiopicum* leaf ethyl acetate extract, MALM=*M. aethiopicum* leaf methanolic extract, MASC=*M. aethiopicum* stem bark chloroform extract, MASE=*M. aethiopicum* stem bark ethyl acetate extract, MASM=*M. aethiopicum* stem bark methanolic extract, MARC=*M. aethiopicum* root bark chloroform extract, MARE=*M. aethiopicum* root bark ethyl acetate extract, MARM=*M. aethiopicum* root bark methanolic extract, LCLC=*L. capassa* leaf chloroform extract, LCLE=*L. capassa* leaf ethyl acetate extract, LCLM=*L. capassa* leaf methanolic extract, LCSC=*L. capassa* stem bark chloroform extract, LCSE=*L. capassa* stem bark ethyl acetate extract, LCSM=*L. capassa* stem bark methanolic extract, LCRC=*L. capassa* root bark chloroform extract, LCRE=*L. capassa* root bark ethyl acetate extract, LCRM=*L. capassa* root bark methanolic extract.

**Table 2** Antibacterial activity of *A. anthelmintica* and *M. salicifolia* (leaf, stem and root bark).

Plant Extracts	Minimum inhibitory concentrations (MICs) in mg/ml						
	<i>P. aeruginosa</i>	<i>K. oxytoca</i>	<i>P. mirabilis</i>	<i>K. pneumoniae</i>	<i>S. kisarawe</i>	<i>S. typhi</i>	<i>E. coli</i>
AALC	6.25	12.5	25	6.25	12.5	3.125	6.25
AALE	6.25	3.125	6.25	3.125	3.125	6.25	6.25
AALM	3.125	6.25	6.25	6.25	12.5	6.25	6.25
AASC	12.5	6.25	6.25	6.25	12.5	12.5	12.5
AASE	6.25	12.5	6.25	6.25	6.25	6.25	12.5
AASM	12.5	6.25	12.5	6.25	25	12.5	12.5
AARC	6.25	12.5	6.25	12.5	6.25	12.5	12.5
AARE	12.5	12.5	6.25	6.25	6.25	12.5	12.5
AARM	12.5	6.25	12.5	12.5	12.5	6.25	12.5
MSLC	6.25	3.125	3.125	3.125	6.25	3.125	1.562
MSLE	6.25	3.125	3.125	3.125	6.25	1.562	6.25
MSLM	1.562	3.125	1.562	1.562	3.125	1.562	1.562
MSSC	1.562	12.5	3.125	12.5	12.5	6.25	12.5
MSSE	0.39	25	1.562	6.25	12.5	6.25	6.25
MSSM	3.125	3.125	0.781	0.781	3.125	6.25	1.562
MSRC	6.25	6.25	6.25	12.5	12.5	3.125	12.5
MSRE	12.5	12.5	6.25	12.5	12.5	3.125	6.25
MSRM	3.125	6.25	0.781	0.781	3.125	1.562	1.562
Gentamycin	0.0078125	0.015625	0.125	0.0625	0.03125	0.0625	0.0625

**Key:** AALC=*A. anthelmintica* leaf chloroform extract, AALE=*A. anthelmintica* leaf ethyl acetate extract, AALM=*A. anthelmintica* leaf methanolic extract, AASC=*A. anthelmintica* stem bark chloroform extract, AASE=*A. anthelmintica* stem bark ethyl acetate extract, AASM=*A. anthelmintica* stem bark methanolic extract, AARC=*A. anthelmintica* root bark chloroform extract, AARE=*A. anthelmintica* root bark ethyl acetate extract, AARM=*A. anthelmintica* root bark methanolic extract, MSLC=*M. salicifolia* leaf chloroform extract, MSLE=*M. salicifolia* leaf ethyl acetate extract, MSLM=*M. salicifolia* leaf methanolic extract, MSSC=*M. salicifolia* stem bark chloroform extract, MSSE=*M. salicifolia* stem bark ethyl acetate extract, MSSM=*M. salicifolia* stem bark methanolic extract, MSRC=*M. salicifolia* root bark chloroform extract, MSRE=*M. salicifolia* root bark ethyl acetate extract, MSRM=*M. salicifolia* root bark methanolic extract.

and methanolic extracts exhibited antibacterial activity against *S. typhi* and *P. aeruginosa* respectively with MIC value of 3.125 mg/mL. It is therefore surprising that besides leaf extracts which had MIC value of 3.125 mg/mL, stem and root extracts had MIC value above 6.25 mg/mL.

## Discussion

Validation of ethnomedical information of plants commonly used by ethnic groups has been a strategy in the discovery of novel bioactive secondary metabolites [16]. Despite these efforts, there are still some medicinal plants that have never been validated. That is why this study is reporting antibacterial activity of *M. aethiopicum*, *L. capassa*, *A. anthelmintica* and *M. salicifolia* growing in Tanzania. According to Rios and Recio [15], the maximum plant extract concentration of interest should be less than 1 mg/mL. Therefore, extracts reported in this study which displayed antibacterial activity with minimum inhibition concentration (MIC) of 0.39 and 0.781 mg/mL are hence regarded as potential drug template sources.

In this regard, *Lonchocarpus capassa* leaf ethyl acetate extract which demonstrated antibacterial activity against *Salmonella kisarawe* and *Salmonella typhi* with MIC values of 0.39 and 0.781 mg/mL respectively are potential source of drug leads for the management of diseases caused by these pathogens. Likewise, *L. capassa* root bark ethyl acetate extract demonstrated a potential source of drug leads for the treatment of *S. typhi* and *E. coli* as

evidenced by MIC values of 0.39 and 0.781 mg/mL respectively (**Table 1**). These results validate the previous reported traditional uses of *L. capassa* root bark in Shinyanga region, Tanzania for the treatment of abdominal problems [17].

The *Myroxylon aethiopicum* leaf and root bark chloroform extracts selectivity inhibited the growth of *S. kisarawe* and *S. typhi* respectively with MIC value of 0.781 mg/mL just like *L. capassa* leaf ethyl acetate. It is therefore assumed that these extracts are potential source of drug leads for the management of infections caused by *S. kisarawe* and *S. typhi*. These results ratify the traditional uses of *M. aethiopicum* by Pare people in the Northern Tanzania in treating hemorrhagic diarrhea [6]. However, the antibacterial principle candidates from *M. aethiopicum* were reported in Kenya to be tannins, flavonoid, saponins and alkaloid [18]. However, studies on phytochemicals have showed that, plants rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids have been found *in vitro* to have antimicrobial properties [19]. Results emanated from this study which focused on *M. aethiopicum* growing in Tanzania, and the reported data for the same plant growing in Kenya indicate that the tannins, flavonoid, saponins and alkaloid are responsible for the *S. kisarawe* and *S. typhi* respectively.

It was observed that *Myrica salicifolia* had high selectivity against *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella pneumoniae* to warrant the discovery of possible drug leads for management of diseases associated with these pathogens.

This is deduced from the antibacterial activity of *M. salicifolia* stem bark ethyl acetate against *P. aeruginosa* with MIC value of 0.39 mg/mL. *Myrica salicifolia* stem bark methanolic and root bark methanolic extracts selectively inhibited the growth of *P. mirabilis* and *K. pneumoniae* with MIC value of 0.781 mg/mL. The high susceptibility shown by *P. aeruginosa*, *P. mirabilis* and *K. pneumoniae* towards stem and root bark extracts suggesting that the stem and root bark might contain secondary metabolites that can be used in the management of infections caused by these pathogens. It also validates the traditional uses of *M. salicifolia* in treating chest congestion, pneumonia and respiratory diseases [20].

## Conclusion

The extracts from *M. aethiopicum* L. *capassa*, *A. anthelmintica* and *M. salicifolia* exhibited antibacterial activities against seven tested Gram negative bacterial strains namely *P. aeruginosa*, *K. oxytoca*, *P. mirabilis*, *K. pneumonia*, *S. kisarawe*, *S. typhi* and *E. coli*. Thus the use of plant materials as alternative to antibiotic

resistant pathogens has shown promising results which could be useful in the treatment of various infectious diseases. Results from this study therefore suggest the potential source of antibacterial drug leads hence viable tool for the drug discovery. However, antibacterial secondary metabolites from these plants which could significantly contribute in drug discovery remained unveiled thus need phytochemical investigations.

## Conflicts of Interests

Authors have declared that no competing interests exist.

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