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Antimicrobial Activity and Safety of *Maesa lanceolata* for the Treatment and Management of Selected Bacterial Pathogens

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Authors' contributions

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

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ABSTRACT

Aim: *In vitro* antibacterial activity and safety of aqueous, dichloromethane and methanolic extracts of *Maesa lanceolata* against the selected bacteria.
Methods and Results: Efficacy of air-dried leaves roots, stem bark extracts from *M. lanceolata* and phytochemicals were determined at the Center for Traditional Medicine and Drug Research laboratory, Kenya Medical Research Institute. Antibacterial activity was tested against; *Staphylococcus aureus* ATCC (American Type Culture Collections) 25923, *Escherichia coli* ATCC 27853, *Shigella dysentriae* and *Pseudomonas aeruginosa* using broth dilution technique. Stem bark methanolic extracts registered higher activity with zone inhibition diameter (ZID) of 21 mm and Minimum Inhibitory Concentration (MIC) value of 3.91 mg ml^{-1} against *S. aureus*. *E. coli* showed the least activity of 6.3 mm ZID and 250 mg ml^{-1} MIC. Phytochemicals present included alkaloids, phenols, terpenoids, anthraquinones and tannins. The selected leaves (dichloromethane and

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methanol) and stem bark (dichloromethane and aqueous) extracts displayed cytotoxicity concentration (CC_{50}) on Vero E6 cell lines from 206 µg ml^{-1} to 684 µg ml^{-1}.
Conclusion: Activity of *M. lanceolata* extracts confirms its use in folklore traditional medicine.
Significance and Impact of the Study: The findings from this study validate the claim that extracts of *M. lanceolata* possess antibacterial activity and justifies their use in herbal medicine.

Keywords: Maesa lanceolata; bacteria; antibacterial activity; extracts.

1. INTRODUCTION

A remedy to bacterial infections is principally the use of antibiotics. In the recent past, most antibiotics have lost their potency due to the emergence of resistant strains attributed to expression of resistance genes [1]. The need to develop alternative antibacterial drugs for the treatment of infectious diseases from various sources such as medicinal plants is critical. *Maesa lanceolata* flourishes on stream banks and cliff tops in both terrestrial and coastal regions to about 1500 m above sea level [2]. In Kenya, the plant is traditionally used for the therapy of helminthic and bacterial infections [3]. In Marakwet community, the stem bark is sliced to pieces, boiled and used to treat dermatophytic conditions [4]. Biological activity studies revealed MIC values of 100 µg ml^{-1} for *Vibrio cholera* and 125 µg ml^{-1} for *Salmonella typhi* [5].

Traditional medicine as an alternative form of therapy has motivated researchers to scrutinize the antimicrobial activity of several medicinal plants [6, 7and 8]. The current research focused on the evaluation of *in-vitro* antibacterial properties and cytotoxicity of methanol, aqueous and dichloromethane extracts of *M. lanceolata.*

2. MATERIALS AND METHODS

2.1 Plant Materials

Maesa lanceolata roots, leaves and stem bark were collected from Kapsowar, Elgeyo Marakwet county of Kenya based on the indigenous knowledge of the locals. Taxonomical identification was done at University of Eldoret herbarium by a plant taxonomist and voucher specimen (MU/0038/87) deposited.

2.2 Experimental Animals

Thirty-nine healthy male Swiss albino mice eight weeks old bearing a mean body weight of 20±2 g bred at KEMRI, Nairobi, Kenya were used fed with pellets (Mice pellets UNGA® feeds) and water. Guidelines on care and handling of the animals were observed as specified by the Animal Care and Use Committee-KEMRI (ACUC-KEMRI, Kenya).

2.3 Extraction Procedure

The plant parts (roots, leaves and stem bark) were air dried at room temperature (25 °C) beneath shade for two weeks, crushed using a laboratory mill (Christy and Norris Ltd., Chelmsford, England) and packed in airtight polythene bags at the Center for traditional medicine and drug research (CTMDR). From the 200g of each powdered plant material, the percentage extract yield was calculated. Out of these, fifty grams of the powdered plant material was extracted using 500 ml distilled water in a shaking water bath set at 70°C for two h, filtered, dried using a freeze dryer (Edwards freeze dryer Modulyo) then weighed and stored. Equally, soaking of the powdered plant material was done using methanol and dichloromethane for 24 h each, and the organic solvents evaporated to dryness by vacuum evaporation using a rotary evaporator (Buchi Rotavapor R-114). Percentage yield was calculated as follows:

Percentage yield =

$$\frac{\text{Weight of extract obtained}}{\text{Weight of powdered material}} \times 100\ \%$$

2.4 Bacterial Test Cultures

The bacterial strains used were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 27853, *Shigella dysentriae* ATCC 13313 and *Pseudomonas aeroginosa.* All the strains were obtained from the KEMRI culture collection and maintained as stock cultures in 50% glycerol in Eppendorf® tubes at -30°C until use.

2.5 Determination of Antibacterial Activity

Disc diffusion method [9] was used to evaluate the antibacterial activity of *M. lanceolata* against

Staphylococcus aureus ATCC 25923, *Escherichia coli* ATCC 27853, *Shigella dysentriae* ATCC 13313 and *Pseudomonas aeroginosa* using Mueller Hinton agar (Oxoid) at 37 °C for 24h. The Mueller Hinton agar test plates were set and inoculated on their surface with a cell suspension of the test bacteria (1.5 x 10^8 c.f.u ml^{-1}) in sterile normal saline. All the test assays were carried out in a Class II Biological Safety Cabinet. Sterile Whatman's No.1 (6mm diameter) discs were impregnated with 20 µl of the extracts from the stock solution of 100 mg ml^{-1} and utilized for the disc diffusion assay. The discs were then aseptically placed on the MH agar. Gentamicin discs (25 µg) were used as the reference drug while discs containing sterile distilled water were used as negative controls. The test plates were incubated at 37°C for 24 h. Each assay was done in triplicates. The zones of inhibition diameters were measured in millimeters and the findings expressed as mean inhibition zones ± standard deviation.

The minimum inhibitory concentration (MIC) was determined for extracts exhibiting inhibition zone diameter of ≥10 mm against the test microorganism using broth dilution technique. Serial dilutions of the extract were done using distilled water resulting in a working concentration range from 500 mg ml¯1 to 3.91 mg ml¯1. Sterile filter paper discs containing 20µl of the dissolved extracts were placed aseptically on the surface of MH media with inoculated test bacteria. MIC was pronounced as the lowest concentration of the extract that exhibited a clear zone of inhibition [10].

2.6 Phytochemical Screening

Phytochemical screening of active extracts was done to determine the phytocompounds present in different extracts separated by thin layer chromatography (TLC) (Kieselgel 60 F254 0.2 mm, Merck). TLC plates were developed with Ethyl acetate: petroleum spirit (3:7) as the solvent system for dichloromethane extracts while dichloromethane: methanol (9.5:0.5) solvent system was employed for methanol extracts [11]. Separated constituents were visualized under ultra violet light (254nm and 365 nm) then sprayed with visualizing agents for the colorimetric view.

2.7 Cytotoxicity Assay

The most active plant extracts were examined for *in vitro* cytotoxicity according to the modified rapid calorimetric assay [12] using Vero E6 cancer cell lines acquired from American Type Culture Collections (ATTC). The Vero cells were maintained in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 2 mol L-glutamine. Approximately 2 x 105 cell ml^{-1} suspensions were seeded on 96- well microtiter plates and incubated in a humified atmosphere with 5% CO_2 at 37°C for 12 h. Test extracts were added to the cultured cells over a concentration range of 1000 µg ml^{-1} to 1.23 µg ml^{-1}. The plates were incubated at 37°C, 5% CO2 for 48 h following which 10 µL of (3-(4, 5-dimethylthiazol-2-yr)-2, 5-diphenyltetrazolium bromide) MTT dye was added to each well. Incubation of the plates was effected for another four h, and subsequently, the media detached from the wells and 100 µL of Dimethylsulfoxide (DMSO) were added [12]. The plates were read (color absorbance) using an ELISA scanning Multiwell spectrophotometer (Multiskan Ex Labssytems) at 562 nm and 620 nm as reference. The percentage cell viability (CV) was calculated using the formula:

> % C V = [{(Average abs of duplicate drug wells − Average abs of blank wells) x 100%} / Average abs of control wells]

Data were entered into Microsoft Office Excel 2007 software and expressed as a percentage of the untreated controls. Cytotoxic concentration responsible for lysis and death of 50% of the cells was determined using Microsoft Office Excel 2007 software by linear regression analysis.

2.8 Determination of Acute Toxicity

Thirty-nine Swiss male albino mice were used in the *in vivo* acute toxicity study with permission granted by the KEMRI Animal Care and Use Committee (ACUC). Healthy mice (weight 20±2g) were randomly divided into groups of three in each cage. The mice were allowed access to water and food, except for a short fasting period of 12 h before oral administration of the test sample. The active extracts suspension were administered orally at a logarithmic dose of 5.0 mg kg^{-1}, 300.0 mg kg^{-1} and 2000.0 mg kg^{-1} body weight [13] The general behavior of mice was observed continuously for thirty minutes after treatment then intermittently for four hours and after that over a period of 24 hours [14,15]. Further observations were made up to 14 days for any sign of restlessness and death whereby the lethal dose was determined. Dead mice were

discarded according to KEMRI biosafety guidelines and the following completion of the experiment all the mice were sacrificed using chloroform and the carcasses incinerated.

3. RESULTS

3.1 Extraction of the Plant

The percentage yields resulting from different solvent extracts are summarized in Table 1.

Water extracts presented relatively higher extract yields followed by methanol and DCM the least. Stem bark produced the higher extract yield, followed by roots and leaves the least. Aqueous stem bark presented the highest yields of 12.1% while dichloromethane leaves presented the least yield of 2.2% (Table 1).

3.2 Antibacterial Bioassay

All extracts showed antibacterial activity against the selected bacteria to varying levels of activity. *Maesa lanceolata* against *Staphylococcus aureus* (Fig. 1).

Generally, there was a significant difference between extracts for each plant part for a specific bacteria (Table 2). Classically, stem bark methanolic extracts registered the highest antibacterial activity generating zone diameter inhibition of 21 mm and MIC value of 3.91 mg ml^{-1} against *S. aureus* ATCC 25923. Minimum inhibitory concentration (MIC) was considered for extracts with zone diameter inhibition greater than 10 mm. Least activity was observed against *E. coli* displaying a zone diameter inhibition ranging between 6.3±0.6 mm and 9.3±0.6 mm. Antibacterial activity against *P. aeruginosa* and *S. dysenteries* were relatively moderate as judged by the zone diameter inhibition between 7.4 mm and 19.5mm (Table 2).

3.3 Phytochemical Screening

Preliminary screening of phytoconstituents displayed the presence of phenols, terpenoids, anthraquinones, flavonoids, saponins and alkaloids on the leaves, roots and stem bark extracts resulting from water and dichloromethane while their presence was moderate about methanol extracts. Tannins were lacking in methanol derived extracts of the leave, roots and stem bark (Table 3).

3.4 Cytotoxicity Studies

Cell cytotoxicity studies of the selected extracts indicated by their MIC value ≤125 mg ml^{-1} were determined using Vero E6 cell lines whose cytotoxic concentration (CC_{50}) values were determined using the microplate reader software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA). The cytotoxicity concentration (CC_{50}) of the four extracts were determined, and their values were as follows: Dichloromethane leave, methanolic leave and dichloromethane stem bark extracts were lowly toxic with CC_{50} values of 684.995 µg ml^{-1}, 546.86 µg ml^{-1} and 322.08 µg ml^{-1} respectively. Aqueous stem bark extract was moderately toxic with a CC_{50} value of 206.45 µg ml^{-1} (Table 4).

3.5 Acute Toxicity Studies

In vivo toxicity studies involved the use of male Swiss albino mice.

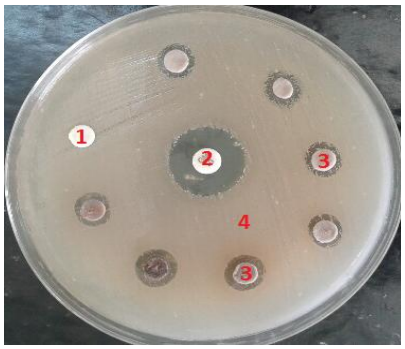

Fig. 1. Disks bearing *M. lanceolata* extracts seeded against *Staphylococcus aureus* on a Petri plate showing clear zones of inhibition

Key: 1- Negative control 2 - Positive control, 3 - Test extract, 4 - Staphylococcus aureus

Table 1. Percentage yields for plant extraction

Plant part	Percentage yield (%)		
	Dichloromethane	Methanol	Water
Leaves	2.2	2.65	4.6
Roots	1.4	5.1	5.2
Stem bark	1.8	4.8	12.1

Table 2. *In-vitro* activity of *M. lanceolata* and minimum inhibitory concentration against *E. coli* and *Shigella dysenteriaea*

Bacteria	Part	ZD (mm) ± SD and MIC (mg ml^{-})			
		Methanol	DCM	Water	Gentamicin 10µg ml^{-1}
Staphylococcus aureus	L	12.30±0.60^{cd} (125)	6.30±0.60^{a} (*)	10.30±0.60^{c}(125	15.00± 0.00^{de}
	R	16.00±1.00^{de} (7.81)	8.70±0.60^{b} (*)	11.00±1.0^{c}(62.5	15.00± 0.00^{de}
	SB	20.70±0.60^{f} (3.91)	8.70±1.20^{bc} (*)	9.30±0.60^{bc} (*)	14.30± 0.60^{cd}
Shigella dysenteriaea	L	9.70±0.60^{cd} (*)	13.30±0.60^{e} (31.25)	7.30±0.60^{a} (*)	17.00± 1.00^{gh}
	R	14.70±0.60^{f} (31.25)	11.30± 0.60 (31.25)	9.30±0.60^{bc} (*)	16.30± 0.60^{g}
	SB	18.70±0.60^{h} (15.63)	16.30± 0.60 (15.63)	8.30±0.60^{ab} (*)	16.00± 0.00^{g}
Escherichia coli	L	6.30±0.60^{a} (*)	6.70± 0.60^{ab} (*)	8.00±0.00^{c} (*)	19.70± 0.60^{e}
	R	7.30±1.20^{ab} (*)	6.70± 0.60^{ab} (*)	8.00±0.00^{c} (*)	19.00± 1.00^{e}
	SB	7.00±1.00^{ab} (*)	7.60± 0.60^{ab} (*)	9.30± 0.60^{d} (*)	18.30± 0.60^{e}
Pseudomonas aeruginosa	L	9.30±1.50^{bc} (*)	10.00±1.70^{bc} (62.5)	7.30±0.60^{a} (*)	17.00± 0.50^{ef}
	R	9.70±2.00^{abc} (*)	11.30±2.50^{bcd} (31.25)	9.00±1.60^{b} (*)	17.30± 0.50^{ef}
	SB	13.00±1.00^{d} (31.25)	12.30± 1.50^{cd} (31.25)	12.30±1.50^{cd} (31.25)	16.00± 1.00^{e}

Key: *L – Leaves, R – Roots and SB- Stem bark. (ZD) Zone diameter of microbial inhibition, (MIC) Minimum inhibitory concentration, (*) MIC value not considered for extracts with ZD<10mm (little or no activity against the selected pathogens); 6mm is the diameter of the disc (no activity). Means followed by the same letter within a row are not significantly different at P=.05.*

Table 3. Phytochemical constituents present in *M. lanceolata* extracts

Solvent	Plant part	Tannins	Flavonoids	Phenols	Saponins	Terpenoids	Alkaloids	Anthraquinones
Water	L	++	+++	+++	+++	+++	+++	+++
	R	+++	+++	+++	+++	+++	+++	+++
	SB	++	++	+++	+++	+++	+++	+++
DCM	L	++	+++	+++	+++	+++	+++	+++
	R	++	++	++	++	+++	+++	++
	SB	+	++	+++	+++	+++	+++	+++
Methanol	L	-	++	++	+++	+++	++	+++
	R	-	+	+	++	++	++	++
	SB	-	++	++	++	+	++	++

Key: L -Leaves, R - Roots, SB - Stem bark, DCM - Dichloromethane. +++ Abundant, ++ Moderate, + Trace, - Absent

Table 4. Cytotoxicity activity of *M. lanceolata* extracts against Vero E6 cell lines

Plant extract	MIC value(mg ml^{-1})	CC_{50} values ± SD (µg ml^{-1})
DCM leave	125	684.995±0.332[d]
Aqueous stem	125	206.445±1.874[a]
Methanolic leave	31.25	322.08±0.679[b]
DCM stem bark	62.5	564.86±1.249[c]

CC 50 values; (mean ± SD) µg ml^{-1} Means followed 4by the same letter within a column are not significantly different at P=.05.

Table 5. Acute toxicity results of *Maesa lanceolata* extracts on Swiss albino mice

Plant species	Extract	Conc. (mg kg^{-1})	Survivors	% Mortality
Control	Distilled water	10% Tween 80	3⁄3	0
Leaves	Methanol	5	3⁄3	0
		50	3⁄3	0
		300	3⁄3	0
		2000	3⁄3	0
Leaves	Dichloromethane	5	3⁄3	0
		50	3⁄3	0
		300	3⁄3	0
		2000	3⁄3	0
Stem bark	Water	5	3⁄3	0
		50	3⁄3	0
		300	3⁄3	0
		2000	3⁄3	0

4. DISCUSSION

Antibacterial activity of *Maesa lanceolata* against the bacteria tested was obtained for each of the three extracts under investigation. Greater activity was observed with methanol extracts against the selected bacteria followed by dichloromethane extracts with water extracts taking the least. This can be attributed to the amphiphilic nature and relatively high polarity index 5.1 of methanol thereby many phytoconstituents dissolve in it with a greater degree of freedom.

Maesa lanceolata methanol stem bark extracts were particularly active against *Staphylococcus aureus* ATCC 25923 and *Shigella dysenteriaea* ATCC 13313 with zone inhibition diameter and MIC values of 21 mm, 3.91 mg ml^{-1} and 19 mm, 15.625 mg ml^{-1} respectively. The little activity of water extracts against most bacterial strains is in agreement with previous studies disseminating that aqueous extracts of plants that showed little or no antimicrobial tendencies [16]. In East Africa, fruits of *M. lanceolata* are widely used to treat multiple ailments such as a sore throat, tapeworms, hepatitis and cholera [17]. [18] Reported that *M. lanceolata* extracts had been used for the treatment of helminthes, fungal and bacterial infections. The Marakwet community utilizes cut pieces of stem bark to treat skin rashes and dermatophytic infections [4]. In their findings, [19] stated that *M. lanceolata* roots are used as a purgative to remove pimples.

Phytoconstituent screening of the *M. lanceolata* extracts showed the presence of phenols, tannins, flavonoids, alkaloids and anthraquinones and that antibacterial activity is attributed to their occurrence. Even though phytochemicals are claimed to be safe, numerous phytometabolites are very toxic and potentially pose serious side effects [20].

Toxicity studies are vital in determining the effectiveness of medicinal plants. In this regard, MTT [3-(4, 5-dimethylthiazol-2-yr)-2, 5-diphenyltetrazolium bromide] colorimetric assay established by [12] served to monitor the cytotoxic activity of all extracts. Extracts whose MIC values were 1.25×10^5 µg $ml^{-1}$and below were considered potent, and their cell toxicity was investigated. The *In vitro* cytotoxicity of the crude extracts of the four extracts of *M. lanceolata* are presented in Table 4. The inhibitory concentration at 50% (CC_{50}) is the concentration of the extracts that inhibits 50% proliferation of the Vero E6 cell lines. The extracts had varying degrees of toxicity on the Vero E6 cell lines with IC_{50} values ranging from 206.45 µg ml^{-1} to 684.76 µg ml^{-1} (Table 4).

The standards used to substantiate the activity of *M. lanceolata* extracts against Vero E6 cell lines was based on CC_{50} values modified from those of National Cancer Institute (NSI) and [21,22] as follows: IC_{50} value ≤ 20 µg ml^{-1} = extremely toxic (very active), CC_{50} 21-200 µg ml^{-1} = highly toxic (moderately active), IC_{50} 201-500 µg ml^{-1} =moderately toxic (weakly active), CC_{50} ≥501-1000 µg ml^{-1} = lowly toxic (inactive), CC_{50} ≥1000 µg ml^{-1} = particularly non-toxic. Based on this, DCM extracts of *M. lanceolata* stem bark and leaves were lowly toxic or inactive against Vero E6 cell lines with CC_{50} of 546.86±1.249 µg ml^{-1} and 684.0.332±0.332 respectively. Similarly, aqueous stem extracts and methanol leave extracts of *M. lanceolata* were weakly active and that moderately toxic with CC_{50} values of 322.08±0.679 µg ml^{-1} and 206.445±1.874 µg ml^{-1} respectively. In general, the *in vitro* cytotoxicity determined on Vero cells indicated that most of the extracts were relatively non-toxic.

No mortality was observed within 24 hours of the mice that received the extracts in all dose levels. However, signs such as unkempt fur, crowding in a cage and moderate activity were observed for mice treated with the highest dose (2000 mg kg^{-1} body weight) particularly about dichloromethane derived leave extracts of *M. lanceolata* Similarly, there was an increase in weight in all groups of mice treated with the extracts in all dose levels and that their weight increase was not significantly different from that of control mice (P=.05). This suggests that *M. lanceolata* extracts can be safe as the antimicrobial agent. These results are in tandem with the findings of [23] who discovered that crude extracts of *M. lanceolata* possess chemotherapeutic compounds that can serve to protect other plants against microbial infections.

5. CONCLUSION

The methanol and aqueous root and stem bark extracts of *M. lanceolata* assayed exhibited a promising activity against *Staphylococcus aureus, Shigella dysenteries, E. coli, Pseudomonas aeruginosa* and this coupled to their relative non-toxic nature support their traditional uses. The need to clarify which particular photo component responsible for one or both activities is crucial.

5.1 Availability of Data and Material

The datasets on zone diameter of inhibition, phytoconstituent analysis and MIC for bioassays analyzed during the study are accessible from the corresponding author on reasonable request. Data on acute toxicity studies generated during this study are included in this published article.

ETHICAL APPROVAL

All authors at this moment declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed coupled to guideline adherence and examination by the Kenya Medical Research Institute Animal Care and Use Committee (ACUC).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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