



Phytochemical Screening, Antibacterial Activity and Bioautography of *Sorindeia madagascariensis*, *Mucuna stans*, and *Albizia harveyi*

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Abstract: This study investigated the antibacterial activities of crude extracts of three Tanzanian plants; *Sorindeia madagascariensis*, *Mucuna stans* and *Albizia harveyi*, following reports on their ethnomedicinal applications and those of their related species. The reported ethnomedicinal applications of the selected plants include treatment of; tuberculosis, urinary tract infections and bacterial infections of the skin among other applications. Plant material were collected from Njombe, Iringa and Pwani regions of Tanzania. Phytochemical screening and bioautography were conducted as per adopted methods. Screening for antibacterial activity was done by broth microdilution assay against the standard and clinical isolates of bacteria. Phytochemical screening revealed the presence of phenolics, tannins, flavonoids, terpenoids and glycosides among the plant extracts. Antibacterial activity-study displayed weak to moderate antibacterial activities of the plant extracts, whereby *S. madagascariensis* leaf extract displayed the highest activity against; *Staphylococcus aureus* (ATCC 25923), clinical isolate of *S. aureus* and a methicillin-resistant *S. aureus* (MRSA) isolate, at a minimum inhibitory concentration (MIC) of 192±0.00 µg/mL. Bioautography of *S. madagascariensis* indicated this antibacterial activity to be associated with polar compounds. MICs observed due to *M. stans* ranged from 770 to 3080 µg/mL against all tested bacterial species whereas the observed MICs due to *A. harveyi* ranged from 1283 to > 3080 µg/mL. These findings reveal the antibacterial activities of the selected plants, corroborating their ethnomedicinal applications. Bioautography-guided isolation of compounds from these plants particularly *S. madagascariensis*, may give leads for newer antibacterial agents.

Keywords: Phytochemical Screening, Antibacterial Activity, Bioautography, *S. madagascariensis*, *M. stans*, *A. harveyi*

1. Introduction

Infectious diseases are responsible for high rates of morbidity and mortality in low income countries [1]. It has been cautioned that, the current success in containment of such diseases will ultimately be lost as a result of the rapidly emerging antimicrobial resistance [2, 3], since bacteria resistant to drugs of last resort, such as carbapenems and

colistin are being reported [4-6].

To curb the problem; scaling up of research and development of newer antibacterial agents is one of the highly advocated counter-measures [3, 7-9]. To streamline the process, the World Health Organization (WHO) has named the priority bacterial pathogens against which new drugs should be developed [5].

Among other sources, searching for antibacterial drugs from plants is of paramount advantage for several reasons.

Shortly; the plants' biodiversity is presently unexhausted since it is estimated that, only 10% of plant species have been explored. Within that small portion, some species have given compounds with direct antibacterial activities while others possess compounds with modulating actions on the resistance profiles of bacteria [10-12]. This means, continuous exploration of plants will ultimately give successful leads for newer antibacterial agents.

Driven by this context, we investigated three Tanzanian medicinal plants namely; *A. harveyi* Fourn (Fabaceae), *M. stans* Baker (Fabaceae) and *S. madagascariensis* Baill (Anacardiaceae) for antibacterial activities. This decision was propelled by, primarily an ethnobotanical report by Mbunde *et al.*, (2017) and other literature on the related plant species [13-19]. Generally, the selected plants range from multi-stem shrubs to erect tall, native trees of tropical Africa. In Tanzania, they are well distributed along the coastal regions especially Pwani, and the Southern highlands particularly Njombe and Iringa [13, 20].

Among others, the published ethno-medicinal uses of the selected plants include treatment of; tuberculosis for *S. madagascariensis* and urinary tract infections for *A. harveyi* [13, 21]. Ethno-medicinal applications of *M. stans* are not well reported. However, reports on the antibacterial activity of *M. pruriens* [15, 16, 22], a related species to *M. stans* raised anticipation for the antibacterial activity of the latter, considering the possible phylogenetic relatedness of plants belonging to the same genus [23].

Considering such ethnomedicinal profiles of the plants, we anticipated their antibacterial activities. It was therefore the aim of this study to investigate the selected plants for antibacterial activities, aiming to come up with lead information in the course of discovery of antibacterial agents.

2. Materials and Methods

2.1. Plant Material Collection and Drying

Following identification by a botanist at the University of Dar es Salaam, leaves of *A. harveyi* and *M. stans*, as well as the leaves and roots of *S. madagascariensis* were collected from Njombe, Iringa and Pwani regions of Tanzania in October 2018.

The collected plant samples were cut into small pieces and allowed to dry under shade [24, 25]. The dried samples were then separately pulverized into coarse powders. Voucher specimens were concurrently processed for storage in the herbarium at the Institute of Traditional Medicine of the Muhimbili University of Health and Allied Sciences (MUHAS).

2.2. Extraction of the Plant Samples

The powders were extracted by cold maceration with 80% ethanol for 96 hours accompanied with 12 hourly agitations. The resulting extracts were filtered, and concentrated *in vacuo* (Buchi®, Switzerland) at 55°C. The obtained semi-solid crude extracts were freeze-dried (BenchTop Pro®), kept in airtight

containers and refrigerated at 4°C until further investigations [26-28].

2.3. Phytochemical Screening of the Extracts

Standard qualitative phytochemical procedures as adopted from literature [24, 25, 29, 30] were carried to detect the presence of tannins, saponins, flavonoids, steroids, terpenoids, phenols, alkaloids, and glycosides.

2.4. Antibacterial Activity Study

2.4.1. Selection of Study Bacteria

Bacterial strains were selected based on the WHO priority list [5] and availability. These included; *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (ATCC 8385) and *Staphylococcus aureus* (ATCC 25923). Also included were the clinical isolates of the same bacteria and a methicillin-resistant *Staphylococcus aureus* (MRSA) strain.

2.4.2. Determination of the Minimum Inhibitory Concentrations

Minimum inhibitory concentrations (MICs) were determined by broth microdilution assay as described in the National Committee for Clinical Laboratory Standards (NCCLS) [31] and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) [32] guidelines. Mueller Hinton broth (MHB), (Oxoid, UK) was used as the nutrient medium and vehicle for the extracts and test controls. Solubilization of extracts was aided by 20% dimethylsulfoxide (DMSO), (Fisher Scientific, UK) in MHB. Ciprofloxacin (Sigma-Aldrich) at a concentration range of 0.5 to 51.5 µg/mL [2, 33] and 20% DMSO in MHB were used as positive and negative controls respectively.

The extracts were tested across a concentration range of 24 to 3,080 µg/mL [34, 35] and before setting for MICs determination, the test bacteria were initially sub-cultured for 24 hours on a fresh Nutrient Agar, Techno Pharmchem, India (NA) for their reactivation [31, 32].

Upon MIC determination, 100 µL of autoclave-sterilized Muller Hinton broth was added into all wells of microtitre plates using a multi-channel micropipette. Subsequently, 100µL of crude extracts at the concentration of 12,320µg/mL were added in the first wells of the plates and mixed to make total volumes of 200µl in each well. From such wells, 100µL were drawn and added to the wells in the next row. The process continued down to the wells in the last row to constitute the 2-fold microdilutions whereby the final 100µL were discarded [31, 32, 36].

0.5 McFarland-equivalent (approximately 1×10^8 cfu/mL) suspensions of the respective bacteria in saline were prepared by adjustments of turbidity to that of the 0.5 McFarland turbidity standard (Remel, USA). The resulting suspensions were diluted to approximately 1×10^6 cfu/mL by mixing 0.1mL of the bacterial suspensions with 9.9mL of MHB. 100µL of the final suspensions were then added to each; test and control wells of the microtiter plates [32]. The plates

were then incubated at 37°C for 24 hours, after which they were observed for inhibition of bacterial growth [31, 32].

Detection of growth inhibition was by observing the colour changes after addition of iodinitrotetrazolium (INT) chloride salt (Sigma-Aldrich) indicator, whereby 30µl of 0.4mg/mL INT were added into the wells followed by re-incubation at 37°C for 30 minutes [37]. Formation of purple or pink colour signified the presence of actively growing bacteria as opposed to inhibited growth in which the indicator remained colourless. The lowest extracts' concentrations showing complete inhibition of bacterial growth were taken as the MICs. The MICs were determined in triplicates and the whole exercise was repeated once. The results were expressed as the mean values and standard deviations of the readings [31, 32].

2.5. Bioautography

This was done only for the leaf and root extracts of *S. madagascariensis*, following their observed superior antibacterial activities. Elution characteristics of the compounds responsible for the antibacterial activity were studied aiming to later guide isolation of such compounds.

Agar overlay bio-autography method as described in several literature was adopted [38-40]. Two bacteria namely; *S. aureus* ATCC 25923 and the MRSA strain were preferentially used following their prior observed high susceptibility to the respective extracts. 10cm x 5cm aluminium-backed thin layer chromatography (TLC) plates (Silica gel 60 F₂₅₄, Merck, Germany) were used. Upon spotting, 16 µg (10mg/ml) of extracts were added per spot, by using 4 µL micro-capillaries in four cycles of spotting [41, 42].

The spots were eluted into the respective chromatograms using the pre-developed mobile phases. Ciprofloxacin in methanol (10mg/mL) was spotted on some plates as the positive control. Before mounting of Muller-Hinton agar (MHA) on the chromatograms, mobile phase solvents were evaporated in an oven at 50°C for six hours. Following mounting, the hardened MHA layers on TLC plates were refrigerated at 4°C overnight to allow diffusion of compounds from the silica layer into the MHA layer, while limiting microbial growth [40].

The agar surfaces were thereafter inoculated with 1 x 10⁶ cfu/mL bacterial suspensions in saline, and incubated at 37°C for 24 hours. Determination of areas of inhibited growth was carried out by spraying with 0.4 mg/mL INT solution and re-incubation for 30 minutes. The areas that had purple or pink

colour signified active growth of bacteria and those with yellow or white colour signified growth inhibition. The resulting bioautograms were matched with their corresponding chromatograms to characterize the spots with bioactive compounds [38].

3. Results

3.1. Phytochemical Screening of Extracts

Phytochemical screening results are presented in Table 1. Difference in colour intensities among the tested samples were visually compared to give impression on the relative amounts of the detected phytoconstituents among the plant extracts. Generally tannins, flavonoids, saponins, terpenoids phenolics, and glycosides were the most detected phytochemical groups among the plant extracts.

3.2. Antibacterial Activities of the Extracts

Antibacterial activities of the extracts expressed as mean values of the minimum inhibitory concentrations (MICs) and their respective standard deviations are presented in Table 2.

S. madagascariensis leaf extract was the most active among the extracts and inhibited all test-bacteria at MICs between 192 and 1283.3 µg/mL, exhibiting the best activity (MIC=192±0.00 µg/mL) against *S. aureus* (ATCC 25923), *S. aureus* (clinical isolate) and the MRSA. The root extracts of the same plant generally trailed the leaf extracts, inhibiting all bacteria at MICs from 385 to 1283.3 µg/mL.

The leaf extract of *M. stans* was as well active against all bacteria, exhibiting MICs from 770 to 3080 µg/mL. *A. harveyi* leaf extracts inhibited most bacteria at MICs from 1283 to 3080 µg/mL, but failed to inhibit *P. aeruginosa* (ATCC 27853) and a clinical isolate of *E. coli* at the highest test-concentration (3080 µg/mL).

All test-bacteria were inhibited within the set concentration range (0.5 to 51.5 µg/mL) of the control drug (ciprofloxacin) except the MRSA, whose growth was detected up to the well with the highest test-concentration (51.5 µg/mL).

3.3. Bioautography

The results for bioautography of *S. Madagascariensis* extracts are presented in Figure 1, whereby the bioautograms have been matched with their parent chromatograms. Generally, the polar compounds were observed to be responsible for the antibacterial activity as growth inhibition appeared to be confined to the tailing and hardly eluted spots.

Table 1. Phytochemical groups detected in the plant extracts.

Phytochemical group	<i>Sorindeia madagascariensis</i> (leaf extract)	<i>Sorindeia madagascariensis</i> (root extract)	<i>Mucuna stans</i> (leaf extract)	<i>Albizia harveyi</i> (leaf extract)
Tannins	+	+	++	+
Saponins	++	+	+	+
Flavonoids	+	-	+	+
Steroids	-	-	+	+
Terpenoids	++	+	+	+
Phenolics	++	+	+	+
Alkaloids	-	-	-	-

Phytochemical group	<i>Sorindeia madagascariensis</i> (leaf extract)	<i>Sorindeia madagascariensis</i> (root extract)	<i>Mucuna stans</i> (leaf extract)	<i>Albizia harveyi</i> (leaf extract)
Glycosides	++	++	-	+

+ means the phytochemical group was detected.

++ means the observed colour was so intense to signify the high amount of the phytochemical group detected compared to the other samples.

- means the phytochemical group was not detected.

Table 2. Minimum inhibitory concentrations (microGram/mL) of crude extracts of *S. madagascariensis*, *M. stans* and *A. harveyi* against selected bacteria.

Bacteria	<i>Sorindeia madagascariensis</i> (leaf extract)	<i>Sorindeia madagascariensis</i> (root extract)	<i>Mucuna stans</i> (leaf extract)	<i>Albizia harveyi</i> (leaf extract)	Ciprofloxacin
<i>S. aureus</i> (ATCC 25923)	192±0.00	385±0.00	770±0.00	1283±444.6	6.4±0.00
<i>E. coli</i> (ATCC 25922)	641.7±222	770±0.00	770±0.00	2053±889.1	< 0.5
<i>K. pneumoniae</i> (ATCC700603)	770±0.00	770±0.00	1026±444.6	2053.3±889.1	12.9±0.00
<i>S. typhi</i> (ATCC 8385)	770±0.00	1026.7±444.6	1283.3±444.6	1540±0.00	3.2±0.00
<i>P. aeruginosa</i> (ATCC 27853)	1026.7±444.6	1283.3±444.6	3080±0.00	>3080	2.1±0.9
Clinical isolate <i>S. aureus</i>	192±0.00	385±0.00	770±0.00	1540±0.00	12.9±0.00
MRSA	192±0.00	385±0.00	1026.7±444.6	1540±0.00	> 51.5
Clinical isolate <i>P. aeruginosa</i>	1283.3±444.6	1283.3±444.6	2053.3±889.1	2566.6±889.1	1.6±0.00
Clinical isolate <i>S. typhi</i>	513.3±222.2	1026.7±444.6	1540±0.00	3080±0.00	4.3±1.85
Clinical isolate <i>K. pneumoniae</i>	770±0.00	1540±0.00	2053.3±889.1	1540±0.00	2.4±0.00
Clinical isolate <i>E. coli</i>	385±0.00	770±0.00	1283.3±444.6	>3080	3.2±0.00

> means bacterial growth inhibition was not observed up to the well with the highest concentration.

< means bacterial growth inhibition was observed up to the well with the lowest concentration.

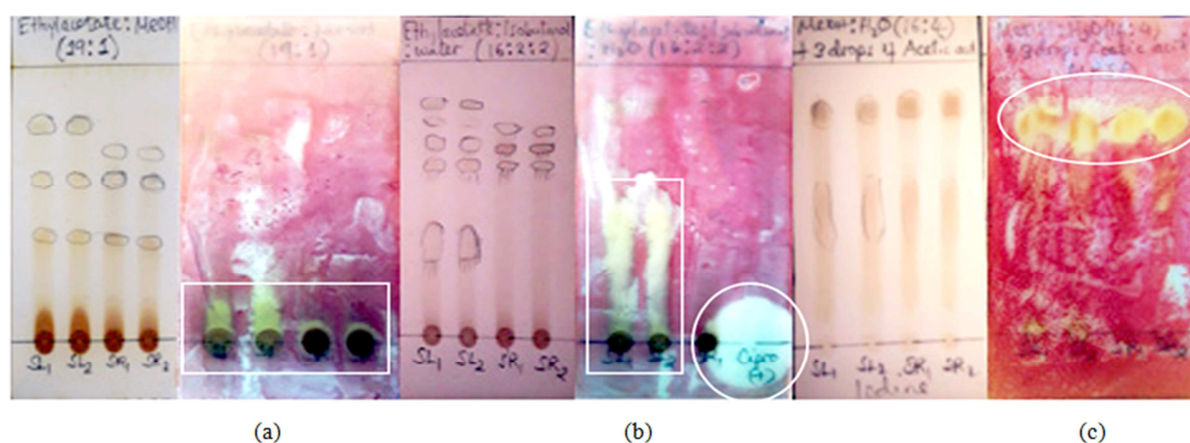


Figure 1. Chromatogram/bioautogram pairs labelled (a) to (c). The bioautograms are stained in purple/pink to show the areas with bacterial growth and those with bacterial growth inhibition. Purple/pink colour means active growth of bacteria, whereas, yellow/white colour means inhibition of bacterial growth. The reference chromatograms in the right of each bioautogram display the corresponding active spots.

SL=*S. madagascariensis* leaf extract, SR=*S. madagascariensis* root extract and Cipro=Ciprofloxacin.

(a) and (b) are bioautograms on which the test bacterium was *S. aureus* (ATCC 25923), at first elution (ethyl acetate: methanol, 19:1) and second elution (ethyl acetate: Isobutanol:H₂O, 16:2:2), respectively.

(c) is the bioautogram on which the test bacterium was MRSA, at the most polar elution (Methanol: H₂O 16:4 plus 3 drops of glacial acetic acid).

4. Discussion

4.1. Phytochemical Screening

Phytochemical screening revealed a wide array of chemical groups among the plant extracts. Tannins, saponins, flavonoids, terpenoids, phenolics and glycosides were detected in the leaf extract of *S. madagascariensis*. With exception to flavonoids, similar phytochemicals were detected in the root extract of the same plant, while steroids and alkaloids were not detected in both extracts. Comparable findings have been reported for other *Sorindeia* species. Specifically, flavonoids, tannins, and saponins have been

intensely detected in a leaf extract of *S. grandifolia* [43]. Similarly, a fruit extract of *S. juglandifolia* has been found to possess phenolics, tannins, flavonoids, saponins, glycosides, triterpenes and anthraquinones but not alkaloids [19, 44, 45].

The leaf extract of *M. stans* appeared to possess tannins, saponins, flavonoids, steroids, terpenoids, and phenolics. Similar phytochemicals have variably been reported in the leaves, roots and seeds of other *Mucuna* species. However, we report non-detection of alkaloids and glycosides in the leaf extract *M. stans*, contrary to the reports on the other species [46-51]. This can be ascribed to; methodological faults, phylogenetic differences, and geo-climatic influences [23, 24].

With exception to alkaloids, all tested phytochemicals were detected in the leaf extract of *A. harveyi*. This is in conformity with other reports whereby, together with other phytochemicals, saponins and flavonoids have been largely detected in several organs of most *Albizia* species [18, 52-54].

4.2. Antibacterial Activity

The plant extracts exhibited moderate ($100 < \text{MIC} < 625 \mu\text{g/mL}$) to weak ($\text{MIC} > 0.625 \mu\text{g/mL}$) antibacterial activities based on classification system by Kuethe *V.*, (2010) [34]. The leaf and root extracts of *S. madagascariensis* exhibited moderate activity against; *S. aureus* ATCC 25923, a clinical isolate *S. aureus* and the MRSA. Despite being novel for *S. madagascariensis*, these findings are in agreement with what is reported of the other *Sorindeia* species [19, 44, 55]. Together with other phytochemicals, the detected phenolics in the extracts of *S. madagascariensis*, may be largely implicated in its antibacterial activity, since similar compounds, namely; 2, 3, 6-trihydroxybenzoic acid and 2, 3, 6-trihydroxymethylbenzoate have been isolated from, and found to be responsible for anti-mycobacterial activity of the fruits of a related species, *S. juglandifolia* [19].

The leaf extracts of both *M. stans* and *A. harveyi* exhibited weak antibacterial activities. Despite their novelty for the two plant species, these findings parallel several reports on the antibacterial activities of the related species in the respective genera. For instance; a few reports exist on the antibacterial activities of *M. pruriens*, a prototype of the *Mucuna* species. The leaves [15, 22] and seeds [16] of *M. pruriens* have been reported to exhibit weak antibacterial activities. The tannins and phenolics detected in the leaf extract of *M. stans* may be responsible for its observed activity as the latter are highly linked to the antibacterial activities of the reported *Mucuna* species [17].

On the other side, the antibacterial activities of *Albizia* species have been reported with some species exhibiting significant to moderate antibacterial activities. Specifically; *A. julibrissin*, *A. odoratissima* and *A. lebeck* have been reported to exhibit antibacterial activities at MICs of $65 \mu\text{g/mL}$, $136 \mu\text{g/mL}$, and $10 \mu\text{g/mL}$ respectively [56-58]. The tannins, saponins and particularly flavonoids, detected in the leaf extract of *A. harveyi*, may be associated with the activity of the extract since a flavonoidal fraction has been linked to the antibacterial activity of a related species; *A. julibrissin* [56].

Furthermore, ciprofloxacin inhibited all test bacteria, except the MRSA which was resistant up to the highest set concentration of the drug ($51.5 \mu\text{g/mL}$). The same MRSA was susceptible to all plant extracts and was highly inhibited by the leaf extract of *S. madagascariensis* ($192 \pm 0.00 \mu\text{g/mL}$). This means bioactive compounds from *S. madagascariensis* may give lead molecules against the multi-drug resistant bacteria particularly the MRSA strains.

4.3. Bioautography

Bioautography of the leaf and root extracts of *S. madagascariensis* indicated the most polar compounds are

responsible for its antibacterial activity. This is depicted by the confinement of activity around the tailing and hardly eluted spots. This is in concordance with the prior intense detection of polar phytochemicals; tannins, phenolics and saponins in the extracts among which, much of the extracts' activity is possibly claimed from [19].

Adding on that, it is well known that, during both contact and agar overlay bioautography techniques, there should be spared enough time for diffusion of molecules from silica layer of the chromatogram into the agar layer. Shorter diffusion periods, may allow the growth of microorganisms to outpace the diffusion of active phytochemicals from the silica gel into the agar, the ultimatum of which is zero growth inhibition [38].

Despite such sensitivity, most literature highlight shorter diffusion periods of a few hours [38-41], implying the lack of standard. In our case, only periods beyond 12 hours (overnight) registered successful inhibition. This can be explained by slow diffusion of the highly polar, bioactive compounds from the polar silica layer into the agar layer.

This informs that, in order to capture all spots with bioactive compounds, diffusion periods should not only be ascertained from literature, but also be optimized by predicting the polarities of the extract-components. A guiding phytochemical screening as well as an effective TLC profiling can be of help in this regard. Generally, longer diffusion periods should be allowed for extracts displaying larger proportions of polar compounds during phytochemical screening and vice versa.

5. Conclusion

The crude extracts of all plants exhibited antibacterial activities justifying their ethno-medicinal applications. The leaf and root extracts of *S. madagascariensis* exhibited the best activities against all test-bacteria. Polar compounds in these extracts were found to be responsible for the antibacterial activity. These findings will greatly focus isolation of the bioactive compounds in the subsequent studies which may later serve as leads for new antibacterial agents.

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