**In vitro Antibacterial and Antifungal Activities of Sorindeia madagascariensis and Albizia harveyi Stem Barks**

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**ABSTRACT**

The emerging multi-drug-resistant pathogens urge continuous searches for new antimicrobial agents. This study investigated the *in vitro* antibacterial and antifungal activities of the stem barks of two plants, *Sorindeia madagascariensis* and *Albizia harveyi*. Broth microdilution assay was used to determine the minimum inhibitory concentrations (MICs) of hydroethanolic extracts of the stem barks against selected bacteria and fungi. Both plant extracts exhibited activity against all tested microorganisms and their minimum inhibitory concentrations (MICs) against bacteria and fungi were from 1.67 to 5.00 mg/mL and from 1.67 to 10.00 mg/mL, respectively. This study reports the antibacterial and antifungal activities of the hydroethanolic extracts of the stem barks of both plants. Antifungal activity of *A. harveyi* is being reported for the first time. We therefore suggest further investigation of bioactive compounds from stem barks of *A. harveyi* and *S. madagascariensis* with antibacterial and antifungal activities.

**Keywords**: antibacterial; antifungal; Albizia harveyi; Sorindeia madagascariensis; stem bark

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**INTRODUCTION**

Antimicrobial agents applications are not only in direct treatment but also are central to several medical procedures that require their prophylactic use (CDC, 2019). Their irrational use in human medicine and unregulated applications in animal husbandry and crop production continue to rise. All these have substantial contributions to the ever-increasing problem of antimicrobial resistance (AMR) which continues to narrow the arsenal of antimicrobial agents (Berger et al., 2017; CDC, 2019). AMR threatens all breakthroughs in the medical practice, not only cause treatment failures, but also impediment on several specialized medical procedures that require prophylactic uses of antimicrobial agents.

For several decades, AMR stewardship has focused mostly on pathogenic bacterial, owing to their ubiquity and notoriety in resistance development. However, the pathogenic fungi that were previously considered less problematic are now turning disastrous. Recent data show that some pathogenic fungi, particularly *Candida albicans* and *Aspergillus* species are increasingly getting resistant to the most antifungals (Berger et al., 2017; CDC, 2019). Of more concern is the recent discovery of *Candida glabrata* and *Candida auris* that are resistant to most of the current antifungal drugs, proving to be deadly (CDC, 2019). The situation is alarming and together with other measures, searching for new antimicrobial agents is inevitable.

Preliminary searches for antimicrobial agents may entail the exploration of nature for potential leads. Among the natural sources, plants prove to be reliable and practical, especially in resource-constrained parts of the world. Their attributes of high biodiversity and long historical uses by the traditional societies in the treatment of ailments, make them a trustable source of bioactive compounds (Katiyar et al., 2012; Petrovska, 2012; Rates, 2001).

In this study, the stem barks of two medicinal plants, *Sorindeia madagascariensis* and *Albizia harveyi* were tested for antibacterial and antifungal activities. This was mainly induced by recent reports, whereby the leaf extracts of both plants have been reported to exhibit antibacterial activities (Makoye et al., 2020). Moreover, a leaf extract of *S. madagascariensis* has been reported to exhibit antifungal activity against clinically important fungi (Mbunde et al., 2019).

Although the antifungal activity of *A. harveyi* has not been reported, there are several reports on the antifungal activity of other *Albizia* species, hence raised expectation for the activity of *A. harveyi* (Ghaly et al., 2010; Maroyi, 2018; Samoylenko et al., 2009; Thippeswamy et al., 2014). We therefore studied the antibacterial and antifungal activities of the stem barks of *S. madagascariensis* and *A. harveyi* as part of our continued investigations on the two species against selected pathogenic bacteria and fungi (Makoye et al., 2020; Mbunde et al., 2019).
**METHODS**

**Plant Identification and Collection**
The stem barks of *S. madagascariensis* and *A. harveyi* were collected from Chalinze district of Tanzania. Specimens of their leaves were as well collected and used for confirmation of identity of the plants by a botanist at the Institute of Traditional Medicines, Muhimbili University of Health and Allied Sciences, Tanzania. The collected stem barks were size-reduced and dried under shade, after which they were separately pulverized into coarse powders using a milling machine (locally made).

**Extraction**
The obtained stem bark powders were thoroughly extracted with 80% ethanol by cold maceration for four days in closed containers. This process was followed with twelve hourly agitations. The obtained extracts were filtered using Whatman filter paper no. 1 and concentrated in vacuo (Buchi®) at 55℃. The concentrates were then freeze-dried, kept in airtight containers and stored at 4℃.

**Phytochemical Evaluation**
Standard qualitative methods were adopted from literature (Rao et al., 2016; Shah & Seth, 2013) and used to detect the presence of phytochemical classes include: tannins, saponins, alkaloids, phenols, phytosterols, glycosides, terpenoids, triterpenoids and flavonoids present in the hydroethanolic extracts of the stem barks of both plants.

**Antimicrobial Activities Study**
Broth microdilution assay as described in the European Committee for Antimicrobial Susceptibility Testing (EUCAST) guidelines of 2003 (European Committee for Antimicrobial Susceptibility Testing, 2003) was used to screen and quantify the antibacterial and antifungal activities of the extracts. The minimum inhibitory concentrations (MICs) of the extracts against selected standard and clinical isolates of bacteria and fungi were measured.

**Preparation of extract stock solutions and test standards**
The extracts stock solutions were prepared by dissolving 200 mg of each extract in separate 8 mL of Muller Hinton broth (Oxoid, UK) (MHB) and Sabouraud dextrose broth (Oxoid, UK) (SDB) for antibacterial and antifungal testing respectively. In each mixture, 2 mL of dimethyl sulfoxide (DMSO) (Fisher Scientific, UK) were added to aid dissolution of the extracts. This constituted 20 mg/mL stock solutions of the extracts. Ciprofloxacin (Sigma-Aldrich, Germany) and fluconazole (Lincoln Pharmaceuticals Ltd, India) were used as positive controls for bacteria and fungi, respectively. Their stock solutions were prepared by dissolving 13.3 mg of ciprofloxacin and 2 g of fluconazole in 100 mL of MHB and SDB respectively. This resulted into stock solutions of 133 µg/mL and 20 mg/mL for ciprofloxacin and fluconazole respectively.

**Test microorganisms and media**
Selection of the test organisms was guided by their medical importance (CDC, 2019; WHO, 2017) and availability. Tested bacteria included the standard *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC700603, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853. Clinical

<table>
<thead>
<tr>
<th>Phytochemical Tested</th>
<th>Name of the Test</th>
<th><em>A. harveyi</em> stem bark</th>
<th><em>S. madagascariensis</em> stem bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wagner’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Sulphuric acid test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>Salkowski’s test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Liebermann Burchard test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Liebermann’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Salkowski’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Liebermann Burchard test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead acetate test</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ the tested phytochemical was detected
- the tested phytochemical was not detected
isolates of the same bacteria and a methicillin-resistant Staphylococcus aureus (MRSA) were included as well. Tested fungi included the standards Candida albicans ATCC 13803, Cryptococcus neoformans ATCC 90112, and Aspergillus niger AZN 8240, as well as the clinical isolates of Candida albicans and Aspergillus niger.

Before activity testing, the test bacteria and fungi were reactivated by sub-culturing at 37 °C for 24 and 48 hours on nutrient agar (Techno Pharmchem, India) and Sabouraud Dextrose Broth (SDB), respectively. Testing of the antibacterial and antifungal activities of the extracts was subsequently performed in Mueller Hinton Broth (MHB) and SDB, respectively.

**Determination of Minimum Inhibitory Concentrations (MICs)**

MICs determinations were carried out in 96-wells microtiter plates (Becton Dickinson Labware, USA). One plate was dedicated for each organism and the respective extracts and positive controls were each tested in three adjacent lanes (columns) of the plates. Initially, all wells of the microtiter plates were added with 100 µL of broth whereby MHB and SDB were added in the plates dedicated for bacteria and fungi, respectively. Furthermore, 100 µL of the prepared solutions for extracts and positive controls were added into the first-row of wells in triplicate. The added solutions were thoroughly mixed with the previously added broth. From those wells, 100 µL of the resulting mixtures were drawn and added to the respective wells in the next row and mixed with the previously added broth again.

This was repeated serially down the plates to the last row whereby the last drawn of 100 µL were discarded. This resulted 2-fold dilutions of both the extracts and positive controls down the columns of the wells. Following that, each well was inoculated with 100 µL of the respective microbial suspensions at 1 x 10^6 cfu/mL which were previously prepared by mixing 0.1 mL of 0.5 McFarland-equivalents (approximately 1 x 10^6 cfu/mL) of bacterial and fungal suspensions with 9.9 mL of MHB and SDB respectively (European Committee for Antimicrobial Susceptibility Testing, 2003).

The inoculated plates were incubated at 37°C for 24- and 48- hours for bacteria and fungi, respectively. Following incubation periods, 30µl of a 0.2 mg/mL iodonitrotetrazolium chloride stain (Sigma-Aldrich) (INT) were added into the wells, and the plates were re-incubated for 30 minutes. Growth inhibition was inferred when no colour changes were observed, whereas formation of purple or pink colour indicated non-inhibition of the growth of the test micro-organisms. The lowest concentrations revealing growth inhibition were regarded as the MICs. The whole experiment was repeated three times in different days and the results were presented as mean values of the MICs with their standard deviations.

**Statistical analysis**

Mean MIC values and their standard deviations were analyzed using Microsoft excel 2013 software. GraphPad Prism 8 software was used to analyze one-way analysis of variance (ANOVA) and subsequently the Tuckey’s honest significant tests of the observed differences in MICs among the extracts and positive controls. The results were graphically elucidated using the GraphPad Prism 8 software as well.

**RESULTS**

**Phytochemical Evaluation**

Phytochemical groups including tannins, saponins,
phenols, phytosterols, glycosides and terpenoids were detected in the extracts of both *A. harveyi* and *S. madagascariensis*, as described in Table 1. Triterpenoids were further detected in *A. harveyi* but not in *S. madagascariensis* extract. In both extracts, alkaloids and flavonoids were not detected.

**Antibacterial Activity**

The extracts of both plants exhibited activity against all tested bacteria at MICs ranging from 1.67 to 5.00 mg/mL. The highest observed activity (1.67 ± 0.72 mg/mL) was exhibited by both extract against *S. aureus* ATCC 25923, a clinical isolate of *S. aureus* and the MRSA. *A. harveyi* extract further displayed this activity against *E. coli* ATCC 25922 and the clinical isolates of *E. coli* and *K. pneumoniae*, as described in Table 2. The rest of the organisms were all susceptible to both extracts, however starting at MICs of 2.5 mg/mL.

Upon one-way analysis of variance, with subsequent Tukey’s multiple comparisons test at 95% confidence level, the antibacterial activities of the two extracts were found to be statistically similar (p = 0.483). However, the antibacterial activities of the two extracts were found to be far inferior to that of the control drug, ciprofloxacin justified by p-values of 0.001 and 0.0002 for *A. harveyi* and *S. madagascariensis* respectively, as depicted in Figure 1.

**Antifungal Activity**

The extracts inhibited all tested fungi at concentration ranges between 1.67 to 6.67 mg/mL and 1.67 to 10.00 mg/mL for *A. harveyi* and *S. madagascariensis*, respectively. They both exhibited the highest activity (1.67 ± 0.72 mg/mL) against a clinical isolate of *C. albicans*. This MIC was further displayed by *A. harveyi* against *C. albicans* ATCC 13803 and *C. neoformans* ATCC 90112. *Aspergillus niger* AZN 8240 and its clinical isolate were the least susceptible to the extracts being inhibited at MICs ranging from 5.00 to 10.00 mg/mL. Moreover, the tested fungi were all susceptible to the control drug, fluconazole and were inhibited at MICs ranging from 0.66 to 10.00 mg/mL, as shown in Table 3.

One-way analysis of variance indicated the antifungal activities of the two plant extracts to be similar to that of the control drug, fluconazole (p = 0.165), as depicted in Figure 2.

**DISCUSSION**

The phytochemical profile of an extract is a key determinant of its bioactivity. Several phytoconstituents have been characterized from nature and their bioactivities are well known today. Tannins, phenolics and saponins, as well as alkaloids, terpenes, and flavonoids for example, are well known for their antibacterial and
Table 3. Minimum inhibitory concentrations of the stem barks of *A. harveyi* and *S. madagascariensis* against fungi

<table>
<thead>
<tr>
<th>Organism</th>
<th><em>A. harveyi</em> stem bark (mg/mL)</th>
<th><em>S. madagascariensis</em> stem bark (mg/mL)</th>
<th>Fluconazole (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em> ATCC 13803</td>
<td>$1.67 \pm 0.72$</td>
<td>$3.33 \pm 1.44$</td>
<td>$1.67 \pm 0.72$</td>
</tr>
<tr>
<td><em>A. niger</em> AZN 8240</td>
<td>$6.67 \pm 2.89$</td>
<td>$10.00 \pm 0.00$</td>
<td>$3.33 \pm 1.44$</td>
</tr>
<tr>
<td><em>C. neoformans</em> ATCC 90112</td>
<td>$1.67 \pm 0.72$</td>
<td>$6.67 \pm 2.89$</td>
<td>$0.63 \pm 0.00$</td>
</tr>
<tr>
<td>Clinical isolate <em>C. albicans</em></td>
<td>$1.67 \pm 0.72$</td>
<td>$1.67 \pm 0.72$</td>
<td>$3.33 \pm 1.44$</td>
</tr>
<tr>
<td>Clinical isolate <em>A. niger</em></td>
<td>$5.00 \pm 0.00$</td>
<td>$6.67 \pm 2.89$</td>
<td>$6.67 \pm 2.89$</td>
</tr>
</tbody>
</table>

Data presented in mean±SD

Figure 1. The overall mean values of minimum inhibitory concentrations of the stem barks of *A. harveyi* and *S. madagascariensis* against selected fungi. Data in mean ± SD, *ns* = the observed difference in activity is statistically non-significant, *p* > 0.05

antifungal activities, among other bioactivities (Arabski et al., 2012; Bahri-Sahloul et al., 2014; Makgatho et al., 2018; Trdá et al., 2019; C. R. Yang et al., 2006; L. Yang et al., 2018; Zacchino et al., 2017; Zhu et al., 2019). With exception to alkaloids and flavonoids, we report detection of tannins, saponins and phenolics, as well as phytosterols, glycosides, and terpenoids in the hydroethanolic stem bark extract of *A. harveyi*. Non-detection of alkaloids in this study is congruent to the published findings on a hydroethanolic leaf extract of *A. harveyi*. However, non-detection of flavonoids in the stem bark extract of the plant, differs from the findings on the same leaf extract (Makoye et al., 2020). This discrepancy can be due to the natural distribution of flavonoids, more to the aerial than the lower parts of plants (Lwashina, 2000), as well as, the intraspecific variations of phytochemical composition resulting from geo-climatic conditions (Omara et al., 2021). Moreover, it is notable that the highest observed antibacterial activity for the stem bark extract ($1.67 \pm 0.72$ mg/ml) of *A. harveyi*, is very slightly lower than the highest activity reported for its leaf extract ($1.28 \pm 0.44$ mg/ml) (Makoye et al., 2020). Considering this resemblance of the activities, and the non-detection of flavonoids in the stem bark extract, it can be proposed that the previously detected flavonoids in the leaf extract had small or no contribution on the antibacterial activity of the leaf extract and the other phytochemicals could be responsible for the activity.
Interestingly, the observed antifungal activity for *A. harveyi* in this study appears to be novel. Together with the other phytochemicals, the detected saponins, terpenes and tannins are probably responsible for this activity, since the antifungal activities for these phytochemical classes are established (Monteiro & Alves dos Santos, 2019). Moreover, the newly observed antifungal activity for the plant is well supported by existing reports on the antifungal activities of other *Albizia* species (Feyera Fufa et al., 2018; Thippeswamy et al., 2014, 2015).

Furthermore, *A. harveyi* displayed higher antifungal activity than the control drug, fluconazole against three organisms namely, *C. albicans* ATCC 13803 (1.67 ± 0.72 mg/mL) and the clinical isolates of *C. albicans* (1.67 ± 0.72 mg/mL) and *A. niger* (5.00 ± 0.00 mg/mL). All these fungi cause mild to severe infections especially in immunologically challenged individuals (Brown et al., 2012; Perlroth et al., 2007; Pfaller et al., 2006; Richardson, 2005). This means if further explored, the plant may give potential leads for effective antifungal agents.

With exception to alkaloids, phytosterols, triterpenoids and flavonoids, the rest of the tested phytochemicals were detected in the 80% hydroethanolic extract of the stem bark of *S. madagascariensis*. With addition to flavonoids, a comparable pattern, with more intense (+++) detections, has been reported for the leaf extract of *S. madagascariensis* (Makoye et al., 2020). Comparing the phytochemical detection color intensities in this study (+), with the previous findings (+++), it can be judged that the stem bark of the plant is phytochemically weaker than its leaf.

The less phytochemical of the stem bark of *S. madagascariensis* as compared to its leaf, may explain that the highest antibacterial activity observed for the stem bark extract in this study (1.67 ± 0.72 mg/mL), is 8-fold less than the reported activity for the leaf extract of the plant (0.192 ± 0.00 mg/mL) (Makoye et al., 2020). Likewise, its highest antifungal activity observed in this study (1.67 ± 0.72 mg/mL) is about 2-fold less than what is reported of its leaf extract (0.625±0.00 mg/mL) (Mbunde et al., 2019).

Moreover, the observed susceptibility trends of the tested bacteria and fungi to the stem bark extract of *S. madagascariensis* are lower than the ones depicted in the studies on its leaf extracts (Makoye et al., 2020; Mbunde et al., 2019). Therefore, these findings not only reveal the activities of the stem bark of *S. madagascariensis*, but also inform that, its stem bark may offer weaker potency compared to its leaf in the quest for antibacterial and antifungal chemical leads.

Furthermore, the antibacterial activity of ciprofloxacin (positive control) was up to 1000 times the activities of both plant extracts, against the respective bacteria. This is a usual occurrence and further processing of the extracts, particularly fractionation may significantly improve their activities. In addition to that, it is notable that the clinical isolates of *C. albicans* and *A. niger*, were generally more susceptible to the plant extracts, than their standard counterparts. This is quite unusual and can be ascribed to several uncontrolled experimental factors. Mutation of the standard/clinical isolated fungi may have contributed to these findings.

**CONCLUSION**

The 80% hydroethanolic extracts of the stem barks of *A. harveyi* and *S. madagascariensis* exhibited antibacterial and antifungal activities. The antifungal activity of *A. harveyi* is hereby reported for the first time. Drawing from these findings, we suggest that the stem bark and other organs of both plants be further investigated for lead compounds with antibacterial and/or antifungal activities.

**ACKNOWLEDGMENT**

We thank the Tanzanian Higher Education Students’ Loan Board (HESLB) for funding this study.

**CONFLICT OF INTEREST**

We declare no conflict of interests.

**REFERENCES**


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E-ISSN 2477-0612


