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Antimicrobial activity and phytochemical profile of *Acacia drepanolobium* Harms Ex Sjostedt (Fabaceae) and *Solanum arundo* Mattei (Solanaceae)

Emburis Mollel[1], Deborah Kiumlo Balira Runyoro*[1], Olipa David Ngassapa[1]

[1]*Department of Pharmacognosy, School of Pharmacy, Muhimbili University of Health and Allied Sciences, P.O Box 65013, Dar es Salaam, Tanzania.*

Abstract

Microbial infections are associated with increased morbidity and mortality worldwide. Management of these infections faces challenges, including resistance, toxicity of some agents, affordability, drug-drug interactions and low production of new agents. These necessitate search for new antimicrobial agents. This study aimed at determining the antimicrobial activity and phytochemicals present in *Acacia drepanolobium* and *Solanum arundo*, used in Arusha region, Tanzania, to treat microbial infections.

Powdered plant materials were extracted by repeated maceration, with methanol and decoctions prepared for plant parts that are used by traditional healers. Antimicrobial activity was determined using broth microdilution method; most active extract was subjected to fractionation with solvents of increasing polarity and the antimicrobial activity of the resultant fractions were evaluated. Phytochemicals present in the active extracts were determined by various qualitative tests.

Acacia drepanolobium exhibited antimicrobial activity against all tested microorganisms, with MICs ranging from 0.3125 to 5 mg/ml. The methanolic stem bark extract was the most active and followed by the stem bark decoction. *Solanum arundo* extracts were inactive at the tested concentrations, except the methanolic leaf extract, which was active against all tested microorganisms, with MICs of 1.25 – 5 mg/ml.

Phytochemical analysis revealed presence of saponins, tannins, steroids, flavonoids and coumarins, with the first two being present in all active extracts. In most cases, fractions exhibited activity which was similar or lower than that of the crude extract and ethylacetate fraction was the most active. Further studies are recommended on *Acacia drepanolobium* stem bark extracts which exhibited promising antimicrobial activity.

Keywords: *Acacia drepanolobium*, antimicrobial activity, phytochemical profile, *Solanum arundo*

INTRODUCTION

There is high prevalence of bacterial and fungal infections in Africa [https://apps.who.int/iris/bitstream/handle/10665/79200/9789241505239_eng.pdf], which are quite common in both young and adult populations. For instance, several neonatal candidemia outbreaks have been previously reported in South Africa [2], while pneumonia and diarrhea are the major causes of death in children below 5 years, accounting for 29% of all deaths and loss of 2 million young lives each year [1].

Persistent bacteria vaginosis (BV) has been commonly reported in sexually active women, at a prevalence of 28% among symptomatic patients [2]. Furthermore, BV is also a risk factor to polymicrobial infections. A recent study revealed the association between bacteria vaginosis and HIV incidence. Bacteria vaginosis caused by non-lactobacillus microorganisms is thought to decrease the efficacy of Tenofovir in post exposure prophylaxis regimen [3].

Bacterial and fungal infections also increase disease burden in patients with communicable and non-communicable diseases. For instance, Opportunistic infections such as tuberculosis, esophageal candidiasis and *pneumocystis jiroveci* pneumonia are common in immuno-compromised patients [4]. In Tanzania 3% of the population suffer from invasive fungal infections annually, while HIV fungal opportunistic infections contribute to 18% of total infections and 80% of all deaths due to fungal infections in the country [5]. Nevertheless, bacterial and fungal infections have also been reported in patients with alzheimers disease [6], diabetes [7] and kidney diseases [8].

There are several factors that hinder effective management of bacterial and fungal infections, including, antimicrobial resistance (AMR). It is a worldwide challenge towards effective management of microbial infections. As a global burden, AMR causes increase in costs of treatment, longer duration of illness, failure of some medical procedures whose success relay on effective antimicrobial agents and high mortality rate in patients with resistant infection. Antimicrobial resistance is estimated to be high in low and middle income countries, causing 25,000 deaths in Europe and 19,000 deaths in USA annually [9].

Affordability of antimicrobial agents is also a challenge to effective microbial treatment, especially in low and middle income countries, where ability to pay for medication is limited. The situation is even worse in cases of resistant infections which require newer antimicrobial agents that tend to be more expensive than the first line antimicrobial agents.

Toxicity of antimicrobial agents, causing life threatening adverse reactions due to impaired drug metabolism or excessive dosing regimen hinders effectiveness of these agents against microorganisms. For instance macrolide antibiotics such as erythromycin, azithromycin and clarithromycin are thought to alter the conductivity of heart muscles causing cardiotoxic effects [10]. Decline in production of new antimicrobial agents, despite the global emergency of antimicrobial resistance, is a challenge to treatment of emerging and reemerging microbial

infections. This may be attributed by decline in pharmaceutical research and development [11].

In an attempt to solve these challenges, WHO has launched various guidelines that enhances and support safe, efficacy and quality use of tradition medicine. Most systems of traditional medicine utilize herbal medicines or traditional procedure based therapies to promote health [https://apps.who.int/iris/bitstream/handle/10665/66783/WHO_EDM_TRM_2000.1.pdf]. Plants have been utilized as medicines for thousands of years [12].

Acacia drepanolobium and *Solanum arundo* are among the medicinal plants used for the treatment of microbial infections in Arusha region, Tanzania. The stem bark decoction of *A. drepanolobium* is used against vaginal candidiasis and gastrointestinal disorders, such as diarrhea. Although the stem bark decoction of *A. drepanolobium* has been reported previously, to be used for uterine cleansing in both humans and animals in Kenya [13, 14, 15] antimicrobial activity of this plant has not been reported. However, related species have been reported to have antimicrobial activity. For example the ethanolic leaf extract of *A. baileyana* and *A. dealbata* from Portugal, were found to be active against *E. coli*, *Bacillus cereus*, *Candida albicans* and *Candida parapsilosis* [16].

Solanum arundo root decoction is used for the treatment of urinary tract infections, tonsillitis and diarrhea. This species has not been reported to possess antimicrobial activity; however, other related species including *S. aculeastrum*, *S. torvum* and *S. incanum* have been reported previously to have antimicrobial activity [17, 18, 19]. These species have been, also, reported to contain steroidal saponins, steroidal alkaloids, pregnane glycosides, terpenes, flavonoids, lignans, sterols, coumarins, phenolic compounds and fatty acids [20].

MATERIALS AND METHODS

Plant collection and authentication

Various plant parts, including leaves, stem barks and roots of *Acacia drepanolobium* and leaves, stems, and roots of *Solanum arundo*, were collected in January 2019, from Makuyuni ward in Monduli District, Arusha region, with the help of a botanist from Tanzania Pesticide Research Institute (TPRI). Identification was further confirmed at the Botany Department, University of Dar es Salaam (UDSM). Herbarium specimens were deposited in the herbaria of the Department of Pharmacognosy at MUHAS, Department of Botany at UDSM and TPRI.

Extraction of plant materials

Plant materials were sorted, cleaned and chopped into smaller sizes, where necessary. They were then dried under the shade and after which they were grinded into appropriate sizes. Dried ground plant material (100g) of each plant part, was extracted exhaustively by maceration with methanol at room temperature, and the extracts were concentrated at 50 °C *in vacuo* using Buchi rotary evaporator.

Furthermore, powdered root of *S. arundo* (50g) and stem bark of *A. drepanolobium* (50g), which are used by traditional healers, were used to prepare decoctions by boiling the materials in water at 100ºC for 30 minutes. The decoctions were dried using a freeze drier and then all dried extracts were kept in air tight containers and stored in a refrigerator at 4 ^{o}C until required for testing.

Screening for Antimicrobial activity

Standard medicines, solvents, reagents and media

Ciprofloxacin, fluconazole, P-iodonitrotetrazolium (INT) chloride dye and Sabouraud Dextrose Broth all from Sigma Aldrich (Germany), methanol (Blulux Laboratories ltd India), ethylacetate, petroleum ether and dichloromethane all from Lobal Chemie ltd India, DMSO (Carlo Erba Spain), Sabouraud Dextrose Agar (Liofilchem Italy) and Mueller Hinton Broth (Oxoid Ltd, UK) were used in this study.

Test organisms

Test organisms included standard strains of *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* (ATCC708903), *Staphylococcus aureus* (ATCC25923), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhi* (ATCC19430), *Proteus vulgaris* (ATCC33420) and *Candida albicans* (ATCC10231).

Inocula

Each organism was cultured separately; bacteria were sub-cultured repeatedly on Mueller Hinton (Oxoid Ltd) agar slants at 37ºC for 24 hrs. *Candida albicans* was subcultured repeatedly on Sabouraud dextrose (Sigma Aldrich) agar slants at 30ºC for 48 hrs. Colonies of each microorganism from the agar slants were suspended in sterile 0.9% NaCl and the turbidity was adjusted to an equivalence of 0.5 McFarland and these were the inocula used in broth microdilution assay.

Test samples

Test samples were prepared by dissolving 100mg of the extract into 1ml of DMSO , this was followed by dilution using 4ml of distilled water to make the highest concentration of 20mg/ml.

Screening for antimicrobial activity

Antimicrobial activity screening was accomplished by determination of the minimum inhibitory concentrations using broth microdilution method [21]. Double and single strength Mueller Hinton and Sabouraud dextrose broth were prepared according to the manufacturer's instructions. Fifty microlitres of the double strength broth was introduced in each well of the first row on the 96 well microtitre plate while 50µl of the single strength broth was introduced into the other wells of the plate.

Various test samples (50µl) were introduced into the first row of the microtitre plate and mixed well with the broth, followed by a two- fold dilution down the column. Ciprofloxacin and fluconazole were used as positive controls for bacteria and *C. albicans*, at highest concentrations of 15.6 µg/ml and 10µg/ml, respectively, while DMSO 5% was used as a negative control.

To each well 50 µl of microorganisms were added to make final extract concentrations range of 5- 0.04 mg/ml , then the plates were incubated at 37ºC and 30ºC, for 24 hours for bacteria and *C. albicans*, respectively. After the incubation period plates were removed and 40µl of 0.02% P-iodonitrotetrazolium (INT) chloride dye was added to each well and reincubated for further 30 minutes. The

lowest concentration in the wells without purple colour was considered as the minimum inhibitory concentration.

Phytochemical analysis

Phytochemical analysis of the active extracts was done using various qualitative methods as described by [22, 23, 24].

Fractionation of the most active extract

The methanol extract of the stem bark of *A. drepanolobium* exhibited the strongest antimicrobial activity; hence bulk extraction was done to obtain more extract for further fractionation. Dried and ground stem bark (1.5 kg) was macerated with methanol for 3 days and the process was repeated twice. The pooled extract was concentrated under vacuum using a rotary evaporator, at a temperature of 50ºC. The methanolic extract (100g) was sequentially fractionated by fractional extraction using solvents of increased polarity, which included petroleum ether, dichloromethane, ethylacetate and methanol, to obtain 4 fractions and the marc.

RESULTS AND DISCUSSION

Percentage yield of the extracts

The percentage yields of extracts were as indicated in Table 1; they ranged from 2.80% for methanol leaf extract of *S. arundo* to 10.77% for the methanol leaf extract of *A. drepanolobium*. The percentage yields for the two plants varied a lot whereby in most cases *A. drepanolobium* gave higher yields, except for the methanolic root extract, for which the yield was lower than that of corresponding extract of *S. arundo*. The yields also varied considerably with the extracting solvents and the plant parts used during extraction.

Antimicrobial activity of various extracts

Results of antimicrobial activity of methanolic and aqueous extracts from various plant parts of *S. arundo* and *A. drepanolobium* were as shown in Tables 2 and 3, respectively. Most extracts of *S. arundo* were not active at the maximum concentration tested, with the exception of the methanolic leaf extract, which exhibited MICs of 1.25mg/ml, 2.5mg/ml and 1.25mg/ml against *E. coli*, *P. aeruginosa* and *C. albicans*, respectively. These findings are being reported for the first time and almost similar activity was previously reported for the fruit methanolic extract of a related species *S. incanum*, from Saudi Arabia [19]. However, the results of this study were different from those reported for another related species, *S. torvum* for which all plant parts of *S. torvum* exhibited antimicrobial activity, with roots being the most active, followed by the stem, the inflorescence and leaf [18].

The susceptibility of *E. coli* and *C. albicans* to the leaf extract is worth mentioning. The extract had a MIC of 1.25mg/ml for both microorganisms. These findings are somehow similar to those reported by [17], who observed high antifungal activity, especially from acetone and methanol leaf extracts of a related species, *S. aculeastrum*, from South Africa. However, the root decoction which is usually given by the traditional healers in Arusha, for the treatment of tonsillitis and gastrointestinal disorders, did not exhibit any antimicrobial activity at the tested concentrations.

All four extracts, of *Acacia drepanolobium*, exhibited antimicrobial activity against all the tested microorganisms, including Gram positive, Gram negative bacteria and yeast with MICs ranging from 0.3125 to 5 mg/ml (Table 3). Similar results have been reported by [25] for ethanolic extract of the aerial parts of related species including *A. laeta, A. hamulosa, A. salicina,* and *A. tortilis* from Saudi Arabia, with MICs of 0.2 to 3.2 mg/ ml.

Table 1: Percentage yield for extracts

Plant species	Plant part	Extracting solvent	Percentage yield(w/w)
S. arundo	Leaf	Methanol	2.80
S. arundo	Stem	Methanol	3.21
S. arundo	Root	Methanol	4.65
S. arundo	Root	Water	4.12
A. drepanolobium	Leaf	Methanol	10.77
A. drepanolobium	Stem bark	Methanol	7.59
A. drepanolobium	Stem bark	Water	7.00
A. drepanolobium	Root	Methanol	3.88

Table 2: Minimum inhibitory concentrations of *Solanum arundo* extracts on various microorganisms

Microorganism	Minimum inhibitory concentrations (mg/ml)					
	a)	b)	c)	d)	e)	f)
E. coli	1.25	>5	>5	>5	0.2×10^{-3}	NA
S. aureus	5	>5	>5	>5	1.95×10^{-3}	NA
K. pneumoniae	5	>5	>5	>5	3.91×10^{-3}	NA
P. aeruginosa	2.5	>5	>5	>5	0.98×10^{-3}	NA
S. typhi	5	>5	>5	>5	0.98×10^{-3}	NA
Proteus species	5	>5	>5	>5	0.1×10^{-3}	NA
C. albicans	1.25	5	>5	>5	NA	0.48×10^{-3}

a): leaf MeOH extract; b): stem MeOH extract; c): root MeOH extract; d): root aqueous extract
e): ciprofloxacin; f): Fluconazole; NA: Not applicable

Table 3: Minimum inhibitory concentrations of *Acacia drepanolobium* extracts against various microorganisms

Microorganism	Minimum inhibitory concentrations (mg/ml)					
	a)	b)	c)	d)	e)	f)
E. coli	2.5	2.5	2.5	5	0.2×10^{-3}	NA
S. aureus	5	0.3125	0.3125	0.625	1.95×10^{-3}	NA
K. pneumoniae	5	1.25	1.25	5	3.91×10^{-3}	NA
P. aeruginosa	2.5	1.25	1.25	5	0.98×10^{-3}	NA
S. typhi	5	0.625	0.625	2.5	0.98×10^{-3}	NA
Proteus species	5	0.625	0.3125	1.25	0.1×10^{-3}	NA
C. albicans	1.25	0.625	2.5	0.625	NA	0.48×10^{-3}

a): Leaf MeOH extract; b): Stem bark MeOH extract; c): Stem bark Aqueous extract ;d): Root MeOH extract ;
e): Ciprofloxacin; f): Fluconazole; NA: Not applicable

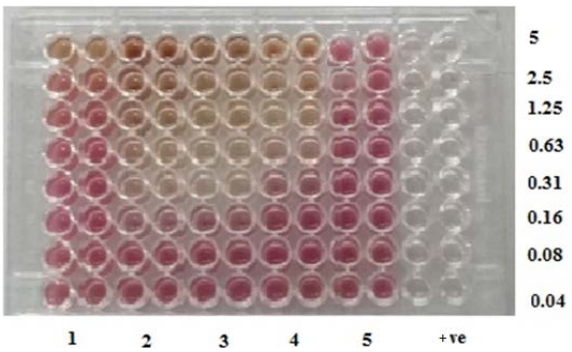

Figure 1: Microtitre plate showing antimicrobial activity of various extracts of *A. drepanolobium* against *S. aureus*

1: *A. drepanolobium* MeOH leaf extract;
2*: A. drepanolobium* MeOH stem back extract;
3: *A. drepanolobium* Aqueous stem back extract;
4*: A. drepanolobium* MeOH root extract,
5: Marc; +ve: Ciproflaxin

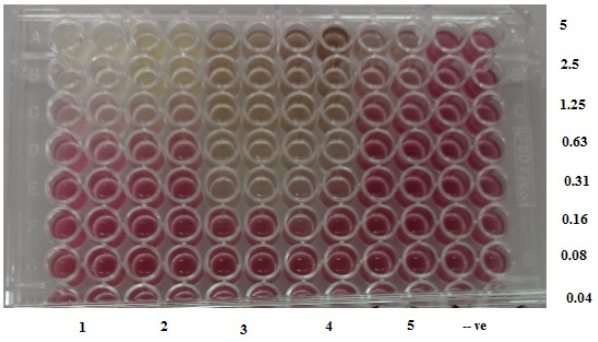

Figure 2: Microtitre plate showing minimum inhibitory concentrations of various fractions against *P.vulgaris*

1: Petroleum ether fraction;
2: Dichloromethane fraction;
3: Ethylacetate fraction;
4: Methanol fraction;
5: Marc; -ve: 5%DMSO.

Table 4: Phytochemical groups present in the active extracts

Phytochemical group	EXTRACTS				
	a)	b)	c)	d)	e)
Alkaloids	-	-	-	-	-
Anthraqunone glycosides	-	-	-	-	-
Coumarins	+	-	+	-	+
Flavonoids	+	+	-	-	-‘
Resins	+	+	-	-	-
Saponins	+	+	+	+	+
Steroids	-	-	+	+	+
Condensed tannins	+	+	+	+	+
Hydrolysable tannins	-	-	-	-	-
Triterpenoids	+	+	-	-	-

a): *S. arundo* leaf MeOH extract;b): *A. drepanolobium* leaf MeOH extract; c): *A. drepanolobium* stem bark MeOH extract; d): *A. drepanolobium* stem bark aqueous extract; e): *A. drepanolobium* MeOH root extract

Table 5: Minimum inhibitory concentrations (MICs) of the crude MeOH extract of *A. drepanolobium* stem bark and its fractions

Microoganism	MIC (mgml)				
	a)	b)	c)	d)	e)
E. coli	2.5	1.25	2.5	1.25	2.5
S. aureus	0.3125	2.5	1.25	0.3125	0.3125
K. pneumoniae	1.25	5	>5	1.25	1.25
P. aeruginosa	1.25	1.25	2.5	0.625	0.625
S. typhi	0.625	>5	>5	2.5	5
P. vulgaris	0.625	5	2.5	0.625	0.625
C. albicans	0.625	>5	>5	5	2.5

a): crude MeOH extract ;b): Pet. ether fraction; c): DCM fraction ;d): EtOAc fraction; e): EtOAc fraction

Staphylococcus aureus was the most susceptible microorganism towards almost all *A. drepanolobium* extracts (Figure 1). The strongest activity was exhibited by the aqueous and methanolic stem bark extracts with MIC of 0.3125mg/ml for both extracts. This observation is slightly similar to results reported for *A. nilotica* by Jabaka et al. [26] who used the disc diffusion method. Higher zones of inhibition were observed for *A. nilotica* stem bark against *S. aureus*. This is an important observation, especially because *S. aureus* is important pathogenic bacteria associated with community and hospital acquired infections including bacteremia, infective endocarditis, pleuropulmonary, osteoarticular, skin and soft tissue infection and device related infections [27]. The methanolic leaf extract of *A. drepanolobium* showed activity against all 7 tested microorganisms, with the strongest activity being exhibited against *C. albicans* (MIC of 1.25 mg/ml). These results are in line with those reported for a methanolic leaf extract of a related species, *A. modesta* that exhibited antimicrobial activity against a number of microorganisms [28].

Aqueous and methanol stem bark extracts of *A. drepanolobium* exhibited almost similar activity against *E. coli, S. aureus, K. pneumoniae, P. aeruginosa* and *S. typhi*. Slight differences in the activity were observed against *Proteus* species and *C. albicans* (Table 3). Overall, the methanolic stem bark extract was the most active against the tested microorganisms.

The methanolic root extract of *A. drepanolobium* exhibited a good activity against *Proteus* species where an MIC of 1.25mg/ml was observed; however, it was not active against *E. coli* at the tested concentrations.

Phytochemical groups present in the active extracts

The phytochemical groups present in the active extracts are indicated in Table 4, which include saponins, tannins, flavonoids, steroids, resins, triterpenoids and coumarins. Some groups of compounds including deoxy sugars, saponins and tannins were detected in all the active extracts (Table 4). It was also noted that the type of phytochemical groups present in the methanolic leaf extract of *S. arundo*, the only active extract for this plant, were also detected in at least one of the active extracts of *A. drepanolobium.*

Generally, the active extracts of these two plants contained similar phytochemical groups of compounds. Most of the groups detected in the leaf extract of *S. arundo* have been previously reported in the genus *Solanum* [29, 30]. However, alkaloids were not detected in the extract of *S. arundo* despite the fact that a steroidal glycoalkaloid had, previously, been isolated from the methanolic root bark extract of this plant [31] also most of Solanaceae plants are known to contain alkaloids [22]. Most probably alkaloids may have been present in small amounts that could not be detected by the method used. The active extracts of *A. drepanolobium* differed slightly in the type of the phytochemical groups they contained. The leaf extract, for example, contained triterpenes, resins and flavanoids, which were absent in both methanol and aqueous stem bark extracts and also in the methanolic root extract. Furthermore, coumarins were present in only the methanolic root and stem bark extracts while steroids were present in both stem bark and root bark extracts. Almost similar groups of compounds were previously detected in an ethanolic bark extract of *A. nilotica* from Nigeria, which was also reported to possess antimicrobial activity [32].

Tannins, steroids, flavonoids and triterpenes were previously reported from the flower and leaf extracts of related Leguminosae species from Malaysia. These species exhibited antibacterial activity against methicillin resistant *S. aureus* infections and possessed antioxidant activity [33]. Similarly, flavonoids were found to be present in various leaf extracts of a related species, *A. nilotica* from India, whereby *S. aureus* was the most susceptible microorganism toward the extracts [34]. These observations from previous studies on related species, somehow agree with the findings of this study.

Fractionation of the most active extract

Acacia drepanolobium stem bark methanolic extract was the most active extract and was subjected to fractionation using fractional extraction using a range of solvents with increasing polarity.

The methanol fraction gave the highest yield of 91.3% while petroleum ether fraction gave the lowest yield of 0.8%. Other fractions yields were 0.9% for dichloromethane, 1.8% for ethylacetate and 1.3% was the remaining marc. This indicates that most of the compounds in this extract were relatively polar. The remaining marc tended to be insoluble in both methanol and water; it could not dissolve even in DMSO at various concentrations. This marc probably contained insoluble fraction of previously reported gum which might have been extracted by methanol [35].

Antimicrobial activity of the fractions

The various fractions obtained were subjected to antimicrobial screening using broth microdilution method and their MICs were as shown in Table 5. The ethylacetate fraction was the most active, followed by the methanolic fraction which exhibited a slightly similar activity. These two fractions exhibited good antibacterial activity for various bacteria including *P. vulgaris* and *P. aeruginosa, K. pneumoniae* and *S. aureus* with similar MICs ranging from 0.3125 -1.25mg/ml (Figure 2). However, the ethylacetate and Petroleum ether fractions exhibited relatively stronger activity against *E. coli* (MIC 1.25mg/ml) when compared to the original MeOH extract (MIC 2.5 mg/ml). Also both ethylacetate and MeOH fractions acted similarly against *P. aeruginosa*, where the activity was also doubled. However, for some fractions the activity against some bacteria was tremendously decreased. For instance Dichloromethane and Petroleum ether extract exhibited a 4 and 8 fold decreases in activity against *S. aureus* while a 4 and 8 fold decrease in activity was observed for dichloromethane and MeOH fractions against *S. typhi*.

The activity of petroleum ether and dichloromethane fractions against *P. aeruginosa* and *K. pneumoniae* were

also very much lower compared to the activity of the original extract. These observations are not surprising since fractionation could either enhance the activity especially when there is antagonism among the components of the extract or could decrease the activity where synergism exists among the components of the extract [36].

CONCLUSION

All extracts of *A. drepanolobium* and the methanolic leaf extract of *S. arundo* possess antimicrobial activity. Phytochemical screening revealed the presence of saponins and tannins in all the active extracts. Other groups of compounds detected included flavonoids, coumarins and resins. The ethylacetate fraction of *A. drepanolobium* crude stem bark extract was the most active against the tested microorganisms.

Most *A. drepanolobium* extracts had a good activity against most of the tested microorganisms. It is therefore recommended that further studies be done on these extracts, including, safety and toxicity studies, isolation of the compounds responsible for antimicrobial activity and phytopharmaceuticals development using both aqueous and methanolic stem bark extract of *A. drepanolobium* which were the most active extracts of this plant.

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