

Full Length Research Paper

Antimicrobial activity of the extracts of *Albizia masikororum* R.Vig., a Fabaceae from Madagascar

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This study is aimed at the assessment of antimicrobial potential of *Albizia masikororum*, a Malagasy Fabaceae. Hexanic and methanolic extracts from fruit pods, stem bark, leaves and seeds were tested by disc diffusion and microdilution methods on 10 pathogenic microorganisms including four Gram positive bacteria, five Gram negative bacteria and one yeast. Only the leaf and seed methanolic extracts, LME and SME respectively, were active on some bacteria. LME and SME had a broad-spectrum activity, with SME more effective. The minimum inhibitory concentration (MIC) values of both extracts were <1000 µg/ml of which 30% <100 µg/ml, 20% between 100 and 500 µg/ml and 50% between 500 and 1000 µg/ml. SME MICs ranked from 6.10 µg/ml (*Streptococcus pneumoniae*) to 781.25 µg/ml (*Yersinia enterocolitica*) and those of LME from 97.65 µg/ml (*Streptococcus pneumoniae*) to 781.25 µg/ml (*Bacillus cereus*, *Streptococcus pneumoniae* and *Yersinia enterocolitica*). LME was bactericidal on all sensitive bacteria whereas SME was bactericidal on some and bacteriostatic on others. Both extracts contained different chemical groups known for their antimicrobial properties, saponins in SME and phenolic compounds in LME.

Key words: *Albizia masikororum*, antimicrobial activity, disc diffusion method, microdilution method, minimum inhibitory concentration, minimum bactericidal concentration.

INTRODUCTION

Globally, the number of multidrug-resistant strains has increased dramatically over the past 10 years (Roca et al., 2015; Sharifi-Rad, 2016) and new more resistant strains may be expected to emerge in the coming years (McGaw et al., 2008).

This situation may exhaust the chemical defenses at

our disposal. Therefore, research programs in alternative therapies including the search for new antimicrobial agents should be encouraged. Natural products still play a major role in the search for new medicines and plants represent a good source for new molecules of interest (Kuethe et al., 2009; Sharifi-Rad et al., 2017a, b, c, d, e;

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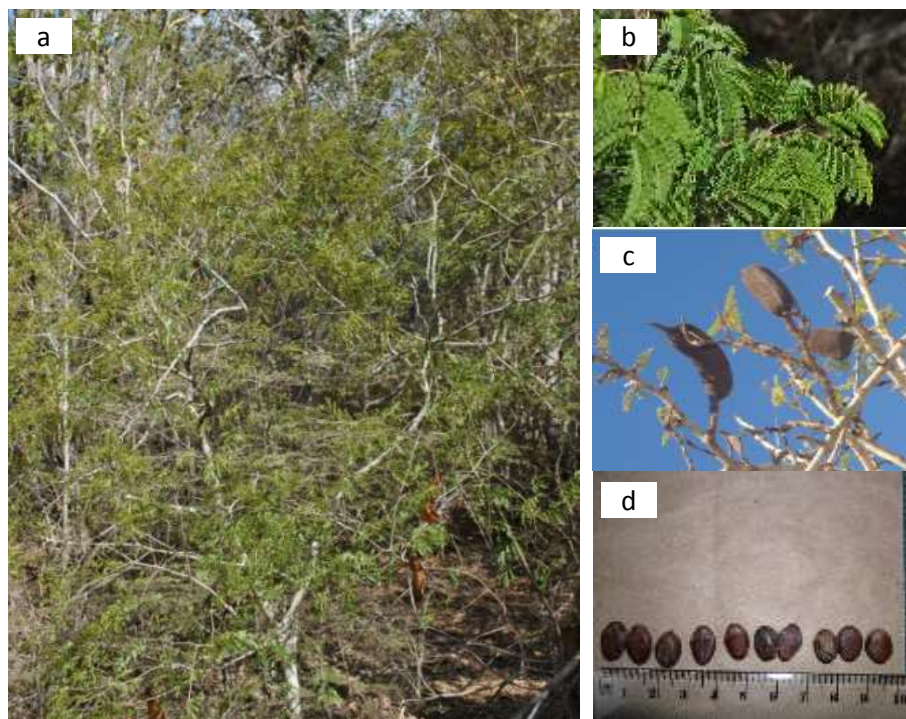


Figure 1. *Albizia masikororum*: (a) the whole plant; (b) leaves; (c) fruits, and (d) seeds. Source: The authors and Silo National des Graines Forestières (SNGF).

Salehi et al., 2017).

Malagasy plants are good candidates in view of the diversity of Malagasy flora whose originality could promise original molecules.

The genus *Albizia* is a group of plants that includes about 150 species and occurs in tropical regions. Worldwide, it has many uses including control of pests such as pathogenic microorganisms (Agyare et al., 2006; Sarkiyayi et al., 2011; Odeyemi et al., 2014). The Malagasy species studied also showed *in vitro* antimicrobial properties (Randriamampianina et al., 2017). In this work, the antimicrobial potential of the endemic species *A. masikororum*, which has already shown appreciable biological properties on mice and chicken (Randrianarivo et al., 2014) was studied.

MATERIALS AND METHODS

Plant materials

A. masikororum is a large shrub or tree up to 25 m tall (Figure 1) growing throughout the western part of Madagascar, from the North to the South. It was collected in Belo-sur-Tsiribihina/Betioky (West of Madagascar). Voucher specimens of the plant were deposited in the herbarium of Silo National des Graines Forestières (SNGF) under the reference number SNGF 873.

Microorganism strains

The 10 microorganisms used in this study included 4 Gram (+)

bacteria, 5 Gram (-) bacteria and 1 yeast (Table 1). They were maintained on agar slants at 4°C and cultured on a fresh appropriate agar plates 24 h prior to any antimicrobial test.

Chemicals for antimicrobial assay

Neomycin (30 µg/ml/disc) (Bio-Rad F-92430, Marnes-la Coquette, France) were used as antibiotic references. Miconazole (500 µg) (Bio-Rad F 92430 Marnes-la-Coquette, France) was used as antifungal reference for yeasts.

Preparation of hexane and methanolic extracts

Ground seeds, leaves, pods and stem bark powder (50 g) were delipidated with hexane (3 X 250 ml), then extracted with methanol (3 X 250 ml). After filtration using a Whatman filter paper, extracts were evaporated to dryness under reduced pressure.

Phytochemical screening

The reactions of chemical group detection were those developed by Marini-Bettolo et al. (1981).

Antimicrobial assays

All the materials and methods used for antimicrobial assay were detailed in a previous study (Randriamampianina et al., 2017).

The results were interpreted using the scale of Ponce et al. (2003) and Celikel et al. (2008). With the disc diffusion method, bacteria are not considered sensitive for an inhibition zone diameter (IZD) less than 8 mm, sensitive for IZD of 9 to 14 mm, very

Table 1. List of microorganisms used.

Strain	Reference	Gram
<i>Staphylococcus aureus</i>	ATCC 25923	+
<i>Bacillus cereus</i>	ATCC 14579	+
<i>Streptococcus pneumoniae</i>	ATCC 6305	+
<i>Streptococcus pyogenes</i>	ATCC 19615	+
<i>Enterobacter aerogenes</i>	ATCC 13048	-
<i>Enterobacter cloacae</i>	ATCC 13047	-
<i>Yersinia enterocolitica</i>	ATCC 23715	-
<i>Pseudomonas aeruginosa</i>	ATCC 10145	-
<i>Clostridium perfringens</i>	ATCC 13124	-
<i>Candida albicans</i>	ATCC 10231	-

Table 2. Extraction yields of the different crude extracts.

Organ	Pod		Bark		Leaf		Seed	
	Hexane	Methanol	Hexane	Methanol	Hexane	Methanol	Hexane	Methanol
Designation	PHE	PME	BHE	BME	LHE	LME	SHE	SME
Yield (% w/w)	2.01	4.61	0.95	2.38	1.17	4.74	3.36	19.01

sensitive for IZD of 15 to 19 mm and extremely sensitive for IZD larger than 20 mm.

For each extract, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined on susceptible strains only. The standards used to interpret MIC results were those of Dalmarco et al. (2010). For crude extracts and fractions, a MIC lower than 100 µg/mL was considered as an excellent effect, from 100 to 500 µg/ml as moderate, from 500 to 1000 µg/mL as weak, and over 1000 µg/ml as inactive.

The extract action is bactericidal or fungicidal when its ratio MBC/MIC or MFC/MIC is ≤ 4 and bacteriostatic and fungistatic when the ratio is >4 (Djeussi et al., 2013; Bouharb et al., 2014; Chamandi et al., 2015).

RESULTS

Extraction yields

The different crude extracts were obtained with yields ranging from 0.95% for BHE to 19.01% for SME (Table 2).

Phytochemical analysis

The major secondary metabolites identified in seed methanolic extract (SME) and leaf methanolic extract (LME) are shown in Table 3. In SME, saponins, unsaturated sterols, triterpenes and cardenolides were present, while alkaloids, tannins, flavonoids, leucoanthocyanins, coumarins, iridoids, steroids, and anthraquinones were not detected. LME contained tannins, flavonoids, leucoanthocyanins and triterpenes, while alkaloids, saponins, unsaturated sterols, cardenolides, steroids, coumarins, iridoids, steroids and anthraquinones were absent.

Antimicrobial activity

Disc diffusion

The effects of the various extracts of seeds, pods, bark and leaves at a concentration of 1 mg/disc on the microorganisms are presented in Table 4.

LME and SME only displayed activity on some bacteria. Their IZD values ranked from 11.5 to 16.5 mm for LME and from 9 to 15 mm for SME. They were less effective than neomycin which is a pure product. None of the extracts was active against *Pseudomonas aeruginosa* and *Candida albicans*.

MIC and MBC values

MIC and MBC values were determined for LME and SME only for active extracts on sensitive bacteria by the disc diffusion method (Table 5).

All the MIC values were <1000 µg/ml: 27.2% were <100 µg/ml, 27.2% between 100 and 500 µg/ml and 45.6% between 500 and 1000 µg/ml. There were more strains sensitive to SME (7 strains) than to LME (4 strains). SME MICs ranked from 6.10 µg/ml (*Streptococcus pneumoniae*) to 781.25 µg/ml (*Yersinia enterocolitica*) and those of LME from 97.65 µg/ml (*Streptococcus pneumoniae*) to 781.25 µg/ml (*Bacillus cereus*, *Streptococcus pneumoniae* and *Yersinia enterocolitica*).

The best activities were observed with SME against *Streptococcus pneumoniae* (6.10 µg/ml) and *Streptococcus pyogenes* (24.41 µg/ml).

LME exerted a bactericidal effect against all bacteria

Table 3. Phytochemical screening of seed powder and organ methanolic extracts.

Chemical group	Test	Seed powder	Methanolic extracts from				
			Seeds	Leaves	Pods	Bark	
Alkaloids	Mayer	-	-	-	-	-	
	Wagner	-	-	-	-	-	
	Dragendorff	-	-	-	-	-	
Tannins	Gelatin 1%	+	-	+	-	+	
	Salted gelatin	+	-	+	-	-	
	FeCl ₃	+	-	+	-	-	
Phenolic compounds	Flavonoides	Flavonols	-	-	+	+	+
		Flavones	-	-	-	-	-
		Flavonones	-	-	-	-	-
	Leucoanthocyanins	-	-	+	+	+	
	Anthraquinones	-	-	nd	nd	-	
	Coumarins	nd	-	nd	nd	-	
Terpenoids	Triterpenes	Liebermann-Burchard	+	+	+	-	-
		Steroids	-	-	-	-	-
	Unsaturated sterols	Salkowski	+	+	-	-	-
	Iridoids	-	nd	nd	-	nd	
Heterosides	Saponins	+	+	-	-	-	
	Cardenolides	nd	+	nd	nd	nd	
	Cyanogenic glycosides	nd	-	nd	nd	nd	

+: Positive test; -: Negative test; nd: Not determined.

Table 4. Effects (IZD in mm) of *A. masikororum* extracts by disc diffusion method at a concentration of 1 mg/disc.

Strain	PHE	PME	BHE	BME	LHE	LME	SHE	SME	Neomycin (30 µg/disc)	Miconazole (500 µg/ml)	
Gram (+) bacteria	<i>Bacillus cereus</i>	6	6	6	6	6	11.5	6	6	21	-
	<i>Staphylococcus aureus</i>	6	6	6	6	6	13	6	10	18	-
	<i>Streptococcus pneumoniae</i>	6	6	6	6	6	16.5	6	9	23	-
	<i>Streptococcus pyogenes</i>	6	6	6	6	6	7.5	6	15	24	-
Gram (-) bacteria	<i>Clostridium perfringens</i>	6	6	6	6	6	7	6	13	22	-
	<i>Enterobacter aerogenes</i>	6	6	6	6	6	6	6	8	19	-
	<i>Enterobacter cloacae</i>	6	6	6	6	6	7	6	12	27	-
	<i>Pseudomonas aeruginosa</i>	6	6	6	6	6	6	6	6	18	-
	<i>Yersinia enterocolitica</i>	6	6	6	6	6	14.5	6	9	20	-
Yeast	<i>Candida albicans</i>	6	6	6	6	6	6	6	nd	-	25

nd: Not determined.

sensitive to it whereas SME had a bactericidal effect on 4 bacteria and bacteriostatic effect on 3 others.

DISCUSSION

Of all the 8 extracts tested, only methanolic extracts from leaves (LME) and seeds (SME) displayed antimicrobial

activity.

The antibacterial compounds in the two extracts did not belong to the same chemical groups: Phenolic compounds for LME and saponins for SME. As these extracts were only semi-purified, the study results did not yet allow the conclusion on the number of active compounds in each one of them.

Table 5. MIC, MBC, MFC ($\mu\text{g/ml}$) and MBC/MIC or MFC/MIC of *A. masikororum* seed extracts.

Strain	Extract	MIC	MBC or MFC	MBC/MIC or MFC/MIC	
Gram (+) Bacteria	<i>Bacillus cereus</i>	LME	781.25	781.25	1
	<i>Staphylococcus aureus</i>	SME	195.31	>1000	>4
		LME	781.25	>1000	2
	<i>Streptococcus pneumoniae</i>	LME	97.65	97.65	1
		SME	6.10	24.41	4
Gram (-) Bacteria	<i>Streptococcus pyogenes</i>	SME	24.41	48.82	2
	<i>Clostridium perfringens</i>	SME	195.31	>1000	>4
	<i>Enterobacter aerogenes</i>	SME	195.31	>1000	>4
	<i>Enterobacter cloacae</i>	SME	781.25	>1000	>4
	<i>Yersinia enterocolitica</i>	SME	781.25	781.25	1
LME		781.25	>1000	4	

LME was active against 4 and SME against 7 of the 10 strains tested but unlike other Malagasy *Albizia* species so far studied (Rakoto et al., 2011; Randriamampianina et al., 2017), both extracts were not active against *Candida albicans*. Generally, the strain sensitivity varied significantly with the extract. For example, *Clostridium perfringens* and *Enterobacter cloacae* were sensitive to SME but resistant to LME whereas *Bacillus cereus* was sensitive to LME but not to SME. This difference could lie in the fact that the two extracts contained very different chemical groups. SME was rich in saponins and LME in phenolic compounds. Both chemical groups are known for their antimicrobial properties.

LME and SME displayed an excellent effect against *Streptococcus pneumoniae* and *Streptococcus pyogenes*. Those two bacteria, because of their high prevalence in infectious diseases and their resistance to various antibiotics, have become a serious clinical and health problem in many countries (Nueremberger and Bishai, 2004; Ardanuy et al., 2014; Lu et al., 2017; Sekizuka et al., 2017; Zhao et al., 2017).

In comparison with antimicrobial activity of other malagasy *Albizia* assessed in similar conditions (Randriamampianina et al., 2017), LME and SME gave MIC values much lower than 1000 $\mu\text{g/ml}$, and therefore far more effective compared to the seed extracts from *A. aurisparsa* against *B. cereus* (MIC = 1980 $\mu\text{g/ml}$) and from *A. mahalao* (MIC = 3750 $\mu\text{g/ml}$) and *A. polyphylla* (MIC = 2420 $\mu\text{g/ml}$) against *Staphylococcus aureus*. However, their activities were more or less similar to those of *A. bernieri* (Randriamampianina et al., 2017) against the same sensitive bacteria.

There is no consensus on the standard scale for interpreting antimicrobial activity of natural products (Benko and Crovella, 2010). According to the scale of Dalmarco et al. (2010), used in this work, 2 bacteria were very sensitive, 3 moderately sensitive and 4 weakly sensitive. With standards used by other authors, plant extracts with MICs values higher than 500 $\mu\text{g/ml}$

(Aligianis et al., 2001) and even much higher than 1000 $\mu\text{g/ml}$ (Maregesi et al., 2008; Abubakar and Majinda, 2016; Touaibia and Chaouch, 2017) were classified as having strong antimicrobial activity. For comparison, LME and SME had excellent activities on all the bacteria tested.

Based on the results obtained with the strains tested, LME and SME have a relatively broad activity spectrum. The effect of SME on sensitive bacteria was bactericidal whereas SME exerted a bactericidal effect on some strains and bacteriostatic effect on others.

In conclusion, *Albizia masikororum* exerted antibacterial effect. LME and SME could constitute good alternatives to synthetic antibiotics, particularly against *Streptococcus*. Further studies should be undertaken to determine the number and the identities of active principles responsible of this property and to know whether or not they are new products.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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