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Antibacterial potential of the *Albizia mahalao* Capuron extracts, a Fabaceae from Madagascar

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Abstract

The aim of this study was to assess the antibacterial potential of *Albizia mahalao*, a Madagascar Fabaceae. Leaf methanolic extracts (LME), root bark methanolic extract (RME), stem bark methanolic extract (SME), and alkaloids extracted from leaves under basic and acidic conditions were used. All the methanol extracts contained alkaloids and saponins. The antimicrobial activity was tested against many bacteria spp. including; *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Enterobacter aerogenes*, *Salmonella enterica*, *Shigella flexneri*, and *Vibrio fischeri*, using the Disc diffusion and the Microdilution assays. With the exception of the SME which is inactive, the other extracts exhibited broad spectrum potential against all the tested bacteria. The alkaloids are efficient against nearly all the bacteria under both of the basic and acidic conditions, with an inhibition zone diameter (IZ) of >17 mm, and minimum inhibitory concentration (MIC) of <100 µg/ ml. RME is the least efficient (IZ ≤ 10 mm, and 100< MIC < 1000 µg/ ml). *S. enterica* is the most sensitive bacterium (IZ= 23 mm, and MIC= 47 µg/ ml), whereas, *Staph. aureus* (IZ= 10 mm, and MIC >12000 µg/ ml) is the more resistant species. The majority of the extracts expressed bactericidal potency against the tested bacterial spp. Current results revealed the antibacterial potential of the *Albizia mahalao* leaves and root bark extracts thus could be used to treat infectious diseases.

Keywords: *Albizia mahalao*, Methanol extracts, Alkaloids, Antibacterial activity, MIC, MBC

1. Introduction

In order to address the problem posed by the increase of the number of multidrug-resistant strains, search for new molecules from natural resources such as plants extracts grew and diversified.

Albizia is a very cosmopolitan botanical genus including about 150 species extensively distributed in tropical and subtropical regions. Several *Albizia* spp. were traditionally used to treat human infectious

diseases such as; syphilis (*A. adianthifolia*) (De Wet *et al*., 2012), malaria (*A. anthelmintica*) (Kareru *et al*., 2008), and pneumonia (*A. schimperiana*) (Samoylenko *et al*., 2009).

Among the 30 spp. growing in Madagascar, 24 were endemic, 3 native and 3 were introduced (DU PUY *et al*., 2002). According to Rakoto *et al*., (2011); Randriamampianina *et al*., (2017); Razafindrakoto *et al*., (2018), no traditional medicinal use of *Albizia* was so far known; however, many studies of several Malagasy species recorded its in vitro antimicrobial properties. The present work was designed to assess the antibacterial potential of *Albizia mahalao*, which is one of the endemic Malagasy species.

2. Material and methods

2.1. Plant materials

A. mahalao Capuron is a tree growing up to 5 m high. It was collected in Tsimanampetsotsa (South west of Madagascar) with the following geographical coordinates: 24°02'19.13" latitude; 43°45'08.28" longitude; 7 m altitude.

2.2. Microbial strains

Seven pathogenic bacterial strains supplied by the National Center for Application of Pharmaceutical Research (CNARP), Madagascar, including 3 Gram positive bacteria; *Listeria monocytogenes* (ATCC 19114), *Staph. aureus* (ATCC 25923), *Clostridium perfringens* (ATCC 13124), and 4 Gram negative mainly; *Enterobacter aerogenes* (ATCC 13048), *S. enterica* (ATCC 13076), *Shigella flexneri* (ATCC 12022), and *V. fischeri* (ATCC 49387), were used for the antibacterial potential assay.

2.3. Preparation of the *A. mahalao* plant extracts

All the used *A. mahalao* plant parts including; leaves, stems and root barks were air-dried out of direct sunlight, powdered and then stored in dark bottles at room temperature. All the extracts were prepared from these powdered organs.

2.3.1. Preparation of solvents extracts

The method of Randrianarivo *et al*., (2014) was used. Leaf powder (100 g) was depigmented using acetone (4 x 700 ml). The leaves, stems and root barks were successively extracted with hexane (3 x 700 ml), ethyl acetate (3 x 700 ml), and methanol (4 x 700 ml). Each of these obtained solutions was filtered using Whatman No. 1 filter paper, and then evaporated to dryness under reduced pressure. The resulting residues were dissolved in dist. water to give the hexanic, ethyl acetate and methanolic extracts, respectively.

2.3.2. Extraction of alkaloids

The methods used for alkaloids extraction under basic and acidic conditions were detailed in our previous paper (Rajemiarimoelisoa *et al*., 2016).

2.3.2.1. Alkaloids extraction under basic conditions

Sixty grams of leaf powder were moistened with 20 ml of NH_4OH (20 %), and then suspended in 700 ml of dichloromethane. The mixture was stirred for 72 h at room temperature. After filtration, the solution was reduced to 100 ml with a rotary evaporator under reduced pressure at 40°C, mixed with 20 ml of acidified water (pH 2-3), and then extracted with ether to remove lipophilic, acidic and neutral compounds. After adjusting its pH to 9-10 with NH_4OH (20 %), the aqueous solution was extracted with dichloromethane (100 ml). The organic phase was washed three times with dist. water, dehydrated with anhydrous Na_2SO_4, and then evaporated to dryness under reduced pressure. The resulting residue was the crude total alkaloids designed as Alk1.

2.3.2.2. Alkaloids extraction under acidic conditions

Sixty grams of leaf powder were moistened with 30 ml of diluted HCl (1 M), suspended in 700 ml of methanol, and then stirred for 24 h at room temperature. After filtration, the solution was evaporated to dryness under reduced pressure. The aqueous acidic solution was alkalinized with NH_4OH (20 %) to pH= 9-10, and then extracted with

dichloromethane (3 x 20 ml). The organic phase was dehydrated with anhydrous Na_2SO_4, and then evaporated to dryness under reduced pressure to obtain the crude total alkaloid named as Alk2.

2.4. Phytochemical screening

The chemical analysis of the different *A. mahalao* plant extracts were detected according to the methods used by Firdouse and Alam, (2011).

2.5. Antibacterial assays

2.5.1. Antibacterial activity test

The antibacterial test was carried out using the disc diffusion method as detailed in a previous study of Andriamampianina *et al*., (2016). Results were interpreted according to IZ scales of Ponce *et al*., (2003); Celikel and Kavas, (2008). The bacteria are not considered sensitive to a certain plant extract for an inhibition zone diameter of (IZ) ≥ 8 mm, sensitive for IZ of 9-14 mm, very sensitive for IZ of 15-19 mm, and extremely sensitive for an IZ ≥ 20 mm. Negative controls were prepared by using the same extracting solvents, while Imipenem (30 µg/ disk) was used as a standard antibiotic (positive control). All experiments were performed in triplicates, and repeated three times.

2.5.2. Determination of the Minimum inhibitory concentration (MIC), and the Minimum bactericidal concentration (MBC)

The MIC and the MBC were evaluated using the microdilution method of Kuete *et al*., (2009). The stock concentration of each extract was adjusted to 12 mg/ ml. This was serially diluted two-fold to obtain concentration ranges of 0.023-12 mg/ml. Approximately, 100 µl of each extract concentration was deposited in a 96-well microplate containing 95 µl of Muller Hinton Broth medium, and 5 µl of each of the tested bacterial inoculum individually (adjusted to 1.5 x 10^6 cells/ ml). Three wells were used for each concentration. The positive controls were inoculated with each bacterial inoculum only, whereas the negative controls were inoculated with sterile water. Thereafter, 40 µl of p-iodonitrotetrazolium chloride or INT (0.2 mg/ ml) was added to each well, and then the microplates were incubated at 37°C for 30 min. The tests were repeated two times in triplicates.

The positive result was recorded as a change in the color of the well from yellow to pink, caused by the viable non-affected bacteria. The MIC was estimated as the lowest concentration of the plant extract which showed no color change, due to the inhibition of the bacterial growth according to (Nielsen *et al*., 2012). For crude extracts, the MIC values below 8 mg/ ml were considered to have non-significant antibacterial potential. For MBC determination, 5 µl of the solution in those wells which expressed no color change were inoculated into Mueller Hinton agar plates and then incubated at 37°C for 24 h. The lowest extract concentration which showed no bacterial growth on the plates was considered to be the MBC. According to Djeussi *et al*., (2013); Chamandi *et al*., (2015), the MBC/ MIC ratio defined the action for each extract. Thus the extract activity is considered bactericidal if this ratio is ≤ 4, and bacteriostatic if the ratio is > 4.

2.6. Statistical analysis

The results obtained by the disk diffusion method (IZ) were expressed as mean values ± standard deviations of three separate replicates. One-way analysis of variance (ANOVA) which was followed by Newman Keuls comparison test with Staticf® software was used for the statistical analysis of results of determining the of MIC and MBC. The statistical estimates were made at confidence interval of 95%.

3. Results

3.1. Extraction yields

The yields of the different parts of the *A. mahalao* plant extracted with different solvents are variable. LME recorded (13.21%), RME (16.11%), SME (11.4%), Alk1 (0.42%), and Alk2 (1.28%).

3.2. Phytochemical analysis

The major secondary metabolites detected in all the methanolic extracts, Alk1 and Alk2 are registered in Table 1. Alkaloids, saponosides and desoxyoses are present in LME, RME and SME, whereas flavonoids and polyphenols are recorded in LME but not detected in RME and SME. Leucoanthocyanins, coumarins, tannins, anthraquinones, steroids, iridoids, triterpenes, unsaturated steroids and amino acid are not detected in all extracts. The Alk1 and Alk2 were non-contaminated with the other non-alkaloid molecules.

Table 1: Phytochemical screening of *A. mahalao* plant extracts

Chemical groups	Tests*	LME	RME	SME	Alk1	Alk2
Alkaloids	Mayer	+++	+	+	+++	+++
	Wagner	+++	+	+	+++	+++
	Dragendorff	+++	+	+	+++	+++
Saponins	Foam test	++	+	+	-	-
Flavonoids	Willstätter	+	-	-	-	-
Desoxyoses		+	+	+	-	-
Leucoanthocyanins	Bate-Smith	-	-	-	-	-
Coumarins		-	-	-	-	-
Tannins and polyphenols	Gelatin 1%	-	-	-	-	-
	Gelatin-salt	-	-	-	-	-
	$FeCl_3$	+	-	-	-	-
Anthraquinones	Borntrager	-	-	-	-	-
Steroids	Liebermann-Burchard	-	-	-	-	-
Iridoids	Hot HCl	-	-	-	-	-
Triterpenes	Liebermann-Burchard	-	-	-	-	-
Unsaturated sterol	Salkowsky	-	-	-	-	-
Amino acide	Ninhydrin	-	-	-	-	-

Where; +: positive test; -: negative test. *: The method of Firdouse and Alam, (2011) was used.

3.3. The antibacterial potential

Using the Disc diffusion method; at 1 mg/ disk which is a concentration generally used to assess the antibacterial potential of the plants according to Sandeep *et al*., (2010); Govindappa *et al*., (2011); Linthoingambi and Mutum, (2013), the active IZ of the extracts ranged from 9 to 23 mm. Alk1 and Alk2 displayed antibacterial activity with IZ ranging from 10 mm (*Staph. aureus*) to 23 mm (*S. enterica*), while LME expressed IZ diameter ranging from 10 mm (*Listeria monocytogenes*) to 16 mm (*V. fischeri*). RME inhibited the growth of *Staph. aureus* and *Clostridium perfringens* only, with IZ of 9 and 10 mm, respectively. On the other hand, SME does not affect either the Gram positive or the Gram negative bacteria as shown in Table (2). The reference antibiotic Imipenem (30 µg/ disk) is more efficient than all extracts as it recorded higher IZ ranges of 28-45 mm.

3.4. Detection of the MIC, MBC and MBC/MIC ratio of the *A. mahalao* plant extracts

Table 2: *In vitro* antibacterial potential of the *A. mahalao* plant extracts using the Disk diffusion assay

	Bacterial strains	Diameter of IZ in mm (1000 µg/ disk)					Imipenem 30 µg/ disk
		LME	RME	SME	Alk1	Alk2	
Gram (+)	*Listeria monocytogenes*	12±1	7±0	6±0	17±1	18±1	30 ±0
	Staph. aureus	10±1	9±1	7±0	10±1	10±0	45±4
	Clostridium perfringens	14±2	10±2	6±0	23±1	23±1	31±0
Gram (-)	*E. aerogenes*	8±1	7±1	6±0	18±1	19±2	31±2
	S. enterica	11±1	8±1	7±0	23±1	23±1	33±1
	Shigella flexneri	15±1	8±1	7±0	19±1	20±1	33±1
	V. fischeri	16±2	8±1	6±0	19±0	22±1	28±2

-Results are averages of three replicates. (±): represent standard deviations.

The MIC values ranged from 47-12000 µg/ ml. They are < 100 µg/ ml for Alk1 (85.71%), Alk2 (85.71%) and LME (14.29%); however, between 100 and 500 µg/ ml for LME (42.85%), RME (42.85%) and Alk2 (14.29%), LME (28.57%), RME (14.29%), Alk1 (14.29%). On the other hand, MIC ranged from 500-1000 µg/ ml for LME (14.29%) and > 1000 µg/ ml for RME (42.85%). Alk1 and Alk2 expressed the best antibacterial potencies against all the tested strains, especially *S. enterica* (MIC= 47 µg/ ml).

The LME displayed bactericidal effect (MBC/MIC≤ 4) against *Staph. aureus*, *S. enterica* and *E. aerogenes*, and bacteriostatic activity against *Listeria monocytogenes*, *Clostridium perfringens*, *Shigella flexneri* and *V. fischeri*. The Alk2 showed bacteriostatic potential against *E. aerogenes*, but exhibited bactericidal effect against all the tested bacterial strains. RME and Alk1 presented bactericidal potency against all the tested bacteria (Table 3).

4. Discussion

All the extracts except for SME showed selective inhibitory activity against all the tested bacterial strains. However in some cases, the disk diffusion and the microdilution assays gave different results. This could be attributed to the differences in the behavior of the active principles in the solid and the broth medium.

Using the disk diffusion method at 1000 µg/ disk, the IZ ranged from 8 to 23 mm in all the extracts against the tested bacteria. However, all the extracts are less efficient than the Imipenem reference antibiotic at 30 µg/ disk, which may be due to the fact that Imipenem is a pure compound, while the tested extracts are still mixtures of different compounds.

Currently, LME (IZ= 14 mm), RME (IZ= 10 mm) and the alkaloids (IZ ranging from 19-23 mm), are more active than the methanolic extract and alkaloids from A. *bernieri* seeds against *Clostridium perfringens* (IZ= 9 mm) and *E. aerogenes* (IZ= 11 mm); respectively, in agreement with the previous study of Randriamampianina *et al*., (2017). In the present study, LME (IZ= 11 mm) is more effective than the methanolic extract of *A. lebbeck* which has no activity against *Salmonella* sp. (Seyydnejad *et al*., 2010). The Alk1 and Alk2 (IZ= 10 mm) displayed the same activity as the alkaloids from *A. polyphylla* (IZ= 11 mm) against *Staph. aureus*, but are more effective than the alkaloids from *A. boivinii* and *A. odorata* in accordance with Rajemiarimoelisoa, (2016). Results recovered in the liquid medium showed that the used concentrations of the extracts have significant influence on the growth of the tested strains ($p < 0.05$). Regarding the interpretation of the results obtained by the microdilution method, Benko and Crovella, (2010)

Table 3: The MIC, MBC (µg/ ml) and the MBC/ MIC values of the *A. mahalao* plant extracts

	Bacterial strains	Extracts	MIC*	MBC	MBC/MIC
Gram (+) Bacteria	*Listeria monocytogenes*	LME	750	6000	8
		RME	1500	3000	2
		SME	>12000	>12000	ND
		Alk1	93	187	2
		Alk2	187	187	1
	Staph. aureus	LME	375	375	1
		RME	375	375	1
		SME	>12000	>12000	ND
		Alk1	750	750	1
		Alk2	93	187	2
	Clostridium perfringens	LME	750	6000	8
		RME	1500	6000	4
		SME	>12000	>12000	ND
		Alk1	93	93	1
		Alk2	93	93	1
Gram (-) Bacteria	*S. enterica*	LME	1500	1500	1
		RME	750	750	1
		SME	>12000	>12000	ND
		Alk1	47	93	1.97
		Alk2	47	93	1.97
	E. aerogenes	LME	375	375	1
		RME	375	375	1
		SME	>12000	>12000	ND
		Alk1	93	93	1
		Alk2	93	750	8
	Shigella flexneri	LME	93	12000	129
		RME	187	187	1
		SME	>12000	>12000	ND
		Alk1	93	93	1
		Alk2	93	187	2
	V. fischeri	LME	187	12000	64
		RME	1500	6000	4
		SME	>12000	>12000	ND
		Alk1	93	187	2
		Alk2	93	187	2

Where; ND: exact value not determined but it is much higher than 4. *Number of replicates: n = 9 and p-value < 0.05

reported that there is no consensus on the inhibition level for the natural products. According to Fabry *et al*., (1998), with MIC values below 8 mg/ ml, all the extracts except for SME are considered having noteworthy antibacterial activity against all the tested bacterial strains.

In reference to Dalmarco *et al*., (2010), the MIC values lower than 100 µg/ ml are considered having an excellent antibacterial potential, from 100-500 µg/ ml as moderate, from 500-1000 µg/ ml as weak. Conversely, those MIC values over 1000 µg/ ml are regarded as being inactive. According to this scale of interpreting the current results, the LME presented an excellent effect against *Shigella flexneri*, moderate effect against *V. fischeri*, *E. aerogenes* and *Staph. aureus*, and weak potential against *Listeria monocytogenes* and *Clostridium perfringens*. However, it showed no activity against *S. enterica* (MIC> 1000 µg/ ml). On the other hand, RME expressed moderate effect against *Shigella flexneri*, *E. aerogenes* and *Staph. aureus*, weak potential against *S. enterica*, but it is inactive against *V. fischeri*, *Listeria monocytogenes* and *Clostridium perfringens*. Meanwhile, SME is totally ineffective against all tested strains. The Alk1 and Alk2 with MICs below 100 µg/ ml are considered to have an excellent effect against almost all the tested strains; however, *S. enterica* (MIC= 47 µg/ ml) exhibited high sensitivity to these alkaloids.

Although the current Alk1 and Alk2 are considered as having an excellent antibacterial efficacy with MIC of 93 µg/ ml against *E. aerogenes* and *Clostridium perfringens*, however they are less active than the same alkaloids from *A. bernieri* seeds having MICs of 62.5 µg/ ml on the same tested bacteria (Randriamampianina *et al*., 2017).

In the present study, all extracts of *A. mahalao* have mainly bactericidal potential against the tested bacterial strains. This may be attributed to the direct action of the bioactive compounds present in these extracts on the bacterial cytoplasmic membrane resulting in bacterial cell lysis and then death. However, further chemical studies are needed to identify these active principles.

All the methanol extracts including; LME, RME and SME, contained alkaloids and saponins which are chemical groups known for their antibacterial properties. As a result of the recorded high potential of the Alk1 and Alk2, they are suspected to be largely responsible for the significant antibacterial efficacy of the *A. mahalao* extracts. The noticeable difference between the antibacterial activities of the different methanolic extracts could be attributed to the difference in the chemical composition and\ or concentration of their bioactive compounds.

The overall results of the present study provide baseline information for the possible use of the *Albizia mahalao* leaves and root barks extracts to treat infectious diseases. However, as several extracts of the *Albizia* spp. are known to be toxic, thus preliminary toxicological studies of these extracts are recommended.

Conclusion

Leaves and root barks of *A. mahalao* plant extracts showed interesting inhibitory activity against several pathogenic bacterial strains. The high potency of the alkaloids is in favor of their strong involvement in this property. Accordingly, *A. mahalao* extracts could be used as alternatives of the synthetic antibiotics if their safeties are confirmed.

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Conflict of interest

The authors declare no conflict of interests.

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