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(RESEARCH ARTICLE)

Antimicrobial activity of *Albizia tulearensis*, an endemic Fabaceae from Madagascar

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Abstract

The antimicrobial activity of methanolic extract, butanolic and saponosidic fractions of different organs (leaf, seed, stem bark, root bark) from *Albizia tulearensis* were tested against 14 pathogenic germs including 5 bacteria Gram-positive (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes*), 6 Gram-negative (*Clostridium perfringens, Enterobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, Yersinia entericolitica*) and 3 yeasts (*Candida albicans, Candida tropicalis, Cryptococcus neoformans*) using disc diffusion and microdilution methods. At the concentration of 1 mg/disc, the methanolic extracts and fractions of seeds, stem and root bark exhibited selective antimicrobial activity with Inhibition Zone Diameters ranking from 8.67 to 16.5 mm. When using microdilution method, all the methanolic extracts and fractions displayed higher antimicrobial activities while saponosidic fractions were by far the most efficient. Saponosides from leaves (Lsap), seeds (Ssap), stem bark (Bsap) and root bark (Rsap) also exhibited excellent effects with Minimum Inhibitory Concentrations lower than 100 µg/mL against 71.4 %, 21.4 %, 7.14 % and 64.3 % of the germs tested respectively. The most sensitive germs were: *S. aureus* (4.87 µg/mL) with Rsap and *S. pyogenes* (9.75 µg/mL) with Lsap in Gram-positive bacteria; *E. coli* and *Y. entericolitica* (19.5 µg/mL) with Lsap in Gram-negative bacteria and *C. albicans* (4.87, 19 and 19.5 µg/mL) with Lsap, Ssap and Rsap respectively in yeasts. The saponosidic fractions had bactericidal and fungicidal effects against the vast majority of the microorganisms tested. The phytochemical screening carried out on plant organs powders revealed the presence of desoxyoses, saponosides, triterpens, unsaturated sterols and phenolic compounds.

Keywords: *Albizia tulearensis*; Antimicrobial Activity; Disc Diffusion Method; Methanolic Extracts; Microdilution Method; Saponosides.

1. Introduction

Antibiotic resistance is currently a major public health problem. Inappropriate and excessive use of synthetic antibiotics have promoted the resistance of pathogenic microorganisms to drugs, making therapeutic treatments less effective [1]. Facing this scourge, many research groups worldwide are screening for plant extracts to detect new active molecules that can eliminate infectious germs without detrimental side effects. Secondary plant metabolites represent a considerable alternative for the exploration of these new healing molecules [2].

Among the many plants investigated for their therapeutic properties, the species of the genus *Albizia* (Fabaceae) have proved to be interesting targets. Indeed, in most of the countries where they grow, these plants are widely used in traditional medicine for the treatment of various diseases. As examples, *Albizia adiantifolia* was used for the treatment of syphilis [3] and bronchitis [4], *Albizia schimperiana* for the treatment of pneumonia [5] and diarrhea [6] and *Albizia odoratissima* for the treatment of dysentery and rhinitis [7]. Most research has shown that the antimicrobial activity of *Albizia* extracts [8, 9] and several active ingredients were already isolated [10]. In Madagascar, 24 species of *Albizia*

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were found to be endemic [11]. Our previous studies demonstrated the antimicrobial potential of several of these Malagasy species [12] and more specifically *A. bernieri* [13], *A. masikororum* [14], *A. polyphylla* [15] and *A. mahalao* [16].

In the present study we investigated the antimicrobial activities of the methanolic extracts, butanolic and saponosidic fractions from different organs of *Albizia tulearensis,* another Malagasy species which has not been studied so far.

2. Material and methods

2.1. Plant and microbial materials

Albizia tulearensis is a tree which is found throughout the southern part of Madagascar and can grow up to 15 m high (figure 1). Plant organs were collected in August, 2018 from the Tsimanampetsotsa Reserve (Western South of Madagascar) during the fruiting period. The plant was identified by the Herbarium of Botanical and Zoological Parc of Tsimbazaza (PBZT) where voucher specimens were conserved under reference number 3719.

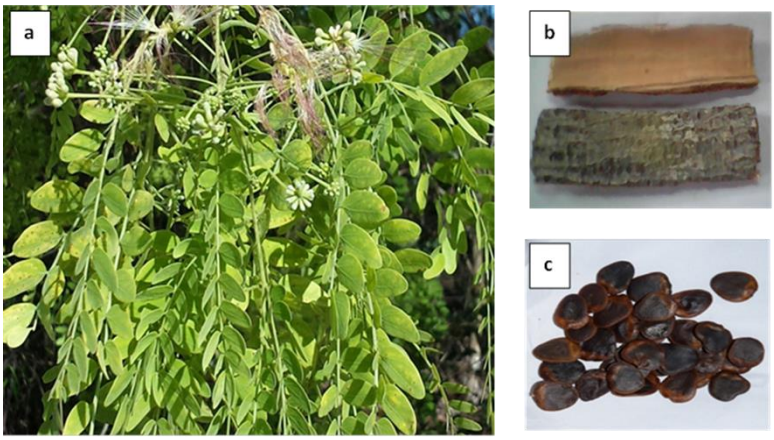

Figure 1 *Albizia tulearensis*: a) a leafy branch; b) stem bark and c) seeds.

The microorganisms used in this study are presented in Table 1. They included 6 Gram-negatives (-) and 5 Gram-positives (+) bacteria, as well as 3 pathogenic yeasts.

Table 1 List of the bacteria and yeasts strains used for the antimicrobial assessment.

Microorganisms		**Strains**	**Reference**
Bacteria	Gram (+)	*Bacillus cereus*	ATCC14579
		Listeria monocytogenes	ATCC19114
		Staphylococcus aureus	ATCC25923
		Streptococcus pneumoniae	ATCC6305
		Streptococcus pyogenes	ATCC19615
	Gram (-)	*Clostridium perfringens*	ATCC13124
		Enterobacter aerogenes	ATCC13048
		Escherichia coli	ATCC25922
		Pseudomonas aeruginosa	ATCC10145
		Salmonella enterica	ATCC13076
		Yersinia entericolitica	ATCC23715
Yeasts		*Candida albicans*	ATCC10231
		Candida tropicalis	ATCC1369
		Cryptococcus neoformans	ATCC76484

These strains were purchased from the collections of Laboratory of Chemistry of Natural Substances and Food Sciences (LCSNSA) of La Réunion University or came from the microorganism collection of the Laboratory of Applied Biochemistry to Medical Sciences of the Fundamental and Applied Biochemistry Department, Faculty of Sciences, University of Antananarivo. They were maintained on agar slant at 4 °C and cultured on a fresh appropriate agar plate during 24 h prior to antimicrobial tests.

2.2. Preparation of methanolic extracts

The air-dried plant organs were ground into powder which was delipidated with hexane (1/10 w/v) for 24 h under stirring at room temperature. After settling and filtration of the supernatant through Whatmann No. 1 filter paper, the filtrate was set aside while the non-soluble residues were recovered and then treated several times with the same solvent, until the extraction solvent was no longer colored. After drying, they were extracted with methanol under the same conditions. The filtrates were combined and concentrated to dryness by evaporation under reduced pressure to give a residue called methanolic extract.

2.3. Preparation of n-butanolic fractions

Each methanolic extract brought into solution in distilled water (150 mL) and an equal volume of n-butanol were vigorously shaken in a separatory funnel. After decanting, the butanolic phase was recovered and the same treatment was repeated twice with the aqueous phase. The butanolic fractions were collected and evaporated to dryness to give a residue called butanolic fraction.

2.4. Preparation of saponosidic fractions

Each butanolic fraction was first dissolved in methanol. In an ice bath, a mixture of acetone and diethyl ether (v/v) were gradually added to the resulting solution until the precipitate appeared. After a few minutes of maceration, the resulting suspension was centrifuged (1000 rpm during 10 minutes at +4 °C). The supernatant was treated several times using the same process until there was no more precipitation. All the collected pellets were dissolved in distilled water, and then evaporated to dryness under reduced pressure to give a residue called saponosidic fraction.

The methanolic extracts and the butanolic and saponosidic fractions generated from the different plant organs used in this work are summarized in Table 2.

Table 2 List and designation of the methanolic extracts, butanolic and saponosidic fractions from the different plant organs used

Extracts/fractions	Methanolic	n-Butanolic	Saponosidic
Leaves	LMet	Lbut	Lsap
Seeds	SMet	Sbut	Ssap
Stem bark	BMet	Bbut	Bsap
Roots	RMet	Rbut	Rsap

2.5. Phytochemical screening

The reactions of chemical group detection were those developed by Firdouse and Alam [17].

2.6. Antimicrobial assays

For all the antimicrobial assays, the methanolic extracts and the butanolic and saponosidic fractions were used as aqueous solutions.

The disc diffusion method described by Pyun and Shin [18], Ngameni *et al.* [19] and Favel *et al*. [20] was used to test the antimicrobial activity. Each methanolic extract, and butanolic and saponosidic fraction was prepared by dilution of the stock solutions (100 mg/mL) using distilled water. Dried and sterilized filter paper discs (6 mm diameter from Bio-Rad) were then impregnated with 10 µl/disc of the test substances. The impregnated discs were then carefully deposited on top of the nutrient agar medium, previously seeded uniformly with the pathogenic test microorganisms Standard antibiotic discs (Amoxicillin 25 µg/disc, chloramphenicol 30 µg/disc, penicillin 6 µg/disc, miconazole 50 µg/disc) from Liofilchem (Italy) and blank discs impregnated with distilled water were used as positive and negative controls. The plates were then incubated at 37 °C for bacteria and 30 °C for yeasts to allow growth of the cells. Diffusion of the aqueous

test solution generated a gradual change in concentration in the media surrounding discs. A clear and distinct zone of microbial growth inhibition was observed for the test solutions having antibacterial activity. This activity was determined by measuring the Inhibition Zone Diameters (IZD) expressed in millimeters. The results were interpreted using the scale of Ponce *et al*. [21] and Celikel *et al*. [22]: meaning that bacteria are were considered as not sensitive for an inhibition zone diameter IZD ≤ 8 mm, sensitive for IZD= 9-14 mm, very sensitive for IZD= 15-19 mm and extremely sensitive for IZD > 20 mm.

2.7. MIC, MBC and MFC determination

The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were determined using microdilution method according to Kuete *et al.* [23]. The initial concentration of each extract was set at 10 mg/mL. This was serially diluted two-fold to obtain concentration ranges from 0.0048 to 10 mg/mL. Each concentration was added in a well (96-well microplate) containing 95 µl of Mueller-Hinton broth (MHB) and 5 µl of microbial inoculum (standardized at 0.5 MacFarland). A positive control containing bacterial culture without extract and a negative control containing only the medium were also analyzed. The microtiter plates were then covered and incubated at 37 °C (bacteria) for 24 h or at 30 °C (yeasts and molds) for 48 h. The MIC of each extract was detected using p-iodonitrotetrazolium chloride colorimetric test which consisted in addition of 40 µl of 0.2 mg/ml mL of p-iodonitrotetrazolium chloride and incubation at appropriate temperature depending on microorganism as discussed above. Viable bacteria generated a change in the medium color from yellow to pink. MIC was defined as the lowest sample concentration that prevented this change and exhibited complete inhibition of bacterial growth.

For the MBC and MFC determination, 5 µl from each well that showed no change in color was transferred on Mueller-Hinton Agar or Sabouraud plate and incubated at 37 °C (bacteria) or at 30 °C (yeasts) for 24 h and 48 h respectively. The lowest concentration at which no growth occurred on the agar plates corresponded to the MBC or MFC. The ratios MBC/MIC and MFC/MIC were calculated for each extract, to determine the nature of the effect. The extract was considered as bactericidal or fungicidal for MBC/MIC or MFC/MIC ≤4 and as bacteriostatic or fungistatic when the ratios were >4 [24, 25, 26.

2.8. Statistical analysis

The results were expressed as average values ± standard deviations from three separate determinations. One-way variance analysis (ANOVA) with XLSTAT 2014 software was used for statistical analysis. Statistical estimates were made at the 95 % confidence interval.

3. Results

3.1. Extraction yields and qualitative phytochemical analysis of the fractions

The different extracts from leaf, seed, bark and root of *A. tulearensis* plant were obtained with yields ranging from 1.7 % for Bbut to 15.6 % for Smet (Table 3).

Table 3 Extraction yields of the different organs of *A. tulearensis* of methanolic extracts and butanolic and saponosidic fractions**.**

Extracts	**Leaf**			**Seed**			**Stem bark**			**Root bark**		
	Lmet	Lbut	Lsap	Smet	Sbut	Ssap	Bmet	Bbut	Bsap	Rmet	Rbut	Rsap
Yields (%)	14.6	7.0	9.3	7.8	1.7	2.1	11.5	7.0	9.4	15.6	8.4	9.0

The phytochemical analysis of the different organs powder from *A. tulearensis* is presented in Table 4.

Table 4 Phytochemical screening of organs powder of *A. tulearensis*

Chemical groups	Tests	Powder of			
		Seeds	Leaves	Stem bark	Root bark
Saponins	Foam test	+	+	+	+
Iridoides	Hot HCl	-	-	-	-
Tannins and Polyphenols	Gelatin 1 %	-	-	+	+
	Gelatin-salt 10 %	+	+	+	+
	$FeCl_3$	+	+	+	+
Desoxyoses	Keller-Kiliani	+	+	+	+
Flavonoids	Willstäter	-	-	-	-
Leucoanthocyanins	Bate-Smith	-	-	-	-
Alkaloids	Mayer	-	-	-	-
	Wagner	-	-	-	-
	Dragendorff	-	-	-	-
Steroids	Salkowski	+	+	+	+
Triterpenes	Liebermann-Burchard	+	+	+	+
Anthraquinones	Bornträger	-	-	-	-

+: positive test; -: negative test

The results revealed the presence of saponins, polyphenols, desoxyoses, steroids and triterpens and the absence of alkaloids, flavonoids, leucoanthocyanins and anthraquinones.

3.2. Antimicrobial activity

The antimicrobial effects of the various extracts normalized at a concentration of 1 mg per disc were investigated against a selection of food pathogenic microorganisms (Table 5).

Except for all the leaf methanolic extract, butanolic and saponosidic fractions that were inactive against all microorganisms tested, the methanolic extracts, butanolic and saponosidic fractions of the three other organs (namely seeds, stem bark and root bark) exhibited a selective antimicrobial activity. The IZD values ranged from 8.67 to 14.5 mm for seed extracts, from 9 to 14 mm for stem bark extracts and from 10 to 16.5 mm for root bark extracts. In most cases, the saponosidic fractions were more efficient than methanolic extracts and butanolic fractions. Bsap impacted the growth of the highest number of microorganisms meaning 11 out of 14 germs with IZD ranking from 9 to 14 mm. Rsap was the most active against all sensitive germs tested with IZD ranking from 12 mm against *C. tropicalis* to 16.5 mm against *E. coli*. The yeast *C. albicans* was sensitive to all the methanolic extracts and fractions of seeds, stem bark and root bark with IZD ranking from 10.3 to 14.5 mm. However, at 1 mg/disc all these methanolic extracts and fractions were less efficient than the reference antibiotics used as positive controls.

In addition, all these methanolic extracts and fractions exhibited broad antimicrobial activity. The germs *Y. entericolitica* and *C. neoformans* were resistant to all the plant extracts. The antimicrobial activity was precisely assessed using microdilution method and the results are shown in Tables 6 – 8.

In accordance with the results obtained by the disc diffusion method, the saponosidic fractions from the different plant organs had proved to be the most efficient against microbial proliferation. The corresponding MICs ranked from 4.87 µg/mL for *S. aureus* with Rsap and *C. albicans* with Lsap to 1250 µg/mL for *E. coli, P. aeruginosa* and *C. neoformans* with Bsap. Lsap and Rsap were the most active extracts against most of the germs tested with respectively 71.4 % and 64.3 % of MICs< 100 µg/mL, while Bsap was relatively the less efficient with almost all MICs >100 µg/mL.

With respect to MBC/MIC or MFC/MIC ratios, the activity effect depended upon the extracts and the microorganisms. A bactericidal or fungicidal action (MBC or MCF / MIC ≤4) was observed for Lsap, Ssap, Bsap and Rsap against 85.7 %, 78.6 %, 71.4 % and 85.7 % of the germs, respectively.

Table 5 Effects (IZD in mm) of methanolic extracts and, butanolic and saponosidic fractions of *A. tulearensis* organs by disc diffusion method

Strains		Leaf			Seed			Stem bark			Root bark			Positive control			
		Lmet	**Lbut**	**Lsap**	**Smet**	**Sbut**	**Ssap**	**Bmet**	**Bbut**	**Bsap**	**Rmet**	**Rbut**	**Rsap**	**A**	**C**	**P**	**M**
Bcteria Gam (+)	*B. cereus*	6	6	6	7.5±1.52	7±0.57	7±0.01	11±0.511	12.5±0.53	13±0.01	7±0.01	7±0.12	7±0.01	15	38	15	-
	S. aureus	6	6	6	11.3±0.57	6	6	12.3±0.52	13±0.57	13.5±0.51	13±0.01	15.5±0.51	16±0.70	37	30	35	-
	S. pyogenes	6	6	6	8±0.01	7±0.01	8±0.01	11.3±0.01	14±0.01	14±0.01	7±0.01	7±0.01	8±0.01	32	22	25	-
	S. pneumoniae	6	6	6	8.67±0.58	8±0.01	8±0.01	12±0.53	12±0.01	12.3±0.51	6	8±0.01	8±0.01	26	25	23	-
	L. monocytogenes	6	6	6	6	6	6	7±0.01	9±0.51	10±0.01	7±0.51	10.3±0.70	12±0.51	-	30	-	-
Bcteria Gam (-)	*E. aerogenes*	7±0.01	6	6	7±0.57	8±0.01	9±0.01	12±0.01	11 ±0.01	10±0.01	12±0.01	11.5±0.51	13±0.01	-	25	-	-
	C. perfringens	6	6	6	6	6	6	10±0.01	11±0.01	11±0.01	7±0.01	7±0.01	7.5±0.51	27	30	-	-
	E. coli	6	6	6	6	6	6	7 ±0.01	8±0.01	9±0.01	7±0.01	15±0.01	16.5±0.51	23	30	-	-
	P. aeruginosa	6	6	6	7±0.01	8±0.01	8±0.01	6	6	6	6	11±0.01	13±1.40	10	15	-	-
	S. enterica	6	6	6	6	6	6	6	6	6	7±0.51	7.3±0.12	7.5±0.01	27	32	-	-
	Y. entericolitica	6	6	6	11±1	12±0.51	12.5±0.51	7.5±0.56	7±0.01	9.5±0.51	6	6	6	10	38	-	-
Yeats	*C. albicans*	7±1.40	6	6	14±0.01	14.5±0.57	14±0.01	10.3±0.53	12±0.50	14±0.58	13±0.01	14±0.57	15±0.41	-	-	-	18
	C. tropicalis	6	6	6	6	6	6	8±0.50	8.5±0.58	10±0.01	7	11.3±1.40	12	-	-	-	18
	C. neoformans	6	6	6	6	6	6	6	6	7±0.01	6	6	6	-	-	-	-

A: Amoxicillin 25 µg/disc; C: Chloramphenicol 30 µg/disc; P: Penicillin 6 µg/disc, M: Miconazole 50 µg/disc
Results are averages of three replicates; (±), represent standard deviations; $p < 0.05$

Table 6 MIC, MBC or MFC (µg/mL) of the *A. tulearensis* methanolic extracts

	Strains	**Lmet**			**Smet**			**Bmet**			**Rmet**		
		MIC	MBC or MFC	MIC/MBC MIC/MFC	MIC	MBC or MFC	MIC/MBC MIC/MFC	MIC	MBC or MFC	MIC/MBC MIC/MFC	MIC	MBC or MFC	MIC/MBC MIC/MFC
Bcteria Gam (+)	*B. cereus*	156.2	156.2	1	312	625	2	1250	5000	4	625	2500	4
	S. aureus	156.2	625	4	625	1250	2	1250	1250	1	312.5	1250	4
	S. pyogenes	156.2	156.2	1	1250	2500	2	625	5000	>4	625	1250	2
	S. pneumoniae	312.5	625	2	1250	2500	2	1250	2500	2	625	1250	2
	L. monocytogenes	156.2	156.2	1	312.5	625	2	1250	10000	>4	312.5	625	2
Bcteria Gam (-)	*E. aerogenes*	312.5	312.5	1	1250	2500	2	1250	2500	2	156.2	1250	>4
	C. perfringens	312	312	1	312	625	2	625	2500	4	625	5000	>4
	E. coli	625	1250	2	1250	1250	1	2500	10000	4	1250	1250	1
	P. aeruginosa	1000	>10000	>4	5000	10000	2	10000	nd	nd	10000	nd	nd
	S. enterica	625	625	1	312.5	1250	4	1250	2500	2	1250	5000	4
	Y. entericolitica	156.5	625	3	156.2	625	4	1250	1250	1	1250	10000	>4
Yeasts	*C. albicans*	78.1	78.1	1	78.1	78.1	1	625	625	1	156.2	625	4
	C. tropicalis	156.2	156.2	1	2500	2500	1	625	1250	2	625	2500	4
	C. neoformans	312.5	625	2	5000	nd	nd	10000	nd	nd	10000	nd	nd

nd : not determined

Table 7 MIC, MBC or MFC (µg/ml) of the *A. tulearensis* butanolic extracts

Strains		Lbut			Sbut			Bbut			Rbut		
		MIC	MBC or MFC	MIC/MBC MIC/MFC	MIC	MBC or MFC	MIC/MBC MIC/MFC	MIC	MBC or MFC	MIC/MBC MIC/MFC	MIC	MBC or MFC	MIC/MBC MIC/MFC
Bcteria Gam (+)	*B. cereus*	39	78.1	2	156.2	625	4	625	2500	4	312.5	625	2
	S. aureus	156.2	312.5	2	312.5	625	2	312.5	1250	4	78.1	78.1	1
	S. pyogenes	19.5	156.5	>4	625	1250	2	312.5	2500	>4	312.5	625	2
	S. pneumoniae	156.5	625	3	625	625	1	625	1250	2	156.2	625	4
	L. monocytogenes	78.1	312.5	4	312.5	625	2	625	1250	2	156.2	312.5	2
Bcteria Gam (-)	*E. aerogenes*	312.5	312.5	1	625	1250	2	312.5	1250	4	156.2	312.5	2
	C. perfringens	39	78.1	2	312.5	625	2	625	5000	>4	625	2500	4
	E. coli	312.5	625	2	625	1250	2	1250	10000	>4	78.1	312.5	4
	P. aeruginosa	625	1250	2	5000	10000	2	5000	nd	nd	1250	>4	>4
	S. enterica	625	2500	4	312.5	625	2	625	1250	2	312.5	1250	4
	Y. entericolitica	78.1	156.2	2	156.2	312.5	2	1250	1250	1	625	1250	2
Yeasts	*C. albicans*	39	156.2	4	39.01	78.1	2	312.5	625	2	78.1	156.2	2
	C. tropicalis	312.5	312.5	1	156.2	1250	>4	312.5	1250	4	156.2	625	4
	C. neoformans	156.2	156.2	1	2500	10000	4	625	1250	2	2500	10000	4

nd : not determined

Table 8 MIC, MBC or MFC (µg/ml) of the *A. tulearensis* saponosidic fractions

	Strains	**Lsap**			**Ssap**			**Bsap**			**Rsap**		
		MIC	MBC or MFC	MIC/MBC MIC/MFC	MIC	MBC or MFC	MIC/MBC MIC/MFC	MIC	MBC or MFC	MIC/MBC MIC/MFC	MIC	MBC or MFC	MIC/MBC MIC/MFC
Bcteria Gam (+)	*B. cereus*	19.5	39	2	78.1	312.5	4	625	1250	2	78.1	312.5	4
	S. aureus	78.1	78.1	1	156.2	312.2	1	156.2	1250	>4	4.87	9.75	2
	S. pyogenes	9.75	78.1	>4	312.2	625	2	312.5	625	2	156.2	312.5	2
	S. pneumoniae	78.1	156.2	2	156.2	625	4	156.2	625	4	39	78.1	2
	L. monocytogenes	78.1	312.5	4	156.2	312.2	1	156.2	1250	>4	9.75	39	4
Bcteria Gam (-)	*E. aerogenes*	78.1	78.1	1	312.5	625	2	312.5	1250	4	78.1	156.2	2
	C. perfringens	19.5	39	2	312.5	625	2	156.2	1250	>4	312.5	625	2
	E. coli	156.2	156.2	1	156.2	625	4	1250	5000	4	39	312.5	>4
	P. aeruginosa	625	1250	2	1000	nd	nd	1250	5000	4	625	2500	4
	S. enterica	625	1250	2	312.5	625	2	312.5	625	2	312.5	625	2
	Y. entericolitica	19.5	19.5	1	78.1	312.5	4	625	1250	2	78.1	312.5	4
Yeasts	*C. albicans*	4.87	78.1	>4	19.5	78.1	4	78.1	156.2	2	19.5	78.1	4
	C. tropicalis	19.5	19.5	1	156.2	1250	>4	156.2	312.5	2	78.1	156.2	2
	C. neoformans	156.2	156.2	1	1000	nd	nd	1250	10000	>4	156.2	1250	>4

nd : not determined

4. Discussion

Our results revealed that the *A. tulearensis* organs contained saponins, polyphenols and triterpens which are known for their antimicrobial properties. Unlike other Malagasy *Albizia* such as *A. polyhylla* [15], *A. bernieri* [13] and *A. mahalao*) [16], *A. tulearensis* did not contain alkaloids.

The overall results of the present work provided evidence that the different organs from *A. tulearensis* possess antibacterial and antifungal properties.

As already noted in the case of extracts from other *Albizia* species namely *A. bernieri* [13] and *A. mahalao* [16], some results from the 2 methods used (Disc diffusion and microdilution methods) were different. For example, Lsap was inactive (IZD < 8 mm) against all the germs tested in solid medium but displayed excellent inhibitory effects (MIC<100 µg/mL) against the majority of strains in liquid medium. We think that this might be to the fact that some bioactive compounds diffused poorly in solid medium whereas in liquid medium as compared to liquid medium where cells are in direct contact with germs. This might also suggest that the molecules involved in the antimicrobial activity were not necessarily the same in the different *A. tulearensis* parts.

Lmet and Bmet were respectively the highest and the lowest active methanolic extracts against all the germs tested. Moreover, our results clearly demonstrated that the increase in the antimicrobial activity is related to the subsequent fractionation of the *A. tulearensis* methanolic extracts. For instance, the saponosidic fractions exhibited the highest antimicrobial activity and Lsap and Rsap were the most efficient extracts with MIC values ranking from 4.87 to 625 µg/mL, while Bsap was the least active with MICs values ranking from 78.1 to 1250 µg/mL.

The pathogenic yeast *C. albicans* was the most sensitive of the germs tested with MIC values ranking from 4.87 µg/mL (Lsap) to 78.1 µg/mL (Bsap) while *P. aeruginosa* was the least sensitive with MICs from 625 µg/mL (Lsap, Rsap) to 1250 µg/mL (Bsap).

In comparison with available comparable data on the antimicrobial activities of other Malagasy *Albizia* we previously studied (Table 9), Lmet (MIC = 156.2 µg/mL) and Smet (MIC = 625 µg/mL) were more efficient than respectively the leaf extract from *A. masikororum* and *A. divaricata*. In addition, the saponosidic fractions from seeds (Ssap) were much more effective than those from *A. bernieri* against *Y. entericolitica, B. cereu*s and *C. albicans* where the MIC values of Ssap were respectively 78.1, 78.1 and 19.5 µg/mL.

Table 9 MIC values of other Malagasy *Albizia* species

Germs	*Albizia* species	Extract	MIC (mm)	References
Y. entericolitica	*A. masikorum*	Leaf extract	781.25	[13]
	A. bernieri	Saponosidic fraction	250	[14]
B. cereus	*A. aurisparsa*	Seed extract	1980	[13]
	A. bernieri	Saponosidic fraction	500	[14]
C. albicans	*A. bernieri*	Saponosidic fraction	125	[14]
	A. bernieri	Seed extract	1000	[14]
	A. arenicola	Leaf extract	15620	[14]
P. aeruginosa	*A. divaricata*	Seed extract	2420	[14]
S. aureus	*A. polyphylla*	Seed extract	2420	[14]

Comparison of the methanolic extracts and fractions from *A. tulearensis* with *Albizia* species from other countries was not easy because antimicrobial activity was assessed under different conditions namely the plant part, the extraction solvent, the assessment method etc. For illustrative purposes only, we give IZD values of extracts from different *Albizia* species against the same microorganisms against which *A. tulearensis* were also tested (Table 10). For instance, *A. tulearensis* extracts displayed similar activities than *A. ferruginea* and *A. odoratissima* against *S. aureus* but was generally less active against *C. albicans*. *A. zigia* and *A. lebbeck* against *P. aeruginosa* and *E. coli* were also more efficient than *A. tulearensis* axtracts.

Table 10 Comparison of the IZD values of the *A. tulearensis* methanolic extracts and fractions with solvent extracts from *Albizia* species from other countries.

Germs	*Albizia* species	Extract	IZD (mm)	Ref.	IZD value range of *A. tulearensis* (mm)
S. aureus	*A. ferruginea*	ethyl alcoholic stem bark	13	[27]	11.3 (Smet) - 16 (Rsap)
	A. odoratissima	methanolic bark	14	[28]	
P. aeruginosa	*A. zygia*	methanolic stem bark	17	[29]	11 (Rbut) - 13 (Rsap)
E. coli	*A. zygia*	methanolic stem bark	22	[29]	15 (Rbut) - 16.5 (Rsap)
	A. lebbeck	butanolic	19.8	[30]	
C. albicans	*A. ferruginea*	alcoholic stem bark	15	[27]	10.3 (Bmet) - 15 (Rsap)
	A. odoratissima	methanolic bark	18	[28]	

Ref.: Reference

If comparison was based on MIC values (Table 11), the *A. tulearensis* extracts and fractions were significantly more active than leaf and root bark methanolic extracts of *A. chevalieri* against *S. aureus,* methanolic bark extract of *A. lebbeck* against *P. aeruginosa* and *E. aerogenes* and against *S. aureus* and methanolic stem bark extract of *A. zygia* against *E coli*, *P. aeruginosa* and *C. albicans*. However, the *A. tulearensis* methanolic leaf extract was less active than the *A. odoratsissima* methanolic leaf extract against *S. aureus*, *E. coli* and *P. aeruginosa*.

Table 11 Comparison of the MIC values of *A. tulearensis* with those of other Malagasy *Albizia* species

Germs	*Albizia* species	Extract	MIC (µg/mL)	Ref.	MIC value range of *A. tulearensis* (µg/mL)
S. aureus	*A. chevalieri*	methanolic organs	6250	[31]	156 (Lmet) – 1250 (Bmet)
	A. lebbeck	methanolic bark extract	1560	[30]	
	A. odoratsissima	methanolic leaf extract	136	[7]	
E. aerogenes	*A. lebbeck*	methanolic bark extract	3120	[30]	312.5 (Lmet) – 1250 (Bmet)
P. aeruginosa	*A. lebbeck*	methanolic bark extract	3120	[30]	1000 (Lmet) – 10000 (Bmet)
	A. zygia	methanolic stem bark	3100	[29]	
	A. odoratsissima	methanolic leaf extract	546	[7]	
E. coli	*A. odoratsissima*	methanolic leaf extract	273	[7]	625 (Lmet) – 2500 (Bmet)
	A. zygia	methanolic stem bark	2.100	[29]	
C. albicans	*A. zygia*	methanolic stem bark	1800	[29]	78.1 (Lmet) – 625 (Bmet)

Ref.: References

Saponosides which were the main secondary metabolites found in all organs extracts and fractions of *A. tulearensis* are also suspected to be the main responsible for their antimicrobial activity. It is worth noting that saponosides were the main compounds present in numerous *Albizia* species [32, 33] and showed many other pharmacological properties as antioxidant, antidiabetic, anthelmentic, antibacterial, hepato protective, anti-inflammatory and cytotoxic. Our results showed that most of the MBC/MIC ratios observed were lower than 4, highlighting the bactericidal and fungicidal properties of the methanolic extracts, butanolic and saponosidic fractions from *A. tulearensis* organs. This could probably be attributed to the direct action of saponosides on the cytoplasmic membrane resulting in microorganisms cell lysis and death [16].

5. Conclusion

This study demonstrated the antimicrobial potential of *Albizia tulearensis.* This plant might be useful in the development of new drugs against many microorganisms and also help address the problems of access to medicines in developing countries. However, further toxicological and pharmacological studies are still required to confirm these hypotheses. A further extensive chemical and biological study of *A. tulearensis* secondary metabolites will be necessary to allow determining the structure, the number, the originality, the distribution of the active principles in the different plant parts and their possible role in antimicrobial activity. In addition, these results constitute additional useful informations on the potentials of Malagasy *Albizia*.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interests.

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