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Antibacterial Activity of Astrotrichiliaparvifolia(J.-F. Leroy &Lescot)Leaf Extracts (Meliaceae)

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Abstract: *This work was designed to assess the antibacterial activity of Astrotrichiliaparvifolia, a Malagasyendemic Meliaceae used in traditional medicine.Ethyl acetate and methanol extracts from leaves were tested against pathogenic germs including 4 Gram(+) and 4Gram (-) bacteriausing disk diffusion and micro dilution methods.At 1000µg/disk, both extracts showedan inhibitory activityagainstGram(+)but not Gram (-)bacteria. Bacillus cereus was the most sensitive germ with an Inhibition Zone Diameter (IZD) of 13mm and 14mm with ethyl acetate and methanol extracts respectively and Streptococcus pneumoniae the less sensitive with an IZD of 9mm and 10mm. With Minimum Inhibitory Concentration (MIC) and Minimum bactericidal concentration (MBC) values ranging from 230 to 930µg/ml, the two extracts displayed significant activity on Staphylococcus aureus, Bacillus cereus andStreptococcus pyogenes. Theyhad the same level of activity againstStreptococcus pyogenes but the methanol extract was more efficient against Staphylococcus aureus and Bacillus cereus.They hada bactericidal actionagainst thosebacteria. Antimicrobial activity might be due to saponins, flavonoids, triterpenes, polyphenols, tannins and leucoanthocyanins present in leaves.This work demonstrated for the first time the antibacterial activity of theAstrotrichiliagenus.*

Keywords: *Astotrichiliaparvifolia, antibacterial activity, Meliaceae,MIC, MBC.*

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I. Introduction

Nowadays, the resistance of pathogenic microorganisms to antibiotics is one of the most serious threats for health in the world. The search of novel antibiotics becomes one of the important occupations of health specialists. One alternative would be the research of active compounds from vegetal species scientifically unexploited and generally used in traditional medicine with the purpose to promote biological products and minimize the use of synthetic antimicrobials [1].

Madagascar is well-known for the richness of its plant biodiversity with high endemismandtherapeutic potentials.Many of those plants are deemed to have therapeutic potentials but their effects are not always scientifically proven. However, a large majority of the Malagasy population has used many of them for a long time to treat diverse diseases.

The main reasons which convinced us to conduct research on *Astrotrichiliaparvifolia*(Meliaceae) were becauseit isa well-known traditional medicine plant used to treat wounds in the highland regions of Madagascar, then no research has been carried out onthe antimicrobial activity of the whole *Astrotrichilia* genus whereas several members of the Meliaceae family from different countries were reported displaying antibacterial properties [2] andfinally, it is an endemic plant from Madagascar.

This studywasmainly intended to testantibacterialactivity of *A. Parvifolia*leafextract on pathogenic germsin order to check the scientific basis of its traditional uses.

II. Materialsand METHODS

2.1. Plant material

A. parvifolia is a tree up to 10m tall(Fig. 1) well-known as "bibilahy" in Ambatoloana and can be found in Moramanga, Mananaraavaratra, Vavatenina, and Fénérive-Est. The botanical identification of the plant was carried out by FOFIFA research institution where a herbarium sample was deposited under number 20-R-10.
The *A. parvifolia*leaves were harvested on April 2011 fromAmbatoloana forestin the MandrakaRegion (Province of Antananarivo), whose geographical coordinates are S: 18°59'03, 8"; E: 047°56'03, 6"; Alt: 902m).

After washing and drying in the shade, leaves(Fig. 1)were ground intofine powder.

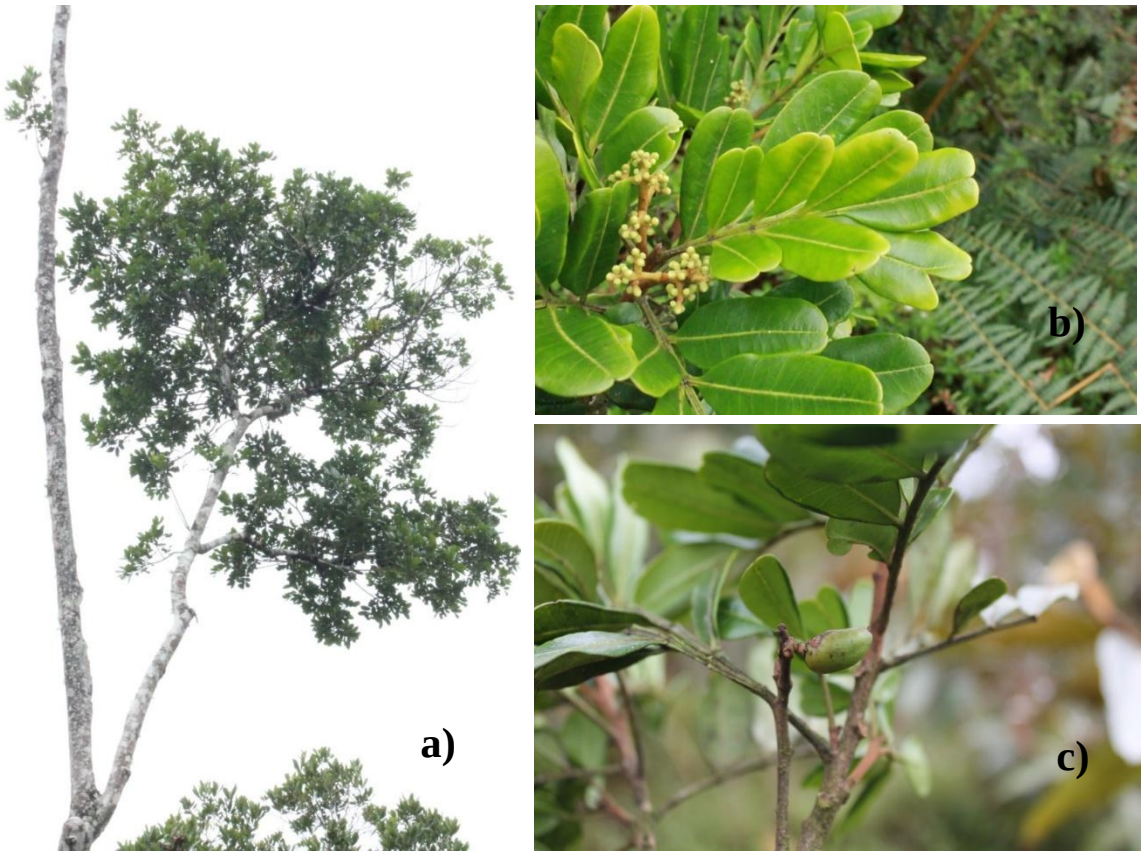

Figure 1: *Astrotrichiliaparvifolia: a) the whole plant; b) leaves and floral buds; c) leaves and fruit*
Source: the authors

2.2 Preparation of extract

In a soxhlet, 100g of leafpowder was delipidated with 200ml of hexane, then successively extracted with 200ml of ethyl acetate and 200ml of methanol. The resulting ethyl acetate and methanol solutionswere evaporated to dryness under reduced pressure. The residues thereby obtained,dissolved in sterile distilled water,were respectively named ethyl acetate extract and methanol extract.

2.3 Phytochemical Screening

Phytochemical screening of *A. parvifolia* leaf crude extracts was performed according tothe methods of Fong *et al*., [3] and Marini-Bertolo*et al*. [4].

2.4Antibacterial assay

2.4.1 Bacterial strains

Four Gram(+)and fourGram (-)bacteria were used for antibacterial assays (TABLE 1).

TABLE 1: List of germs used

Strains	**Reference**	**Gram**
Staphylococcus aureus	ATCC25923	+
Bacillus cereus	ATCC 14579	+
Streptococcus pneumoniae	ATCC 6305	+
Streptococcus pyogenes	ATCC 19615	+
Enterobacteraerogenes	ATCC 13048	-
Enterobactercloacae	ATCC 13047	-
Yersinia enterocolitica	ATCC 23715	-
Pseudomonas aeruginosa	ATCC 10145	-

2.4.2 Antibacterial activity test

I*n vitro* antibacterial activity of the ethyl acetate and methanol extracts was determined using disk diffusion method [5, 6]. Two ml of germs-tests suspension corresponding to 0.5MacFarland (10^8CFU/ml) were uniformly spread on the surface of Columbia Agar medium (for *Streptococcus*) and Mueller-Hinton Agar (for the other bacteria). Sterilized filter paper disks (6mm diameter) (BioMérieux, REF 54991) were impregnated with 10µl of each extract at the concentration of 1mg/ml (1000µg/disk), a concentration often used in antimicrobial activity assessment of plant extracts[7, 8, 9, 10]. Impregnated disks were then placed on the agar surface. After 24 hours of incubationat 37°C, the inhibition zone diameter (IZD) was measured and the results were interpreted according to the Celikel*et al.*criteria[11]:bacteria are not sensitive for IZD less than 8mm, sensitive for IZDfrom 9 to 14mm, very sensitive for IZD of 15 to 19mm and extremely sensitive for IZD higher than 20mm. Gentamycin was used as reference antibiotic. All the experiments were performed in triplicate.

2.4.3 MIC and MBC determination

The MIC (Minimum inhibitory concentration) and MBC (Minimum bactericidal concentration) were evaluated according to the microdilution method described by Kuete*et al.*[12].Methanol and ethyl acetate extracts weredissolved in 2ml of sterile distilled waterwitha final concentration 15mg/ml.Two fold serial dilutions were then carried out and 100µl of each concentration were poured intowells of 96wells microplates containing 95µl of Mueller-Hinton broth and 5µl of inoculum (1.5×10^6cfu/ml). A positive control withbacterial culture only and a negativecontrol with culture medium only were also prepared.Thereafter, 40µl of p-iodonitrotetrazolium chloride (0.2mg/ml) were added and the microplate was incubated at 37°C for 30min.Viable bacteria reduced the yellow dye to a pink color. MIC was estimated as the lowest extract concentration which showed no change of color due to the inhibition of bacterial growth. MIC lower than 100µg/mL was considered as an excellent effect, from 100 to 500µg/ml as moderate, from 500 to 1000µg/mL as weak, and over 1000µg/ml as inactive [13].For MBC determination, 5µlof solution in wells which did not showany change of color were transferred ontoMueller Hinton agar platesand incubated at 37°C for 24h. The lowest concentration which showed no bacterial growth on the plates corresponded to the MBC. The MBC/MIC ratio was calculated for each extract. The extract is bactericidal if the ratio MBC/MIC ≤ 4 and bacteriostatic if MBC/MIC >4[14, 15, 16].

III. Results

3.1 Extraction yield

Extraction yields of *A. parvifolia* leaf extracts were 7.54% for ethyl acetate extract and 11.39% for methanolic extract.

3.2 Phytochemical results

As shown in TABLE 2, the phytochemical screening of the *A. parvifolia*crude extracts revealed the presence of flavonoids, leucoanthocyanins, tannins, polyphenols and triterpenes in the ethyl acetate extract. The same compounds in greater quantity (larger amount) with in addition saponosides were found in methanolic extract.

TABLE 2: Phytochemical screening of *A. parvifolia*leaf extracts

Chemical groups	Tests	Ethyl	Methanolic
Leucoanthocyanins	Bate-Smith	+	++
Flavonoids	Willstätter	+	++
Saponins	Foam test	-	++
Tannins and Polyphenols	Gelatin 1%	+	++
	Gelatin-salt 10%	+	++
	FeCl3	+	++
Quinones	Borntrager	-	-
Steroids	Liebermann-Burchard	-	-
Triterpenes		+	++
Iridoïds	Hot HCl	-	-
Alkaloids	Mayer	-	-
	Wagner	-	-
	Dragendorff	-	-
Insaturated sterols	Salkowsky	-	-

+: positive; -: negative

3.3. Effects of extracts on bacteria

At the concentration of 1mg/disk, *A. parvifolia*extracts inhibited Gram (+) bacteria growth with IZD values from 9 to 14mm (Table 3). Methanolic extract (IZD = 10-14mm) was found to be slightly more effective than ethyl acetate extract (IZD = 9-13mm). The most sensitive germs were *S. aureus and B. cereus*. The two extracts did not show any effects against Gram (-) bacteria. The antibiotic (Gentamycin) used as reference in this study was more efficient than the extracts.

TABLE 3: Antibacterial activity of *A. parvifolia*leafextracts (1mg/disk) on pathogenic bacteria

Bacterial strains		**Inhibition Zone Diameter (mm)**		
		Ethyl acetate extract (1000µg/disk)	Methanolic extract (1000µg/disk)	Gentamycin (10µg/disk)
Gram(-)	*Enterobacteraerogenes*	6	6	22
	Enterobacter cloacae	6	6	20
	Pseudomonas aeruginosa	6	6	19
	Yersinia enterocolitica	6	6	24
Gram(+)	*Bacillus cereus*	13	14	26
	Staphylococcus aureus	12	14	24
	Streptococcus pneumoniae	9	10	26
	Streptococcuspyogenes	11	12	26

MIC, MBC, and MBC/MIC ratio values are presented in TABLE 4. MIC ranged from 230 to 470 µg/mL and MBC from 230 to 930 µg/ml. The MBC/MIC ratios were all less than 4 which meant that ethyl acetate and methanol extracts had a bactericidal action against*Bacillus cereus, Staphylococcus aureus* and *Streptococcus pyogenes*.

TABLE 4:MIC and MBC values of *A. parvifolia* leafextracts on Gram(+) bacteria

Strains	**Extracts**	**MIC (µg/ml)**	**MBC (µg/ml)**	**MBC/MIC**
Bacilluscereus	Ethyl acetate	230	230	1
	Methanol	470	470	1
Staphylococcus aureus	Ethyl acetate	470	470	1
	Methanol	230	230	1
Streptococcuspyogenes	Ethyl acetate	470	930	1.98
	Methanol	470	930	1.98

IV. Discussion

The result of this study showed that leaf extracts of *A. parvifolia*inhibited the growth of the Gram (+) bacteria tested. Antimicrobial activity gradually increased from *Streptococcus pneumoniae*(IZD = 9 – 10 mm) to*Streptococcus pyogenes*(IZD = 11 – 12 mm),*Staphylococcus aureus*(IZD = 12 – 14 mm),and *Bacillus cereus*(IZD = 13 – 14 mm). At 1 mg/disk, theywere less efficient than gentamycin at 10µg/disk used as reference. All the Gram (-) bacteria tested were resistant to extracts. This could be explained by the structure of the cell envelopesof the bacteria which are composed of a thin peptidoglycancell wallsandwiched between an inner cytoplasmiccell membrane and a bacterial outer membrane. This structure constitutes an efficient barrier to biological substances for Gram (-) bacteria [17].

MIC values were between 230 and 470µg/ml, butfor the interpretation of these values it is important to note that there was no consensus on the acceptable level of inhibition for natural products [18].According to the classification of Dalmarco *etal*. [13], the two*A. parvifolia* leafextracts exhibited moderate activity (CMI between 100 and 500 µg/ml) against the bacteria tested. However, according to Aligianis*et al*. [19] an extract with a MIC value less than 500µg/ml has strong microbial activity. Compared to other Meliaceae species, the activity of *A. parvifolia*extracts were higher than distilled water extract from *Walsurarobusta*, *Sandoricumindicum* and

Xylocarpusgranatum against *Streptococcus pyogenes* (MIC = 1000, 500 and >1000µg/ml respectively) [20].However, the methanolic extract from *Melia azedarach* was more efficient with a MIC of 22.4µg/ml and 42.4µg/ml against *B. cereus* and *S. aureus* respectively. In comparison with antibacterial activities of other plant extracts studied under the same conditions inour laboratory,*A. parvifolia*extracts were less efficient than the leaf methanol extract from *Crotalariabernieri*against *Bacillus cereus*(MIC = 97 µg/ml), *Staphylococcus aureus* and *Streptococcus pyogenes* (MIC = 195 µg/ml)[21]. However, they were more efficient than seed methanolic extract from *Albizia bernieri*against *Bacillus subtilis* (MIC = 500 µg/ml) [22] and ethyl acetate leaf extract from*Dilobeathouarsii* against *Bacillus cereus* and *Staphylococcus aureus* (MIC =. 12500 µg/ml) [23].
All the *A. parvifolia*extracts were bactericidal against the sensitive germs.

The antibacterial effect of *A. parvifolia* extracts could be attributed to the chemical compounds present in the extracts such as phenolic compounds (flavonoids, tannins, leucoanthocyanins and polyphenols), saponins and triterpenes. Flavonoids and leucoanthocyanins are known as antimicrobial agents acting through various mechanisms such the inhibition of nucleic acid synthesis, cytoplasm membrane function and energy metabolism [24].Tannins are antimicrobial agents which could inhibit microorganism growth by precipitating microbial protein and thus depriving them of nutritional proteins necessary to their growth and development [25].Saponins also have antimicrobial properties [26]. Triterpenoids from *Cabraleacanjerana*(Meliaceae)were reported to exhibit an activity against *Mycobacterium tuberculosis*[27].

V. Conclusion

This is the first study demonstrating that the genus of*Astrotrichilia* contains antibacterial substances. It provides scientific basis on the antibacterial property of the plant. Ethyl acetate and methanol extracts of *A. parvifolia*leaves exhibited antibacterial activity against Gram (+) bacteria. They could be used to treat different types of infections, including angina, pneumonia, gastrointestinal infections and skin diseases caused by the germs tested.Further chemical and toxicological researcheswill be needed to identify the active principles and to investigate other properties.

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