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Original Article

LANNEA BARTERI ENGL. (ANACARDIACEAE) PLANT USED IN THE TREATMENT OF URINARY TRACT INFECTIONS IN IVORY COAST: BIOLOGICAL AND CHEMICAL STUDIES OF THE AQUEOUS EXTRACT

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ABSTRACT

Objective: This research aims to evaluate the antibacterial activity and determine the chemical composition of the aqueous extract of the bark of *Lannea barteri Engl.* (DA) used in the traditional treatment of urinary tract infections in the Ivory Coast.

Methods: The material is composed of DA, the bacterial strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* isolated from the urine of patients from different hospitals and subsequently stored. The qualitative analysis was performed using color-based detection tests and thin layer chromatography (TLC) reactions and the quantification of total phenols, flavonoids, flavone aglycones and anthocyanins using the method of Folin Ciocalteu. The method of diffusion on Mueller Hinton (MH) agar medium has been used for sensitivity tests.

Results: The phytochemical screening of DA has revealed the presence of polyphenols, terpenes, and derivatives, coumarins, tannins, flavonoids, and alkaloids. Furthermore, the quantification of some polyphenols such as flavonoids, flavone aglycones, and anthocyanins was determined. The total polyphenols found was 0.757±0.003 mg/g MS representing respectively; 0.230±0.01 for flavonoids; 0.028±0.02 for flavone aglycones and 0.016±0.02 mg/g MS for anthocyanins. DA is bactericidal against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, which are mainly responsible for urinary tract infections.

Conclusion: The bark of *Lannea barteri Engl.* (DA) is rich in flavonoids, flavone aglycones, and anthocyanins which are probably responsible for its antibacterial properties on *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* This research thereby supports the use of this plant in the treatment of urinary tract infections.

Keywords: *Lannea barteri engl.*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii,* Ivory Coast

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INTRODUCTION

Nosocomial infections are currently a major public health problem in some developing countries. In Ivory Coast, the prevalence is estimated at about 12% [1]. These infections are partly due to hygienic conditions in the hospitals. Thus, this situation promotes the proliferation of different kinds of bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Because of their high resistance to antibiotics, these bacteria are mostly responsible for the transmission of many hospital pathologies including urinary tract infections [2-6]. This multidrug-resistant antibiotic limits all possible therapeutic choices, leading to an increase in the morbidity and mortality rate [2].

From this fact, the search for new bioactive molecules appears to be essential through other treatment methods such as traditional medicine. *Lannea barteri Engl.* was chosen for this work, it is a dioeciously tree of the family Anarcadiaceae, used in traditional Ivorian medicine to treat various conditions, including urinary tract infections. The studies carried out on this plant have reported its antimicrobial, antibacterial and antifungal properties [7]. However, these results remain insufficient for an efficient evaluation of *Lannea barteri Engl*. Thus, this research is a contribution to the valorization of Ivory Coast flora and especially of *Lannea barteri Engl.* through phytochemical and biological studies on multi-resistant strains.

MATERIALS AND METHODS

Chemical study

Plant material

The plant material consists essentially of the bark of *Lannea barteri Engl.* (Anarcadiaceae). These barks come from ethnobotanical surveys carried out among traditional healers and herbalists in various markets of Abobo and Adjamé in the autonomous district of Abidjan (Southern Ivory Coast). Firstly we have identified the plant sample at the National Center of Floristic (CNF) located at University Felix Houphouet Boigny (Abidjan, Cocody). A voucher specimen was deposited in the Herbarium (UCJ 000 967). Secondly, the barks were harvested in the month of April 2017 in Brofodoumé, the town of the city of Alépé (Southern Ivory Coast). The organs were cleaned, dried under permanent air conditioning at 18 °C for 14 d, then pulverized using an electric grinder (NASCO type electric grinder (Model No: UBC-25) to give fine powders.

Preparation of the decoction

100 g of fine powder was dissolved in 1000 ml of distilled water in an Erlenmeyer flask. The flask was surmounted by an ascending condenser and boiled for 30 min. After filtration under vacuum, the filtrate was concentrated using a rotary evaporator and then dried in an oven at 50 ° C for 2 d to give the aqueous decoction of *Lannea barteri Engl.* (DA).

Qualitative analysis

The qualitative analysis was performed using color-based detection tests and thin layer chromatography (TLC) reactions [8-11]. The development (mobile phase) used consists of the following solvent system: toluene/ethyl acetate/acetic acid (9.7/3/0.3, v/v/v) used by adding 2 drops of ammonia. The reagents of Liebermann-Bürchard, Dragendorff, Neu, 5% lead (II) acetate and 2% iron (III) chloride were used to reveal and characterize the main classes of chemical compounds.

Quantitative analysis

The quantification of total phenols, flavonoids, flavone aglycones, and anthocyanins was performed by spectrophotometry, using respectively the methods of Folin Ciocalteu [10], Hariri *et al.* (1991) and Lebreton *et al.* (1967) [11].

Antibacterial activities

Biological material

The biological material is composed of bacterial strains provided by the Laboratory of Bacteriology-Virology, Unit of Antibiotics, Natural Substances and Monitoring of Microorganisms Resistance to Anti-Infective (ASSURMI) of the Pasteur Institute, Ivory Coast. The strains were essentially those of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from the urine of patients from different hospitals and subsequently stored. The codes and phenotypes are shown in table 1.

Sterility test of the extract da

0.1 g of the aqueous extract (DA) were tested by adding 10 ml of thioglycolate in Petri dishes then incubated at 37 °C for 24 h. The mixture was inoculated into some Petri dishes containing Mueller Hinton agar (MH) and incubated under the same conditions. DA is thereby declared sterile if no colony is found or detected in the different agar plates after 72 h [12].

Table 1: Code and phenotype of bacterial strains

Bacterial strains	Code	Phenotype
Pseudomonas aeruginosa	19UB/17CNRa	Wild phenotypes with carbapenems, fluoroquinolones; cephalosporinases of the low resistance level
	151PP/17CNRa	Wild phenotype with aminoacids; Penicillinase of the high level of resistance; cephalosporinases of the low resistance level
	316UB/17CNRa	Wild phenotype of cephalosporine Crossed resistances of fluoroquinolone
Acinetobacter baumannii	45LC/17CNRa	Wild phenotypes with aminoacids, carbapenems; cephalosporinases of low resistance level and Penicillinase of low level
	248UB/17CNRa	Carbapenems; Penicillinase; Cephalosporinases; crossed resistance to ticarcillin and piperacillin
	354UB/17CNRa	Resistance to fluoroquinones and Cephalosporinases

Preparation of the concentration range of da

The bacterial strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were grown in Petri dishes containing nutrient agar. After 18 h of incubation at 37 °C, bacterial suspensions were taken using a platinum loop; homogenized in 10 ml of Mueller-Hinton Broth (BMH) and incubated for 3 h at 37 °C. 0.1 ml of the opalescent pre-culture broth was removed and diluted with 10 ml of BMH. The bacterial suspension obtained made it possible to have about 106 CFU/ml (standard condition), which constituted the bacterial dilution inoculum 100 or the pure inoculum [13].

Enumeration of the bacterial inoculum

The bacterial inoculum was homogenized and then diluted from 10 to 10 till 10^{-4} to obtain four decimal dilutions of 10^{-1} to 10^{-4}. The initial bacterial inoculum and the four dilutions were inoculated with a loop calibrated at 2 µl in 5 cm long streak on an MH agar, then incubated for 24 h at 37 °C. This preparation was labeled as box A which will be used to determine the minimum bactericidal concentration (MBC) [14].

Preparation of the concentration range of the extract (DA)

An initial solution (100 mg/ml) of DA was prepared. From the stock solution, a series of double dilution in geometrical progression of ratio ½ was performed to obtain five concentration ranges (100; 50; 25; 12.5 and 6.25 mg/ml) [14].

Antibacterial test

The antibacterial tests were carried out according to the dilution method in a liquid medium in six experimental tubes including a control tube [15, 16]. 1.8 ml of the bacterial inoculum was distributed to all tubes. 0.2 ml of the different DA concentrations were dispensed into the different tubes starting from the lowest to the highest concentration except for the control tube which received only 0.2 ml of sterile distilled water. The contents of the transparent tubes (not cloudy) were inoculated by streak 5 cm long on the MH agar starting with the MIC tube and incubated at 37 °C under CO_2 (10%) for 24 h. This box constituted the box B. The minimum bactericidal concentration was determined by comparing the density of streaks of box B to that of box A previously prepared.

Statistical analyzes

All assays were performed in triplicate; a spectrophotometer (AL800/SPECTER DIRECT) was used for the determination of the inhibition diameters and antibacterial parameters (MIC and MBC) as well. The statistical analysis of all the data was computed using Microsoft Office Excel.

RESULTS AND DISCUSSION

Chemical study

Qualitative analysis

With an extractive value of 7.75 %, the study of the chemical composition of the aqueous extract of *Lannea barteri Engl.* bark (DA) performing the detection tests by color reactions indicated the presence of several secondary metabolites (table 1, fig. 2). Among which, we count polyphenols, flavonoids, tannins, coumarins, alkaloids, terpenes, and derivatives. In addition, the presence of these phyto-compounds was confirmed by thin layer chromatography (TLC) (fig. 1 and table 3). Indeed, the flavonoids are colored in yellow after the spots revelation using Neu's reagent, in red, fluorescent yellow and blue under UV/366 nm [17]. These are the following retardation factor ratios (Rf): Rf = 0.18; 0.26; 0.33; 0.38; 0.46; 0.54.

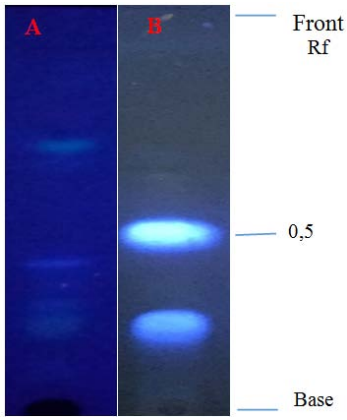

Fig. 1: **TLC Chromatography fingerprints of DA extract**

In addition, some flavonoids appear in blue under UV/366 nm without any revelation, and the color of these spots intensified after

spraying the reagent of Neu; they have been identified as methylated flavonoids: Rf = 0.26 [8]. Coumarins are detected by the lead (II) acetate in fluorescent blue, violet, yellow with Rf = 0.1; 0.26; 0.31; 0.4. The reagent of Liebermann Bürchard made it possible to highlight on the one hand sterols with Rf = 0.13; 0.15; 0.2; 0.69; 0.8 in brown and green in the visible and in yellow and yellow-green under UV at 366 nm [18-19]. On the other hand, the terpenes are detected at Rf = 0.1; 0.69; 0.8 in blue and violet in the visible and yellow-orange under UV/366 nm [17]. Finally, the tannins and alkaloids were identified respectively with iron trichloride in gray or black with Rf =; 0.0; 0.32; 0.51; 0.55 and with Dragendorff's reagent in a yellow spot at Rf = 0.0 [8].

The aqueous decoction of the bark of *Lannea barteri Engl.* (DA) was analyzed in 3 replicates (n=3) and identical Rf was obtained after comparison. Fig. 1A: Solvent system (Developing); Toluene/ethyl acetate/acetic acid+2 drops of ammonia (9.7/3/0.3; v/v/v), visualized in the visible. Fig. 1B: Developing; Toluene/ethyl acetate/acetic acid+2 drops of ammonia (9.7/3/0.3; v/v/v), Developer (revealer): NEU and visualized at UV 366 nm.

Table 2: Phytocompounds detected in DA extract by colored test

Type of compound	Test	Observed color	Reaction
Polyphenols	$FeCl_3$	Black	+
Flavonoïds	Shinoda,	Orangey-red	+
	Lead acetate	Yellow	
Coumarins	Lactone cycle	Yellow	+
Tannins	$FeCl_3$ Bromine water	Black	+
Sterols and polyterpenes	$CH_3CO_3CH_3/H_2SO_4$	Purple-blue	+
Alkaloïds	Dragendorff	Orangey-red (crystal deposit)	+

The tests were repeated 3 times (n=3) to ensure accuracy and reproducibility,+indicates positive reactions (presence of compound).

Table 3: Secondary metabolites detected by thin-layer chromatography (TLC) in DA

EXT	Without revealing (a)				$FeCl_3$ (b)				NEU (c)				Libermann-Büchard (d)				Lead acetate (e)				Dragendorff (f)				Type of possible compounds
	Visible		UV 366		Visible		UV 366		Visible		UV 366		Visible		UV 366		Visible		UV 366		Visible		UV 366		
	Co	R_f	Co	R_f	Co	R_f	Co	R_f	Co	R_f	Co	R_f	Co	R_f	Co	R_f	Co	R_f	Co	R_f	Co	R_f	Co	R_f	
DA					Gris	0															Orange	0			Tannins (b), alkaloïds(f)
															Blue	0.1			Green	0.1				Coum(e),terpene(d)
													Gray	0.13											sterols (d)
															Yellow	0.15									Sterols (d) terpenes(d)
											Orange	0.18													Flavonoïds (c)
													Gray	0.2											sterols (d)
			Blue	0.26							Blue	0.26			Blue	0.26			Green	0.26					Flavonoïds (a,c),coumarins (e)
			Blue	0.31															Green	0.31					Coumarins (e)
					Gray	0.32																			tanins (b)
											Green	0.33													Flavonoïds (c)
											Purple	0.37													NI
											Blue	0.38													Flavonoïds (c)
			Blue	0.4															Blue	0.4					Coumarins (e)
											Orange	0.46													Flavonoïds (c)
					Gray	0.51																			tanins (b)
											Orange	0.54													Flavonoïds (c)
					Gray	0.55																			tanins (b)
															Yellow	0.69									Sterols (d); terpenes(d)
															Yellow	0.8									Sterols (d) terpenes(d)

DA: Aqueous extract; Co: Colour; R_f.: Retardation factor; NI: Not identified; Coum: Coumarins; n=3

Quantitative analysis

Fig. 2 describes the amount of different phenolic compounds embedded in *Lannea barteri Engl*. The results highlight that the overall polyphenols content is 0.757±0.03 mg AG/g DM and the number of total flavonoids is found to be 0.230±0.01 mg/g DM representing 30.38 %. Furthermore, flavone aglycones and anthocyanins were found with respective amounts of 0.028±0.02 mg/g DM and 0.016±0.02 mg/g DM. The

obtained results are clear indications of the richness of the studied extracts in different classes of flavonoids.

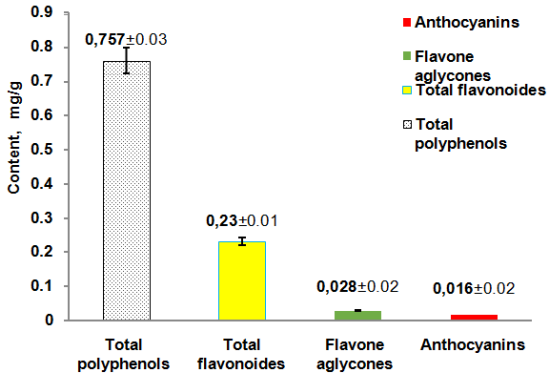

Fig. 2: Content of total phenols and flavonoids, flavones aglycones, anthocyanins extracts of *Lannea barteri Engl.* (DA), values are expressed as mean±SD, n=3

The pharmacological potentials of the same extracts were investigated performing specific antibacterial tests, and the obtained results are thereafter described.

Antibacterial test

According to the sterility tests, no evidence of DA contamination was observed as attested by the absence of bacterial colonies on the different agar plates, after 24 h. Table 4 fully describes the results of the sensitivity of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* to DA extracts. The inhibition diameters recorded for *Pseudomonas aeruginosa* strains vary from 11.7±0.5 to 15.3±1.1 and from 7±1.0 to 14±0.6 for 100, and 50 mg/ml of DA applied. Concerning the strains of *Acinetobacter baumannii*, the obtained inhibition diameters are between 10.6±0.5 and 15±0.0 for the concentration of 100 mg/ml and 7±1.7 and 12.6±0.0 for 50 mg/ml. Finally, for the concentration of 25 mg/ml, the inhibition diameters observed are all equal to 6±0.0 upon all the studied strains. Additionally, the antibiotics gave some inhibition diameters comprised between 6±0.0 and 34±0.7. The inhibition zones were greater than 8 mm; it was then appropriate to calculate and determine the antibacterial parameters (MBC and MIC) and their ratio MBC/MIC (table 5, fig. 3).

Table 4: Inhibition zone (in mm) of the bacterial strains used

Bacterial strains	Code	Concentration (C in mg/ml±SD)				Antibiotics	
		C_1 (100)	C_2 (50)	C_3 (25)	Tm	CAZ (10µg)	TIC (75µg)
Pseudomonas aeruginosa	19UB/17CNRa	11.7±0.5	8±1.0	6±0.0	6±0.0	26±1.4	15±0.7
	151PI/17CNRa	15.3±1.1	14±0.6	6±0.0	6±0.0	33±1.4	23±2.8
	316UB/1CNRa	14.3±0.5	7±1.0	6±0.0	6±0.0	30±2.8	18±0.7
Acinetobacter baumannii	45LC/17CNRa	15±0.0	12.6±0	6±0.0	6±0.0	22.6±0.5	28.3±1.3
	248UB/17CNRa	13.3±1.5	7±1.7	6±0.0	6±0.0	34±0.7	21±2.1
	354UB/17CNRa	10.6±0.5	8±1.0	6±0.0	6±0.0	23±0.4	6±0.0

CAZ: Ceftazidime; TIC: Ticarcilline; Tm: control; values are expressed as mean±SD, n=3

Table 5: Influence of DA and antibacterial parameters; MIC, MBC, MIC/MBC

Bacterial strains	Code	MIC (mg/ml)	MBC (mg/ml)	(MIC/MBC)	Activity
Pseudomonas aeruginosa	19UB/17CNRa	6.25±0.0	6.25±0.0	1	absolute bactericidal
	151PI/17CNRa	21.87±6.2	21.87±6.2	1	absolute bactericidal
	316UB/17CNRa	25±17.6	25±17.6	1	absolute bactericidal
Acinetobacter baumannii	45LC/17CNRa	43.75±1.2	43.75±1.2	1	absolute bactericidal
	248UB/17CNRa	34.37±18.7	34.37±18.7	1	absolute bactericidal
	354UB/17CNRa	25±0.0	25±0.0	1	absolute bactericidal

MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration, Tests were performed 3 times (n=3) and the concentrations expressed as mean±SD.

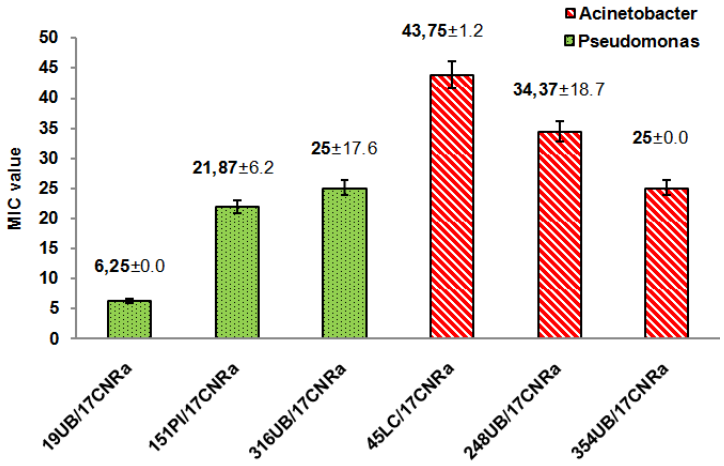

Fig. 3: MIC value of bacterial strains, all the experiments were triplicated, and the results plotted as mean±SD

DISCUSSION

The phytochemical screening identified the presence of sterols, terpenes, alkaloids, tannins, coumarins, and flavonoids in the extracts of *Lannea barteri Engl.* (DA). These results are similar to those obtained by Kone and his research team in 2011 working on the same species acclimated in Ivory Coast [7]. As highlighted by the quantitative analyses; a significant amount of total polyphenols (0.757 mg AG/g DM) was found in DA, these phenolic compounds are main classes of flavonoids as mentioned above with probable presence of coumarins and tannins in the extracts. Comparing these results with those of Kone *et al.* who found 0.25446 mg AG/g MS [7], it appears that the species acclimated in the locality of Alépé (South of Ivory Coast) is richer in polyphenols than the one of Ferkessedougou (northern Ivory Coast). This may be justified by the vegetation, climate and soil types which are important factors in the distribution and content of secondary metabolites in plant species [20]. The amounts of total flavonoids and flavonol aglycones are respectively 0.230 mg/g DM and 0.028 mg/g DM; the values reflect the abundance of flavonoids in *Lannea barteri Engl*.

Antibacterial tests have demonstrated the sensitivity of the tested strains to DA as a gradual increase in the inhibition zone was noticed with increasing concentrations of the extracts. It comes out that the diameters of inhibition obtained using 100 mg/ml are greater than the limiting diameter (10 mm), we can, therefore, affirm that DA is efficient at this concentration of 100 mg/ml [21]. This is an indication of its effectiveness on *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Indeed, according to Ponce *et al.* (2003), a bacteria is said to be resistant to an extract when the inhibition diameter induced by this extract is less than 8 mm. An extract is considered effective if it is able to induce some inhibition diameters comprised between 9 and 14 mm. For diameter between 15 to 19 mm, it is considered very sensitive and extremely sensitive for all diameters greater than 20 mm [22].

In addition, the antibiotics used are shown to be very sensitive to all the tested bacterial strains compared to DA. However, DA has signed a better activity on *Pseudomonas aeruginosa;* and the determination of the antibacterial parameters indicated that DA has bactericidal activities upon all the tested strains. According to the reported findings of Fauchere (2002); when the ratio MBC/MIC = 1, the extract is called "absolute bactericidal", if MBC/MIC<2, the extract is "bactericidal", and when MBC/MIC>2, the extract is simply called "bacteriostatic" [23].

It appears from our study that the decoction of *Lannea barteri Engl.* has an antibacterial potential at 100 mg/ml on the strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. This activity could be related to the presence of sterols, terpenes, coumarins, flavonoids, tannins, and alkaloids found in the tested extracts [14]. Thus, *Lannea barteri Engl.* could be used to reduce some nosocomial infections and especially urinary infections in hospitals.

CONCLUSION

This study is a scientific contribution for a better understanding of the chemical composition and biological potentials of *Lannea barteri Engl.* (DA), a plant used in traditional medicine to treat urinary tract infections. From our findings, the chemical investigation conducted on the aqueous decoction of DA has highlighted the presence of many phytocompounds; such as sterols, terpenes, coumarins, flavonoids, tannins and alkaloids with predominance in phenolic compounds (0.757±0.03 mg AG/g DM) and particularly flavonoids (0.23±0.01 mg AG/g DM). Moreover, the study of the antibacterial potential has revealed an absolute bactericidal character of *Lannea barteri Engl.* on *Pseudomonas aeruginosa* and *Acinetobacter Baumannii*. These bactericidal activities are potentially due to the richness of the plant in various phytochemical compounds as described in this work. Thus, the traditional use of *Lannea barteri Engl.* against urinary infections seems to be justified based on our scientific results.

Further useful chemical researches are planned for the isolation of valuable active ingredients and new pharmacophores that could be used against some nosocomial infections.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Authors have equal contribution in this work and declare no conflict of interests.

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