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Bauhinia thonningii Schumach & Thonn (*Fabaceae*): Phytochemical Screening and Evaluation of the Antibacterial Activity of Barks

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The aim of this study was to identify the groups of chemical compounds present in the aqueous extract of *B. thoninginii* bark from Ivorian flora and to assess its antibacterial activity against multi-resistant strains of *P. aeruginosa* and *A. baumannii*. Phytochemical sorting was used to identify polyphenols, flavonoids, tannins, coumarins, alkaloids, terpenes and derivatives. The levels of polyphenols, flavonoids, flavone aglycones, anthocyanins and condensed tannins were respectively 0.748 ± 0.03, 0.091 ± 0.01, 0.0094 ± 0.03, 0.0359 ± 0.01 and 0.117 ± 0.02 mg EAG /g MS. Sensitivity tests showed that BT was ineffective against multi-resistant strains of *P. aeruginosa* and *A. baumannii*.

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1. INTRODUCTION

Conventional medicine uses antibiotics to relieve bacterial infections. However, bacterial strains have developed resistance, despite the use of several classes of antibiotics, including broad-spectrum antibiotics [1]. They continue to cause suffering in the population, despite the molecules offered by modern medicine. Among the microorganisms responsible for infections, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* occupy a preponderant place in contamination. They are transmitted by direct contact between two people, directly or indirectly, or between an object and a person in a hospital environment or not. The rate of morbidity and mortality associated with them is 20-60% [2]. The death rate from nosocomial infections with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* varies between 17% and 46%. It can reach 70% for pneumonia [3]. In the case of urinary tract infections, the death rate varies from 11.9% to 21% [4]. That's why it's essential to find new molecules through traditional medicine. Traditional medicine uses plants as the mainstay of its therapies. Ivorian flora is abundant in medicinal plants. Previous phytochemical and biological studies on the plants of the genus *Bauhinia* revealed the presence of several classes of secondary metabolites including terpenoids, flavonoids and other phenolic compounds with various biological activities [5,6,7,8,9]. Among these plants, *Bauhinia thonningii Schumach & Thonn*, is employed in traditional medicine to treat various illnesses, including urinary tract infection [10]. *B. thonningii* represent a species of Fabaceae found in the savannah and forests of West Africa. The leaves have anti-inflammatory, antiseptic and antidiarrheal properties [11,12], the barks possess anthelmintic properties [12]. They were selected following ethnobotanical surveys of traditional herbalists. The general aim of this study is to highlight the antibacterial properties of the aqueous extract of the bark of *Bauhinia thonningii Schumach & Thonn* (Fabaceae) against multi-resistant bacterial strains. To perform this, we will identify the chemical groups of the secondary metabolites present in the aqueous extract using phytochemical screening and evaluate the antibacterial activity against multi-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant material

The barks of *Bauhinia thonningii Schumach & Thonn* (Fabaceae), were selected following ethnobotanical surveys of traditional herbalists in the various markets of the communes of Adjamé and Abobo in the District of Abidjan and identified at the national floristic center in Abidjan (Identification code: (AA13847; AA 15937). The bark was harvested at Dimbokro (6° 39' North, 4°42' West) in the N'zi region of central Côte d'Ivoire. They were then cleaned and dried at 18 °C for 14 days and pulverized.

2.1.2 Biological material

The biological material consisted of multi-resistant bacterial strains from the Antibiotics, Natural Substances and Surveillance of Microorganisms and Anti-infective Unit (ASSURMI) of the Bacteriology and Virology Department of the Pasteur Institut of the Ivory Coast. These are the multi-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from the urine of various patients in some health centers in the city of Abidjan (Ivory Coast), presented in Table 1.

2.2 Methods

2.2.1 Preparation of the aqueous extract

100 g of powder from the plant organ are boiled in 1 L of distilled water for 30 min on a heating cap. The resulting mixture was filtered. The operation was rehearsed three times. The filtrate was collected and concentrated using a rotary evaporator under reduced pressure. It was then dried in an oven at 50° C to produce the aqueous crude extract of *Bauhinia thonningii* (BT).

2.2.2 Qualitative analysis

It was carried out on BT, using color reaction detection tests and thin layer chromatography. (TLC) [13,14,15]. The eluent Toluene / Ethyl acetate / Acetic acid + 2 drops of ammonia (9.7/3/0.3; v/v/v) was chosen. We used Liebermann-Bürchard, Dragendor'ff and Neu

Table 1. Codes and biological products for bacterial strains

Bacterial strains	Codes ASSURMI	Phenotypes
Pseudomonas aeruginosa	19UB/17CNRa	Wild phenotypes to carbapenems and fluoroquinolones ; very low level cephalosporinases
	151PI/17CNRa	Wild aminoglycoside phenotype; High level penicillinase resistance ; Cephalosporinases with very low levels of resistance
	316CO/17CNRa	Wild phenotypes to cephalosporins; Cross-resistance to fluoroquinolones
Acinetobacter baumannii	45LC/17CNRa	Wild phenotypes to aminoglycosides, carbapenems ; Cephalosporinases with very low levels of resistance; low-level penicillinase
	248UB/17CNRa	Carbapenems; Penicillinase ; Cephalosporinases ; Cross-resistance to ticarcillin and piperacillin
	354UB/17CNRa	Fluoroquinolone resistance; Cephalosporinases

reagents, 5% potassium hydroxide (KOH) and 2% iron (III) chloride solutions as revealing.

2.2.3 Quantitative analysis

Total polyphenol content: Total polyphenol levels were determined employing the Folin-Ciocalteu colorimetric method [16].

Total flavonoid content: Total flavonoids were determined using the method of Hariri and al [17].

Anthocyanins and flavonoid aglycones content: Anthocyanins, flavanols and flavones were measured using Lebreton and al, methodology [18].

Condensed tannin content: Condensed tannins were measured using the methodology of Broadhurst and Jones (1978), Heimler and al [19].

2.3 Antibacterial Activity

Antibacterial tests were carried out according to the methodology described by Bredou and al [20].

2.3.1 Statistical analysis

In triplicate, all assays were carried out. All data were analysed using ANOVA one way in Origin Pro 9.1 software. The results obtained were expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Phytochemical screening

Secondary metabolites were identified using color reactions to identify polyphenols, flavonoids, tannins, coumarins, alkaloids, terpenes and derivatives (Table 2). To confirm these results, thin layer chromatography (TLC) was performed using the appropriate reagents [13,14,15]. Thus, sulfuric vanillin revealed sterols and terpenes in the visible as violet, pink and orange at R_f = 0,45 ; 0,51 ; 0,59 ; 0,72 ; 0,9. 5% (w/v) methanolic KOH solution was used to detect coumarins at UV 366 nm, in the form of several blue, green and yellow fluorescent spots at R_f =. 0,21 ; 0,35 ; 0,55 ; 0,69 ; 0,81. Flavonoids were revealed by Neu reagent, which color intensifying at UV / 366 nm, at R_f = 0,03 ; 0,05 ; 0,45 ; 0,53 ; 0,59 ; 0,65 ; 0,71. The tannins were revealed using a solution of iron III trichloride ($FeCl_3$), which presents them as grey spots in the visible at R_f = 0,40 ; 0,51. We also detected the alkaloids using the Dragendor'ff reagent in the form of orange spots at. R_f = 0,65; 0,75; 0,88 (Table 3).

3.1.2 Quantitative analysis

Groups of compounds known to have antibacterial properties were quantified. These are total polyphenols, flavonoids, flavone aglycones, anthocyanins and condensed tannins. The results obtained are reported in Fig. 1. Thus, the total polyphenol content of BT was 0.748 ± 0.03 mg EAG/g DM, while total flavonoids were 0.091 ± 0.01 mg EQ/g DM. The content of flavonic aglycones and anthocyanins were 0.0094 ± 0.03 mg EQ/g DM and 0.0359 ± 0.01 mg EQ/g DM respectively. The content of condensed tannin was 0.117 ± 0.02 mg EC/g DM.

3.1.3 Antibacterial activity

The antibacterial activity of BT was evaluated by determining the diameters of the inhibition zones of the strains of *P. aeruginosa* and *A. baumannii*. The range of different concentrations gave inhibition zone diameters less than or equal to 8 mm against the two strains of multi-resistant bacteria compared to the reference antibiotics. (Ceftazidime and Ticarcillin). The results obtained are recorded in Table 4.

Table 2. Phytocompounds detected

Compounds	Tests	Coloration	Results
Polyphenols	$FeCl_3$	Black	Presence
Flavonoids	Schinoda, KOH (5 %)	Red-orange Yellow	Presence
Coumarins	Lactone cycle	Yellow	Presence
Tannins	$FeCl_3$ Bromine water	Black	Presence
Sterols and polyterpenes	$CH_3CO_3CH_3$ / H_2SO_4	Blue-violet	Presence
Alkaloids	Dragendor'ff	Red-orange (crystal deposit)	Presence

Table 3. Secondary metabolites detected in the aqueous crude extract of *Bauhinia thonningii* (BT) bark

EXT	Without developer (a)				Neu (b)				KOH (5%) (c)				$FeCl_3$ (d)		Libermann Büchard (e)				Sulfuric Vanillin (f)		Draggendorff (g)		Compounds
	Visible		UV 366		Visible		UV 366		Visible		UV 366 nm		Visible		Visible		UV 366		Visible		Visible		
BT	Co	Rf	Co	Rf	Co	Rf	Co	Co	Rf	Co	Rf	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	
							green	0,0			blue	0,0	grey	0,0			y-o	0,0	r-o	0,00			flav[b], coum[c], tan[d], terpenes[f,e] alkaloid[g]
			green	0,03																			NI
									yellow	0,21													NI[a] coumarins[c]
											green	0,35					yellow	0,35	yellow	0,35			sterols[f,e]
							blue	0,45											grey	0,45			flavonoid[b], terpenes[f]
																			grey	0,51			NI terpenes[f]
							green	0,53															flavonoids[b]
			green	0,55					yellow	0.55	green	0,55											NI coumarins[c,a]
																							NI
			y-o	0,59											violet	0,59			gr-vi	0,59			NI sterols[e], terpenes[f]
													grey	0,61									NI tannins[d]
			yellow	0,65																	orange	0,65	terpenes[a,f]
											blue	0,69											coumarins[c]
							green	0,71															flavonoid[b],
															blue	0,72			violet	0,72			sterols[e], terpenes[f]
																					orange	0,75	alkaloid[g]
											green	0,81											coumarins[c]
																					orange	0,88	alkaloid[g]
																			grey	0,90			terpenes[f]

BT: Aqueous extract; Co: Color; y: yellow; gr: grey; g: green; o: orange; r: red; vi: violet; flav: flavonoids; coum: Coumarins; tan: Tannins; alc: Alkaloids; NI: Not identified; Rf: Retention factor

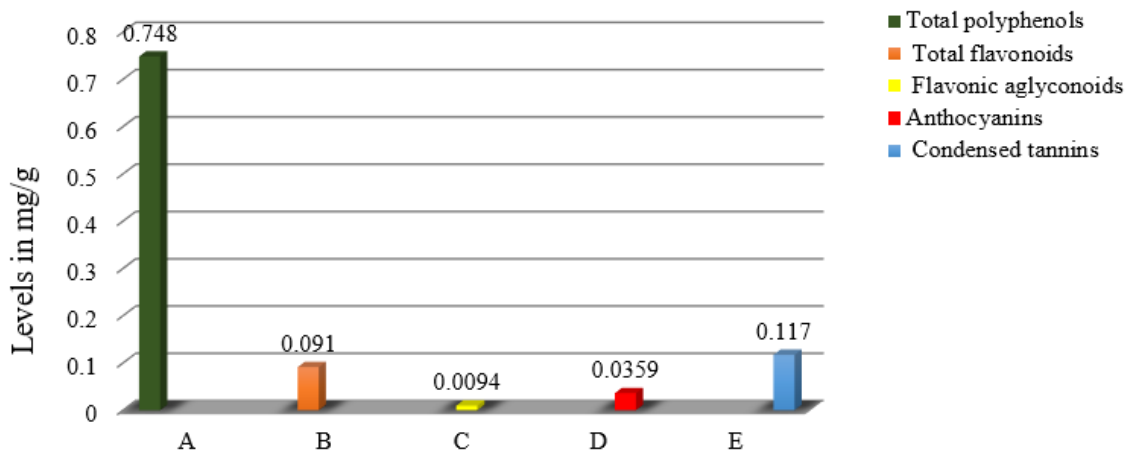

Fig. 1. Contents of total polyphenols (A), total flavonoids (B), flavonic aglycones (C), anthocyanins (D) and condensed tannins

Table 4. Diameter of inhibition zones (mm) of bacterial strains

		Concentration BT (mg/mL)				Antibiotics (µg)	
Bacterial strains	**Strain Codes**	**C_1 (100)**	**C_2 (50)**	**C_3 (25)**	**Wit**	**CAZ (10)**	**TIC (75)**
P. aeruginosa	19UB/17CNRa	6±0,01	6±0,0	6±0,00	6±0,00	33±0,14	26±0,07
	151 PI/17CNRa	6±0,53	6±0,0	6±0,00	6±0,00	31±0,21	6±0,70
	316CO/17CNRa	7,2±0,12	6±0,50	6±0,01	6±0,00	33±1,40	23±0,80
A. baumannii	45LC/17CNRa	8±0,35	6±,01	6±0,00	6±0,00	30,5±0,7	20±0,28
	248UB/17CNRa	7±0,50	6±0,30	6±0,	6±0,00	30,5±0,7	26±0,07
	354UB/17CNRa	6±0,30	6±0,0	6±0,00	6±0,00	32±0,0	6±0,00

CAZ: Ceftazidime; TIC: Ticarcillin; Wit: Witness

3.2 Discussion

Detection tests are based on color variations perceptible in the visible range and at UV 366 nm, when secondary metabolites are subjected to the action of appropriate reagents. Thus, the tests identified polyphenols, flavonoids, tannins, coumarins, alkaloids, terpenes and derivatives in BT. The results of the staining tests are in accordance with those of the TLC. These results do not corroborate with those of Adiko *and al* (2013), which reported an absence of alkaloids [21]. In addition, an absence of flavonoids, sterols and terpenes in the aqueous extract of *Bauhinia thonningii* leaves was observed by Ouattara *and al*. (2020) [22]. This difference could be explained by the variability in the composition of the organs of the same plant due to biotic and abiotic factors and the extraction solvents used. Quantification of the groups of chemical compounds showed that BT was rich in total polyphenols and condensed tannins. However, when compared with the content obtained from dates (5660 µg EAG/g) [23], *Bauhinia thonningii* relatively low in polyphenols, flavonoids and tannins. The BT extract showed inhibition zone diameters less than or equal to 8 mm against multi-resistant strains of *P. aeruginosa* and *A. baumannii*.

According to Ponce (2003), a strain is resistant if the diameter of the inhibition zone is less than 8 mm. Sensitive if the diameter is between 9 and 14 mm, very sensitive if it is between 15 and 19 mm, and extremely sensitive if it is greater than 20 mm. Therefore, BT is ineffective against multi-resistant strains of *P. aeruginosa* and *A. baumannii*, despite the co-presence of these groups of chemical compounds.

4. CONCLUSION

The aim of this study was to identify the groups of chemical compounds present in the aqueous extract of *B. thoninginii* bark from Ivorian flora and to assess its antibacterial activity against multi-resistant strains of *P. aeruginosa* and *A. baumannii*. Thus, Phytochemical screening by

color reactions and TLC allowed identifying polyphenols, flavonoids, tannins, coumarins, alkaloids, terpenes and derivatives. The assay showed that *B. thoninginii* is rich in total polyphenols and condensed tannins. Regarding antibacterial activity, BT is ineffective against multi-resistant strains of *P. aeruginosa* and *A. baumannii*. Nevertheless, the identification of these groups of chemical compounds could justify the use of *B. thoninginii* in the traditional treatment of infectious diseases in Côte d'Ivoire.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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