***Bauhinia thoninginiiSchumach & Thonn (Fabaceae)*, a medicinal plant of the Ivorian flora: phytochemical screening and evaluation of the antibacterial activity of barks.**

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*.

Keywords: *Bauhinia thoninginii*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*

1. Introduction

Conventional medicine uses antibiotics to relieve bacterial infections. However, bacterial strains have developed resistance, despite the use of several classes of antibiotics available, including broad-spectrum antibiotics[**1**].They continue to cause suffering in the population despite the molecules offered by modern medicine. 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 rich in medicinal plants. Among these plants,*Bauhinia thonningiiSchumach& Thonn,* used in traditional medicine to treat a number of illnesses[2].*B. thonningii*is a species of Fabaceae found in the savannahs and forests of West Africa. The leaves have anti-inflammatory, antiseptic and antidiarrheal properties [3 ;4],the barks have anthelmintic properties [4]. The general aim of this study is to highlight the antibacterial properties of the aqueous extract of the bark of *Bauhinia thonningiiSchumach& Thonn* (Fabaceae) against multi-resistant bacterial strains. To do 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. Material and methods

2.1. Material

2.1.1. Plant material

The barks of *Bauhinia thoninginiiSchumach& 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 centre 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 pulverised.

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-Infectives Unit (ASSURMI) of the Bacteriology and Virology Department of the Pasteur Institut of Ivory Coast. These are the multi-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* presented in Table I.

TableI: Codes and biological products for bacterial strains

Bacterial strains	**Codes ASSURMI**	**Phenotypes**
Pseudomonas aeruginosa	19UB/17CNRa	Wild phenotypes to carbapenems and fluoroquinones;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; Very low level penicillinase
	248UB/17CNRa	Carbapenems; Penicillinase ; Cephalosporinases ; Cross-resistance to ticarcillin and piperacillin
	354UB/17CNRa	Fluoroquinone resistance; Cephalosporinases

2.2. Methods

2.2.1. Preparation of the aqueousextract

100 g of powder from the plant organ were added to an Erlenmeyer flask containing 1 L of water. The resulting mixture was boiled for 30 min on a heating cap. The resulting decoctate was vacuum filtered using a Büchner. The operation was repeated three times. The filtrate collected was pooled and concentrated in a rotavapor under reduced pressure. It was then dried in an oven at 50°C to produce the aqueous crude extract of *Bauhinia thoninginii* (BT).

2.2.2. Qualitative analysis

It was carried out on BT, using colour reaction detection tests and thin layer chromatography. (TLC) [5-6, 7]. 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 reagents, 5% potassium hydroxide (KOH) and 2% iron (III) chloride solutions as revealing.

2.2.3. Quantitative analysis

2.2.3.1. Total polyhenols content

Total polyphenol levels were determined using the Folin-Ciocalteu colorimetric method [8 ;12].

2.2.3.2. Total flavonoids content

Total flavonoids were determined using the method of Hariri *et al*[12; 9].

2.2.3.3. Anthocyaninsandflavonicaglycones content

Anthocyanins, flavanols and flavones were measured using Lebreton*et al*, methodology [12; 10].

2.2.3.4. Condensed tanins content

Condensed tannins were measured using the methodology of Broadhurst and Jones (1978), Heimler *et al* (2006) [12 ; 11].

2.3. Antibacterial activity

Antibacterial tests were carried out according to the methodology described by Bredou*et al* (2019) [12].

2.3.1. Statiscal analysis

All assays were carried out in triplicate using the spectrophotometer (AL800/SPECTRE DIRECT), as was the determination of the diameters of the zones of inhibition. 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

The identification of phytocompounds by colour reactions enabled to identify polyphenols, flavonoids, tannins, coumarins, alkaloids, terpenes and derivatives (**Table II**). To confirm these results, thin layer chromatography (TLC) was carried out using the appropriate reagents [5-6 ;7]. Thus, sulphuric 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.The methanolic KOH solution at 5% (w/v) 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 (**TableIII**).

Table II: 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 et polyterpenes	$CH_3CO_3CH_3$ / H_2SO_4	Blue-violet	Presence
Alkaloids	Dragendor'ff	Red-orange (crystal deposit)	Presence

Table III: Secondary metabolites detected in the aqueous crude extract of *Bauhinia thoninginii* (BT) bark.

EXT	Without developer (a)				Neu (b)				KOH (5%) (c)				$FeCl_3$ (d)		LibermannBüchard (e)				SulfuricVanillin (f)		Draggendorff (g)		Compounds
	Visible		UV 366		Visible		UV 366		Visible		UV 366 nm		Visible		Visible		UV 366		Visible		Visible		
	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]
																							NI
			green	0,03																			NI[a]
									yellow	0,21													coumarins[c]
											green	0,35					yellow	0,35	yellow	0,35			sterols[f,e]
							blue	0,45											grey	0,45			flavonoid[b], terpenes[f]
																							NI
																			grey	0,51			terpenes[f]
							green	0,53															flavonoids[b]
																							NI
BT			green	0,55					yellow	0.55	green	0,55											coumarins[c,a]
																							NI
																							NI
			y-o	0,59											violet	0,59			gr-vi	0,59			sterols[e], terpenes[f]
																							NI
													grey	0,61									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 ; c**oum**: Coumarins ; **tan** : Tannins ; **alc**: Alkaloids ; **NI** : Not identified ; **Rf** : Retention factor

3.1.2. Quantitative analysis

The groups of compounds recognised as having antibacterial properties have been quantified. These are total polyphenols, flavonoids, flavone aglycones, anthocyanins and condensed tannins.The results obtained are shown in Figure 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 condensed tannin content was 0.117 ± 0.02 mg EC/g DM.

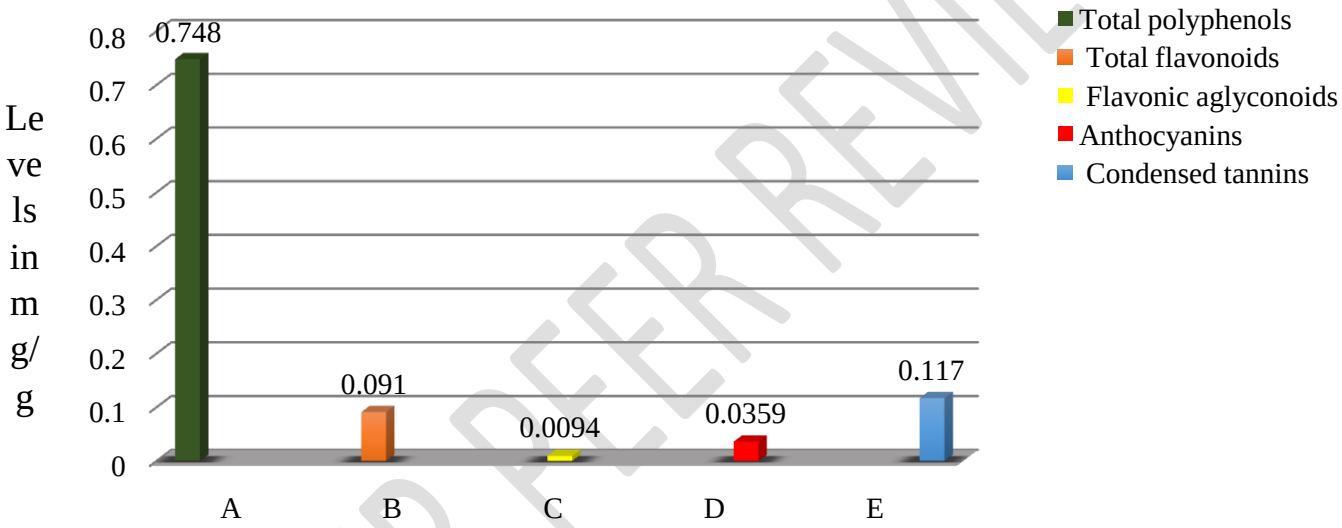

Figure 1: Contents of total polyphenols (A), total flavonoids (B), flavonic aglycones (C), anthocyanins (D) and condensed tannins.

3.13. 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 IV**.

TableIV: Diameter of inhibition zones (mm) of bacterial strains.

Bacterial strains	StrainCodes	Concentration BT (mg/mL)				Antibiotics (µg)	
		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 colour variations perceptible in the visible range and at UV 366 nm, when phytocompounds are subjected to the action of appropriate reagents. Thus, the tests identified polyphenols, flavonoids, tannins, coumarins, alkaloids, terpenes and derivatives in BT. These results do not corroborate whit those of Adiko *et al* (2013), who reported an absence of alkaloids **[13]**.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 is rich in total polyphenols and condensed tannins.However, when compared with the content obtained from dates (5660 µg EAG / g) [14], *Bauhinia thoninginii* is a plant that is 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 colour reactions and TLC allowedidentifying 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.

References

[1]Seok Y., Bae I. K., Jeong S. H., Kim S. H., Lee H., Lee K. (2011). Dissemination of IMP-6 metallo-β-lactamase-producing Pseudomonas aeruginosasequence type 235 in Korea. J. Antimicrob. Chemother. 66; 2791-2796.

[2] Fernandez de la Pradilla C. (1988). Medicinal plants againsthepatitis. Pabre, Ouagadougou, Burkina Faso. 62p.

[3] Lemessa D. (2010). Piliostigmathonningii (Schumach.) Milne-redh. In: Brink M, & Achigan-Dako EG (editors). Prota16:Fibers/fiber plants. [CD-ROM]. PROTA, Wageningen (Netherlands) 10p.

[4] Jean-Louis P. (2004). Medicinal plants fromAfrica. How to recognize and use them. Edi south. Aix en Provence. 288p.

[5] Konan F, Guessennd N, Oussou K. (2014), Antibacterialeffect of the aqueousextract of the bark of TerminaliaglaucescensnPlanch ex Benth (combretaceae) on the in vitro growth of enterobacteriaproducingextended-spectrum beta-lactamases (EBLSE). Int. Journal Biologic Chemistry Sci. 8(3): 1192-1201.

[6] Ladiguina EY, Safronitch LN, Otriachenkova VE, Balandina IA and Grinkevitch; Chemical analysis of medicinal plants. Edition 1983. Moskva, VischayaChkola.

[7]Bekro Y-A, Mamyrbekova-Békro J, Boua B, Tra Bi F and Ehilé E. (2007); Ethnobotanicalstudy and phytochemical screening of Caesalpiniabenthamiana (Baill.) Herend ET Zarucchi (Caesalpiniaceae). Science &Nature; 4(2): 217-225

[8] Singleton V, Ortofer R, Lamuela-Raventos R; (1999) Analysis of total phenols and otheroxidationsubstrates and antioxidants by means of Folin-Ciocalteureagent; Packer L Methods in enzymology. Orlando Academic PressLondon;edition: 152-178.

[9] Hariri E., Sallé G., Andary C. (1991). Involvement of flavonoids in the resistance of twopoplar cultivars to mistletoe (Viscum album L.). Protoplasma: 162(1); 20-26.

[10] Lebreton P., Jay M., Voirin B. (1967). On the qualitative and quantitative analysis of flavonoids. AnalyticaChimica Acta Paris: 49(7); 375-383.

[11] Heimler D., Vignolini P., Din M., Vinueri F., Ronani A. (2006). Antiradicalactivity and polyphenol composition of local Brassiccaceaeediblevarieties. Food Chemistry: 99; 464-469.

[12] Bredou J B, Boua B B*, Konan K F. (2019). Phytochemical and antibacterial investigations of medicinal plants used in the traditionaltreatment of urinary infections in Ivory Coast: case of LanneabarteriEngl. (Anarcadiaceae). J.Soc. West-Afr. Chem. 047; 8 - 15

[13]Adiko N., Okpekon A., Bony F., Koffi K., Kablan B. (2013). Ethnobotanicalsurvey and phytochemical screening of plants used in traditionalophthalmology, listed on the Abidjan markets. J.sci. pharm. biol.: 14(1); 10-21.

[14] Bessas A. (2008). Biochemical dosage of phenolic compounds in dates and honeyharvested in southern Algeria. End of study dissertation, DjillaliLiabesUniversity -Sidi Bel Abbes 57 p.