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Phytochemical Composition, Antibacterial Activities against Multi-Resistant Strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* of the Bark Extract of *Ficus platyphylla* Dell. Holl.

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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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ABSTRACT

Ficus platyphylla Ficus platyphylla belongs to the Moraceae family which contains nearly 1400 species divided into around forty genera. It is used to manage several diseases in folklore medicine. This study focused on the phytochemical screening and evaluation of the antibacterial potential against *Pseudomona aeruginosa* and *Acinetobacter baumannii* of hydroethanolic extract from *Ficus platyphylla* (FP) bark. Phytochemical and antibacterial activity were carried out according to the literature procedure. Qualitative analyses of FP revealed the presence polyphenols, flavonoids, coumarins, tannins, alkaloids, sterols and terpenes. Quantitative analysis by spectrophotometry showed a phenolic compound content to be 0.878 ± 0.02 mg EAG/g DM. The flavonoids content was 0.084 ± 0.02 mg EQ/g DM, while flavonic aglycones, anthocyanins and condensed tannins were 0.014 mg EQ/g DM, 0.018 mg EQ/g DM and 0.189 mg EC/g DM, respectively. *In vivo* antibacterial activity showed that (FP) was ineffective against six (06) multi-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Keywords: Ficus platyphylla; phytochemical screening; P. aeruginosa; A. baumannii.

1. INTRODUCTION

Medicinal plant are used since ancient times to manage and treat various diseases, because they constitute a renewable source of nutrients and bioactive principles [1,2]. Although progress in the field of medicine and pharmacology had allowed the discovery of certain therapeutics, the use of medicinal plants in the form of decoctions, herbals teas and several other formulations are still used today. They present themselves as an alternative to complex and urgent population health problems [3,4].

“Indeed, plants contain several bioactive compounds such as alkaloids, phenolic compounds (phenol acids, coumarins, flavonoids, tannins), These compounds, known to protect plants, play an essential therapeutic role in humans” [5,6].

“Ficus platyphylla Dell. Holl is a medicinal plant belonging to the Moraceae family. It is widely distributed throughout the savannah region of the West African coast. In Côte d'Ivoire, it is used to treat a number of illnesses. A decoction of stem bark and roots is used to treat anemia” [7]. Leaves and stem bark are used to treat dysmenorrhea and urinary and intestinal schistosomiasis [8]. Pharmacological studies carried out on *F. platyphylla* have shown that it has antinocupressive, antimalarial, antibacterial, antifungal, anti-inflammatory and gastrointestinal activities [9,10]. “Anthocyanin, known for its anti-angiogenic activity, was first extracted from *Ficus benghalensis*. Phytotoxins such as furanocoumarins have been reported in many species of the *Ficus* genus. *Ficus benghalensis* methanol extract exhibits various antibacterial, wound healing, pollution inhibitory and fungicidal effects” [11,12].

“The resistance of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* to antibiotics frequently used in conventional medicine represents a real therapeutic challenge worldwide, according to the World Health Organization (WHO). Indeed, the WHO (2017) has classified these two bacteria as priority agents in the search for new effective molecules” [2]. However, the search for active substances of natural origin could contribute to effectively combating this bacterial resistance. That's why we've undertaken this project, which is part of our ongoing search for new active substances in medicinal plants. The aim of this study is to demonstrate the antibacterial properties of *Ficus platyphylla* from the Ivorian flora against multi-resistant bacterial strains. To do this, we will identify the chemical groups of secondary metabolites present in the hydroethanol extract by phytochemical screening and evaluate antibacterial activity on multi-resistant strains of *P. aeruginosa* and *A. baumannii*.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant material

Ficus platyphylla Del. Holl (FP), were harvested from Dimbokro (Central Côte d’Ivoire, N 6° 39', W 4° 42'), selected following ethnobotanical surveys of herbalists in the various markets from Abidjan. It was authenticated at the national floristic center of Abidjan (NFC) (Identification code: MAA 3964). After cleaning, they were dried

for 14 days at 18°C, then pulverized and packaged.

2.1.2 Biological material

The biological material consists of six (06) 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 Institut Pasteur of Côte d'Ivoire (IPCI). These are the *P. aeruginosa* and *A. baumannii* strains isolated from the urine of patients from Abidjan health centers, whose profiles are presented in Table 1.

2.2 Methods

2.2.1 Hydroethanolic extract

100 g of powder were boiled in 1000 mL of ethanol (80%), for 30 min. After vacuum filtration, the filtrate was concentrated on a rotary evaporator and oven-dried at 50°C for 2 days to obtain the hydroethanolicl extract from *Ficus platyphylla* (FP).

2.2.2 Qualitative analysis

It was carried out on DF, using detection tests with color reactions and thin-layer chromatography (TLC). [13-22]. Toluene / Ethyl acetate / Acetic acid +2 drops of ammonia (9.7/3/0.3; v/v/v) was chosen as eluent. We used Liebermann-Bürchard, Dragendor'ff and Neu reagents, 5% potassium hydroxide (KOH) and 2% iron (III) chloride solutions as revealing.

2.2.3 Total polyphenol content

Total polyphenol levels were determined employing the Folin-Ciocalteu colorimetric method [14,18].

2.2.4 Total flavonoid content

Total flavonoids were determined using the method of Hariri and al [15,11].

2.2.5 Anthocyanins and flavonoid aglycones content

Anthocyanins, flavanols and flavones were measured using Lebreton *and* al, methodology [16].

2.2.6 Condensed tannin content

Condensed tannins were measured using the methodology of Broadhurst and Jones (1978), Heimler *and* al [17].

2.2.7 Antibacterial activity

Antibacterial tests were carried out according to the methodology described by Bredou *and* al [18].

2.2.8 Statistical analysis

All assays were performed in triplicate using the brand's spectrophotometer (AL800/SPECTRE DIRECT), as was the determination of inhibition zone diameters. All data were analyzed using ANOVA-one-way variance analysis with Origin Pro 9.1 software. Results were expressed as mean ± standard deviation.

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

3. RESULTS

3.1 Phytochemical Composition

Color reactions revealed the presence of polyphenols, flavonoids, tannins, coumarins, terpenes and derivatives and alkaloids in FP (Table 2). [18,19].

In addition, the presence of these secondary metabolites was confirmed by TLC with appropriate reagents [18-22]. The results are shown in Table 3. Coumarins were revealed by 5% (m/v) potassium hydroxide at UV 366 nm in blue, green and yellow fluorescent with R_f = 0.36; 0,5; 0,60; 0, 69; 0,81; 0,90 (Fig. 1A). Terpenes and derivatives have been identified in the visible by Libermann-Bürchard in blue, yellow, green and brown R_f = 0,67; 0,76; 0,83; 0,86; 0,96. Iron III trichloride was used to identify tannins. They appear in the visible as grey or black at R_f = 0,13; 0,18; 0,38; 0,51; 0,71; 0,83 (Fig. 1C). Flavonoids were revealed by Neu's reagent as blue, green, violet and red spots at UV 366 nm at R_f = 0,02; 0,06; 0,12; 0,22; 0,29; 0,34; 0,39; 0,44; 0,47; 0,51;0,56; 0,61; 0,71; 0,86; 0,94. Considering their blue at UV 366 nm without prior treatment, they could be methylated flavonoids (Fig. 1B) [19-22]. The TLC results corroborate with those from the color reaction revelation tests.

Table 2. Phytocompounds detected

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

+ = Present, - = Absent

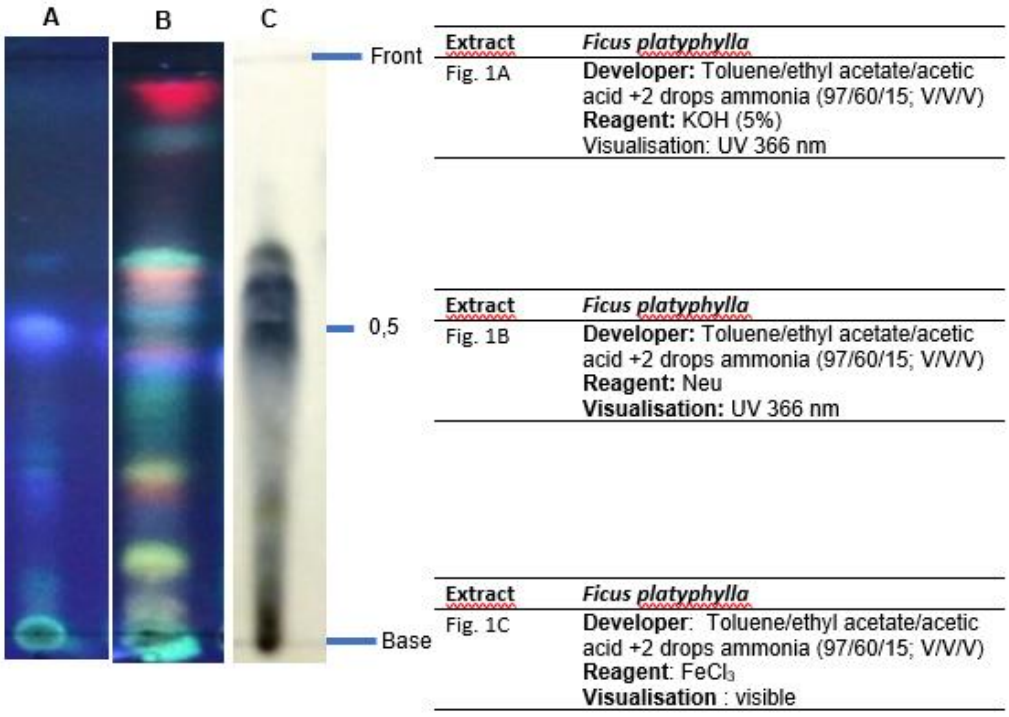

Fig. 1. TLC of *F. platyphylla* bark extract

Table 3. Secondary metabolites detected in ethanolic extract of *Ficus platyphylla* (FP) bark

EXT	Without reagent (a)				Neu (b)				KOH (5%) (c)				$FeCl_3$ (d)		Libermann Büchard (e)				Sulfuric Vanilline (f)		Draggendorff (g)		Compounds
	Visible		UV 366		Visible		UV 366		Visible		UV 366		Visible		Visible		UV 366		Visible		Visible		
FP	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	
							blue	0,02															flavonoid
											blue	0,05											coumarins[c,a]
							green	0,06															flavonoid,
																					orange	0,11	alkaloid
							blue	0,12					gr-v	0,13									flavonoid, tannin[s]
									yellow	0,18			grey	0,18									coumarins[a], tannin[s]
							yellow	0,22															flavonoid,
					yellow	0,29	green	0,29															flavonoid,
																							NI
							orange	0,34															flavonoid·
											green	0,36											coumarins[c]
							blue	0,39					green	0,38									flavonoid, Phenols
							orange	0,44															flavonoid
																							NI
							bleu	0,47															flavonoid
					j-v	0,51	green	0,51					grey	0,51									flavonoid, tannin[s]
							green	0,53															flavonoid
									yellow	0,54													coumarins[c]
							blue	0,56															flavonoid
											green	0,60											coumarins[c]
					yellow	0,61	yellow	0,61															flavonoid
															yellow	0,67			green	0,67	orange	0,66	Sterols, alkaloid
											blue	0,69											coumarins[c]
							green	0,71					green	0,71									flavonoid, Phenols
																					orange	0,73	alkaloid
															blue	0,76			violet	0,76			Terp[f], sterols
											blue	0,81			blue	0,81							coumarins[c], sterols
													grey	0,83					green	0,83			tannin[s], sterols
							green	0,86							violet	0,86			violet	0,86			flavonoid, sterols,
											green	0,90											coumarins[c]

***FP**: Hydro ethanolic extract; **Co**: Color; **y:** yellow; **gr**: grey; **g**: green; **o:** orange; **r**: red; vi: violet; **NI**: Not identified; **Rf**: Retention factor*

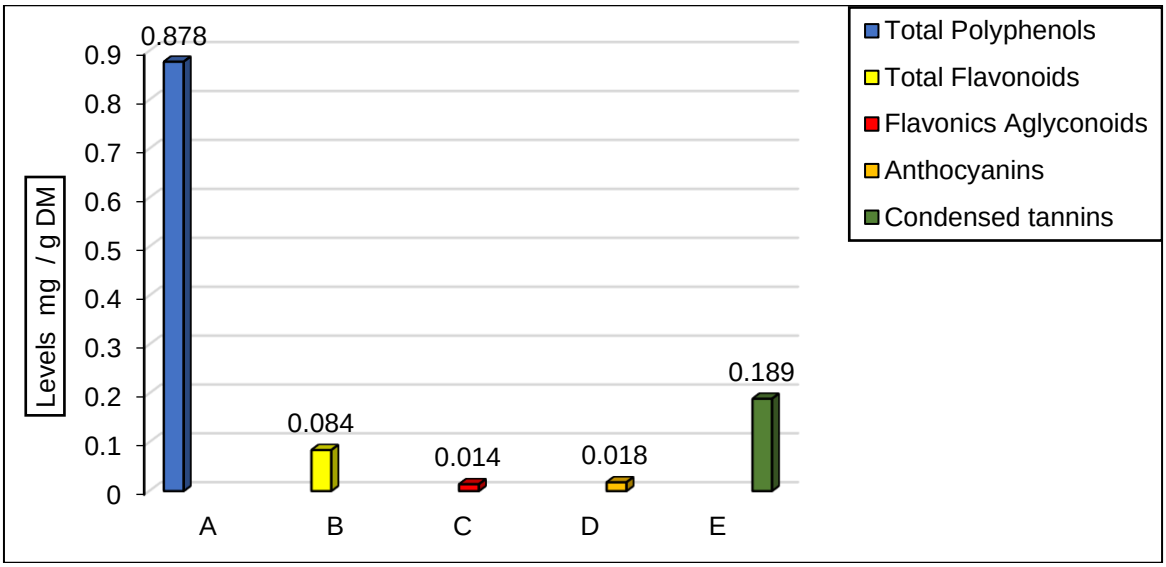

Fig. 2. Contents of total polyphenols (A), total flavonoids (B), flavonic aglycones (C), anthocyanins (D) and condensed tannins

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

		Concentration FP (mg/mL)				Antibiotic (µg)	
Bacterial strains	**Strain codes**	**C_1 (100)**	**C_2 (50)**	**C_3 (25)**	**Ct**	**CAZ (10)**	**TIC (75)**
P. aeruginosa	19UB/17CNRa	6±0,00	6±0,00	6±0,00	6±0,00	33±0,14	26±0,07
	151PI/17CNRa	8±0,10	6±0,00	6±0,00	6±0,00	31±0,21	6±0,70
	316CO/17CNRa	6±0,00	6±0,00	6±0,00	6±0,00	33±1,40	23±0,80
A. baumannii	45LC/17CNRa	6,5±0,02	6±0,00	6±0,00	6±0,00	30,5±0,7	20±0,28
	248UB/17CNRa	6±0,00	6±0,00	6±0,00	6±0,00	30,5±0,7	26±0,07
	354UB/17CNRa	7,3±0,01	6±0,00	6±0,00	6±0,00	32±0,0	6±0,00

***CAZ**: Ceftazidime; **TIC**: Ticarcillin; **Ct**: control*

3.2 Quantitative Analysis

The content of total polyphenols, flavonoids, flavonic aglycones, anthocyanins and condensed tannins are reported in Fig. 2: The total polyphenol content of FP was 0.878 ± 0.02 mg/g EAG DM. Flavonoids: 0.084 ± 0.02 mg EQ/g DMS. Concerning flavonic aglycones and anthocyanins, the recorded contents are respectively 0.014 mg EQ/g and 0.018 mg EQ/g of dry matter. As for condensed tannins, the content is 0.189 mg EC/g DM (Fig. 2).

3.3 Antibacterial Activity

The multi-resistant bacterial strains tested were resistant to different concentrations of FP extract. The results obtained are reported in Table 4. The diameters of the zones of inhibition were less than or equal to 8 mm. Compared with FP extract, reference antibiotics were sensitive.

4. DISCUSSION

Phytochemical screening by color reaction and TLC showed the presence of flavonoids, alkaloids, tannins, sterol, coumarins and terpenes in the hydro ethanol extract of *F. platyphylla* bark. These results corroborate those obtained by Adeshina during preliminary phytochemical analyzes using the ethanolic extract of *Ficus platyphylla* and *Ficus sycomorus* [23], but differ from those of Gbogbo et al. In fact, his work has shown that flavonoids are absent in the ethanolic extract of *F. platyphylla* leaves and stem bark harvested in the Bassar locality (Kara region) in Togo. [10]. This difference could therefore be attributable to the diversity of vegetation, climate and soil type, which are important factors in the distribution and content of secondary metabolites in plant species. [24]. Phytoconstituents including terpenoids, triterpenes, ketones, coumarin esters, furocoumarins, flavonols, flavonoids, sterols and

carbohydrates with important medicinal values have been revealed in various *Ficus benghalensis* extracts [11]. By comparing total polyphenol contents (878 µg EAG / g DM) of *F. Platyphylla* bark with those obtained from certain plants or plant organs known to be rich in polyphenols, including dates (5660 µg EAG / g) [25], grape seeds (7500 µg EAG / g) [26], parsley (2802 µg EAG / g), Brussels sprouts (2571 µg EAG / g), lychee (2223 µg EAG / g), broccoli (989 µg EAG / g) and celery (847 µg EAG / g) [27], we can confirm that *F. Platyphylla* bark is relatively rich in total polyphenols. This could justify the use of Ficus Platyphylla bark in the traditional treatment of several pathologies in Côte d'Ivoire. Regarding to antibacterial activity, the diameters of the inhibition zones gave values less than or equal to 8 mm. Consequently, according to Ponce, FP is ineffective against multidrug-resistant strains of *P. aeruginosa* et *A. baumannii* [28]. This ineffectiveness could be explained by natural resistance or resistance acquired by bacterial strains. However, antibacterial activity was observed in the *Ficus* genus. Indeed, methanol extract of *Ficus benghalensis* and aqueous extract of *Ficus benghalensis* showed antibacterial effects against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosae, E. coli and Klebsiella pneumonia* [11]. Hexane, chloroform and methanol fractions of *Ficus benghalensis* exhibited antibacterial action against Gram-negative and Gram-positive bacteria [29,30].

5. CONCLUSION

Ficus platyphylla is a medicinal plant of the Ivorian flora used in the traditional treatment of several pathologies. Phytochemical sorting by color reaction and TLC of the hydroethanolic extract of *F. Platyphylla* bark identified polyphenols, alkaloids, tannins, coumarins, flavonoids, sterols and terpenes. In addition, the assay showed that the hydroethanol extract of *F. Platyphylla* bark contained 0.878 mg EAG/g phenolics, 0.084 mg EQ/g flavonoids, 0.014 mg EQ/g flavone aglycones, 0.018 mg EQ/g anthocyanins and 0.189 mg EC/g condensed tannins in dry matter. Despite the co-presence of these groups of chemical compounds, *F. Platyphylla* is ineffective against multi-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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