Original Research Article

Comment [D1]: Please add some importance of medicinal plant. Add conclusion with some recommendations and mention key compound of ficus.

Phytochemical composition, antibacterial activities against multi-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* of thebark extract of*Ficus platyphylla Dell. Holl*.

ABSTRACT

This study focused on phytochemical screening and evaluation of the antibacterial potential of the hydroethanol extract from *Ficus platyphylla* (FP) bark.The phytochemical study revealed polyphenols, flavonoids, coumarins, tannins, alkaloids, sterols and terpenes.Quantitative analysis by spectrophotometry showed a phenolic compound content of 0.878 ± 0.02 mg EAG/g DM, flavonoids of 0.084 ± 0.02 mg EQ/g DM, flavonic aglycones of 0.014 mg EQ/g DM, anthocyanins of 0.018 mg EQ/g DM and condensed tannins of 0.189 mg EC/g DM.In addition, sensitivity tests 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

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 [1]. 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. *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 [**2**]. Leaves and stem bark are used to treat dysmenorrhea and urinary and intestinal schistosomiasis [**3**]. Pharmacological studies carried out on *F. platyphylla* have shown that it has antinocupressive, antimalarial, antibacterial, antifungal, anti-inflammatory and gastrointestinal activities [**4** ;**5].** 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*.

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2.MATERIAL AND METHODS

2.1. Material

2.1.1. Plant material

Ficus platyphylla Del. Holl (FP), were harvested fromDimbokro (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.

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
Acinetobacterbaumannii	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

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). [6-12,13,15]. Toluene/Ethyl acetate /Acetic acid +2 dropsof 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 [7 ; 11].

2.2.4. Total flavonoid content

Total flavonoids were determined using the method of Hariri *and al*[8 ; 11].

2.2.5.Anthocyanins and flavonoid aglycones content

Anthocyanins, flavanols and flavones were measured using Lebreton*and al,* methodology [9].

2.2.6.Condensed tannin content

Condensed tannins were measured using the methodology of Broadhurst and Jones (1978), Heimler *and al* (2006)[10 ; 11].

2.2.7 Antibacterial activity

Antibacterial tests were carried out according to the methodology described by Bredou*and al* (2019) [11].

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.

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). [12, 13].

Table 2.Phytocompounds detected

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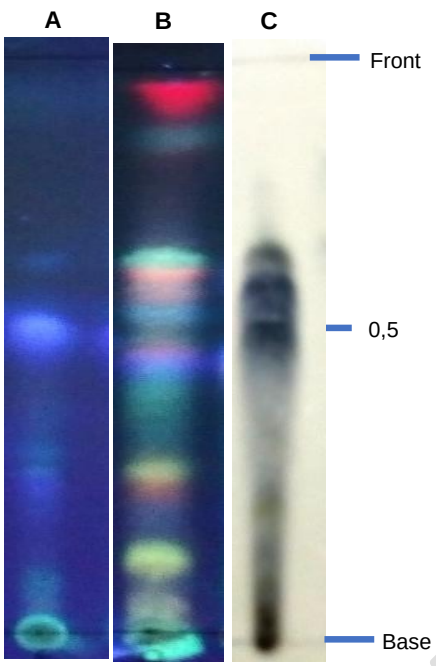
Compounds	Tests	Coloration	Results
Polyphenols	$FeCl_3$	Black	Presence
Flavonoids	Schinoda, KOH (5 %)	Red-orange Yellow	Presence
Coumarins	Lactone cycle	Yellow	Presence
Tannins	$FeCl_3$ Bromine water	Black	Presence
Sterols and polyterpenes	$CH_3CO_3CH_3$ / H_2SO_4	Blue-violet	Presence
Alkaloids	Dragendor'ff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

In addition, the presence of these secondary metabolites was confirmed by TLC with appropriate reagents [12,13,14 ,15,]. 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) [12,13,15]. The TLC results corroborate with those from the color reaction revelation tests.



Extract :	*Ficus platyphylla*
Fig. 1A :	**Developer:** Toluene/ethyl acetate/acetic acid +2 drops ammonia (97/60/15; V/V/V) **Reagent:** KOH (5%) Visualisation: UV 366 nm
Fig. 1B :	**Developer:** Toluene/ethyl acetate/acetic acid +2 drops ammonia (97/60/15; V/V/V) **Reagent:** Neu **Visualisation:** UV 366 nm
Fig. 1 C	**Developer**: Toluene/ethyl acetate/acetic acid +2 drops ammonia (97/60/15 ; V/V/V) **Reagent**: $FeCl_3$ **Visualisation** : visible

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

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

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

3.2. Quantitative analysis

The content of total polyphenols, flavonoids, flavonic aglycones, anthocyanins and condensed tannins are reported in Figure 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).

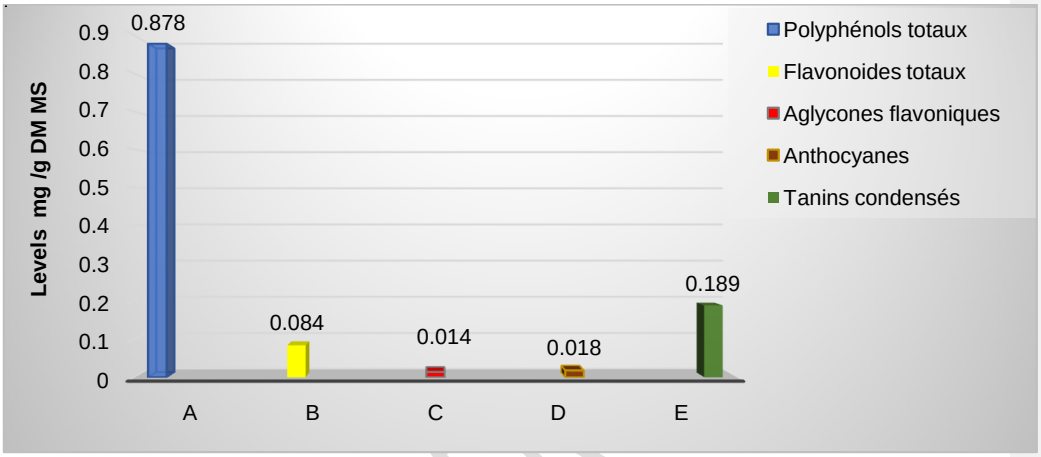

Fig. 2.Contents of total polyphenols (A), total flavonoids (B), flavonic aglycones (C), anthocyanins (D) and condensed tannins.

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.

Table4.Diameter of inhibition zones (mm) of bacterial strains.

Bacterial strains	Strain codes	Concentration FP (mg/mL)				Antibiotic (µg)	
		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

4.Discussion

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Phytochemical screening by color reaction and TLC showed the presence of flavonoids, alkaloids, tannins, sterol coumarins and terpenes in the hydroethanol extract of *F. platyphylla* bark.These results corroborate those obtained by Adeshina et *al.* (2010) [16],but differ from those of Gbogbo et *al.*(2013). 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. [5]**.**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.[17]. 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)[18], grape seeds (7500 µg EAG / g) [19], 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) [20], 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 *et al* (2003), FP is ineffective against multidrug-resistant strains of *P. aeruginosa* et *A. baumannii* [21].This ineffectiveness could be explained by natural resistance or resistance acquired by bacterial strains.

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*.

REFERENCES

1. . World Health Organization, Geneva (02/27/2017). French.
2. . Koné M, Koffi A, Bomisso E, Tra Bi F. Ethnomedical study and iron content of some medicinal herbs used in traditional medicine in Cote d'Ivoire for the treatment of anemia. Afr J Tradit Complement Altern Med. 2012; 9 (1): 81-87.
3. . Sanogo R. Medicinal plants traditionally used in Mali for dysmenorrhea. Afr J Tradit Complement Altern Med. 20118; (5): 90-96. French.
4. . Isma'il S, Amlabu E, Andrew J. Antimalaria Effect of the Ethanolic Stem Bark Extracts of Ficus platyphylla Del. Journal of Parasitology Research. 2011; p. 5. doi:10.1155/2011/618209.
5. . Gbogbo K, Dourma M, Akpavi S, Batawila K, Akpagana K. Evaluation of the antifungal activity of Ficus platyphylla del. (moraceae). European Scientific Journal. 2013; Edition: 9 (33). ISSN: 1857 – 7881.French.
6. . Konan F, Guessennd N, Oussou K. Antibacterial effect of the aqueous extract of the bark of Terminalia glaucescensn Planch ex Benth (combretaceae) on the in vitro growth of extended-spectrum beta-lactamase-producing enterobacteria (EBLSE). Int. Journal Biologic Chemistry Sci. 2014; 8 (3): 1192-1201. French.
7. . Singleton V, Ortofer R, Lamuela-Raventos R. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent; Packer L Methods in enzymology. Orlando Academic Press London.1999; edition 1999, 152-178.
8. . Hariri E, Sallé G, Andary C. Involvement of flavonoids in the resistance of two poplar cultivars to mistletoe (Viscum album L.). Protoplasma. 1991; 162 (1):20-26.
9. . Lebreton P, Jay M, Voirin B. On the qualitative and quantitative analysis of flavonoids. Analytica Chimica Acta Paris. 1967; 49 (7): 375-383. French
10. . Heimler D, Vignolini P, Din M, Vinueri F, Ronani A. Antiradical activity and polyphenol composition of local Brassiccaceae edible varieties. Food Chemistry. 2006; 99:464-469.
11. . Bredou JB, Boua BB, Konan KF. Phytochemical and antibacterial investigations of medicinal plants used in the traditional treatment of urinary infections in Ivory Coast: case of Lannea barteri Engl. (Anarcadiaceae). J.Soc. West-Afr. Chem. 2019 ;047: 8 – 15. French
12. . Ladiguina EY, Safronitch LN, Otriachenkova VE, Balandina IA and Grinkevitch. Chemical analysis of medicinal plants. Moskva, Vischaya Chkola. 1983 edition.
13. . Bekro Y-A, Mamyrbekova-Békro J, Boua B, Tra Bi F and Ehilé E. Ethnobotanical study and phytochemical screening of Caesalpinia benthamiana (Baill.) Herend ET Zarucchi (Caesalpiniaceae). Science & Nature. 2007;4(2):217-225.
14. . Kabran GR, Ambeu N, Mamyrbekova-Békro J, Békro Y-A. Total phenols and flavonoids in organic extracts of ten plants used in traditional therapy for breast cancer in Ivory Coast. European Journal of Scientific Research. 2012; 68 (2): 182-190.

15. . Boua B, Bekro Y-A, Mamyrbekova-Békro J. Phytocompounds of the extracts of four medicinal plants of Côte d'Ivoire and assessment of their antioxidant potential by thin layer chromatography. European Journal of Scientific Research. 2008; 24(2): 219-228.
16. . Adeshina G, Okeke C, Osuagwu N, Ehinmidu J. Preliminary in-vitro antibacterial activities of ethanolic extracts of Ficus sycomorus Linn. and Ficus platyphylla Del. (Moraceae). African Journal of Microbiology Research. 4(8), 2010; 598-601.
17. . Adiko N, Okpekon A, Bony F, Koffi K, Kablan B. Ethnobotanical survey and phytochemical screening of plants used in traditional ophthalmology, listed in the Abidjan markets. J. sci. pharm. biol. 2013;14(1): 10-21. French.
18. . Ahmed Bessas. Biochemical dosage of phenolic compounds in dates and honey harvested in southern Algeria; End of cycle study dissertation; Djillali Liabes University -Sidi Bel Abbes, p 57, 2008.
19. . Popovici C, Saykova I, Tylkowski B. Evaluation of the antioxidant activity of phenolic compounds by reactivity with the free radical DPPH; Technical University of Moldova, Kisinew, Moldova; University of Chemical Technology and Metallurgy, Sofia, Bulgaria. Industrial Engineering Journal. 2009;4:25-39. French.
20. . Akowah G, Zhari I, Norgyati I, Sadikun A. and Khamsah S. The effects of different extraction solvents of varying polarities on polyphenols of Orthosiphon stamineus and evaluation of the free radical-scavenging activity. Food chemistry. 2005; 93(2):311-317.
21. . Ponce A, Fritz R, Del Alle C, Roura S. Antimicrobial activity of essential oil on the native microflora of organic Swiss chard. Lebensmittel-Wissenschaft und Technologic. 2003; 36:679-684.