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Phytochemical analysis and antibacterial activity of extracts from the bark of *Cnestis ferruginea* (Vahl ex De Cantolle)

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Abstract
This study aim is to highlight the antibacterial properties of the aqueous extract of *Cnestis ferruginea* bark from Ivorian flora against multidrug-resistant bacterial strains. Phytochemical composition, dosages, and antibacterial tests were performed to accomplish this. Thus, phytochemical screening showed polyphenols, flavonoids, tannins, coumarins, alkaloids, terpenes and derivatives. Quantitative phytochemical analyses showed the polyphenols flavonoids, flavonics aglycones, anthocyanins and tannins contents were 632±0.03; 9±0.01; 7±0.03±0.03; 12±0.01 and 135±0.02 µg/g respectively. The result of the *in vitro* study showed that *C. ferruginea* extract is ineffective against multidrug-resistant strains of *P. aeruginosa* and *A. baumannii*.

Keywords: Phytochemistry, *Cnestis ferruginea*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*

1. Introduction
Nowadays, the resistance of bacteria to antibiotics used in modern medicine is on the rise However, herbal remedies could be an alternative. Indeed, plants have always been a potential source of antimicrobial compounds and/or inhibitors of bacterial antibiotic resistance mechanisms. Many plant-derived compounds have already demonstrated antimicrobial properties by acting through several mechanisms [1]. This is the case, for example, with Echinaforce®, an antiviral, antibacterial and immunomodulatory product, derived from *Echinacea purpurea Moench* (Asteraceae) [2], The bark of *Cinchona* sp (Rubiaceae) is the source of quinine and its derivatives, while Artemisinin and its derivatives come from *Artemisia annua* (Asteraceae) [3]. The Ivorian flora is full of several plants with pharmacological properties. Among these plants is *Cnestis ferruginea* (Vahl ex De Cantolle) Connaraceae. This plant is widespread in the forests and savannahs of tropical Africa. In Ivory Coast, it is used to treat several diseases including gonorrhea, cystitis, dysmenorrhea and skin infections [4, 5, 6]. The aim of this study is to highlight the antibacterial properties of *C. ferruginea* from Ivorian flora against multidrug-resistant bacterial strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

2. Materials and Methods
2.1. Material
2.1.1. Vegetal material
The bark of *C. ferruginea* (CF), were harvested from Dimbokro (center of Ivory Coast, 6° 39' North, 4° 42' West). They were selected following ethnobotanical surveys conducted among traditional therapists and herbalists in the District of Abidjan. The identification and authentication were carried out at the National Floristic Centre in Abidjan (Identification Code: (MAA 3964). After cleaning, they were dried for 14 days at 18°C and then ground to powder and packaged.

2.1.2. Biological material
Six (06) multidrug-resistant bacterial strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were obtained from the Unit for Antibiotics, Natural Substances and the Surveillance of Microorganisms and Anti-Infective (ASSURMI) of the Department of Bacteriology and Virology of the Pasteur Institute of Ivory Coast (PIIC). The profiles are presented in Table 1.

Table 1: Bacterial Strain Codes and Biologics

Bacterial strains	Codes ASSURMI	Phenotypes
P. 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
A. 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. Extract preparation (CF)

100 g of powder from the bark of *C. ferruginea* were extracted in 1 L of distilled water. The mixture was brought to a boil for 30 minutes at 100° C. The resulting mixture is vacuum-filtered with Büchner. The operation was repeated three times. The collected filtrates are concentrated under the vacuum in a rotary evaporator. They are then dried in an oven at 50° C for tree (03) days to obtain the aqueous extract (CF).

2.2.2. Phytochemical screening

It was performed on CF, using stained reaction detection tests and thin-layer chromatography (TLC) [7, 8, 9, 10]. Toluene / Ethyl Acetate / Acetic Acid + 2 drops of ammonia (9.7/3/0.3; v/v/v) was chosen. The reagents of Liebermann-Bürchard, Dragendor'ff, Neu, solutions of potassium hydroxide (KOH) at 5%, iron chloride (III) ($FeCl_3$) at 2% are the developers we used.

2.2.3. Quantitative phytochemical analysis

2.2.3.1. Polyphenols content

The total polyphenol content was determined using the Folin-Ciocalteu colorimetric method (1999) [11, **Error! Bookmark not defined.**].

2.2.3.2. Total Flavonoid content

The method described by Hariri et *al*. method (1991) [12, **Error! Bookmark not defined.**], were used with modifications.

2.2.3.3. Flavonic, Anthocyanins and Aglycones content

The content of anthocyanins, flavanols and flavones was done according to the methodology of Lebreton *et al.* (1967) [13, **Error! Bookmark not defined.**].

2.2.3.4. Condensed tannin content

Condensed tannins were measured according to the methodology of Broadhurst and Jones, (1978), Heimler et *al*. (2006) [14, **Error! Bookmark not defined.**].

2.2.3. Antibacterial Activity

Antibacterial tests were performed according to the methodology described by Bredou et *al*. (2019) [15].

2.2.4. Statistical analyses

All analyses were performed in triplicate. The analysis of all the data was done using the ANOVA-one way variance of the Origin Pro 9.1 software. The results obtained were expressed as a mean±standard deviation.

3. Results and Discussion

3.1. Phytochemical study

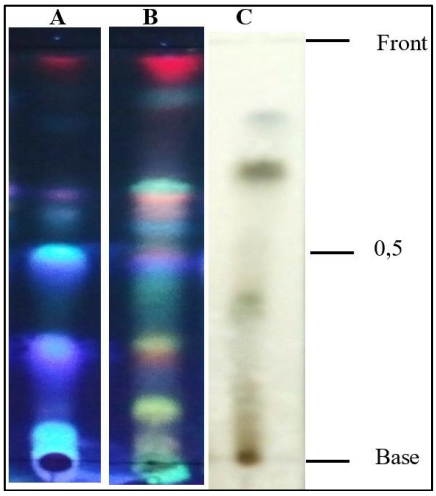
3.1.1. Phytochemical screening

CF has an extractive value of 4.98%. Polyphenols, flavonoids, tannins, coumarins, alkaloids, terpenes and derivatives are the groups of chemical compounds identified by coloured reactions (Table 2). In addition, thin-layer chromatography (TLC) screening, using appropriate reagents [**Error! Bookmark not defined.**-**Error! Bookmark not defined.**; **Error! Bookmark not defined.**] confirmed the presence of these groups of chemical compounds in CF (Table 3). Thus, sulphuric vanillin reveals sterols and terpenes in the visible light in the form of purple, pink and orange spots at Rf = 0.06; 0.44; 0.47; 0.54; 0.64; 0.69; 0.76; 0.77. The 5% (m / V) methanolic solution of KOH was used to detect coumarins at UV / 366 nm, in the form of several blue, green, yellow fluorescent spots at Rf =. 0.11; 0.56; 0.65; 0.79 (Figure 1B). Flavonoids were revealed by Neu's reagent, which stains in the form of spots of variable color intensifying at UV / 366 nm, at Rf = 0.01; 0.04; 0.08; 0.29; 0.55; 0.60; 0.71; 0.94 (Figure 1A). The tannins were revealed by means of a solution of iron III trichloride ($FeCl_3$) which presents them as grey spots in the visible range at Rf = 0.31; 0.48 (Figure 1C) (Table 3). The results of the TLC screening are consistent with those of the stained reactions with the exception of alkaloids. They contradict those made by some researchers. Indeed, Allabi et *al*. (2016) showed the presence of alkaloids, free anthracene derivatives and anthraquinones. In addition to the compounds identified in stem bark except for phenols [16]. Adiko et *al*. (2013) showed an absence of flavonoids and alkaloids in the methanolic extract of the leaves [17]. This difference could be explained by the variability in the composition of the organs of the same plant and the extraction solvents used.

Table 2: Phytocompounds

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
Phenols	$FeCl_3$	No staining	Absence

Anthracenes	Lactone cycle	No staining	Absence



Extract	Decoctate of the bark of *C. ferruginea*
Figure 1A	Mobile phase: Toluene/Ethyl Acetate/Acetic Acid +2 drops Ammonia (97/60/15; V/V/V) Reagent: Neu Visualization: UV 366 nm
Figure 1B	Mobile phase: Toluene/Ethyl Acetate/Acetic Acid +2 drops Ammonia (97/60/15; V/V/V) Reagent: KOH (5 %) Visualization: UV 366 nm
Figure 1 C	Mobile phase: Toluene/Ethyl Acetate/Acetic Acid +2 drops Ammonia (97/60/15; V/V/V) Reagent: $FeCl_3$ Visualization: UV 366 nm

Fig 1: Thin-layer chromatograms of (CF) decoctate from *C. ferruginea* bark.

Table 3: Secondary metabolites detected in the aqueous crude extract of *Cnestis ferruginea* (CF) bark.

EXT	Without developer (a)				Neu (b)				KOH (5%) (c)				$FeCl_3$ (d)		Libermann Büchard (e)				Sulfuric Vanillin (f)	Possible Compounds
	Visible		UV 366		Visible		UV 366		Visible		UV 366		Visible		Visible		UV 366		Visible	
	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	Co	Rf	
CF							yellow	0,01			green	0,00	grey	0,0			j-o	0,0	or	0,0	flav[b], coum[c], tan[d], Terpenes[f,e]
							green	0,04													
			green	0,06															grey	0,06	flavonoids[b], Terpenes[a,f]
							green	0,08													
			blue	0,09													blue	0,09			flavonoids[b]
											blue	0,11									Sterols[a,e]
							blue	0,29													coumarins[c]
													grey	0,31							flavonoids[b]
																			grey	0,44	tannins[d]
			blue	0,45																	Terpenes[f]
															blue	0,47			violet	0,47	NI[a]
													grey	0,48	jaune	0,48					Sterols[e], Terpenes[f]
			green	0,53															Grey	0,54	tannins[d], Sterols[e]
							blue	0,55													Terpenes[a,f]
											green	0,56									flavonoids[b]
																					coumarins[c]
																					NI
			y-g	0,59																	NI[a]
							green	0,60													flavonoids[b]
																					NI
			blue	0,63											violet	0,64			violet	0,64	Sterols[e,a], Terpenes[f,a]
			green	0,65							blue	0,65									coumarins[c]
															blue	0,69			violet	0,69	Sterols[e], Terpenes[f]
			or	0,71			blue	0,71													flavonoids[b,a]
			blue	0,75													blue	0,76	g-v	0,76	Sterols[e,a], Terpenes[f,a]
																			grey	0,77	Terpenes[f]
											green	0,79									coumarins[c]
			green	0,85																	NI
							green	0,94													flavonoids[b]
															blue	0,96			Gr-v	0,96	Sterols[e], Terpenes[f]

CF: 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

3.1.2. Quantitative phytochemical analysis

The content of total polyphenols, total flavonoids, flavonics aglycones, anthocyanins and condensed tannins levels were

showed in Figure 1. The result showed the total polyphenol content to be 632±0.03 10^{-3} mg / g. The total flavonoids content was estimated to be 9±0.01 10^{-3} mg /g, while Flavonics aglyconones and anthocyanins are 7±0.03 10^{-3} mg / g and 12±0.01 10^{-3} mg /g, respectively. The condensed tannins content were 135±0.02 10^{-3} mg /g. If we compare our results with those obtained with certain plants or plant organs known for their richness in phenolic compounds, including grape seeds (7500 µg EAG / g) [18], persil (2802 µg EAG / g) [19], CF has a relatively low content of total polyphenols and total flavonoids.

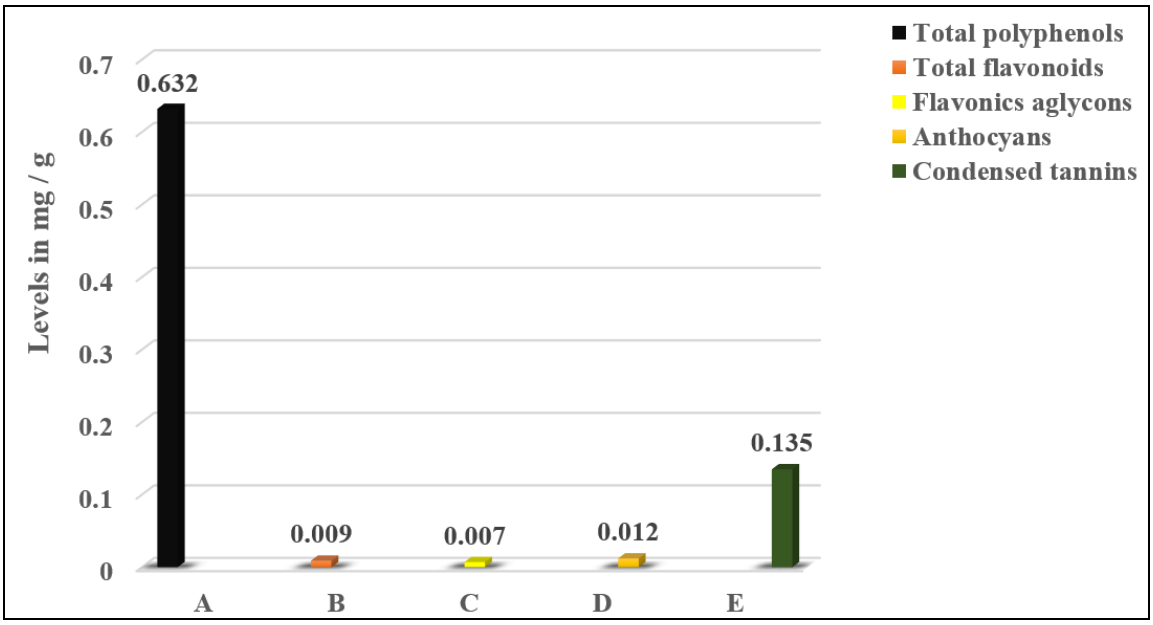


Fig 2: Contents of total polyphenols (A), total flavonoids (B), flavonics aglycones (C), anthocyanins (D) and condensed tannins (E).

3.2. Antibacterial activity

The result obtained from antibacterial activity of CF was assessed by determining the diameters of the inhibition zones of *P. aeruginosa* and *A. baumannii* strains. The range of different concentrations resulted in inhibition zone diameters less than or equal to 8 mm for the two multidrug-resistant bacterial strains compared to the reference antibiotics (Ceftazidime and Ticarcillin) (Table 4). Ponce (2003) found that CF was ineffective against multidrug-resistant strains of *P. aeruginosa* and *A. baumannii* [20]. In addition, this resistance of *P. aeruginosa* and *A. baumannii* to the *C. ferruginea* extract could be explained either by acquired resistance or by natural resistance of bacterial strains [21, 22].

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	7,3±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	6,2±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	6,05±0,30	6±0,0	6±0,00	6±0,00	32±0,0	6±0,00

CAZ: Ceftazidime; **TIC**: Ticarcillin; **Wit**: Witness

4. Conclusion

The objective of this study is to highlight the antibacterial properties of *C. ferruginea* in the Ivorian flora against multidrug-resistant strains of *P. aeruginosa* and *A. baumannii*. To do this, phytochemical sorting and antibacterial tests were carried out. Thus, phytochemical sorting by TLC and color reactions made it possible to identify polyphenols, flavonoids, tannins, coumarins, alkaloids, terpenes and derivatives. As for the dosage, it showed that CF is relatively low in total polyphenols, total flavonoids and condensed tannins with regard to the contents. With respect to antibacterial activity, CF is ineffective against multidrug-resistant strains of *P. aeruginosa* and *A. baumannii*. Nevertheless, the identification of groups of secondary metabolites could justify the use of C. ferruginea in the traditional treatment of pathologies in Ivory Coast.

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