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Comparative Study of Hydroethanolic and Aqueous Extracts of Two Medicinal Plants from Côte d'Ivoire

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ABSTRACT

In order to highlight the antibacterial potential of the barks of *Bauhinia thonningii* and *Cnestis ferruginea* from the Ivorian flora, a new phytochemical and antibacterial investigation was conducted on their hydroethanolic extracts against multidrug-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. However, these extracts were also found to be ineffective. Nevertheless, the phytochemical screening identified polyphenols, flavonoids, tannins, coumarins, alkaloids, and terpenes. Quantification showed that the concentrations of these compounds in the hydroethanolic extracts of the plant species were relatively lower than in the aqueous extracts. However, the identification of these chemical compound groups could justify the use of these plant species in the traditional treatment of certain diseases.

Keywords: *Bauhinia thonningii, Cnestis ferruginea, Pseudomonas aeruginosa, Acinetobacter baumannii*

1. INTRODUCTION

Bauhinia thonningii Schumach & Thonn (Fabaceae) and *Cnestis ferruginea* (Vahl ex De Cantolle) (Connaraceae) are plant species found in the savannas and tropical forests of West Africa [1]. In Côte d'Ivoire, they are mainly found in the northern and central regions. Various parts of *B. thonningii* are used by indigenous populations to treat different ailments. In the north, the trunk bark is used to treat foot-and-mouth disease, the leaves are used to combat diarrhea, dysentery, and cough, while the roots are employed to treat dermatoses [2,3]. The decoction of leafy stems is used to treat cystitis [4]. Furthermore, pharmacological studies have revealed that the leaves of *B. thonningii* possess anti-inflammatory, antiseptic, and antidiarrheal properties [5,6]. Additionally, bark extracts have demonstrated anthelmintic properties [5]. Regarding *C. ferruginea*, its parts are traditionally used to treat gonorrhea, cystitis, dysmenorrhea, and skin infections [7,8,9]. It is also known for its antioxidant, antimicrobial, hypoglycemic, aphrodisiac, analgesic, and anti-inflammatory properties [10,11] Although these plants are commonly used in traditional medicine to treat various infectious diseases, their antibacterial potential remains to be demonstrated. It is within this perspective that Bredou *et al.* conducted investigations to highlight the antibacterial activity of the crude aqueous extracts from the barks of *B. thonningii* and *C. ferruginea* [12,13] The results showed that these extracts were ineffective against multidrug-resistant strains of

Pseudomonas aeruginosa and *Acinetobacter baumannii*. To further investigate their antibacterial potential, a new study was conducted on the crude hydroethanolic extracts of *B. thonningii* and *C. ferruginea*. This study aimed to identify the chemical groups of secondary metabolites present in the extracts through phytochemical screening and to evaluate their antibacterial activity against multidrug-resistant strains of *P. aeruginosa* and *A. baumannii*. Additionally, we compared the hydroethanolic extracts of the two plant species with the previously studied aqueous extracts.

2. MATERIAL AND METHODS

2.1. Material

2.1.1. Plant material

The plant material consists of the barks of *B. thonningii* and *C. ferruginea*, selected following ethnobotanical surveys conducted among traditional healers and herbalists in various markets in the communes of Adjamé and Abobo in the District of Abidjan. The species were identified at the National Floristic Center of Abidjan with the respective identification codes: *B. thonningii* (AA13847; AA15937) and *C. ferruginea* (MAA3964). The barks of *B. thonningii* and *C. ferruginea* were collected in Dimbokro (DD: 5.51358, -3.93072; DMS: 5° 30' 49" N, 3° 55' 51" W) in the N'zi region, located in central Côte d'Ivoire. They were then cleaned, dried at 18°C for 14 days, and pulverized.

2.1.2. Biological material

The biological material consists of multidrug-resistant bacterial strains obtained from the Antibiotics, Natural Substances, and Microorganism Surveillance Unit for Anti-Infectives (ASSURMI) of the Department of Bacteriology and Virology at the Pasteur Institute of Côte d'Ivoire. These include multidrug-resistant strains of *P. aeruginosa* and *A. baumannii*, as presented in Table I.

Table I: Codes and biological products of bacterial strains

bacterial strains	Codes ASSURMI	Phenotypes
P. aeruginosa	19UB/17CNRa	Wild Phenotypes to Carbapenems and Fluoroquinolones; Very Low-Level Cephalosporinases
	151PI/17CNRa	Wild type phenotype to aminoglycosides; High-level penicillinase resistance; Very low-level cephalosporinase resistance
	316CO/17CNRa	Wild type phenotypes to cephalosporins; Cross-resistance to fluoroquinolones
A. baumannii	45LC/17CNRa	Wild type phenotypes to aminoglycosides, carbapenems; Very low-level cephalosporinase resistance; Very low-level penicillinase resistance
	248UB/17CNRa	Carbapenems; Penicillinase; Cephalosporinases; Cross-resistance to ticarcillin and piperacillin
	354UB/17CNRa	Resistance to fluoroquinolones ; Cephalosporinases

2.2. Methods

2.2.1. Hydroethanolic decoctions Preparation

In two round-bottom flasks, each containing 200 ml of ethanol (80%), 20 g of finely powdered plant species were added. Each mixture was topped with a reflux condenser and brought to a boil for 30 minutes. After vacuum filtration, each filtrate was concentrated using a rotary evaporator and then dried in an oven at 50°C for 48 hours to obtain hydroethanolic decoctions of *B. thonningii* (BTH) and *C. ferruginea* (CFH).

2.2.2. Qualitative analysis

Qualitative analysis of the crude BTH and CFH extracts was performed using color reaction tests and thin-layer chromatography (TLC) [14-15,16]. The solvent system used was Toluene/Ethyl acetate/Acetic acid and 2 drops of ammonia (9.7/3/0.3 ; v/v/v). Liebermann-Bürchard reagent, Dragendorff reagent, Neu's reagent, 5% potassium hydroxide (KOH) solution, and 2% ferric chloride ($FeCl_3$) solution were used as detection agents.

2.2.3. Quantitative analysis

2.2.3.1. Total polyphenolic content

Total polyphenol content was determined using the Folin-Ciocalteu colorimetric method [17;21].

2.2.3.2. Total flavonoid content

Total flavonoid content was determined using the modified Hariri *et al.* method [18;21].

2.2.3.3. Anthocyanin and flavonic aglycone content

Anthocyanin, flavanol, and flavone content were determined following the methodology of Lebreton *et al.*, [19;21].

2.2.3.4. Condensed tannin content

Condensed tannin content was determined using the methodology of Broadhurst and Jones, Heimler et *al.* [20;21].

2.3. Antibacterial activity

Antibacterial tests were conducted following the methodology described by Bredou et *al.* [21].

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. Phytochimical study

3.1.1. Phytochimical screening

The phytochemical screening of the crude extracts CFH and BTH, performed using tube reactions, revealed the presence of polyphenols, flavonoids, tannins, coumarins, alkaloids, and terpenes. These results are consistent with those obtained during screening using thin-layer chromatography (TLC) (Table II). Sterols and terpenes were identified using Liebermann-Burchard and sulfuric vanillin reagents [14,16]. Yellow molecular fingerprints visible to the naked eye, revealed by the 5% (w/v) methanolic KOH solution, indicate the presence of coumarins [15]. This coloration may intensify or shift to blue or green under UV light at 365 nm. Tannins appear as gray spots in the visible spectrum when revealed by ferric chloride ($FeCl_3$) solution [15,16]. Variable-colored molecular fingerprints intensifying under UV/366 nm, detected using Neu's reagent, indicate the presence of flavonoids [15]. Dragendor'ff reagent presents alkaloids as orange spots [15,16]. Overall, these results corroborate those obtained by Bredou et al. Indeed, they identified the same groups of compounds in aqueous extracts of the barks of *B. thonningii* and *C. ferruginea* [12.13].

Table II: Secondary metabolites detected in crude hydroethanolic extracts

Extracts		Rf, color, possible compound family
BTH	Visible	0,24 J^e Fl; 0,47 J^e Fl; 0,47 J^e Fl; 0,2 Gr^h Tan; 0,35 Gr^h Tan; 0,62 Gr^h Tan; 0,05 R^d Ter; 0,11 Gr^d Ter; 0,24 V^d Ter; 0,43 Gr^d Ter; 0,60 Vi^d Ter; 0,68 V^d Ter; 0,75 Vi^d Ter; 0,80 V^d Ter; 0,83 Vi^d Ter; 0,88 Gr^d Ter; 0,92 V^d Ter; 0,98 Vi^d Ter; 0,02 Or^g Al; 0,53 Or^g Al; 0,67 Or^g Al; 0,86 O^g Al
	UV 366 nm	0,02 V^e Fl; 0,04 Or^e Fl; 0,05 J^e Fl; 0,06 V^e Fl; 0,14 Or^e Fl; 0,24 J^e Fl; 0,29 V^e Fl; 0,40 Bl^e Fl; 0,47 Or^e Fl; 0,52 V^e Fl; 0,56 J^e Fl; 0,68 V^e Fl; 0,88 V^e Fl; 0,96 R^e Fl; 0,06 Bl^b Cou; 0,13 J^b Cou; 0,30 J^b Cou; 0,34 V^b Cou; 0,42 J^b Cou; 0,52 V^b Cou; 0,58 J^b Cou; 0,65 Or^b Cou; 0,69 B^b Cou; 0,72 J^b Cou; 0,76 V^b Cou; 0,88 Bl^b Cou; 0,96 R^b Cou
CFH	Visible	0,29 J^e Fl; 0,47 J^e Fl; 0,65 J^e Fl; 0,20 J^b Cou; 0,28 V^b Cou; 0,48 J^b Cou; 0,04 Gr^h Tan; 0,5 Gr^h Tan; 0,63 Gr^h Tan; 0,18 Bl^c St; 0,36 Bl^c St; 0,55 Vi^c St; 0,72 J^c St; 0,84 Bl^c St; 0,90 J^c St; 0,96 Vi^c St; 0,2 Vi^d Ter; 0,36 Vi^d Ter; 0,47 Gr^d Ter; 0,55 Vi^d Ter; 0,66 V^d Ter; 0,7 V^d Ter; 0,78 Gr^d Ter; 0,82 Vi^d Ter; 0,06 J^g Al; 0,3 J^g Al; 0,66 J^g Al
	UV 366 nm	0,02 J-V^e Fl; 0,04 Bl^e Fl; 0,06 Bl^e Fl; 0,12 V^e Fl; 0,16 Bl^e Fl; 0,29 Bl^e Fl; 0,31 V^e Fl; 0,47 Or^e Fl; 0,51 B^e Fl; 0,56 V^e Fl; 0,59 Bl^e Fl; 0,66 Bl^e Fl; 0,68 J^e Fl; 0,75 Or^e Fl; 0,04 V^b Cou; 0,12 J^b Cou; 0,20 V^b Cou; 0,31 J^b Cou; 0,36 J^b Cou; 0,43 J^b Cou; 0,48 Or^b Cou; 0,54 V^b Cou; 0,6 Or^b Cou; 0,65 Bl^b Cou; 0,69 Or^b Cou

J/yellow; Vi/violet; Or/orange; Bl/blue; V/green; R/red; J/yellow-green; Br/brown; Gr/gray; St/sterol; Ter/terpene; Fl/flavonoid; Cou/coumarin; Al/alkaloid; Tan/tannins; b/ KOH; c/ Liebermann-Burchard; d/ Sulfuric vanillin; e/ Neu; g/ Dragendorff; h/ $FeCl_3$

3.1.2. Quantitative analysis

Secondary metabolites known for their antibacterial properties were quantified, including total polyphenols, total flavonoids, flavonics aglycones, anthocyanins, and condensed tannins. The results obtained are presented in Figures 1 and 2. In the BTH extract, the total polyphenol content was 0.568 ± 0.03 mg GAE/g DM, while the total flavonoid content was 0.057 ± 0.01 mg QE/g DM. The contents of flavonics aglycones and anthocyanins were 0.009 ± 0.03 mg QE/g DM and 0.01 ± 0.01 mg QE/g DM, respectively. The condensed tannin content was 0.1 ± 0.02 mg ECAT/g DM (Figure 1). In the CFH extract, the total polyphenol content was 0.546 ± 0.02 mg GAE/g DM, and the total flavonoid content was 0.083 ± 0.01 mg QE/g DM. The contents of flavonic aglycones and anthocyanins were 0.008 ± 0.01 mg QE/g DM and 0.016 ± 0.02 mg QE/g DM, respectively. The condensed tannin content was 0.192 ± 0.02 mg ECAT/g DM (Figure 2). Comparing these values to those reported by Bredou et al., the concentrations in aqueous extracts, particularly total polyphenols (CFA: 0.632 ± 0.03 mg/g; BTA: 0.748 ± 0.01 mg/g), were higher than those in hydroethanolic extracts. Additionally, the total flavonoid content in the aqueous extract of *B. thonningii* (BTA: 0.091 ± 0.01 mg/g) was higher than in hydroethanolic extracts. Regarding condensed tannins, the contents in hydroethanolic extracts were similar to those in aqueous extracts (CFA: 0.135 ± 0.02 mg/g; BTA: 0.117 ± 0.02 mg/g). However, these results confirm the presence of compounds with antibacterial properties in these plant species [12,13].

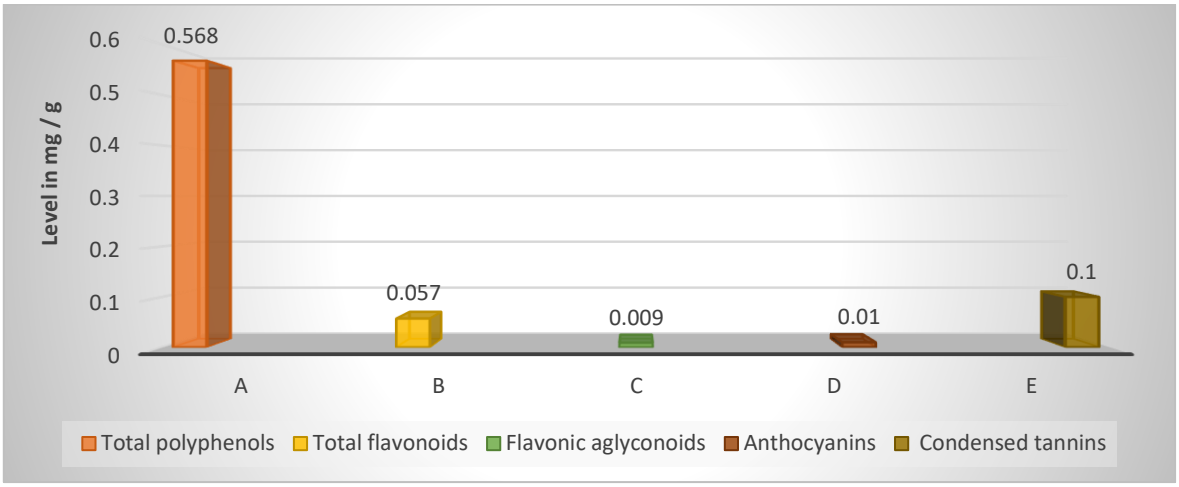

Figure 1: Contents of total polyphenols (A), total flavonoids (B), flavonic aglycones (C), anthocyanins (D), and condensed tannins of BTH.

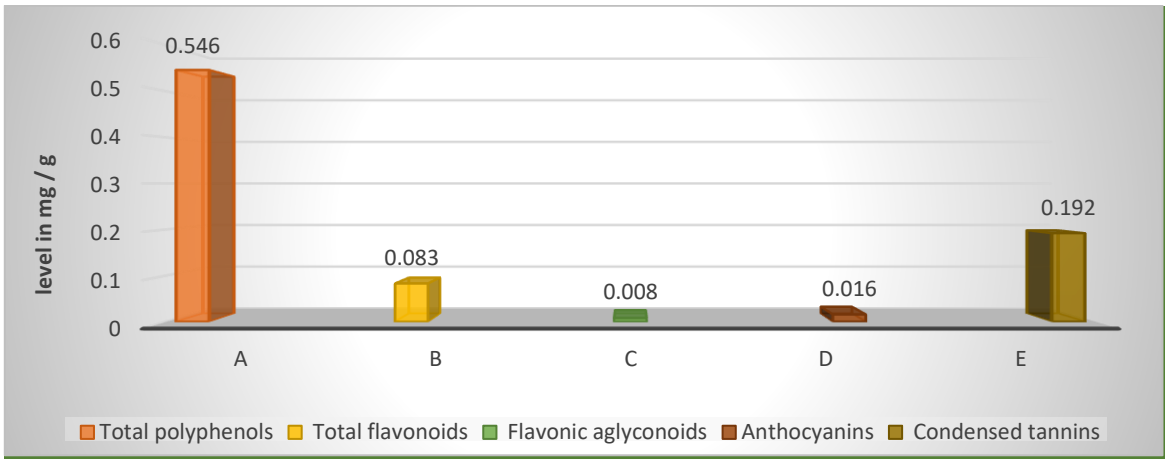

Figure 2: Contents of total polyphenols (A), total flavonoids (B), flavonic aglycones (C), anthocyanins (D), and condensed tannins (E) of CFH.

3.2. Antibacterial tests

The BTH and CFH extracts showed inhibition zone diameters of ≤8 mm against multidrug-resistant strains of *P. aeruginosa* and *A. baumannii* (Table III-IV). According to Ponce (2003), a strain is resistant if the inhibition zone diameter is less than 8 mm, sensitive if the diameter is between 9 and 14 mm, very sensitive if between 15 and 19 mm, and extremely sensitive if greater than 20 mm [22]. Consequently, the BTH and CFH extracts were ineffective against multidrug-resistant strains of P. aeruginosa and A. baumannii. These results align with those obtained for aqueous extracts by Bredou et *al*., [12,13]. Indeed, they demonstrated that aqueous extracts of the two plant species were also ineffective against the same bacterial strains. The ineffectiveness of CFH and BTH extracts could be attributed to acquired or natural bacterial resistance.

Table III: Diameter of inhibition zones (mm) of bacterial strains.

		Concentration BTH (mg/ml)				Antibiotics (µg)	
Bacterial strains	**Strain codes**	**C_1 (100)**	**C_2 (50)**	**C_3 (25)**	**Co**	**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	7±0,53	6±0,0	6±0,00	6±0,00	31±0,21	6±0,70
	316CO/17CNRa	6±0,12	6±0,50	6±0,01	6±0,00	33±1,40	23±0,80
A. baumannii	45LC/17CNRa	7±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; Co: Control

Table IV: Diameter of inhibition zones (mm) of bacterial strains.

		Concentration CFH (mg/ml)				Antibiotics (µg)	
Bacterial strains	**Strain codes**	**C_1 (100)**	**C_2 (50)**	**C_3 (25)**	**Co**	**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±0,03	6±0,0	6±0,00	6±0,00	31±0,21	6±0,70
	316CO/17CNRa	6,2±0,6	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	7,2±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: Ticarcilline; Co: Control

CONCLUSION

The objective of this study was to highlight the antibacterial properties of *B. thonningii* and *C. ferruginea*, two species from the Ivorian flora. To achieve this, phytochemical screenings and antibacterial tests of hydroethanolic extracts against multidrug-resistant strains of *P. aeruginosa* and *A. baumannii* were conducted. The phytochemical screening using tube reactions and TLC identified polyphenols, flavonoids, tannins, coumarins, alkaloids, terpenes, and their derivatives. Quantification showed that the total polyphenol and flavonoid contents in hydroethanolic extracts of *B. thonningii* and *C. ferruginea* were lower than those in aqueous extracts, while condensed tannin contents were similar. The antibacterial activity of hydroethanolic extracts was ineffective against multidrug-resistant strains of *P. aeruginosa* and *A. baumannii*. However, the identification of secondary metabolites could justify the traditional use of *B. thonningii* and *C. ferruginea* in the treatment of diseases in Côte d'Ivoire.

Declaration by Authors

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