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Contribution to the phytochemical, cytotoxic, and antibacterial activity study of aqueous extracts of *Blighia sapida* and *Trichilia emetica* (Côte d'Ivoire)

Assoman Serge Alain Kouakou [1,*], Yao Bertin Kouakou [2], Marius Lébri [3], Charles Bodet [4] and Bakary Coulibaly [5]

[1] *Laboratory of Environmental Sciences and Technologies, Faculty of Environment, Jean Lorougnon Guédé University of Daloa, P.O. Box 150, Daloa, Côte d'Ivoire.*
[2] *Faculty of Agroforestry, Jean Lorougnon Guédé University of Daloa, P.O. Box 150, Daloa, Côte d'Ivoire.*
[3] *Laboratory of Microbiology and Biotechnology, Ecology Research Center, Nangui Abrogoua University, P.O. Box 109, Abidjan 08, Côte d'Ivoire.*
[4] *Laboratory of Inflammation, Epithelial Tissues, and Cytokines (LITEC), UR15560, University of Poitiers, Poitiers, France*
[5] *Enval Group Laboratory, P.O. Box 977 Abidjan 26, Côte d'Ivoire.*

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Abstract

The present study aims to scientifically valorize medicinal plants of West Africa by evaluating the bioactive properties of aqueous extracts of *Blighia sapida* (stem bark) and *Trichilia emetica* (root bark). The extraction yields obtained with distilled water were satisfactory, at 12.96% and 13.26%, respectively. Phytochemical screening revealed a more diversified composition for *T. emetica*, including flavonoids, alkaloids, tannins, and volatile oils, compared to a more restricted composition for *B. sapida*. Cytotoxicity tests conducted on the HaCaT keratinocyte cell line indicated moderate toxicity of the extracts, with IC50 values of 205 µg/mL (EABS) and 140 µg/mL (EATE). Antibacterial assays showed significant activity of T. emetica against Staphylococcus aureus (inhibition diameter: 9.16 mm), with confirmed bactericidal effect (MIC = MBC = 100 µg/mL), while no activity was observed against Pseudomonas aeruginosa. These results suggest that *Trichilia emetica*, due to its chemical richness and biological efficacy, is a promising candidate for the development of natural antimicrobial formulations. The study highlights the interest of an integrated approach for the valorization of local floristic resources for pharmaceutical and therapeutic purposes.

Keywords: *Blighia sapida*; *Trichilia emetica*; Secondary metabolites; Cytotoxicity; Antibacterial activity

1. Introduction

The floristic diversity of tropical zones, particularly in West Africa, constitutes an invaluable treasure for scientific research and the development of innovative solutions in health [1], agri-food, and cosmetic fields. Among local medicinal plants, *Blighia sapida* [2] and *Trichilia emetica* [3] hold a prominent place due to their traditional use in treating various ailments, such as bacterial infections, inflammatory disorders [4], and metabolic pathologies. However, despite their widespread use in traditional medicine [5], their chemical and pharmacological properties remain underexplored in a rigorous scientific context. *Blighia sapida*, known as "ackee" [6], is a fruit tree from the Sapindaceae family, native to West Africa. Traditionally, its different parts, especially the seeds, leaves, and barks, are used for their medicinal virtues [7]. On the other hand, *Trichilia emetica,* belonging to the Meliaceae family, is renowned for its antimicrobial [8] and anti-inflammatory properties, and its oil extracted from seeds is commonly used in body care [9]. In a global context marked by a resurgence of bacterial infections [10-12] resistant to antibiotics and the search for new bioactive molecules from natural sources, the in-depth study of medicinal plants represents a strategic issue. Phytochemical analysis allows detecting secondary metabolites responsible for biological activities, while cytotoxic and antibacterial tests offer an evaluation of the therapeutic and safety potential of plant extracts [13-15]. This study aims to contribute

* Corresponding author: Assoman Serge Alain Kouakou

to elucidating the bioactive properties of *Blighia sapida* and *Trichilia emetica* through a multidisciplinary approach. It will focus on three main axes: phytochemical characterization to identify potential bioactive compounds, an evaluation of their toxicity on model cells to determine their safety, and an analysis of their antibacterial activities to explore their potential as natural alternatives to classical antimicrobial agents. The expected results of this study aim not only to enrich scientific knowledge about these plants but also to open perspectives for their valorization in pharmaceutical and agri-food fields, in Côte d'Ivoire and beyond.

2. Materials and Methods

2.1. Plant Material

The plant material consists of stem barks and root barks, respectively, of *Blighia sapida* and *Trichilia emetica*. These barks were identified by botanists from Jean Lorougnon Guédé University of Daloa and collected in the Haut-Sassandra region (Daloa), Côte d'Ivoire, in February 2024. The barks were cleaned, then air-dried away from sunlight. Finally, they were ground into powder to perform a solid-liquid extraction of secondary metabolites.

2.2. Biological Material

Biological tests were performed on two distinct matrices: HaCaT cells (immortalized human keratinocytes) for cytotoxicity evaluation, and two bacterial strains, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923, for the antibacterial activity study. These matrices were provided by the Inflammation, Epithelial Tissues, and Cytokines Laboratory (LITEC) of the Faculty of Medicine and Pharmacy of the University of Poitiers, France.

The preparation of extracts and phytochemical screening were carried out at the Environmental Science and Technology Laboratory (LSTE) of Université Jean Lorougnon Guédé de Daloa. As for biological tests, they were performed at the Inflammation, Epithelial Tissues, and Cytokines Laboratory (LITEC) of the Faculty of Medicine and Pharmacy of the University of Poitiers, France.

2.3. Preparation of Extracts

The preparation of extracts was carried out according to the maceration procedure described by Sylla et al. (2021) [16]. Fifty (50) g of stem and root barks, respectively, of *Blighia sapida* and *Trichilia emetica* were macerated in 500 mL of distilled water solution for 24 hours. The obtained solution was filtered, and the filtrate was evaporated in an oven at 50°C. The resulting dry extracts constitute the aqueous extracts. The operation was repeated several times to obtain a sufficient quantity of extract for the experiments.

2.4. Phytochemical Analysis

Qualitative phytochemical screening was performed on the aqueous filtrates using the standard method based on coloration and precipitation reactions as described by Houghton and Raman (1998) [17] and modified by Lébri et al., (2015) [18]. Table 1 indicates the different chemical groups sought and the tests for their detection.

Table 1 Detection tests and observations of phytochemical compounds

Phytochemical Compounds	**Detection Test**	**Observation**
Anthocyanins	HCl and 50% NH_3	Red-purple color
Tannins	Ferric chloride	Blue-black color
Catechic Tannins	Stiasny reagent	Pink precipitate
Gallic tannins	Ferric chloride + sodium acetate	Blue tint
Volatile oils	Iodine vapor test	Brown color
Coumarins	Alkaline reaction with NaOH	Intense yellow turning red with acid
Sterols and triterpenes	Liebermann-Burchard reaction	Green or purple color
Cardiac glycosides	Kedde reaction with alkaline dinitrobenzoquinone solution	Violet coloration

Free quinones	NaOH addition	Red, yellow, or violet depending on the type
Anthraquinones	Bornträger test: alkaline extraction with KOH or NH_4OH	Red coloration
Alkaloids	Mayer's reagent / Dragendorff's reagent	Yellow-white or orange-red precipitate
Mucilages	Absolute alcohol test	Flocculent precipitate
Saponins	Foam index test	Significant foam of at least 1 cm
Flavonoids	Shinoda test with magnesium powder	Orange coloration

2.4.1. Evaluation of Cytotoxicity on Skin Cells (HACAT)

Aqueous plant extracts of the studied plants were dissolved in 1% DMSO and PBS to obtain stock solutions with concentrations of 400 µg/mL. Using the double dilution method in liquid medium (PBS solvent), dilutions were made in test tubes, giving the following dilution range: 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.125 µg/mL for each extract. HaCaT cells were seeded in 96-well plates at an appropriate density (4×10^4 cells/well) and incubated for 24 hours to allow adhesion. Plant extracts were added according to the dilution range in wells (according to a plate plan), and cells were incubated for 24 hours. After incubation, the XTT reagent (50 µL/well) was added, and plates were incubated again for 2 hours. Optical density (OD) was measured at specific wavelengths of 492 nm and 620 nm (reference to eliminate background noise) using a microplate spectrophotometer. Tests were performed in triplicate for each concentration, and the average of the three optical measurements was calculated. Formula (1) below was used to calculate cell viability percentages.

(%) Cell Viability = [(DO Treated) - (DO Blank)] / [(DO Untreated) - (DO Blank)] × 100 (1)

From the cell viability percentages, inhibitory concentrations (IC_{50}) of plant extracts were determined graphically, and extracts were classified according to their cytotoxic potential (Table 2).

Table 2 IC_{50} Toxicity Correspondence Table

IC_{50}	**Toxicity**
IC_{50} > 100 µg/mL	Non-toxic
IC_{50} between 10 and 100 µg/mL	Moderately toxic
IC_{50} < 10 µg/mL	Very toxic

2.5. Determination of Antibacterial Activities

2.5.1. Disc Diffusion Method

To determine sensitivity, in sterile Petri dishes, 20 ml of Mueller-Hinton agar were poured and left to rest for 20 minutes. After solidification, 1 ml of bacterial suspension of 10^8 CFU (Colony Forming Unit)/ml was inoculated on the entire surface of each culture medium. Paper disks (diameter: 6 mm) were impregnated with 5 µl of extracts (100 µg/ml) and placed on the surface of the solidified and infected medium. Petri dishes were then incubated at 37°C for 48 hours in an incubator. Bacterial sensitivity to extracts was estimated by measuring the diameter (mm) of the inhibition zone induced by different concentrations around the disks, and each experiment was repeated three times. Cefoxitin and ceftazidime were employed as positive controls (distilled water as a negative control), and bacteria-containing plates were incubated for 24 h at 37°C. Bacterial sensitivity to extracts was estimated by measuring the diameter (mm) of the inhibition zone induced by different concentrations around the disks, and each experiment was repeated three times [19].

2.5.2. Determination of MIC by Dilution in Liquid Medium

Minimum Inhibitory Concentrations (MIC) were determined using the broth microdilution method in Mueller-Hinton, according to protocols described by Dieye et al. (2022) [20]. Aqueous extracts of the studied plants were prepared at an initial concentration of 200 µg/mL. In a sterile 96-well microplate, 100 µL of sterile Mueller-Hinton broth was distributed in each well. Then, 100 µL of each extract at 200 µg/mL was added to the first wells of lines A and B for *Blighia sapida*, and lines E and F for *Trichilia emetica*. After homogenization, a sequential two-fold dilution was

performed by transferring 100 µL of the mixture from one well to the next until reaching a minimum concentration of 3.125 µg/mL. Subsequently, 100 µL of standardized bacterial suspensions at 10^6 CFU/mL (*S. aureus* and *P. aeruginosa*) were added to each well. Control wells were included: Positive controls (lines C and D), containing 100 µL of bacterial suspensions and 100 µL of Mueller-Hinton broth, to verify bacterial growth. Negative control (line G), containing 200 µL of Mueller-Hinton medium, to ensure the sterility of the culture medium. After incubation at 37°C for 24 hours, the lowest concentration showing no visible turbidity corresponded to the Minimum Inhibitory Concentration (MIC).

2.5.3. Determining the Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) is the lowest concentration of substance that leaves at most 0.01% of surviving germs. First, using a calibrated loop at 2 µl, the contents of wells showing no turbidity were sampled and streaked onto Mueller-Hinton agar starting from the MIC well. Streaking was done in parallel stripes 5 cm long on the agar surface (Box B). Then, four successive 1/10th dilutions, ranging from 10^{-1} to 10^{-4}, were performed from the initial inoculum. These dilutions, along with the starting inoculum, were then streaked in 5 cm bands on MH agar plates (Box A). After 24 hours of incubation at 37°C, the number of colonies on the streaks was compared to those on the inoculum count plate (Box A). Thus, the first experimental tube whose number of germs on its streak is less than or equal to that of the 10^{-4} dilution corresponds to the MBC [21]. The MBC/MIC ratio specifies the mode of action of a substance [22-27]. Modes are indicated in Table 3 below.

Table 3 Bacterial power of extracts

MBC/MIC Ratio	Bacterial Power
MBC/MIC ≤ 4	The substance is said to be bactericidal
MBC/MIC > 4	The substance is said to be bacteriostatic

2.6. Statistical Analysis

Statistical analysis was performed using two-way ANOVA to compare the activity of plant extracts with the negative control (distilled water) and positive controls (cefoxitin [FOX] and ceftazidime [CAZ]), according to bacterial species. Results are expressed as mean ± standard deviation. A probability value $P < 0.05$ was considered statistically significant. All analyses were performed using GraphPad Prism 5 software.

3. Results

3.1. Percentage Yields of Aqueous Extracts of *Blighia sapida* and *Trichilia emetica*

The results of the different extraction yields are given in Table 4. Values vary from 6.48 ± 0.01 to 6.63 ± 0.01.

Table 4 Percentage Yields of Aqueous Extracts

Plants	Extracts	Mass (g)	Yields (%)
Bligha Sapida	AEBS	6.48 ± 0.01	12.96
Trichilia emetica	AETE	6.63 ± 0.01	13.26

AEBS: Aqueous Extract of *Blighia sapida*, AETE: Aqueous Extract of *Trichilia emetica*

3.2. Phytochemical Study

The families of compounds tannins, flavonoids, coumarins, mucilages, free quinones, anthraquinones, alkaloids, sterols-triterpenes, saponins, anthocyanins, volatile oils, and cardiac glycosides were studied in the aqueous extracts of *Blighia sapida* and *Trichilia emetica*. The obtained results are recorded in Table 5. In general, all studied compounds were found present in the aqueous extracts.

Table 5 Phytochemical Compounds Identified in Extracts of Studied Plants

Compounds	AEBS	AETE
Anthocyanins	-	-
Tannins	-	+
Catechic Tannins	-	+
Gallic tannins	-	+
Volatile oils	-	+
Coumarins	+	+
Sterols and triterpenes	+	+
Cardiac glycosides	+	+
Free quinones	-	+
Anthraquinones	+	+
Alkaloids	-	+
Mucilages	+	+
Saponins	+	+
Flavonoids	-	+

(+) = Presence (-) = Absence

3.3. Evaluation of Cytotoxicity on Skin Cells (HACAT)

Inhibitory concentrations CI50 were determined using regression curve equations for each studied species (Table 6).

Table 6 IC_{50} Values of Extracts of Studied Plant Species on HACAT

Extracts	Regression Equation	R^2	IC_{50} (µg/mL)
AEBS	y = -0.1514x + 81.076	R^2 = 0.9186	205
AETE	y = -0.2267x + 81.75	R^2 = 0.9607	140

3.4. Study of Antibacterial Activity

3.4.1. Sterility of Plant Extracts

Sterility tests performed showed that all plant extracts presented no signs of contamination as evidenced by the absence of colonies in the various gelled plates after 24 hours of incubation.

3.4.2. Antibacterial Activity in Solid Medium

The results of inhibition diameters are presented in Table 7. The values of aqueous extracts vary from 00 to 8.16 mm, while those of reference antibiotics vary from 00 to 33 mm.

Table 7 Inhibition Zone Diameters (mm) of Plant Extracts, Cefoxitin, and Ceftazidime on Strains of *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853

Bacterial Strains	Concentrations (100 µg/mL)		EDS	Antibiotic (30 µg)
S. aureus ATCC 25923	AEBS	AETE		FOX
	7,00±0,1	9,16±0,13	00±00	33±0,1
P. aeruginosa ATCC 27853	AEBS	AETE		CAZ
	00±00	00±00	00±00	25±0,1

EDS : Control (Sterile Distilled Water), **FOX** : Cefoxitin, **CAZ** : Ceftazidime

3.4.3. Determination of MIC by Dilution in Liquid Medium

Table 8 presents the Minimum Inhibitory Concentration (MIC) of aqueous extracts of *Blighia sapida* and *Trichilia emetica*, respectively, for sensitive bacterial species. EABS (*Blighia sapida*) shows no significant antibacterial activity against either S. aureus or P. aeruginosa. EATE (*Trichilia emetica*) shows moderate antibacterial activity against S. aureus (MIC = 100 µg/mL), but no activity against *P. aeruginosa*.

Table 8 The minimum inhibitory concentrations (MIC) of the aqueous extracts of the stem bark and root bark, respectively, of *Blighia sapida* and *Trichilia emetica*

Concentrations (µg/mL)	**AEBS**		**AETE**	
	S. aureus	***P. aeruginosa***	***S. aureus***	***P. aeruginosa***
100	+	+	-	+
50	+	+	+	+
25	+	+	+	+
12,5	+	+	+	+
6,25	+	+	+	+
3,125	+	+	+	+

(-) Absence of growth and (+) Presence of growth

3.4.4. Antibacterial Power of Extracts

Table 9 Values of Antibacterial Parameters of Plant Extracts on Strains of S. aureus ATCC

Strain	**Extracts**	**MIC (µg/mL)**	**MBC (µg/mL)**	**MBC/MIC**	**Antibacterial Power**
S. aureus ATCC 25923	AETE	100	100	1	Bactericidal
	AEBS	<100	<100	--	--

MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) were determined only for the bacterial strain *S. aureus* ATCC 25923 - the *P. aeruginosa* ATCC 27853 strain did not yield results. The results are presented in Table 9.

4. Discussion

4.1. Extraction Yield and Phytochemical Screening of Extracts

The extraction yields obtained from the barks of *Blighia sapida* (EABS) and *Trichilia emetica* (EATE) are 12.96% and 13.26%, respectively. These values indicate good extractive power of distilled water as a polar solvent, capable of effectively solubilizing hydrophilic secondary metabolites from both plant species. Yields around 10–15% are generally considered satisfactory for crude extracts, especially when dealing with woody matrices like barks [28-29]. However, beyond the quantitative yield, qualitative analysis of the phytochemical profile highlights marked differences between the two extracts. EATE presents a much greater diversity in secondary metabolites than EABS. It contains flavonoids, volatile oils, alkaloids, free quinones, as well as tannins (catechic and gallic), which are totally absent in EABS. These compounds are known for their broad spectrum of biological activities. Flavonoids, for example, have antioxidant, anti-inflammatory, and antimicrobial properties [30]. Alkaloids, on the other hand, are often associated with microbial growth inhibition by interfering with protein or DNA synthesis [31]. The combined presence of saponins, coumarins, sterols, triterpenes, and cardiac glycosides in both extracts suggests a common base of biochemical activities, but the more diversified profile of EATE could explain a more pronounced biological activity in cytotoxic and antibacterial tests [32]. This phytochemical screening thus constitutes a fundamental step in selecting plant extracts for in-depth pharmacological investigations, as it allows establishing a correlation between the richness in bioactive metabolites and the observed effects in vitro [33-34].

4.2. Cytotoxicity and Antibacterial Activities of Extracts

Evaluation of cytotoxicity on the HaCaT keratinocyte cell line reveals an IC_{50} of 205 µg/mL for AEBS and 140 µg/mL for AETE. Although these values indicate some cellular toxicity, they remain acceptable for crude extracts intended for topical use or further purification [35]. The higher toxicity of AETE could be explained by its concentration of active compounds, particularly flavonoids and free quinones, known for their ability to interact with cell membranes or generate oxidative stress. Regarding antibacterial activities, tests conducted on strains of *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 show exclusive sensitivity of the former to both extracts.

AETE presents an inhibition diameter of 9.16 ± 0.13 mm, higher than that of AEBS (7.00 ± 0.1 mm), although both are lower than cefoxitin (33 ± 0.1 mm), the reference antibiotic. Neither extract shows activity against P. aeruginosa, consistent with the intrinsic resistances of this Gram-negative bacterium, often related to its impermeable outer membrane and efflux pump activity [36]. Minimum inhibitory concentration (MIC) tests show that only AETE manages to inhibit the growth of S. aureus at 100 µg/mL. It also displays bactericidal activity (MIC = MBC = 100 µg/mL; MBC/MIC = 1), while AEBS did not allow determination of a precise inhibition threshold. The absence of bacterial growth at high concentration for AETE suggests interesting potential for antimicrobial use, particularly against Gram-positive strains. The more pronounced activity of AETE is certainly linked to its richer composition in specific antimicrobial metabolites (flavonoids, alkaloids, tannins), reinforcing the idea that the biological efficacy of an extract strongly depends on its chemical diversity [37-38]. Cytotoxic and microbiological results converge towards a biological superiority of the extract of *Trichilia emetica*. These observations justify its potential as a candidate for natural antimicrobial formulations, particularly for external use or after purification of its active compounds.

5. Conclusion

This study highlights the bioactive potential of aqueous extracts of two emblematic plant species of the West African pharmacopoeia: *Blighia sapida* and *Trichilia emetica*. Through an integrated approach combining the evaluation of extraction yield, phytochemical screening, cytotoxicity tests, and antibacterial activity analyses, it was possible to thoroughly characterize the properties of these two plants. The obtained results reveal that *Trichilia emetica* has a more diversified phytochemical profile and more pronounced biological activity than *Blighia sapida*. This richness in secondary metabolites – notably flavonoids, alkaloids, and tannins – gives the extract of *T. emetica* better antibacterial efficacy against *Staphylococcus aureus* as well as proven bactericidal activity, despite slightly higher cytotoxicity. Conversely, *B. sapida*, though presenting a more restricted chemical composition, remains an interesting candidate due to its relative innocuousness and the presence of certain bioactive compounds like saponins and cardiac glycosides. In a global context facing the rise of antimicrobial resistance and the need to diversify therapeutic sources, these results confirm the relevance of valorizing endogenous floristic resources through rigorous scientific investigations. They open promising perspectives for the development of natural antimicrobial formulations, particularly for topical use, from *Trichilia emetica*. In summary, this study contributes to strengthening the scientific basis supporting traditional medicine and encourages the sustainable valorization of tropical medicinal plants for both pharmaceutical and agri-food applications. It calls for the continuation of complementary work, including the isolation of active principles, understanding their mechanisms of action, and evaluating their long-term safety.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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