PHYTOCHEMICAL, CYTOTOXIC, AND ANTIBACTERIAL ACTIVITIES OF ETHANOL EXTRACTS OF *BLIGHIA SAPIDA*K.D. KOENIG(SAPINDACEAE)AND *TRICHILIA EMETICA*VAHL (MELIACEAE)EVALUATED BY XTT ASSAY

Abstract

Phytochemical composition, cytotoxicity and antibacterial activity of ethanol extracts study were made from two medicinal plants native to Côte d'Ivoire: *BlighiasapidaK.D.Koenig*(Sapindaceae) and *Trichiliaemetica*Vahl(Meliaceae). These plants are traditionally used to treat various ailments, including skin infections, fevers and parasitic disorders. The extraction yields obtained were 10.88% for *Blighiasapida* (EEBS) and 11.34% for *Trichiliaemetica*(EETE), slightly lower than those reported for aqueous extracts in previous studies. Phytochemical analysis revealed the presence of several bioactive metabolites, such as flavonoids, sterols, triterpenes, cardiac glycosides, and saponins, with specific differences between the two plants. Notably, EEBS is rich in saponins, while EETE contains anthraquinones and coumarins, which are absent in EEBS.

The evaluation of cytotoxicity on immortalized human keratinocyte cells (HACAT) showed that EETE exhibited a more potent inhibitory activity (IC_{50} = 131 µg/mL) compared to EEBS (IC_{50} = 183 µg/mL). This difference was attributed to the specific anthraquinones and coumarins present in EETE. These results suggest promising therapeutic potential for *Trichiliaemetica*, particularly in the treatment of hyperproliferative diseases or certain cancers.

Regarding antibacterial activity, both extracts demonstrated significant efficacy against Staphylococcus aureus, with slightly higher activity observed for EETE, as confirmed by inhibition zone diameter tests and minimum inhibitory concentrations (MICs). However, no notable effect was observed against *Pseudomonas aeruginosa*, likely due to the intrinsic resistance mechanisms of this bacterium, including its protective biofilms and efflux pumps.

This study highlights the chemical diversity and pharmacological properties of ethanol extracts from *Blighiasapida* and *Trichiliaemetica*. It also underscores the importance of carefully selecting the extraction solvent based on the target metabolites. The findings open new avenues for identifying and clinically exploiting the bioactive compounds present in these plants, particularly for antimicrobial and anticancer applications.

Keywords:*Blighiasapida*, *Trichiliaemetica*, Ethanol extracts, Cytotoxicity, Antibacterialactivity

Introduction

Medicinal plants constitute a valuable source of bioactive compounds traditionally used to treat numerous conditions [1-2]. In West Africa, and more specifically in Côte d'Ivoire, traditional pharmacopeia relies on a wide variety of plants, some of which exhibit therapeutic potential that remains largely unexplored [3-4]. Among these plants, *Blighiasapida* (Sapindaceae), locally known as akpi or ackee, is widely used to treat skin infections, fevers, and certain inflammatory disorders [5, 6]. Similarly, *Trichiliaemetica* (Meliaceae) is traditionally employed for its purgative, antiparasitic, and antimicrobial properties [7-8]. Phytochemical analysis serves to identify groups of secondary metabolites responsible for the observed biological activities, such as flavonoids, alkaloids, saponins, or tannins, which may play an essential role in antimicrobial or cytotoxic activity [9-10]. These secondary metabolites are often extracted using solvents such as ethanol or distilled water, whose efficiency depends on their polarity and ability to solubilize specific compounds. For example, distilled water is optimal for extracting hydrophilic metabolites like polysaccharides and glycosides, while ethanol excels in extracting amphiphilic or lipophilic compounds, such as flavonoids and limonoids [11-12]. In a context where increasing microbial resistances and the limitations of conventional treatments pose serious challenges, the search for new active natural substances becomes crucial [13-14].

The XTT test (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) is a reliable and sensitive colorimetric method used to evaluate the metabolic activity of living cells, whether eukaryotic or prokaryotic. In human cells, such as immortalized keratinocytes (HaCaT), this test allows measuring cytotoxicity induced by foreign substances [15]. In a microbiological context, the same test can assess the viability of metabolically active bacteria, making it a relevant tool for testing the antibacterial activity of natural substances [16]. This study aims to characterize the phytochemical composition of ethanol extracts of *Blighiasapida* and *Trichiliaemetica*, two Ivorian medicinal species, and to evaluate their cytotoxic effects on HaCaT cells as well as their antibacterial activity against pathogenic strains using the XTT assay. The results obtained will provide valuable information on the

biological properties of these plants and could contribute to the identification of new natural therapeutic agents.

Materials and Methods

Biological Material

VegetableMaterial: Bark from stems and roots of *Blighiasapida* and *Trichiliaemetica* were identified and collected by botanists from the University Jean Lorougnon Guédé in Daloa, Haut-Sassandra region, Côte d'Ivoire, in February 2024. After cleaning and grinding, the samples were air-dried and stored away from sunlight for solid-liquid extraction of secondary metabolites.

Cellular Material: Immortalized human keratinocyte cells (HaCaT).

Bacterial Material: Two bacterial strains: *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923.

Cellular and bacterial materials were provided by the Inflammation, Epithelial Tissues, and Cytokines Laboratory (LITEC) at the Faculty of Medicine and Pharmacy, University of Poitiers, France.

Methods

Preparation of Extracts

50 grams of stem and root bark from *Blighiasapida* and *Trichiliaemetica* were macerated in 250 mL of ethanol for 24 hours. After filtration, the filtrates were evaporated at 50°C under reduced pressure to obtain dry ethanol extracts.

Phytochemical Screening

The ethanol extracts of each plant were subjected to phytochemical screening using standard methods described by Houghton and Raman (1998) [17], modified and used by Assoman et al. (2025) [18] (Table 1). Phytochemical tests were conducted at the Laboratory of Environmental Sciences and Technologies (LSTE) of the University Jean Lorougnon Guédé in Daloa.

Table 1. Detection Tests and Observations of Phytochemical Compounds

Metabolites	Test for identification	Positive results
Alkaloids	Mayer's reagent	Yellow-white precipitate

Anthocyanins	HCl and 50% NH_3	Red-purple color
Sterols and triterpenes	Liebermann-Burchard reaction	Green or purple color
Flavonoids	Shinoda test with magnesium powder	Orange coloration
Anthraquinones	Bornträger test: alkaline extraction with KOH or NH_4OH	Red coloration
Saponins	Foam index test	Significant foam of at least 1 cm
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
Cardiac glycosides	Kedde reaction with alkaline dinitrobenzoquinone solution	Violet coloration
Mucilages	Absolute alcohol test	Flocculent precipitate
Free quinones	NaOH addition	Red, yellow, or violet

Cytotoxicity Tests on HaCaTCells

The tests were performed at the LITEC laboratory, affiliated with the Faculty of Medicine and Pharmacy, University of Poitiers, France.

Ethanol extracts were dissolved in DMSO (1%) to prepare stock solutions at a concentration of 1 mg/mL. Serial dilutions were prepared in PBS to obtain the following concentrations: 200, 100, 50, 25, 12.5, and 6.125 µg/mL.For the XTT assay, HaCaT cells were seeded in 96-well plates at a density of 4×10^4 cells/well and incubated for 24 hours to allow adhesion. Different concentrations of the extracts were then added according to a plate layout, and the cells were incubated for an additional 24 hours. After incubation, the XTT reagent was added, and the plates were incubated again for 2 hours. Optical density (OD) was measured at a specific wavelength of 492 nm, with a reference at 620 nm (to eliminate background noise), using a microplate spectrophotometer. Tests were conducted in triplicate for each concentration, and the mean of the three optical measurements was calculated. Cell viability (%) was calculated using the formula:

(%) Viability = [(OD Treated) - (OD Blank)] / [(ODUntreated) - (OD Blank)] × 100

The inhibitory concentrations (IC50) of the plant extracts were determined graphically from dose-response curves, and the extracts were ranked according to their cytotoxic potential.

Determination of Antibacterial Activity

Preparation of Inoculum

Standardized bacterial suspensions of Pseudomonas aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 25923 were prepared in Mueller-Hinton broth. The suspensions were incubated in an orbital shaker incubator at 26°C ± 1°C for 24 hours. They were then adjusted to an optical density corresponding to 0.5 McFarland, which is approximately equivalent to 1.5×10^8 colony-forming units (CFU)/mL.

Disc Diffusion Method

To evaluate bacterial sensitivity to the tested extracts, 20 mL of Mueller-Hinton agar was poured into sterile Petri dishes and allowed to solidify completely after 15-20 minutes. Once solidified, standardized bacterial suspensions at an optical density of 0.5 McFarland (~1.5×10^8 CFU/mL) were uniformly inoculated onto the medium surface. Filter paper discs (diameter 6 mm) impregnated with 5 µL of extract (concentration 100 µg/mL) were placed on the infected medium, and the Petri dishes were incubated at 37°C for 48 hours. Sensitivity was determined by measuring the diameter of the inhibition zones around the discs using a caliper. Positive controls (cefoxitin, ceftazidime) and negative controls (distilled water) were included, and each experiment was repeated three times to ensure reliability of the results.

Determination of MIC by Liquid Medium Dilution

Minimum Inhibitory Concentrations (MIC) were determined using the modified microdilution method in Mueller-Hinton broth by Assoman et al. [8]. In a 96-well microplate, each well received 100 µL of sterile Mueller-Hinton broth (except for wells in the last row). Then, 100 µL of the extract, resuspended in 1% DMSO at the highest concentration (200 µg/mL), was added to the first well. Columns 1 to 6 and 7 to 12 were reserved respectively for *Blighiasapida* and *Trichiliaemetica*. A serial dilution at a 1:2 ratio was performed to obtain a range of concentrations from 3.125 to 100 µg/mL. Finally, 100 µL of bacterial suspensions at 0.5 McFarland were added to the wells according to the previously established distribution. Control wells were included (row H): H1-H3 contained 100 µL of Mueller-Hinton broth and 100 µL of bacterial suspension for the Pseudomonas aeruginosa strain (positive control), and H4-H6 contained 100 µL of Mueller-Hinton broth and 100 µL of bacterial suspension for the Staphylococcus aureus strain (positive control). H7-H9 contained 200 µL of Mueller-Hinton broth (negative control). After incubation at 37°C for 24 hours, bacterial growth was evaluated by observing turbidity. Absence of turbidity indicated

bacterial inhibition, while opacity indicated growth. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of extract preventing visible growth.

Microplate Growth Inhibition Test

Each well of a round-bottom 96-well plate received 100 µL of Brain Heart Infusion (BHI). Plant extracts were initially added at a concentration of 400 µg/mL, distributed as follows: columns 1 to 3 and 4 to 6 received 100 µL of ethanol extract of *B. sapida*, while wells 7 to 9 and 10 to 12 received 100 µL of ethanol extract of *T. emetica* (resulting in a concentration of 200 µg/mL in these wells). This organization was chosen to facilitate reading and reproducibility of the results. A manual serial dilution was then performed to obtain a range of concentrations from 3.125 to 200 µg/mL. Subsequently, 100 µL of bacterial suspensions prepared in BHI (1.5×10^8 CFU/mL) were added to the wells according to the pre-established distribution, except for the wells in row H, which served as positive controls for evaluation (BHI + bacterial inoculum without extract, 100 µL each). The plate was incubated for 4 hours at 37°C before adding XTT (a mixture of 5 mL of XTT and 0.1 mL of electron coupling agent). After a second incubation for 2 hours at 37°C, optical densities (OD) were read at 492 nm, with background correction at 620 nm, using a Tecan Infinite F50 microplate reader controlled by Magellan™ software. The percentage of bacterial inhibition (%) was calculated using the following equation (2):

(%) Inhibition = [(OD Control - ODSample) /ODControl]x 100 (2)

Statistical Analysis

Data were analyzed using GraphPad Prism 5 and expressed as mean ± standard deviation (SD), with graphical representation as mean ± SEM. After verifying data normality, a two-factor ANOVA followed by a post-hoc test was used to compare antibacterial and cytotoxic activities of the extracts. For phytochemical activities, a one-factor Student's t-test was used depending on the number of groups compared. Differences were considered significant for $p < 0.05$.

Results

Extraction Yields of Ethanol Extracts of *Blighiasapida* and *Trichiliaemetica*

The extraction yields obtained are expressed as a percent **(%)** and presented in Table 2

Table 2. Percentage Yields of Ethanol Extracts

Plants	Extracts	Mass (g)	Yields (%)
BlighiaSapida	EEBS	5,44± 0,32	10,88
TrichiliaEmetica	EETE	5,67± 0,27	11,34

EEBS: Ethanol Extract of *BlighiaSapida*,EETE: Ethanol Extract of *TrichiliaEmetica*

Phytochemical Screening

The results of the phytochemical screening are summarized in Table 3. In general, all compounds studied were found in the ethanol extracts, except for anthocyanins and volatile oils, which were absent from both extracts.

Table 3. Phytochemical Compounds Identified in the Extracts of Studied Plants

SecondaryMetabolites	EEBS	EETE
Flavonoides	+	+
Anthocyanins	-	-
Anthraquinones	-	+
Volatile oils	-	-
Sterols and triterpenes	+	+
Coumarins	-	+
Free quinones	+	+
Mucilages	+	+
Saponosides	+	-
Catechic Tannins	+	+
Gallic tannins	+	+
Alkaloids	+	-
Cardiac glycosides	+	+

(+) = Presence (−) = Absence

Evaluation of Cytotoxicity on Keratinocytes (HaCaT)

The IC50 values of the plant extracts were determined using regression equations for each species (Table 4)

Tableau 4.CI50 Values of Plant Extracts on HACAT Cells

Extracts	Regressionequation	R^2	IC_{50}
EEBS	y = -0,1107x + 70,271	R^2 = 0,9224	183
EETE	y = -0,1861x + 74,408	R^2 = 0,9329	131

EEBS: Ethanol Extract of BlighiaSapida , EETE : Ethanol Extract of TrichiliaEmetica

Study of Antibacterial Activity

Sterility Test of Plant Extracts

Sterility tests demonstrated that all plant extracts were free of contamination, as evidenced by the absence of visible colonies on agar plates after 24 hours of incubation.

Antibacterial Test in Solid Medium

Table 5 presents the inhibition zone diameters induced by the ethanol extracts of B. sapida and T. emetica . The diameters ranged from 0 to 12.87 mm, while those of the reference antibiotics ranged from 0 to 33 mm.

Table 5. Inhibition Zone Diameters (mm) of Plant Extracts, Cefoxitin, and Ceftazidime on P. aeruginosa and S. aureus Strains

BacterialStrains	**Concentrations (100 µg/mL)**		**Tem (EDS)**	**Antibiotic(30 µg)**
*P. aeruginosa*ATCC27853	**EEBS**	**EETE**		**CAZ**
	00±00	00±00	00±00	25±0,1
S. aureus ATCC 25923	**EEBS**	**EETE**		**FOX**
	8,66 ±0,83	12,87±1,0	00±00	33±0,1

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

Determination of MIC by Liquid Medium Dilution

The Minimum Inhibitory Concentrations (MIC) of the ethanol extracts of *Blighiasapida* and *Trichiliaemetica* are indicated in Table 6. The MICs are, respectively, 100 and 50 µg/mL for EEBS and EETE against S. aureus. The tests on P. aeruginosa did not yield conclusive results for both extracts.

Table 6: The minimum inhibitory concentrations (MIC) of the ethanolic extracts of the stem bark and root bark, respectively, of *BlighiaSapida*and *TrichiliaEmetica*

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

MicroplateGrowth Inhibition Test

The results showed that the ethanol extracts of *Blighiasapida* (EEBS) and *Trichiliaemetica* (EETE) inhibit the growth of S. aureus, but not P. aeruginosa.

Table7: Inhibition (%) at different concentrations of *B.Sapida*and *T.Emetica*

Extracts	**Strains**	**200**	**100**	**50**	**25**	**12,5**	**6,25**	**3,125**
EEBS	S. aureus	73,29 ± 1,17	67,96 ± 0,84	53,37 ±1,05	48,24 ± 1,12	48,18 ±1,34	47,6 ± 1,15	47,34± 1,08
EETE		76,28 ± 0,90	71,77 ± 0,39	67,17 ± 0,79	57,21± 0,17	62,04 ± 0,34	60,81 ± 0,57	58,42 ± 0,96
EEBS	P. aeurignosa	2,44 ±0,1	2,33 ±0,03	2,0 ±0,12	1,76 ±0,08	1,17 ±0,03	0,91 ±0,11	0,78 ±0,07
EETE		2,58 ±0,59	2,36 ±0,77	2,33 ±0,46	2,03 ±0,38	1,09 ±0,08	1,01 ±0,1	0,86 ±0,05

Discussion

1-Extraction Yield and Phytochemical Screening of Extracts

The extraction yields obtained for the ethanol extracts of *Blighiasapida* (EEBS) and *Trichiliaemetica*(EETE) (Table 2), respectively 10.88% and 11.34%, show a slight superiority for *Trichiliaemetica*. However, these values remain lower than those obtained with aqueous extracts in previous studies by Assoman et al. (2025) [18], who reported values of 12.96% and 13.26%. This difference highlights the crucial influence of the solvent on the extraction of secondary metabolites. Distilled water, highly polar, is optimal for extracting hydrophilic metabolites such as polysaccharides and glycosides, while ethanol excels in extracting amphiphilic or lipophilic compounds, such as flavonoids and limonoids. These observations are well documented by Silva et al. (2019) [19] and Kumar et al. (2022) [20], who respectively demonstrated the capacity of water to extract polysaccharides and ethanol to efficiently isolate limonoids. Beyond quantitative yields, qualitative analysis reveals marked differences between the two ethanol extracts (Table 3). EEBS and EETE share some common metabolites, notably sterols, triterpenes, and cardiac glycosides, known for their antimicrobial and antioxidant properties. This observation is consistent with our previous work [18] and that of Kaur et al. (2020) [21], reinforcing the validity of the methods used. However, each plant presents distinct chemical specificities:EEBS (*Blighiasapida*) stands out for its richness in saponins, indicating a strong presence of hydrophilic polysaccharides. This observation is interesting because it underscores the potential role of these compounds in the plant's natural defense mechanisms.

EETE (*Trichiliaemetica*), on the other hand, is characterized by the presence of anthraquinones and coumarins, involved in laxative and antimicrobial activities. These results confirm the work of Moshi et al. (2020) [22], who highlighted the pharmacological properties of anthraquinones in this species.The notable absence of anthocyanins and volatile oils in the ethanol extracts could be attributed to several factors:Low concentration of these metabolites in the analyzed parts,Incomplete extraction methods,Intrinsic preference of the solvents used for certain classes of metabolites. However, previous studies [19-20] show that distilled water is more effective for extracting volatile oils and mucilages, while ethanol favors amphiphilic or lipophilic metabolites. These results highlight the chemical diversity between *Blighiasapida* and *Trichiliaemetica*, emphasizing the importance of carefully selecting the solvent based on the target metabolites. Binary solvent mixtures, as proposed by Martins et al. (2021) [23], could improve yields while preserving metabolite diversity.

2-Cytotoxicity and Antibacterial Activities of the Extracts

Table 4 reveals that the ethanol extract of *Trichiliaemetica* (EETE) exhibits stronger inhibitory activity on HaCaT cells (IC_{50} = 131 µg/mL) compared to that of *Blighiasapida* (EEBS, IC_{50} = 183 µg/mL), indicating greater efficacy in limiting cell proliferation. This trend is consistent with that observed by Assoman et al. with the aqueous extracts of these plants on HaCaT cells. This difference can be attributed to the specific anthraquinones and coumarins in EETE, known for their cytotoxic properties [24-26]. High determination coefficients ($R^2 > 0.92$) validate the robustness of the data and the observed dose-dependent relationship. These results suggest promising therapeutic potential for *Trichiliaemetica*, particularly in applications such as hyperproliferative skin diseases or certain cancers, while *Blighiasapida*, although less active, remains relevant due to its flavonoids and tannins [27-28]. These observations underscore the need to identify the bioactive compounds responsible and evaluate their mechanism of action to optimize clinical exploitation.The results in Table 6 show selective inhibitory activity and marked differences between the two extracts and target strains. Against S. aureus, both extracts demonstrate antibacterial activity, with slightly better efficacy for EETE. This observation is consistent with the results obtained in the inhibition zone diameter test (Table 5), where EETE generated a larger inhibition zone (12.87 ± 1.0 mm) than EEBS (8.66 ± 0.83 mm). These results corroborate, furthermore, the observations made by Assoman et al. [18], where no inhibitory effect was observed for the two aqueous extracts against P. aeruginosa. The absence of activity or limited activity can be attributed to the complex defense mechanisms of this bacterium, such as its intrinsic resistance, protective biofilms, and efflux pumps that expel potentially toxic molecules [29-30]. Additionally, the compounds present in the extracts may not effectively target the essential biological pathways of P. aeruginosa, unlike their actions against S. aureus.

Finally, the results in Table 7 highlight selective and dose-dependent inhibitory activity of the extracts, with significant differences between the two target strains. The ethanol extracts of *Blighiasapida* (EEBS) and *Trichiliaemetica* (EETE) exhibit maximum inhibitions of 73.29 ± 1.17% and 76.28 ± 0.90%, respectively, at a concentration of 200 µg/mL, with efficacy decreasing progressively at lower concentrations. These results confirm the antibacterial activity of both extracts against S. aureus, with slightly better efficacy for EETE. This trend is consistent with the previous data obtained in the inhibition zone diameter test (Table 5) and the MICs (Table 6), where EETE also demonstrated stronger activity. Conversely, against P. aeruginosa, both extracts show very low inhibitory activity, confirming their ineffectiveness

against this bacterium. These values corroborate the observations made in previous tests (Tables 5 and 6), where no significant effect was observed against P. aeruginosa.

Conclusion

This study characterized the phytochemical, cytotoxic, and antibacterial properties of ethanol extracts of *Blighiasapida* and *Trichiliaemetica*(two Ivorian medicinal plants used in traditional medicine). The results show that these extracts exhibit significant chemical diversity, with specific bioactive compounds for each species: saponins in *Blighiasapida* and anthraquinones and coumarins in *Trichiliaemetica.* These secondary metabolites are essential in biological activities observation.The ethanol extract of *Trichiliaemetica* (EETE) proved particularly active, both cytotoxically and antibacterially. With an IC50 of 131 µg/mL on HaCaT cells, it demonstrates promising therapeutic potential against hyperproliferative diseases or certain cancers. On the other hand, the extract of *Blighiasapida* (EEBS), although less potent, remains relevant due to its flavonoids and tannins, known for their antioxidant and antimicrobial properties.In terms of antibacterial activity, both extracts showed significant efficacy against Staphylococcus aureus, but no notable activity against Pseudomonas aeruginosa. This selectivity could be attributed to the complex defense mechanisms of the latter, including its protective biofilms and efflux pumps.

These results emphasize the importance of selecting the appropriate extraction solvent to maximize yield and target the desired metabolites. They also open promising perspectives for identifying and valorizing the bioactive compounds present in these plants, particularly for human health applications. However, further research is needed to isolate the main active compounds, elucidate their mechanisms of action, and evaluate their safety and efficacy in vivo. These studies could contribute to the development of new natural therapeutic agents to address the challenges posed by microbial resistance and chronic diseases.

Compliance with Ethical Standards

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