



### RESEARCH ARTICLE

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

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### Abstract

Phytochemical composition, cytotoxicity and antibacterial activity of ethanol extracts study were made from two medicinal plants native to Côte d'Ivoire: *Blighia sapida* K.D. Koenig (Sapindaceae) and *Trichilia emetica* 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 *Blighia sapida* (EEBS) and 11.34 % for *Trichilia emetica* (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 ( $IC_{50} = 131 \mu\text{g/mL}$ ) compared to EEBS ( $IC_{50} = 183 \mu\text{g/mL}$ ). This difference was attributed to the specific anthraquinones and coumarins present in EETE. These results suggest promising therapeutic potential for *Trichilia emetica*, 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 *Blighia sapida* and *Trichilia emetica*. 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.

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## Introduction:-

Medicinal plants play a fundamental role in traditional healthcare systems, particularly in West Africa, where they serve as a privileged source of bioactive molecules used for generations to treat a wide array of ailments [1]. In Côte d'Ivoire, traditional pharmacopeia relies on a rich botanical diversity, yet many locally used species remain scientifically underexplored despite their widespread ethnomedicinal application [2]. Among these, *Blighia sapida* K.D. Koenig (Sapindaceae), locally known as akpi, is traditionally employed for its antipyretic, antimicrobial, and anti-inflammatory properties [3,4], while *Trichilia emetica* Vahl (Meliaceae) is commonly used as a purgative, antiparasitic agent, and remedy for skin infections [5,6].

The therapeutic efficacy of these plants is frequently attributed to the presence of secondary metabolites—including flavonoids, tannins, saponins, alkaloids, and terpenoids—whose biological activities are strongly influenced by the extraction solvent used [7,8]. Polar solvents such as water preferentially extract hydrophilic compounds like polysaccharides and glycosides, whereas organic solvents like ethanol demonstrate superior efficiency in recovering amphiphilic or lipophilic constituents, such as aglycone flavonoids, limonoids, and coumarins [9,10]. Consequently, solvent selection is a critical factor in optimizing extraction yield and targeting specific pharmacological activities [11].

In the context of rising global antimicrobial resistance and the limitations of conventional therapies, the exploration of natural products derived from local flora has emerged as a promising strategy for identifying novel therapeutic agents [12,13]. Among the tools available for evaluating the biological activity of plant extracts, the XTT assay [2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide] stands out for its sensitivity and reliability [14]. This colorimetric assay measures the metabolic activity of viable cells—both eukaryotic (e.g., human immortalized keratinocytes, HaCaT) and prokaryotic (bacteria)—thereby enabling simultaneous assessment of cytotoxicity and antibacterial potential [15,16].

This study aims to characterize the phytochemical composition of ethanol extracts of *Blighia sapida* and *Trichilia emetica*, two important medicinal plant species of the Ivoirian pharmacopeia, and to evaluate their cytotoxic effects on HaCaT keratinocytes as well as their antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, using the XTT assay. The findings may provide scientific validation for their traditional uses and contribute to the discovery of new natural compounds with therapeutic potential.

## Material and Methods:-

### Biological Material:

**Plant Material:** Stem and root barks of *Blighia sapida* and *Trichilia emetica* were collected in February 2024 from the Haut-Sassandra region (Daloa, Côte d'Ivoire) by botanists from Jean Lorougnon Guédé University. Following taxonomic identification, samples were cleaned, ground, and air-dried under indirect sunlight prior to extraction.

**Cellular Material:** Human immortalized keratinocytes (HaCaT cells) were used for cytotoxicity assays.

**Bacterial Material:** Two reference bacterial strains were employed: *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923. The cellular and bacterial materials were provided by the Laboratory of Inflammation, Epithelial Tissues and Cytokines (LITEC), University of Poitiers, France.

## Methods:-

### Preparation of Extracts:

Fifty grams of plant powder from each species were macerated in 250 mL of ethanol for 24 hours under moderate agitation. After filtration through Whatman No. 1 filter paper, the filtrates were concentrated by vacuum evaporation at 50 °C until a dry residue was obtained. The ethanol extracts were stored at 4 °C until further analysis.

### Phytochemical Screening:

The detection of major secondary metabolite groups (flavonoids, tannins, saponins, alkaloids, etc.) was carried out according to the protocols described by Houghton and Raman (1998) [17], with modifications based on the methods of Assoman et al. (2025) [18]. All tests were performed at the Laboratory of Environmental Sciences and Technologies (LSTE), University of 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 <sub>3</sub>	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 <sub>4</sub> OH	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 HaCaT Cells:

The extracts were dissolved in DMSO (1% v/v) to prepare stock solutions at 1 mg/mL. Serial dilutions were performed in phosphate-buffered saline (PBS) to obtain final concentrations ranging from 6.125 to 200 µg/mL. HaCaT cells were seeded into 96-well plates at a density of  $4 \times 10^4$  cells/well and incubated for 24 hours at 37 °C to allow cell adhesion. Increasing concentrations of the extracts were then added, and the plates were further incubated for an additional 24 hours. Subsequently, the XTT reagent was added, followed by a 2-hour incubation period. Optical density (OD) was measured at 492 nm (reference wavelength: 620 nm) using a microplate reader (Tecan Infinite F50). Each experiment was performed in triplicate.

The percentage of cell viability was calculated using the following formula:

$$\text{Viability (\%)} = \frac{(\text{OD Treated}) - (\text{OD Blank})}{(\text{OD Control}) - (\text{OD Blank})} \times 100$$

The half-maximal inhibitory concentration (IC<sub>50</sub>) was determined graphically from the dose-response curves.

### Antibacterial Activity Evaluation:

**Inoculum Preparation:** Standardized bacterial suspensions were prepared in Mueller-Hinton broth, incubated for 24 hours at  $26 \pm 1$  °C, and then adjusted to a turbidity of 0.5 McFarland standard, equivalent to approximately  $1.5 \times 10^8$  CFU/mL.

**Disk Diffusion Method:** Twenty milliliters of Mueller-Hinton agar were poured into sterile Petri dishes. After solidification, the agar surfaces were uniformly inoculated with the bacterial suspensions. Sterile filter paper disks (6 mm diameter) impregnated with 5 µL of extract (100 µg/mL) were placed on the agar surface. Plates were incubated at 37 °C for 48 hours, after which the diameters of the inhibition zones were measured using a digital caliper. Cefoxitin (FOX) and ceftazidime (CAZ) disks were used as positive controls, while sterile distilled water (SDW) served as the negative control.

### Determination of Minimum Inhibitory Concentration (MIC):

The MIC was determined by microdilution in liquid medium (Mueller-Hinton broth), following a modified method by Assoman et al. [8]. In a 96-well microtiter plate, a two-fold serial dilution was performed starting from an initial

concentration of 200 µg/mL, yielding final concentrations ranging from 3.125 to 100 µg/mL. One hundred microliters of bacterial suspension (0.5 McFarland) were added to each well. Positive controls (bacteria + medium) and negative controls (medium only) were included. After 24 hours of incubation at 37 °C, absence of visible turbidity indicated bacterial growth inhibition. The MIC was defined as the lowest concentration that completely prevented visible bacterial growth.

#### Microplate Growth Inhibition Assay (XTT):

In round-bottom 96-well plates, 100 µL of Brain Heart Infusion (BHI) broth was added per well. Extracts were initially added at 400 µg/mL and then manually diluted to obtain final concentrations ranging from 3.125 to 200 µg/mL. One hundred microliters of bacterial suspension ( $1.5 \times 10^8$  CFU/mL) were added to all wells except those in the last row (positive control: BHI + bacteria). After 4 hours of incubation at 37 °C, a mixture of XTT reagent and electron-coupling agent was added, followed by an additional 2-hour incubation. Optical density (OD) was measured at 492 nm (background corrected at 620 nm). The percentage of inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{(\text{OD Control} - \text{OD Sample})}{\text{OD Control}} \times 100$$

#### Statistical Analysis:

Data were analyzed using GraphPad Prism 5 and expressed as mean  $\pm$  standard deviation (SD). Following confirmation of normality, a two-way ANOVA with post-hoc testing was used to compare antibacterial and cytotoxic activities. For phytochemical comparisons, a one-factor Student's t-test was applied. Differences were considered statistically significant when  $p < 0.05$ .

#### Results:-

##### Extraction Yields of Ethanol Extracts of *Blighia sapida* and *Trichilia emetica*:

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 (%)
<i>Blighia sapida</i>	EEBS	5,44 $\pm$ 0,32	10,88
<i>Trichilia emetica</i>	EETE	5,67 $\pm$ 0,27	11,34

EEBS: Ethanol Extract of *Blighia sapida*, EETE: Ethanol Extract of *Trichilia emetica*

#### 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**

Secondary Metabolites	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 IC<sub>50</sub> values of the plant extracts were determined using regression equations for each species (Table 4)

**Tableau 4. IC<sub>50</sub> Values of Plant Extracts on HACAT Cells**

Extracts	Regression equation	R <sup>2</sup>	IC <sub>50</sub>
EEBS	y = -0,1107x + 70,271	R <sup>2</sup> = 0,9224	183
EETE	y = -0,1861x + 74,408	R <sup>2</sup> = 0,9329	131

EEBS: Ethanol Extract of *Blighia sapida*, EETE: Ethanol Extract of *Trichilia emetica*

**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**

Bacterial Strains	Concentrations (100 µg/mL)		Ctrl (SDW)	Antibiotic (30 µg)
<i>P. aeruginosa</i> ATCC 27853	EEBS	EETE	00±00	CAZ
	00±00	00±00		25±0,1
<i>S. aureus</i> ATCC 25923	EEBS	EETE	00±00	FOX
	8,66 ±0,83	12,87±1,0		33±0,1

Ctrl (SDW): 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 *Blighia sapida* and *Trichilia emetica* 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 *Blighia sapida* and *Trichilia emetica***

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

### Microplate Growth Inhibition Test:

The results showed that the ethanol extracts of *Blighia sapida* (EEBS) and *Trichilia emetica* (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	<i>S.</i> <i>aureus</i>	73,29	67,96	53,37	48,24	48,18	47,6	47,34±
EETE		± 1,17	± 0,84	±1,05	± 1,12	±1,34	± 1,15	1,08
EEBS	<i>P.</i> <i>aeruginosa</i>	76,28	71,77	67,17	57,21±	62,04	60,81	58,42±
EETE		± 0,90	± 0,39	± 0,79	0,17	± 0,34	± 0,57	0,96
EEBS	<i>P.</i> <i>aeruginosa</i>	2,44	2,33	2,0	1,76	1,17	0,91	0,78
EETE		±0,1	±0,03	±0,12	±0,08	±0,03	±0,11	±0,07
EEBS	<i>P.</i> <i>aeruginosa</i>	2,58	2,36	2,33	2,03	1,09	1,01	0,86
EETE		±0,59	±0,77	±0,46	±0,38	±0,08	±0,1	±0,05

### Discussion:-

#### 1-Extraction Yield and Phytochemical Screening of Extracts:

The extraction yields obtained for the ethanol extracts of *Blighia sapida* (EEBS) and *Trichilia emetica* (EETE) (Table 2), respectively 10.88% and 11.34%, show a slight superiority for *Trichilia emetica*. 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 (*Blighia sapida*) 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 (*Trichilia emetica*), 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 *Blighia sapida* and *Trichilia emetica*, 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 *Trichilia emetica* (EETE) exhibits stronger inhibitory activity on HaCaT cells ( $IC_{50} = 131 \mu\text{g/mL}$ ) compared to that of *Blighia sapida* (EEBS,  $IC_{50} = 183 \mu\text{g/mL}$ ), 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 *Trichilia emetica*, particularly in applications such as hyperproliferative skin diseases or certain cancers, while *Blighia sapida*, although less active, remains relevant due to its flavonoids and tannins [27]. 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 ( $12.87 \pm 1.0$  mm) than EEBS ( $8.66 \pm 0.83$  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. 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 *Blighia sapida* (EEBS) and *Trichilia emetica* (EETE) exhibit maximum inhibitions of  $73.29 \pm 1.17\%$  and  $76.28 \pm 0.90\%$ , respectively, at a concentration of 200  $\mu\text{g/mL}$ , 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 *Trichilia emetica* (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 *Blighia sapida* and anthraquinones and coumarins in *Trichilia emetica*. These secondary metabolites are essential in biological activities observation.

The ethanol extract of *Trichilia emetica* (EETE) proved particularly active, both cytotoxically and antibacterially. With an  $\text{IC}_{50}$  of 131  $\mu\text{g/mL}$  on HaCaT cells, it demonstrates promising therapeutic potential against hyperproliferative diseases or certain cancers. On the other hand, the extract of *Blighia sapida* (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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