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GC–MS and HPLC Profiling and *In vitro* Antibacterial Activity of Hydromethanolic Stem-Bark Extract of *Antiaris toxicaria* var. *africana* Scott-Elliot ex A.Chev. (Moraceae)

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ABSTRACT
Medicinal plants continue to attract scientific interest as sources of structurally diverse natural products with potential applications in pharmaceutical research. This study investigated the preliminary chemical profile and *in vitro* antibacterial activity of the 70% methanolic (hydromethanolic) stem-bark extract of *Antiaris toxicaria* var. *africana*. Chemical profiling was performed using gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC), while antibacterial activity was evaluated against six bacterial species using agar well diffusion and minimum inhibitory concentration (MIC) assays. GC–MS analysis yielded 31 chromatographic entries, with n-hexadecanoic acid (25.24%) and (Z)-methyl heptadec-9-enoate (9.03%) recording the largest relative peak areas. Other notable tentative assignments included octadecanoic acid (3.37%), 11-octadecenoic acid methyl ester (3.19%) and 2,4-di-tert-butylphenol (3.13%). HPLC analysis produced peaks tentatively assigned to flavonoids and other phenolic groups, alkaloids, cardiac glycosides and additional chemical classes. The extract produced inhibition zones of 15–17 mm against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Enterobacter cloacae*, but no measurable inhibition against *Bacillus cereus*. Ciprofloxacin produced inhibition zones of 28–40 mm; however, the substantially different concentrations used for the crude extract (15 mg/mL) and ciprofloxacin (1.6 µg/mL) preclude direct comparison of their antimicrobial potency. The observed MIC was 20 mg/mL for the five organisms inhibited by the extract, whereas the MIC for *B. cereus* was greater than 40 mg/mL. These findings indicate preliminary, organism-dependent *in vitro* antibacterial activity of the hydromethanolic stem-bark extract *A. toxicaria* . However, the tentative nature of the chromatographic assignments, absence of validated HPLC calibration data and limited experimental replication constrain interpretation. Further studies involving analytical confirmation of constituent identities, quantitative characterisation, bioactivity-guided fractionation, dose–response evaluation and appropriate toxicity assessment are necessary to clarify the extract's chemical composition, antibacterial properties and safety.

Keywords: *Antiaris toxicaria* var. *Africana*, Stem-bark Extract, GC–MS Profiling, HPLC Analysis, Antibacterial Activity, Minimum Inhibitory Concentration

INTRODUCTION

Antimicrobial resistance (AMR) represents a major global public health challenge, increasingly compromising the effectiveness of conventional antibiotics and complicating the management of bacterial infections. The WHO (2025a) reported that approximately one in six laboratory-confirmed bacterial infections associated with common infections worldwide in 2023 was resistant to antibiotic treatment. The global burden remains substantial, with an estimated 4.71 million deaths associated with bacterial AMR in 2021, including 1.14 million deaths directly attributable to resistance (GBD 2021 Antimicrobial Resistance Collaborators, 2024). The growing prevalence of resistant bacterial pathogens has intensified the search for alternative sources of antibacterial agents, particularly compounds with diverse chemical structures and potentially different mechanisms of action. This has renewed interest in medicinal plants as reservoirs of pharmacologically relevant natural products.

Medicinal plants remain important in traditional healthcare, particularly in developing countries, where their accessibility, affordability and cultural acceptance sustain their widespread use. The World Health Organization (WHO, 2025b) has emphasised the need to strengthen the scientific evidence, quality assurance, safety assessment, and appropriate integration of traditional medicine into health systems. Their therapeutic potential is largely associated with secondary metabolites, including alkaloids, flavonoids, phenolic acids, terpenoids, tannins and glycosides, many of which possess documented antimicrobial properties (Woo *et al*., 2023; Berida *et al*., 2024). These compounds may interfere with microbial membrane integrity, essential enzymes and other cellular processes, making plant-derived products valuable starting materials for antibacterial research (Ugboko *et al*., 2020). Nevertheless, ethnomedicinal application alone does not establish therapeutic efficacy; systematic chemical characterization and experimental evaluation are necessary to substantiate the biological properties of medicinal plants (Aiwonegbe *et al*., 2025; Akpotu & Udi, 2026; Abdul *et al*., 2026).

One species of considerable ethnomedicinal and phytochemical interest is *Antiaris toxicaria* var. *africana* Scott Elliot ex A.Chev. (Moraceae), a large deciduous tree, native to tropical Africa, including Nigeria (Kew Science, 2026). It has a taxonomic synonym (*Antiaris africana* Engl.) which is traditionally associated with the management of

various ailments, including rheumatism, respiratory complaints and skin infections (Ilesanmi *et al*., 2016). However, the presence of potentially toxic cardenolides also warrants careful investigation of its chemical constituents and reinforces the need to distinguish pharmacological potential from safety (Vouffo *et al*., 2010). Its chemical composition is particularly noteworthy because of the occurrence of structurally diverse triterpenoids and cardiac glycosides, some of which possess significant biological activities. Vouffo *et al*. (2010) isolated two new metabolites, antiarol cinnamate and africanoside, from the methanol extract of the stem bark of *Antiaris africana* Engl., together with several known triterpenoids, sterols and cardiac glycosides. This investigation established the chemical diversity of the stem bark but did not provide a comprehensive chromatographic assessment of its antibacterial properties. Subsequently, Ilesanmi *et al*. (2016) investigated the methanolic leaf extract using HPLC coupled with diode-array detection (HPLC-DAD), identifying phenolic constituents including catechin, chlorogenic acid, caffeic acid, ellagic acid, rutin, quercetin and kaempferol. Collectively, these findings establish the phytochemical and pharmacological relevance of the plant. Nevertheless, further investigation is warranted to expand the characterisation of its chemical constituents and evaluate their biological significance, particularly their potential antibacterial properties. These studies provide relevant preliminary evidence of the chemical constituents and antibacterial potential of the species. However, differences in extraction solvent, fractionation and analytical scope make further investigation of the hydromethanolic stem-bark extract worthwhile.

The chemical characterization of complex plant extracts requires complementary analytical approaches because no single technique adequately captures the full range of their constituent metabolites (Aiwonegbe & Ativie, 2023). Gas chromatography–mass spectrometry (GC–MS) is particularly useful for profiling volatile and semi-volatile constituents, whereas high-performance liquid chromatography (HPLC) facilitates the separation and analysis of less volatile and thermally labile compounds. Their combined application can therefore provide broader chemical information than either technique independently (Li, 2023; Çiçek *et al*., 2024). Furthermore, solvent composition influences the extraction of plant metabolites, making hydromethanolic extraction a relevant approach for recovering constituents with different polarities. Integrating these analytical techniques with *in vitro* antibacterial assays provides an opportunity to examine the chemical composition of an extract alongside its experimentally observed antibacterial activity.

Despite previous phytochemical and antibacterial investigations, information specifically integrating GC–MS profiling, HPLC analysis and *in vitro* antibacterial evaluation of the hydromethanolic stem-bark extract of *A. toxicaria* var. *africana* remains limited. The present investigation addresses this gap by providing a complementary chemical and biological assessment of the hydromethanolic extract, thereby extending existing findings beyond methanolic leaf extracts and ethanol-derived stem-bark preparations. The resulting information is expected to provide a basis for further investigation of potentially active constituents, without presuming that individual chromatographically detected compounds are responsible for the observed antibacterial effects. Therefore the aim of this study is to characterize the chemical constituents of the hydromethanolic stem-bark extract of *Antiaris toxicaria* var. *africana* using GC–MS and HPLC and evaluate its *in vitro* antibacterial activity against selected bacterial isolates.

MATERIALS AND METHODS

Sample Collection and Authentication

The stem-bark of *Antiaris toxicaria var. africana* used in this study was collected on March 21st, 2025, from a mature tree in front of the Faculty of Pharmacy (New Building), University of Benin, Ugbowo Campus, Benin City, Nigeria (6°23’36”N 5°37’17”E). Immediately after harvesting, they were placed in sterile polythene bags, labelled with the date and location. The plant sample was authenticated and identified by Prof. E. I. Aigbokhan at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria, and a voucher specimen (EIA02063) was prepared and deposited in the departmental herbarium, ensuring traceability and scientific credibility for this research.

Extraction of *Antaris toxicaria* Stem Bark

After being carefully cleaned with distilled water to get rid of any sand or debris, the harvested stem bark of *Antiaris toxicaria var. africana* was allowed to air dry for two weeks under shade. A clean electric grinder was used to grind the dried bark into a fine powder, which was then moved to a sterile, airtight container and kept at ambient temperatures until it was extracted. A 400 g sample of the finely ground bark was macerated for 120 hours at ambient temperature in 2 × 2.5 L of 70% (v/v) methanol. To enhance solvent penetration, the resulting mixture was shaken every 30 minutes for the first two hours. After that, it was allowed to stand with sporadic hand shaking to guarantee complete extraction. The resulting mixture was separated by decanting and filtered through the Whatman No. 1 filter paper in order to clarify the extract. A hot water bath set at 60°C was used to concentrate the filtrate until it was dry and the solvent had mostly evaporated, leaving behind a crude slurry extract (20.53g). After being moved to labelled sample vials, this extract was kept at 4°C for subsequent analysis (Aiwonegbe *et al*., 2022).

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The chemical constituents of the extract were analysed using a gas chromatography–mass spectrometry (GC–MS) system comprising an Agilent 7890A gas chromatograph coupled to an Agilent 5975C inert mass-selective detector (MSD) equipped with a triple-axis detector (Agilent Technologies, USA). The system was fitted with an automatic injector equipped with a 10 µL syringe. Chromatographic separation was performed using an Agilent HP-5MS capillary column (Part No. 19091-433), with dimensions of 30 m × 250 µm × 0.25 µm and a stationary phase consisting of 5% phenyl methyl siloxane. Helium was used as the carrier gas. The injector temperature was maintained at 300 °C, with injection performed in split mode at a split ratio of 1:50 and an injection volume of 1 µL. The ion source and interface temperatures were maintained at 250 °C and 300 °C, respectively, while the inlet pressure was 16.2 psia. The column temperature programme commenced at 35 °C and was maintained for 5 min, followed by an increase to 150 °C at a rate of 4 °C/min. The temperature was subsequently raised to 250 °C at a rate of 20 °C/min and maintained for a further 5 min. The total chromatographic run time was 43.7.5 min. The remaining instrumental parameters, including carrier gas flow rate, were maintained according to the instrument configuration. The GC–MS system was controlled, and data were acquired and processed using the manufacturer's MassHunter software. The compounds were tentatively identified by comparing their acquired mass spectra with reference spectra in the National

Institute of Standards and Technology (NIST) mass spectral library (NIST II). Compound identification was based on the degree of spectral similarity between the experimental spectra and the library reference spectra.

Extraction of Phytochemicals for HPLC Analysis

The extraction procedure was carried out following the method described by Kaisoon *et al*. (2011), with modifications. Briefly, 0.2 g of the extract was weighed into a test tube, followed by the addition of 15 mL of ethanol and 10 mL of 50% (m/v) potassium hydroxide solution. The mixture was heated in a water bath at 60 °C for 3 h. After the reaction, the contents were transferred into a separating funnel. The test tube was successively rinsed with 20 mL of ethanol, 10 mL of cold water, 10 mL of hot water and 3 mL of hexane, and the respective washings were transferred into the separating funnel. The combined extract was subsequently washed three times with 10 mL portions of 10% (v/v) aqueous ethanol. The ethanol was then evaporated, and the resulting sample was reconstituted in 1,000 µL of pyridine. An aliquot of 200 µL was transferred into an HPLC vial for chromatographic analysis.

High-Performance Liquid Chromatography (HPLC) Analysis

The phytochemical constituents of the extract were analysed using a Shimadzu LC-10AD HPLC system equipped with dual binary pumps, a Shimadzu CTO-10AS column oven and a Shimadzu Prominence SPD-20A UV–visible detector. Chromatographic separation was performed using a reversed-phase Phenomenex Gemini C12 column (5 µm particle size, 200 mm length × 4.6 mm internal diameter), following the chromatographic approach described by Whelan *et al*. (2022). The mobile phase consisted of solvent A (deionised water acidified with acetic acid to pH 2.8) and solvent B (acetonitrile), delivered at a flow rate of 0.8 mL/min. The column temperature was maintained at 38 °C, and the injection volume was 20 µL.

Gradient elution was programmed as follows: 5–9% solvent B from 0 to 5 min; 9% from 5 to 15 min; 9–11% from 15 to 22 min; 11–18% from 22 to 38 min; 18–23% from 38 to 43 min; 23–90% from 43 to 44 min; and 90–80% from 44 to 45 min. The column was subsequently re-equilibrated with the initial mobile-phase composition of 5% solvent B and 95% solvent A from 45 to 55 min. Chromatographic detection was performed at 280 nm. The resulting chromatogram was evaluated based on retention times, integrated peak areas and peak heights. Detected components were tentatively assigned based on the available chromatographic information. Relative peak-area percentages were calculated by expressing the integrated area of each peak as a percentage of the total integrated area of the detected peaks. These values were used to describe the relative chromatographic responses of the detected constituents rather than their absolute concentrations. Accordingly, the findings were interpreted as a preliminary chromatographic profile of the extract.

Collection and Maintenance of Bacterial Isolates

The bacterial isolates used in this study were obtained from the University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria. The test organisms comprised *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterobacter cloacae* and *Bacillus cereus*. The isolates were maintained in nutrient broth supplemented with 20% glycerol and stored frozen until required for analysis. Specific reference strain accession numbers were not available for the clinical isolates.

Preparation and Standardisation of Bacterial Inocula

Before antibacterial testing, the stored bacterial isolates were revived by subculturing onto sterile nutrient agar plates and incubating at 37 °C overnight. Representative colonies from each isolate were subsequently suspended in sterile nutrient broth and incubated for 12 h. The resulting bacterial suspensions were adjusted to a turbidity equivalent to the 0.5 McFarland standard, corresponding to an approximate inoculum density of 10^7 CFU/mL as reported in the original experimental procedure. The standardized suspensions were used for both agar well diffusion and agar dilution assays. However, viable bacterial counts were not independently verified by serial dilution and plate counting.

Antibacterial Susceptibility Testing by Agar Well Diffusion

The antibacterial activity of the hydromethanolic extract of *Antiaris toxicaria* var. *africana* was evaluated using the agar well diffusion method. A stock solution was prepared by dissolving 400 mg of the dried extract in 2 mL of 20% methanol to obtain a nominal concentration of 200 mg/mL. Mueller–Hinton agar (30 mL) was dispensed into sterile Petri dishes and allowed to solidify. The plates were dried in a hot-air oven at 40 °C for approximately 5 min to remove excess surface moisture. Each plate was inoculated with its corresponding standardized bacterial suspension using a sterile inoculating wire loop.

Two wells, each measuring 10 mm in diameter, were bored into each inoculated agar plate using a sterile cork borer. The bases of the wells were sealed with 0.02 mL of molten Mueller–Hinton agar. An aliquot of 0.2 mL (200 µL) of the extract stock solution, corresponding to 40 mg of extract, was introduced into one well, while 0.2 mL of ciprofloxacin solution (1.6 µg/mL) was introduced into the second well as the reference antibacterial control. The plates were incubated at 37 °C for 18–24 h. Following incubation, the inhibition zone diameters (IZDs), including the diameters of the wells, were measured in millimetres. The experiment was conducted once, and the recorded inhibition zones were used to assess the antibacterial activity of the extract against the six bacterial isolates.

Determination of Minimum Inhibitory Concentration by Agar Dilution

The minimum inhibitory concentration (MIC) of the hydromethanolic extract of *Antiaris toxicaria* var. *africana* was determined using the agar dilution method, following the procedure attributed to Afoyan & Meyer (1997). Four concentrations of the extract were prepared using 20% Tween 80 as the diluent. Briefly, 50, 100, 200 and 400 mg of the extract were separately dissolved or dispersed in 1 mL of 20% Tween 80 to obtain individual preparations. Each preparation (1 mL) was transferred into a separate universal bottle containing 9 mL of molten Mueller–Hinton agar and gently mixed to ensure homogeneity. The resulting preparations yielded final extract concentrations of 5, 10, 20 and 40 mg/mL, respectively. Each preparation was poured into a sterile Petri dish and allowed to solidify. The plates were subsequently dried in a hot-air oven at 40 °C for approximately 5 min to remove excess surface moisture.

The base of each plate was divided into six sections corresponding to the six bacterial isolates. Each section was inoculated with its respective standardized bacterial suspension (approximately 10^7 CFU/mL) using a sterile inoculating wire loop, which was flamed after each inoculation to prevent cross-contamination. The plates were incubated at 37 °C for 18–24 h and subsequently examined visually for bacterial growth. The MIC was defined as the

lowest concentration of the extract incorporated into the agar that completely inhibited visible bacterial growth. The experiment was conducted once, without independent biological or technical replication.

RESULTS AND DISCUSSION

GC-MS Analysis

The GC–MS analysis of the hydromethanolic stem-bark extract of *Antiaris toxicaria* var. *africana* produced 35 integrated chromatographic peaks (Figure 1). Applying a conservative NIST14 library-match threshold of ≥80 yielded 17 peak entries for reporting, although some peaks had competing candidate identities.

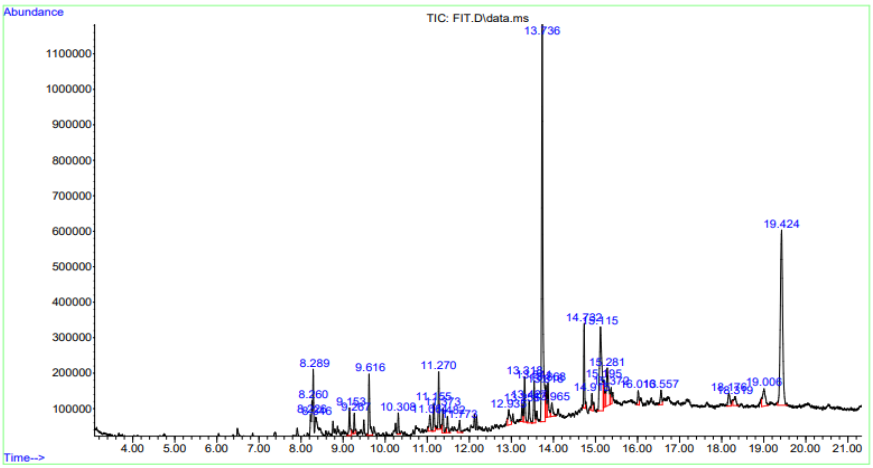


Figure 1: Total Ion Chromatogram (TIC) of the Hydromethanolic stem-Bark Extract of *Antiaris toxicaria* var. *africana* Obtained by Gas Chromatography–mass Spectrometry (GC–MS)

The assignments indicate a preliminary chemical profile comprising fatty acids and their esters, substituted phenolic compounds, hydrocarbons, aldehydes and other oxygenated compounds. These identifications are tentative because they were based on mass-spectral library matching and were not confirmed with authentic reference standards or retention-index data.

Table 1 presents peaks for which the leading NIST14 library assignment had a match quality of at least 80. Where the library returned competing assignments with similar match qualities, they are reported as alternatives rather than treated as confirmed identities. Relative peak areas are chromatographic response percentages, not absolute concentrations or mass percentages.

Table 1: Tentatively Identified Constituents of the Hydromethanolic Stem-bark Extract of *Antiaris toxicaria* var. *africana* by GC–MS

Peak	Retention Time (min)	Tentative library assignment	CAS number(s)	Relative peak area (%)	NIST match quality
5	9.153	Cyclotetradecane / 1-tetradecanol	000295-17-0 / 000112-72-1	1.557	91 / 91
7	9.616	2,4-Di-tert-butylphenol	000096-76-4	3.131	97
8	10.308	Hexadecane	000544-76-3	0.895	96
11	11.270	2-Propenoic acid, pentadecyl ester (pentadecyl acrylate)	043080-23-5	2.502	91
12	11.373	Octacosane	000630-02-4	1.049	91
13	11.482	Tetradecanal	000124-25-4	0.781	91
14	11.773	Hentriacontane	000630-04-6	0.747	90
16	13.255	Octacosane	000630-02-4	0.912	83
17	13.318	Hexadecanoic acid, methyl ester (methyl palmitate)	000112-39-0	2.118	97
18	13.427	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	082304-66-3	1.244	99
19	13.541	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	006386-38-5	2.016	98
20 + 21	13.736 + 13.816	n-Hexadecanoic acid (palmitic acid); two chromatographic peaks combined for summary	000057-10-3	25.242	99 / 93
24	14.732	Methyl octadecenoate isomer(s): 11-octadecenoic acid methyl ester / methyl (E)-9-octadecenoate / methyl (Z)-9-octadecenoate	052380-33-3 / 001937-62-8 / 000112-62-9	3.190	99 (each)

Peak	Retention Time (min)	Tentative library assignment	CAS number(s)	Relative peak area (%)	NIST match quality
25	14.915	Methyl 16-methylheptadecanoate	005129-61-3	0.829	95
26	15.115	Competing assignments: (Z)-methyl heptadec-9-enoate / 2-hydroxycyclopentadecanone / cis-13-octadecenoic acid	014101-91-8 / 004727-18-8 / 013126-39-1	9.033	93 (each)
29	15.372	1-Octadecene	000112-88-9	0.876	90
35	19.424	Bis(2-ethylhexyl) phthalate (DEHP)	000117-81-7	18.201	91

Note: Palmitic Acid (25.242%) Combines Peaks 20 and 21. Peak 28 (Match Quality 60) was Excluded; Peak 26 Remains Unresolved. DEHP May Reflect Plastic-related Contamination.

The most prominent assignment was n-hexadecanoic acid (palmitic acid), represented by two chromatographic peaks at retention times of 13.736 and 13.816 min. Their relative peak areas were 23.753% and 1.489%, respectively, giving a combined area of 25.242%. Other fatty-acid-related assignments included hexadecanoic acid methyl ester (2.118%), methyl octadecenoate isomer(s) (3.190%), and the competing assignments associated with the peak at 15.115 min (9.033%). The latter peak was matched equally to (Z)-methyl heptadec-9-enoate, 2-hydroxycyclopentadecanone and cis-13-octadecenoic acid; consequently, the 9.033% area cannot be attributed confidently to (Z)-methyl heptadec-9-enoate alone. The profile is consistent with the presence of lipid-related constituents in the extract, even though the relative peak areas could not be interpreted as their absolute concentrations or mass fractions.

The detection of 2,4-di-tert-butylphenol (2,4-DTBP), with a relative peak area of 3.131% and a NIST match quality of 97, is noteworthy. Antimicrobial activity has been reported for this compound. Rouvier *et al.* (2024) reported activity against Cutibacterium acnes and Bacillus cereus and investigated its effects on bacterial membrane integrity. Seenivasan *et al*. (2022) isolated and characterised 2,4-di-tert-butylphenol from an endophytic Streptomyces species and investigated its antibacterial properties. Sundar & Arunachalam (2025) also reported activity against multidrug-resistant bacterial pathogens for 2,4-di-tert-butylphenol isolated from *Fusarium oxysporum.* These reports provide a rationale for considering 2,4-DTBP as a candidate for further investigation in relation to the biological activity of the present extract..

Other assignments included methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (2.016%), 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (1.244%), hexadecane (0.895%), cyclotetradecane and/or 1-tetradecanol (1.557%), octacosane (1.049% and 0.912% in two peaks), hentriacontane (0.747%), tetradecanal (0.781%) and 1-octadecene (0.876%). These assignments broaden the preliminary profile of the extract, though their presence and structural identities are yet regarded as provisional until supported by additional analytical evidence.

Bis(2-ethylhexyl) phthalate (DEHP) was assigned to the peak at 19.424 min, representing 18.201% of the total corrected chromatographic area, with a library match quality of 91. This relatively large peak requires particular caution. Phthalate esters are widely used as plasticisers and can enter analytical samples through contact with plastic laboratory consumables, tubing, caps or other materials (Ding *et al*., 20205). DEHP is therefore presented here not as an established natural constituent of *A. toxicaria* since appropriate blank controls and confirmatory analysis are absent.

Overall, the GC–MS results provide a preliminary indication of fatty-acid-related compounds and other organic constituents in the hydromethanolic stem-bark extract of *A. toxicaria* var. *africana*. These findings are expected to guide subsequent isolation and targeted analysis, particularly of 2,4-di-tert-butylphenol and the major fatty-acid-related signals.

HPLC Analysis

The HPLC chromatogram of the hydromethanolic stem-bark extract of *Antiaris toxicaria* var. *africana* is presented in Figure 2, while the corresponding chromatographic data are summarised in Table 2. The chromatogram showed a series of peaks distributed across a retention-time range of 0.100–44.170 min, with 21 components or phytochemical classes listed in the supplied instrument report. The assignments are presented as reported by the original analysis and regarded as tentative rather than definitive chemical identifications.

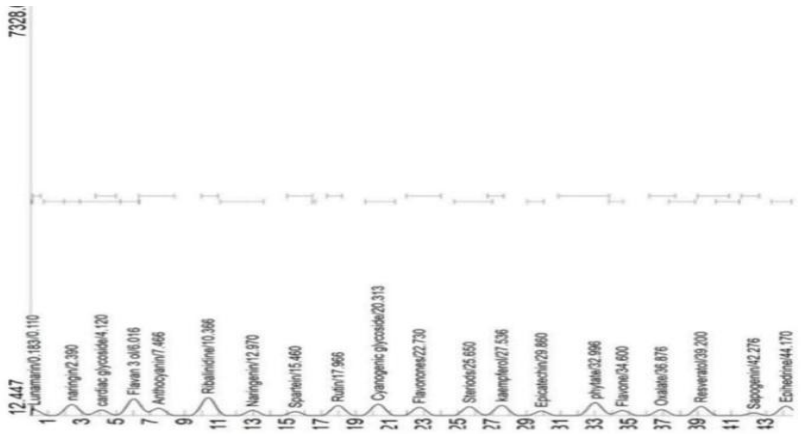


Figure 2: HPLC Chromatogram of the Hydromethanolic Stem-bark Extract of *Antiaris toxicaria* var. *africana*

Peak Labels and Retention Times were Reproduced from the Supplied Chromatogram

Proanthocyanin was among the reported chromatographic assignments, with a retention time of 0.100 min. Other reported phenolic- and flavonoid-related assignments included naringin, with a retention time of 2.390 min and a peak area of 12,468.8678; flavan-3-ol (6.016 min; peak area 18,505.8704); anthocyanin (7.466 min; 8,168.2068); naringenin (12.970 min; 5,983.7040); rutin (17.966 min; 10,773.6850); kaempferol (27.536 min; 11,128.3451); epicatechin (29.860 min; 5,158.3293); flavone (34.600 min; 5,611.0061); and resveratrol (39.200 min; 9,871.3256).

Flavanones were also among the reported assignments, with a retention time of 22.730 min and a corresponding peak area of 9,214.8346. These assignments indicate that the chromatographic profile was annotated with several compounds and classes associated with plant phenolic chemistry. However, their presence could not be considered conclusively established from retention-time information alone.

Other reported chromatographic assignments included lunamarin (0.183 min; peak area 13,787.2370), cardiac glycoside (4.120 min; 6,439.0392), ribalinidine (10.366 min; 19,378.1656), sparteine (15.460 min; 4,717.6830), cyanogenic glycoside (20.313 min; 12,044.9656), steroids (25.650 min; 9,877.4402), phytate (32.996 min; 14,257.1034), oxalate (36.876 min; 6,421.6611), sapogenin (42.276 min; 3,468.1816) and ephedrine (44.170 min; 10,568.1228).

The peak-area and peak-height values are presented in Table 2 as instrument-generated chromatographic responses. They were neither converted into concentrations nor used to establish the relative quantities of the reported constituents. The calibration standards, calibration equations and measurement basis were not available for verification.

Table 2: HPLC Profile of the Hydromethanolic Stem-bark Extract of *Antiaris toxicaria* var. *africana*

S/N	Reported component	Retention time (min)	Peak Area	Peak Height
1	Proanthocyanin	0.100	561.7208	222.208
2	Lunamarin	0.183	13787.2370	124.959
3	Naringin	2.390	12468.8678	203.396
4	Cardiac glycoside	4.120	6439.0392	107.073
5	Flavan-3-ol	6.016	18505.8704	298.351
6	Anthocyanin	7.466	8168.2068	138.998
7	Ribalinidine	10.366	19378.1656	319.450
8	Naringenin	12.970	5983.7040	100.620
9	Sparteine	15.460	4717.6830	79.750
10	Rutin	17.966	10773.6850	181.880
11	Cyanogenic glycoside	20.313	12044.9656	203.087
12	Flavanones	22.730	9214.8346	154.355
13	Steroids	25.650	9877.4402	163.611
14	Kaempferol	27.536	11128.3451	185.848
15	Epicatechin	29.860	5158.3293	87.501
16	Phytate	32.996	14257.1034	233.200
17	Flavone	34.600	5611.0061	96.493
18	Oxalate	36.876	6421.6611	110.381
19	Resveratrol	39.200	9871.3256	165.134
20	Sapogenin	42.276	3468.1816	57.444
21	Ephedrine	44.170	10568.1228	172.796

Note: Phytochemical Assignments are Tentative, Owing to the Absence of Authentic Reference Standards and Validated Calibration Data.

Some of these entries represent broad phytochemical classes rather than individual molecular structures. Their assignments have therefore been cautiously interpreted, as retention time alone cannot reliably distinguish structurally related compounds or unrelated substances that may elute under similar chromatographic conditions.

Overall, the HPLC profile in Figure 2, together with the corresponding data in Table 2, provides a preliminary account of the 21 reported assignments in the hydromethanolic stem-bark extract of *A. toxicaria* var. *africana*. Confirmation of the individual constituents would require comparison with authentic reference standards under the same chromatographic conditions and, where necessary, complementary analytical techniques such as LC–MS/MS. Quantitative determination would additionally require validated calibration curves, clearly defined units and appropriate quality-control procedures. The present results thus are appropriately interpreted as chromatographic observations rather than definitive compound identification or quantitative phytochemical determination.

Antibacterial Activity Based on Inhibition-Zone Diameter

The methanolic stem-bark extract of *Antiaris toxicaria* var. *africana* produced inhibition zones of 16 mm against *Staphylococcus aureus*, 17 mm against *Escherichia coli*, 16 mm against *Pseudomonas aeruginosa*, 15 mm against *Bacillus subtilis* and 15 mm against *Enterobacter cloacae* (Figure 2). No measurable inhibition zone was recorded against *Bacillus cereus*. Thus, under the conditions of the agar well diffusion assay, the extract exhibited detectable growth inhibition against five of the six bacterial species examined.

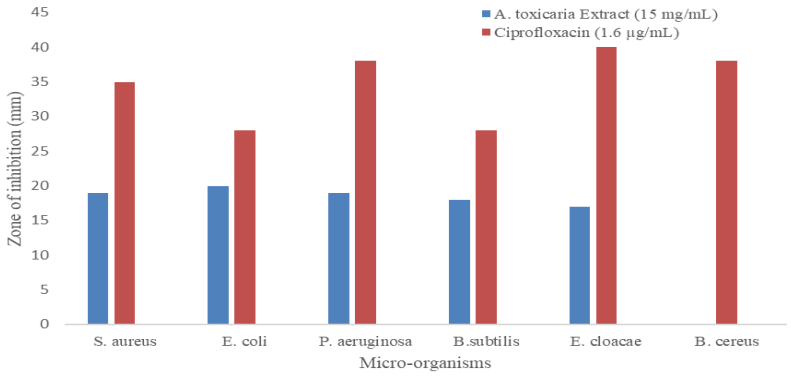


Figure 3: Inhibition-zone Diameters Produced by the Methanolic Stem-bark Extract of *Antiaris toxicaria* var. *africana* and Ciprofloxacin Against the Tested Bacterial Species

Among the tested organisms, *E. coli* recorded the largest inhibition zone (17 mm), while *B. subtilis* and *E. cloacae* recorded the smallest zones (15 mm). The absence of a measurable inhibition zone against *B. cereus* indicates that the tested extract preparation did not produce detectable inhibition of this organism under the assay conditions. These observations represent the recorded inhibition-zone measurements and should not be interpreted as evidence of statistically significant differences in susceptibility, particularly because replicate measurements and associated variability estimates were not presented.

Ciprofloxacin produced inhibition zones ranging from 28 to 40 mm across the six bacterial species, with the largest zone recorded against *E. cloacae* (40 mm) and the smallest against *E. coli* and *B. subtilis* (28 mm each). Although the inhibition zones produced by ciprofloxacin were larger than those of the plant extract, the two preparations were tested at substantially different concentrations: 1.6 µg/mL for ciprofloxacin and 15 mg/mL for the crude extract. Consequently, their inhibition-zone diameters do not provide a direct measure of comparative antimicrobial potency. The difference also reflects the distinct chemical composition and diffusion characteristics of a purified antibiotic and a crude plant extract.

The observed inhibition across both Gram-positive bacteria (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa* and *E. cloacae*) indicates that the extract exhibited activity against representatives of both bacterial groups under the experimental conditions. However, the absence of measurable inhibition against *B. cereus* demonstrates that the response was organism-dependent rather than uniformly distributed across the tested bacteria. Although the outer membrane of Gram-negative bacteria may influence their susceptibility to antimicrobial substances (Xu *et al*., 20206), the observed pattern in this study does not support a general conclusion that either Gram-positive or Gram-negative organisms were more susceptible.

The antibacterial response may be associated with one or more constituents of the extract. The GC–MS profile included fatty-acid-related compounds, such as hexadecanoic acid methyl ester and octadec-9-enoic acid, as well as the phenolic derivative 2,4-di-tert-butylphenol. Antimicrobial properties have been reported for some compounds within these chemical groups (Seenivasan *et al*, 2022; Rouvier, *et al*., 2024; Sundar & Arunachalam, 2025; Venugopal *et al*., 2026), although their occurrence in a chromatographic profile does not establish their contribution to the activity observed in the present study. Furthermore, the possible contribution of interactions among extract constituents remains speculative and would require investigation using isolated compounds and characterised fractions. It has been previously shown that the stem bark of *Antiaris africana* contains a flavonoid fraction with antibacterial effects (Banso & Mann, 2008; Ilesanmi, *et al*., 2021), including activity against selected bacterial species such as *B. subtilis* and *E. coli*. This provides relevant literature support for investigating the antibacterial properties of the plant.

Summarily, the agar well diffusion results provide preliminary evidence of antibacterial activity of the methanolic stem-bark extract against five of the six bacterial species examined. The findings justify further investigation of the extract and its constituent fractions.

Minimum Inhibitory Concentration

The agar dilution results showed no visible bacterial growth at extract concentrations of 20 and 40 mg/mL for *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *E. cloacae*, whereas growth was observed at 5 and 10 mg/mL (Table 3). Accordingly, the lowest tested concentration preventing visible growth for each of these five organisms was 20 mg/mL, representing the minimum inhibitory concentration (MIC) under the conditions of the assay.

Table 3: Minimum Inhibitory Concentration of the Methanolic Stem-bark extract of *A. toxicaria* var. *africana*

Microorganisms	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL	Observed (mg/mL)	MIC
Staphylococcus aureus	NG	NG	G	G	20	
Escherichia coli	NG	NG	G	G	20	
Pseudomonas aeruginosa	NG	NG	G	G	20	
Bacillus subtilis	NG	NG	G	G	20	
Enterobacter cloacae	NG	NG	G	G	20	
Bacillus cereus	G	G	G	G	>40	

Key: NG = No Visible Growth; G =Visibleg Growth

The MIC is defined as the lowest concentration of an antimicrobial preparation that prevents visible microbial growth under specified experimental conditions (Balouiri *et al.*, 2016). The observed endpoints therefore indicate that the extract inhibited visible growth of the five bacterial species at 20 mg/mL, but not at the lower concentrations tested.

In contrast, *B. cereus* exhibited visible growth at all tested concentrations, including 40 mg/mL. Its MIC was therefore greater than 40 mg/mL, indicating that the inhibitory endpoint was not reached within the concentration range examined. This observation is consistent with the absence of a measurable inhibition zone against the same organism in the agar well diffusion assay. The agreement between the two assays provides complementary evidence of the comparatively limited response of *B. cereus* to the extract under the conditions investigated.

The MIC findings were nevertheless interpreted within the limitations of the experimental design. The reported values represent endpoints from the tested concentration series, and no information on replicate variability or statistical analysis. was provided. They should therefore be regarded as observed MIC values rather than statistically validated estimates of antimicrobial susceptibility.

Integrated Interpretation of GC–MS, HPLC, Inhibition-Zone and MIC Results

Considered together, the chromatographic profiles and antibacterial findings provide preliminary evidence of the chemical complexity and antibacterial activity of the methanolic stem-bark extract of *A. toxicaria* var. *africana*. The GC–MS analysis tentatively assigned several compounds, including 2,4-di-tert-butylphenol and fatty-acid-related constituents, while the HPLC analysis provided additional chromatographic assignments. These chemical observations offer candidates for subsequent investigation but do not establish which constituents, individually or collectively, account for the observed antibacterial response.

The identification of 2,4-di-tert-butylphenol is noteworthy because antimicrobial activity has been reported for this compound in other biological materials (Rouvier *et al.*, 2024). Similarly, fatty-acid-related compounds have been investigated for potential antimicrobial properties (Nazzaro *et al*., 2026; Torun *et al*., 2026). However, the tentative nature of the chromatographic assignments, together with the absence of quantitative validation, limits the extent to which these compounds can be linked directly to the antibacterial results. Establishing such relationships would require confirmation of compound identities, quantitative characterisation and antibacterial testing of isolated constituents or well-characterised fractions.

The inhibition-zone and MIC findings provide complementary observations. The agar well diffusion assay demonstrated measurable inhibition against five bacterial species, while the agar dilution assay established an observed MIC of 20 mg/mL for the same organisms. Both methods indicated no detectable inhibitory response against *B. cereus* within the tested conditions. Nevertheless, the different assay principles and the influence of compound diffusion on inhibition-zone formation should be considered when interpreting their outcomes.

Previous investigations have reported antibacterial activity in material derived from *Antiaris africana* (Banso & Mann, 2008), while other studies have documented chemically diverse metabolites from the plant (Vouffo *et al*., 2010). The present findings extend the available observations by presenting GC–MS and HPLC chromatographic profiles alongside agar well diffusion and agar dilution results for the methanolic stem-bark extract. Collectively, these findings provide a preliminary basis for further phytochemical characterisation and bioactivity-guided fractionation. Confirmation of the active constituents, assessment of their individual contributions and evaluation of their antimicrobial potency will require appropriately controlled follow-up investigations.

Toxicological and Safety Evaluation

The toxicological significance of the chromatographic findings warrants attention because *Antiaris toxicaria* is recognised as a poisonous plant, with cardiac glycosides and cardenolides reported among its constituents. For example, Shi *et al*. (2010) isolated several cardiac glycosides from the trunk bark of *A. toxicaria* and demonstrated cardiotonic activity in isolated guinea-pig atria. Similarly, Vouffo *et al*. (2010) reported the isolation of africanoside, a cardenolide, from the methanol extract of the stem bark of *Antiaris africana*. These findings are relevant to the present study because cardiac glycosides can inhibit Na^+/K^+-ATPase, thereby altering ionic homeostasis and cardiac function (Shattock *et al*., 2015). However, the tentative HPLC assignment of a cardiac glycoside in the present extract does not confirm the identity, concentration or biological activity of any specific cardiac glycoside. The other tentative assignments, including cyanogenic glycosides and alkaloids such as ephedrine and sparteine, also require analytical confirmation before their presence or toxicological relevance can be established. Likewise, the detection of flavonoids, tannins and other commonly occurring plant constituents should not be interpreted as evidence that the extract is inherently safe.

Importantly, GC–MS library matches and HPLC peak assignments provide preliminary chemical information; they do not independently establish the toxicity, bioavailability or safe exposure limits of the crude extract. Some compound assignments may be erroneous, while relative GC–MS peak areas cannot be interpreted as concentrations or toxicological doses without appropriate calibration and validation. Consequently, the established toxicological history of *A. toxicaria* provides a sound reason for caution, but does not demonstrate that the particular methanolic extract investigated in this study is toxic. Confirmation of potentially hazardous constituents using appropriate analytical techniques, together with systematic cytotoxicity and dose-dependent toxicity studies, is necessary before the safety or medicinal potential of the extract can be assessed.

CONCLUSION

The methanolic stem-bark extract of *Antiaris toxicaria* var. *africana* exhibited *in vitro* antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Enterobacter cloacae*, with no detectable inhibition of *Bacillus cereus* under the conditions investigated. The corresponding observed MIC was 20 mg/mL for the five susceptible organisms and greater than 40 mg/mL for *B. cereus*. Although GC–MS and HPLC profiling provided preliminary chromatographic assignments, the identities and contributions of individual constituents to the observed activity remain unconfirmed. Interpretation is further constrained by the tentative nature of the chemical assignments, absence of validated HPLC calibration data and limited experimental replication. Further studies should therefore prioritise confirmation of constituent identities, dose–response evaluation, bioactivity-guided isolation and appropriate toxicity and safety assessments before any therapeutic relevance can be inferred.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS
AEA: conceptualization, study design, supervision, writing, formatting, and editing of final manuscript.
TO: procurement and preparation of plant sample and extraction, laboratory analysis, writing of the initial manuscript.
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