Heliyon 10 (2024) e40159

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Comparative phytochemical profile and biological activity of three *Terminalia* species as alternative antimicrobial therapies

Jotham Yhi-pênê N'do [a,*], Dramane Paré [a], Loyapin Bondé [b], Adama Hilou [a]

[a] Laboratory of Applied Biochemistry and Chemistry, Joseph Ki-Zerbo University, 03 BP 7021, Ouagadougou, 03, Burkina Faso
[b] Laboratory of Plant Biology and Ecology, University Joseph Ki-Zerbo, 03 BP 7021, Ouagadougou, 03, Burkina Faso, Burkina Faso

ARTICLE INFO

Keywords:
Antimicrobials
HPLC analysis
Phytochemistry
Antioxidant
Medicinal plants

ABSTRACT

Ethnopharmacological relevance: Medicinal plants can help combat antibiotic resistance by providing novel, active molecules. Three plant species of the *Terminalia* genus are widely used in traditional medicine in the Mouhoun region for the treatment of cutaneous and respiratory diseases. Therefore, it is important to determine the ethnopharmacological potential of bark extracts from the trunks of these three *Terminalia* species.

Aim of the study: This study compared the phytochemical and biological activities of extracts from three *Terminalia* species to determine their ethnopharmacology.

Materials and methods: The medicinal properties of the extracts were assessed based on their ability to inhibit the growth of the following microorganisms: *Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida krusei*, *Candida glabrata,* and *Candida tropicalis*. The significant interest in these medicinal plants among the local communities were elucidated by their antioxidant properties and phytochemical composition, along with the detection key bioactive compounds. Major phytochemical groups and phenolic compounds were determined using high-performance liquid chromatography with a diode array detector. These phytochemical findings were validated by evaluating the antioxidant capacity of the extracts using DPPH, FRAP, and ABTS assays.

Results: Hydroethanolic, ethanolic, and hexane extracts from the bark of three *Terminalia* species inhibited the growth of both bacteria and fungi, as evidenced by their minimum inhibitory concentrations (MICs).

The findings showed that *Terminalia* species were most effective against various tested bacteria and fungi, with MICs ranging from 0.1 to 6.25 mg/mL. *Terminalia avicennioides*, *Terminalia macroptera,* and *Terminalia laxiflora* extracts demonstrated 50 % inhibition of DPPH at concentrations ranging from 0.04 to 0.6 mg/mL. Phytochemical analysis revealed the presence of several families of chemical compounds, such as total phenolics and flavonoids. Phenolic compounds identified by HPLC in ethanolic extracts of *T. avicennioides*, such as isorhamnetin, quercetin, and ferulic acid, are recognised for their antimicrobial and antioxidant properties.

Conclusion: These findings establish an ethnobotany for these three *Terminalia* species, with their chromatographic characteristics facilitating the identification of key molecules of interest. The ethanolic extract of *T. avicennioides* can be used in phytomedicinal formulations against bacterial (*P. aeruginosa* and *S. aureus*) and fungal (*C. albicans* and *C. glabrata*) infections, both of which are recurrently recorded in certain skin and respiratory tract diseases.

* Corresponding author.
E-mail addresses: yhipene2005@yahoo.fr (J.Y.-p. N'do), paredra@yahoo.fr (D. Paré), loyapinbonde@yahoo.fr (L. Bondé), hiloudio@gamil.com (A. Hilou).

https://doi.org/10.1016/j.heliyon.2024.e40159
Received 25 July 2024; Received in revised form 4 November 2024; Accepted 4 November 2024
Available online 6 November 2024

1. Introduction

Globally, infectious diseases rank among the primary causes of mortality [1]. The World Health Organization (WHO) estimates that infectious diseases claim 14 million lives annually, with 43 % of fatalities in developing countries attributed to these diseases [2]. As global outbreaks of infectious diseases increase, developing countries are particularly vulnerable. These countries face major public health challenges because of the severity of infectious diseases [3]. In 2008, Burkina Faso reported that 80 % of the primary causes of mortality in health districts were linked to infectious pathologies, including malaria, diarrhoea, meningitis, acute respiratory infections, and skin diseases [4]. Skin infections are attributed with various pathogens, of which bacterial and fungal species are the most frequently encountered [5]. These diseases are typically caused by bacteria, such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli,* and *Staphylococcus aureus*, and by fungi, such as *Candida albicans*, *Trichophyton tonsurans*, and *Candida glabrata* [6].

The scientific community has discovered various treatments to alleviate the effects of microbial infections [7]; however, healthcare systems in developing countries struggle with significant challenges, including insufficient healthcare coverage and the unavailability of medicines to populations. Approximately 80 % of the population in developing countries uses traditional medicine for their healthcare requirements [8]. These traditional remedies serve as important resources for the discovery of new drug molecules, with medicinal plants recognised by the WHO as effective treatments for managing infections caused by microorganisms [7]. However, the majority of these formulations are used without proper scientific evaluation of their therapeutic properties. Therefore, it is important to scientifically validate traditional recipes to contribute to better therapeutic management for human health. *Terminalia* species, particularly *Terminalia macroptera*, *Terminalia avicennioides,* and *Terminalia laxiflora*, are widely used in traditional medicine to treat infectious diseases in Burkina Faso. Our earlier work highlighted the widespread use of these plant parts in traditional medicine [9]. The use of medicinal plants suffers from poor management, ranging from uncontrolled use to the threat of extinction of certain medicinal plant species, partly because of a lack of reliable scientific information. Consequently, some species are threatened by heavy human pressure. Establishing a reliable scientific base for the use medicinal plants such as *Terminalia* species is an important step. Expanding research on these plant species would significantly contribute to their more efficient and sustainable use [9], while providing opportunities for biotechnology based on their properties.

Numerous studies have thoroughly explored the antimicrobial qualities of these medicinal plants [10]. However, studies comparing these three *Terminalia* species within the same context (harvesting area, post-harvest treatments, and laboratory tests) are limited. Evaluating the biological properties, complemented by phytochemical investigations, across three extraction methods for each plant part (aqueous, ethanolic, and hexane extracts) proved to be highly innovative and significant.

Oxidative stress mechanisms in pathogenic microorganisms during infection are primarily driven by free radicals [11]. Certain secondary metabolites, such as flavonoids, are known to exhibit antioxidant effects on these stress mechanisms induced by pathogenic organisms [12]. Thus, in addition to the antimicrobial properties, the present study assessed the antioxidant capabilities of phytochemical extracts from three selected *Terminalia* species. Phytochemical analysis enabled quantification and characterization of the primary phytochemical groups present. Moreover, high-performance liquid chromatography (HPLC) analysis allowed us to identify phenolic compounds in the extracts**.**

This study highlighted the pharmacological properties of the three medicinal plants species of the *Terminalia* genus.

2. Materials and methods

2.1. Study framework

Phytochemical tests were conducted at the LABIOCA (Biochemistry and Applied Chemistry Laboratory) and UFR/SVT (Joseph Ki-Zerbo University). Antimicrobial tests were performed at LAMBM (Microbiology and Microbial Biotechnology Laboratory).

2.2. Plant material collection and extraction

The bark from the trunks of three *Terminalia* species was used throughout this study. These plant species were identified during a field visit with traditional health practitioners in the Mouhoun region (Burkina Faso) during an ethnobotanical survey conducted between November 2022 and August 2023. Herbarium specimens were created to verify their identities at the Biodiversity Laboratory of Joseph Ki-Zerbo University. The following *Terminalia* plant species were selected: *T. macroptera* Guill. & Perr., *T. avicennioides* Guill. & Perr., and *T. laxiflora* Engl. & Diels. The herbarium samples were registered at the Joseph Ki-ZERBO University Herbarium under the following successive codes: J-16751, J-16752, and J-16753.

Freshly harvested plant material was oven-dried at 40 °C until completely dry. The dried material was immediately powdered and stored in airtight containers in the dark, at room temperature. For biological activities, extraction was carried out non-sequentially using three solvents: water-ethanol (50:50; v:v), ethanol (98 %), and pure hexane [13].

The powders were placed in conical flasks containing the respective solvents in a ratio of 1:10 (g/mL). The mixtures were agitated at room temperature for 24 h and sonicated for 1 h on ice. Extracts were filtered using Whatman filter paper and concentrated under vacuum using a rotary evaporator at 40 °C.

Calculation of extraction yields: The yield is the quantity of extract obtained from a plant material [14], expressed as a percentage of dry matter (plant powder), and calculated according to the formula:

$$R\ (\%) = \mathbf{M1\ x\ 100}/\mathbf{M0},$$

where R is the extraction yield in percentage (%), M1 is the mass of the extract (g), M0 is the mass of the bark powder (g).

The concentrated extracts were then transferred into pre-weighed glass vials, dried under a stream of air at room temperature until a constant weight was achieved, and stored at 10 °C in the dark until use.

2.3. Phytochemical analysis

2.3.1. Phytochemical characterization

The phytochemical characteristics of the bark extracts was estimated to identify the chemical groups present in the active extracts that may be responsible for their antimicrobial activity. The methods described in Ref. [15] were used. Qualitative methods were used to ascertain chemical compounds present in the plants.

2.3.2. Qualitative analysis

Flavonoids were detected using the Shibata reaction. Tannins, polyphenols, saponins, and alkaloids were analysed using the Dragendorff test [16].

2.3.3. Quantitative analysis

2.3.3.1. Determination of total phenolic content. Total phenolic content was measured following a previously described procedure [17], based on the high oxidisability of phenolic compounds. The Folin-Ciocalteu reagent (FCR) reacts with phenolic compounds, reducing phosphomolybdate and sodium tungstate to a mixture of molybdenum and tungsten blue. Gallic acid was used as the standard. After 30 min of reaction at room temperature, absorbance was measured at 765 nm. The tests were performed in triplicates to ensure the reproducibility of the results. The total phenolic content was expressed in mg gallic acid equivalents per gram of sample [18].

2.3.3.2. Determination of total flavonoid content. In a test tube, 1 mL of ethanolic extract of each plant was mixed with 1 mL of 2 % aluminium chloride solution ($AlCl_3$). After incubation for 10 min at room temperature, absorbance was measured at 415 nm. The concentrations of flavonoids was determined in mg quercetin equivalents per gram of dry extract, using a calibration curve obtained under the same reaction conditions with quercetin as the standard [19].

2.3.3.3. Determination of flavonol content. The flavonol content was determined as previously described [20]. In triplicate, 75 µL of extract (0.1 mg/mL) prepared in ethanol and 75 µL of an aqueous solution of $AlCl_3$ (20 %) were mixed. A sample blank was prepared by mixing 75 µL of the extract with 75 µL of ethanol. After 10 min of incubation, the absorbance was measured at 425 nm using a spectrophotometer. The concentrations were calculated with reference to a standard curve prepared using rutin (1–50 µg/mL) [21]. The flavonol content was expressed in µg rutin equivalent per 100 g of dry extract.

2.3.3.4. Determination of total coumarin content. Coumarin content was determined spectrophotometrically. A coumarin calibration curve was plotted according to the method described by Refs. [22,23] with the equation $y = 0.0182x + 0.0277$ ($R^2 = 0.998$). The assay was conducted using 150 µL of the extracts, and absorbance was measured at 290 nm. The contents were expressed in µg coumarin equivalent per 100 mg (µg CE/100 mg) of dry extracts (convertible to mg/mL).

2.3.4. Analysis of bioactive polyphenols using HPLC

A Thermo Scientific Dionex UltiMate 3000 Rapid Separation Liquid Chromatography system (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to identify polyphenols present in the ethanolic extracts. The samples were dissolved in methanol (Table 1).

The system was equipped with a quaternary rapid separation pump (LPG-3400RS), Ultimate 3000RS auto-sampler (WPS-3000), and rapid separation diode array detector (DAD-3000RS). An Acclaim® C18 120 Å (7.5 cm × 4.6 cm x 100 mm, 5 µm) column (Dionix, USA) was used to separate phenolic compounds. Temperature of the column compartment (TCC-3000) was maintained at 30 °C. Dionix Chromeleon software (Version 6.80 RS 10) was used for data acquisition, peak integration, and calibration.

Extracts of 10 mg/mL were prepared for analysis [24]. The samples were filtered using a 0.22 µm Millipore filter, and the UV–visible spectra were recorded at 254, 272, 365, and 200 nm (Table 2). A standard curve was prepared using a stock solution prepared in methanol containing [25] chlorogenic acid, caffeic acid, tannic acid, ellagic acid, syringic acid, gallic acid, ferulic acid,

Table 1
Elution program.

Time (min)	% A (H_2O–ACN 90:10, ATF 0.1 %)	% B (CH_3CN, ATF 0.1 %)
00	95	05
10	95	05
15	85	15
25	85	15
35	75	25
40	95	05

quercetin, isorhamnetin, chrysin, hypersaccharide, and luteolin standards.

2.4. Antimicrobial assay

2.4.1. Microbial strains used for antimicrobial activities

Antimicrobial activity was performed against four microbial genera: 3 g-negative bacteria, 1 g-positive bacterium, and four fungal strains (Table 2). Based on several criteria, the microbial strains, commonly associated with hospital-origin infections in animal and human pathologies (skin and respiratory tract), were used in this study. These strains were chosen according to their resistance to different types of antimicrobial agents.

2.4.2. Minimum inhibitory concentration (MIC)

Antibacterial activity was assessed using 96-well microtiter plates. Plant extracts at different concentrations were prepared in suitable solvents and were placed in different wells (100 μL each), with six replicates for each sample. The solvent was evaporated from each well by placing the plates under a fume hood at 25 °C. The bacteria grown in LB broth was diluted in fresh LB medium, and the optical density (OD) of the dilution was determined at 630 nm using a UV–Vis spectrophotometer. The bacterial culture was further diluted or concentrated to an OD between 0.2 and 0.6.

Bacterial solutions were diluted according to the method described by Amsterdam [26] to obtain a concentration of 2×10^8 cells/mL. Bacterial dilutions were placed in each well (100 μL) under aseptic conditions. Growth control were wells containing only the bacterial inoculum, and the solvent without extract was used as the negative control. The plates were covered with sterile plate sealant and incubated at 37 °C for 48 h. The growth intensity visible in the control and treated wells, as observed with the naked eye, was compared. The tests were performed in triplicate to ensure reliability of the results. The lowest concentration of the test solution that led to growth inhibition was the MIC [27].

2.4.3. Antifungal assay

The antifungal potency of plant extracts was determined using a microtiter plate dilution technique [28]. An overnight culture of each of the four fungal strains was prepared by inoculation with a positive colony and incubating at 37 °C with shaking. In order to prepare a suitable dilution (1:1000), 400 μL of the stock fungal culture (overnight) was mixed with sterile 0.85 % sodium chloride (NaCl) solution. The absorbance was measured at 530 nm using a spectrophotometer, and dilutions were adjusted with sterile saline to correlate with that of 1 mL of 0.5 McFarland solution (0.25–0.28 range). Each well of the 96-well microtitre plate was seeded with 100 μL of sterile distilled water, followed by the addition of a 100 μL aliquot of plant extract (20 mg/mL), in row A of the microtitre plate and diluted twice in series from A to H. The positive control was prepared using the reference drug nystatin at a concentration of 0.25 mg/mL. The fungal cultures (100 μL aliquot) were added to each well of the microtitre plate. As negative controls, the respective extraction solvents were used were YM without fungi, SD broth, and fungal cultures. Plates were covered with parafilm and incubated at 37 °C overnight, after which 50 μL of 0.2 mg/mL 2-(p-iodophenyl)-3-p(nitrophenyl)-5 phenyl tetrazolium chloride (INT) was added, and further incubated for 24 h at 37 °C. The lowest concentration of the plant extract capable of inhibiting fungal growth (no colour change) was recorded as the MIC. Clear wells indicated growth inhibition, whereas pink to red colour indicated growth. The test was performed in triplicate.

2.5. Determination of antioxidant properties

2.5.1. 2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity

The anti-radical activity was evaluated using DPPH [29]. This method is based on the reduction in absorbance at 515 nm of the free radical DPPH in the presence of the radical donor $H^{\cdot}$ [30]. The absorbance measured was used to calculate the percentage inhibition of the DPPH radical, which is proportional to the antiradical potential of the sample. The absorbance was also measured using ascorbic acid as the standard, and the results were expressed with reference to the standard curve in μg ascorbic acid equivalent per 100 mg of extract (μg AAE/100 mg) (Appendix A3). The experiments were performed in triplicate. The samples were tested at different concentrations. The concentrations exhibiting 50 % inhibition (IC50 %) were calculated. Extracts were prepared 24 h before DPPH measurements. Radical-scavenging activity (%) was calculated using the following formula:

Table 2
Microbial strains tested and their references.

Microorganism	Species	References
Gram negative bacteria	*Escherichia coli*	ATCC25922
	Klebsiella pneumoniae	ATCC13883
	Pseudomonas aeruginosa	ATCC27853
Gram positive bacteria	*Staphylococcus aureus*	ATCC43800
Fungal strains	*Candida albicans*	ATCC10231
	Candida krusei	ATCC34135
	Candida glabrata	ATCC2001
	Candida tropicalis	ATCC28707

$$\%\ Scavenging\ activity = \mathbf{1} - (\mathbf{Abss} / \mathrm{Absc} \times \mathbf{100})$$

2.5.2. Ferric reducing antioxidant power (FRAP)

The FRAP assay measured the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) by electron transfer mechanism. In the presence of antioxidants, Fe^{3+} are reduced to Fe^{2+} with an absorption maximum around 700 nm [31]. Absorbance was measured in triplicate against a blank without sample. The concentration (content) of reducing compounds in the extracts was expressed as mg ascorbic acid equivalent per 100 mg of dry extract (mg AAE/100 mg) (Appendix A2). A successive 1/2 dilution series was conducted using a stock solution of 1 mg/mL. The experiment was replicated three times (independent tests), and the IC50 % was determined using the standard curves of anti-radical activity percentages plotted against extract concentrations.

2.5.3. 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) reduction

The ABTS reduction test is based on the decolourisation of the stable radical cation $ABTS^{+}$ in the presence of antiradical compounds [32]. The reducing activity was evaluated by measuring the absorbance at 734 nm because the blue-green colour of the $ABTS^{+}$ reacts with potassium persulfate to form ABTS. The reducing activity was calculated with reference to a standard curve (Appendix 1) and the results were expressed as μg trolox equivalent per 100 mg of extract (μg TE/100 mg).

2.6. In vitro cytotoxicity assay

The *in vitro* cytotoxicity of the extracts on the rabbit corneal epithelial cell line obtained from the National Centre for Cell Science (NCCS), (Pune, India) was assessed using an MTT colorimetric assay. Dulbecco's Modified Eagle's medium (DMEM) medium (Gibco, United States) supplemented with fetal bovine serum (10 %) and antibiotic solution (1 %) was used to culture the cells until reaching 1×10^5 cells/mL concentration in a 96-well culture plate. After incubation at 37 °C for 24–48 h, the cells were washed with sterile PBS and treated with various amounts of plant samples in a serum-free DMEM medium. The plates were then incubated for 24 h at 37 °C with 5 % CO_2. Subsequently, 10 μL of MTT (5 mg/mL) was added to each well and incubated until a purple precipitate appeared as observed under an inverted microscope. The plates were washed with 1X PBS after the spent culture, and MTT was removed. The formazan crystals formed were dissolved in 100 μL of DMSO by shaking the plate for 5 min. Percentages of cell viability were calculated by quantifying the reduction of MTT to formazan crystals by measuring the OD values at 570 nm using a microplate reader (Thermo Fisher Scientific, United States), as described previously [33].

2.7. Data processing and analysis

Data were expressed as mean ± standard deviation (M ± SD) and subjected to a Student's t-test using R. The significance threshold was 5 %. XLSTAT-2019 software was used for principal component analysis (PCA). PCA was used to explore the correlation between the secondary metabolite content and the biological activities of the extracts. The molecules were depicted using ChemDraw, a molecular modelling software.

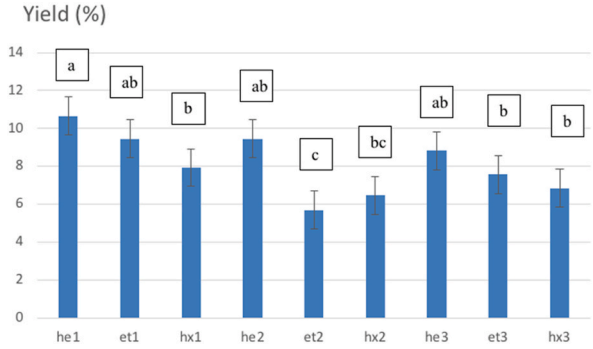


Fig. 1. *Terminalia* species bark extract yields expressed as percentages (%).
Values with the same superscript letters are considered statistically identical. Statistical significance is set at $p < 0.05$.
he1, he2, and he3: hydroethanolic extracts of *T. avicenioids, T. macroptera, T. laxiflora,* respectively; et1, et2, and et3: ethanolic extracts of *T. avicenioids, T. macroptera,* and *T. laxiflora,* respectively; hx1, hx2, and hx3: hexane extracts of *T. avicenioids, T. macroptera,* and *T. laxiflora,* respectively.

3. Results

3.1. Phytochemical analyses

3.1.1. Extraction yields

The bark extraction yields varied depending on the extraction solvent and plant species. The highest yields were obtained in polar solvents, with 10.65 ± 0.01 % and 9.45 ± 0.02 % for the water-ethanol and pure ethanol extracts, respectively. Yields from the three plant species ranged from 10.65 ± 0.01 to 5.69 ± 0.02 %. *T. avicennioides* yielded the highest extract with 10.65 ± 0.01, 9.450 ± 0.02, and 7.93 ± 0.01 % for the hydroethanolic, ethanolic, and hexane extracts, respectively (Fig. 1).

3.1.2. Phytochemical characterization

Phytochemicals in the extracts from the three *Terminalia* species varied substantially. The ethanolic extract of *T. macroptera* (+++) contained high concentrations of flavonoids compared to the other extracts. The ethanolic and hydroethanolic extracts of *T. avicennioides* were rich in tannins and polyphenols, whereas saponins were not detected in the hydroethanolic extract. Weak alkaloids were present in all extracts, except for the ethanolic extract of *T. laxiflora* (Table 3).

3.1.2.1. Quantification of total phenols, flavonoids, flavonols, and coumarin contents in the bark extracts. Analysis of secondary metabolites revealed that the ethanolic extracts contained high contents of total polyphenols, with 158.69 ± 0.12 μg GAE/g in *T. avicennioides*; whereas low phenolic content was recorded in hexane extracts of *T. laxiflora* with 29.43 ± 0.03 μg GAE/g. The total flavonoids in the hydroethanolic and ethanolic solvents were high, indicating the greater extraction capacities of these solvents for these metabolites (Table 4). The content of total flavonoids in the ethanolic extract of *T. macroptera* was the highest (72.25 ± 0.03 μg QE/g), whereas it was lowest in the hexane extract of *T. laxiflora* (8.35 ± 0.02 μg QE/g). The flavonol content displayed the same order as the flavonoids, with high flavonol content in the ethanolic extract of *T. avicennioides* (42.27 ± 0.26 μg RE/100 g), and the lowest in the hexane extract of *T. laxiflora* (3.08 ± 0.02 μg RE/100 g) (Table 4).

Coumarin content was comparable in all the hexane extracts of the three species (10.05 ± 0.03, 9.32 ± 0.02 and 8.46 ± 0.02 μg CE/mg) (Table 4).

3.1.3. Identification of phenolic compounds by HPLC

3.1.3.1. HPLC analysis of the ethanolic extract of T. avicennioides. HPLC analysis revealed that the ethanolic extract of *T. avicennioides* (he1) contained hyperoxides with a real surface area equivalent to 54.15 %. Phenolic acids, such as gallic acid, chlorogenic acid, and ferulic acid, were also identified (Table 5).

3.1.3.2. HPLC analysis of the ethanolic extract of T. macroptera. The HPLC analysis of the ethanolic extract of *T. macroptera* exhibited a notable surface area of 10.22 % for isorhamnetin. Other flavonoids, such as quercetin and luteolin, were detected with relatively smaller surface areas (Table 6).

3.1.3.3. HPLC analysis of the ethanolic extract of T. laxiflora. HPLC analysis showed that the ethanolic extract of *T. laxiflora* was rich in phenolic acids, particularly syringic acid, which accounted for 20.76 % of the total surface area. Chlorogenic and ellagic acids were also detected at varying concentrations (Table 7).

3.2. Antibacterial activity

The antibacterial activity assays revealed that the MIC of the ethanolic extracts varied between 0.10 and 0.20 mg/mL against *E. coli*. The MIC for *P. aeruginosa* was 0.20 mg/mL for the ethanolic extracts, while a value of 0.10 mg/mL was recorded for the hydroethanolic extracts. The results showed that ethanolic extracts exhibited competitive MIC values compared to extracts with other solvents.

In the case of *K. pneumoniae* and *S. aureus*, the hydroethanolic extract of *T. laxiflora* demonstrated strongest inhibitory effect activity

Table 3
Phytochemical composition of bark extracts from three *Terminalia* species.

Plant species	Extracts	Flavonoids	Tannins and polyphenols	Saponins	Alkaloids
T. avicennioides	he1	++	+++	–	+
	et1	++	+++	+	+
	hx1	+	++	++	+
T. macroptera	he2	++	++	+	+
	et2	+++	++	++	–
	hx2	+	+	+	+
T. laxiflora	he3	+	++	+	+
	et3	++	++	+	–
	hx3	+	+	+	+

+++ = strong presence, ++ = medium presence; + = weak presence; - = absence.

Table 4
Quantification of secondary Values with the same letters are statistically identical. A significant difference is considered for $p < 0.05$.

Plant species	Extracts	Total polyphenols (μg GAE/g)	Total flavonoids (μg QE/g)	Flavonols (μg RE/100 g)	Coumarin (μg CE/mg)
T. avicennioides	he1	146.35 ± 0.07^{a}	65.29 ± 0.06^{b}	28.49 ± 0.03^{bc}	13.28 ± 0.02^{a}
	et1	156.31 ± 0.12^{a}	72.25 ± 0.03^{a}	42.27 ± 0.26^{a}	14.29 ± 0.03^{a}
	hx1	56.31 ± 0.02^{d}	49.58 ± 0.12^{d}	23.25 ± 0.03^{d}	9.32 ± 0.02^{b}
T. macroptera	he2	132.39 ± 0.08^{b}	58.25 ± 0.16^{c}	26.31 ± 0.05^{c}	9.32 ± 0.03^{b}
	et2	158.69 ± 0.12^{a}	36.19 ± 0.05^{f}	15.89 ± 0.02^{e}	10.48 ± 0.03^{b}
	hx2	62.41 ± 0.23^{d}	42.29 ± 0.02^{e}	6.09 ± 0.01^{f}	8.46 ± 0.02^{b}
T. laxiflora	he3	93.74 ± 0.26^{cd}	43.25 ± 0.04^{e}	25.61 ± 0.03^{c}	12.39 ± 0.18^{ab}
	et3	103.53 ± 0.08^{c}	48.31 ± 0.05^{d}	32.57 ± 0.08^{b}	6.92 ± 0.01^{c}
	hx3	29.43 ± 0.03^{e}	18.35 ± 0.02^{g}	3.08 ± 0.02^{g}	10.05 ± 0.03^{b}

he1: hydroethanolic extract of *T. avicenioids*; he2: hydroethanolic extract of *T. macroptera;* he3: hydroethanolic extract of *T. laxiflora;* et1: ethanolic extract of *T. avicenioids;* et2: ethanolic extract of *T. macroptera;* et3: ethanolic extract of *T. laxiflora*; hx1: hexanic extract of *T. avicenioids*; hx2: hexanic extract of *T. macroptera;* hx3: hexanic extract of *T. laxiflora*.

Table 5
HPLC analysis of the ethanolic extract of *T. avicennioides* (he1).

Woodpecker names	Height (mAU)	Surface (mAU*min)	Actual surface (%)	Retention time (min)
Gallic acid	5.707	3.154	24.17	3.187
Chlorogenic acid	1.103	1.153	8.45	6.573
Ferulic Acid	0.128	0.096	3.47	12.123
Hyperoxide	4.680	6.558	45.15	16.553
Ellagic acid	0.003	0.000	lq	18.857
Isorhamnetin	0.004	0.000	lq	26.307
Chrysine	0.868	0.776	4.87	28.563

lq: low quantity.

at 3.125 mg/mL, surpassing the efficacy of ethanolic and hexane extracts.

3.3. Antifungal activity

The ability of the extracts to inhibit fungal growth revealed MICs ranging between 0.20 and 6.25 mg/mL. The ethanolic extract of *T. macroptera* exhibited the lowest MIC of 0.20 mg/mL against *C. albicans*, whereas the highest value for MIC of 6.25 mg/mL was recorded for the hexane extract of *T. laxiflora*. The hexane extracts of *T. avicennioides* and *T. macroptera* exhibited an MIC of 0.20 mg/

Table 6
HPLC analysis of the ethanolic extract of *T. macroptera* (he2).

Woodpecker names	Height (mAU)	Surface (mAU*min)	Actual surface (%)	Retention time (min)
Not determined	170.642	16.852	72.64	2.070
Chlorogenic acid	0.005	0.000	lq	2.613
Caffeic acid	0.020	0.003	3.02	3.725
Tannic acid	0.021	0.002	4.01	6.237
Ferulic Acid	0.006	0.000	lq	8.264
Hyperoside	0.005	0.000	lq	13.715
Ellagic acid	0.014	0.002	3.01	15.791
Quercetin	0.009	0.000	2.05	18.673
Luteolin	0.005	0.000	lq	20.759
Isorhamnetin	0.075	0.026	10.22	26.423

lq: low quantity.

Table 7
HPLC analysis of the ethanolic extract of *T. laxiflora (he3)*.

Woodpecker names	Height (mAU)	Surface (mAU*min)	Actual surface (%)	Retention time (min)
Not determined	48.760	35.615	63.76	1.547
Chlorogenic acid	0.033	0.001	lq	2.630
Syringic acid	6.691	6.010	20.76	3.319
Ellagic acid	0.004	0.000	lq	18.967
Isorhamnetin	0.170	1.205	4.16	26.869
Chrysine	0.008	0.000	lq	28.789

lq: low quantity.

mL against *C. krusei*. The ethanolic extracts of *T. avicennioides*, *T. macroptera,* and *T. laxiflora* exhibited MICs of 0.20 mg/mL against *C. glabrata*. The MICs of the extracts for *C. tropicalis* varied between 6.25 and 3.125 mg/mL (Table 8).

3.4. Antioxidant activity

Antioxidant activity tests showed that the ethanolic extract of *T. avicennioides* had the highest ABTS antioxidant activity (56.72 ± 0.49 μg TE/100 mg), whereas the lowest value was recorded for the hexane extract of the same plant with 9.25 ± 0.02 μg TE/100 mg (Table 9).

The hydroethanolic extract of *T. macroptera* exhibited a better Fe^{3+} reduction capacity with 47.38 ± 0.41 μg AAE/100 mg, whereas the hexane extract of *T. laxiflora* exhibited a low capacity with 4.58 ± 0.02 μg AAE/100 mg (Table 9).

The DPPH test showed that the ethanolic extract of *T. avicennioides* had a strong anti-radical activity (84.74 ± 1.04 μg AAE/100 mg), whereas the lowest anti-radical activity was recorded for the hexane extract of the same plant (15.84 ± 0.23 μg AAE/100 mg) (Table 9).

The IC50 % analysis revealed that amongst the plant extracts, the ethanolic extract of *T. avicennioides* demonstrated the most effective concentration at 14.45 μg/mL. In contrast, the hexane extract of the same species exhibited the least potent IC50 % at 665.23 μg/mL (Table 9).

3.5. Cytotoxicity assay

The cytotoxicity test findings showed that the hexane extracts from the three plant species exhibited very high lethal concentrations (LC50 %) compared to the hydroethanolic and ethanolic extracts. Specifically, the hexane extracts of *T. avicennioides*, *T. macroptera,* and *T. laxiflora* demonstrated LC50 % values of 105.67 ± 10.48, 106.82 ± 8.56, and 117.35 ± 9.25 μg/mL, respectively. In contrast, the hydroethanolic extracts of *T. avicennioides*, *T. macroptera,* and *T. laxiflora* showed the lowest values at 55.87 ± 3.45, 52.37 ± 2.1, and 50.74 ± 2.46 μg/mL, respectively (Fig. 2). Based on the National Cancer Institute's classification, the cytotoxicity of these extracts can be classified as moderate (LC50 %: 0.021–0.2 mg/mL).

3.6. Summary of the bioactive polyphenols

The properties of the bioactive polyphenols present in the extracts of the three *Terminalia* species are summarised in Table 10.

Table 8
Antimicrobial activities of the extracts.

(a) Antibacterial activity

Bacteria	MIC (mg/mL)									Imipenem (μg/mL)
	he1	et1	hx 1	he2	et2	hx2	he3	et3	hx3	
Gram-negative										
***E. coli* ATCC25922**	0.20 ± 0.0001	0.10 ± 0.0001	6.25 ± 0.01	0.20 ± 0.0001	0.20 ± 0.0001	3.125 ± 0.02	0.20 ± 0.0001	0.10 ± 0.0002	3.125 ± 0.02	1.56 ± 0.001
***K. pneumoniae* ATCC13883**	6.25 ± 0.02	6.25 ± 0.02	3.125 ± 0.01	6.25 ± 0.02	6.25 ± 0.02	3.125 ± 0.01	3.125 ± 0.01	3.125 ± 0.01	3.125 ± 0.01	1.56 ± 0.001
***P. aeruginosa* ATCC27853**	0.10 ± 0.0001	0.20 ± 0.00010	3.125 ± 0.01	0.10 ± 0.0001	0.20 ± 0.0001	1.56 ± 0.001	0.20 ± 0.0001	0.20 ± 0.0001	1.56 ± 0.001	0.78 ± 0.0005
Gram positive										
***S. aureus* ATCC43800**	6.25 ± 0.02	3.125 ± 0.01	6.25 ± 0.02	6.25 ± 0.02	6.25 ± 0.02	6.25 ± 0.02	3.125 ± 0.01	3.125 ± 0.01	6.25 ± 0.02	1.5 ± 0.0016

(b) Antifungal activity

Fungi	MIC (mg/mL)									Nystatin (μg/mL)
	he1	et1	hx 1	he2	et2	hx2	he3	et3	hx3	
***C. albicans* ATCC10231**	0.39 ± 0.0003	0.39 ± 0.0003	3.125 ± 0.01	0.39 ± 0.0003	0.20 ± 0.0001	3.125 ± 0.01	0.39 ± 0.0003	0.20 ± 0.0001	6.25 ± 0.02	0.78 ± 0.0000005
***C. krusei* ATCC34135**	3.125 ± 0.01	6.25 ± 0.02	0.20 ± 0.0001	4.59 ± 0.03	3.125 ± 0.01	0.20 ± 0.0001	3.125 ± 0.01	1.59 ± 0.003	6.25 ± 0.02	0.78 ± 0.0005
***C. glabrata* ATCC2001**	3.125 ± 0.01	0.20 ± 0.0001	1.56 ± 0.005	3.125 ± 0.01	0.20 ± 0.0001	1.56 ± 0.005	1.56 ± 0.005	0.20 ± 0.0001	1.56 ± 0.005	0.78 ± 0.0005
***C. tropicalis* ATCC28707**	6.25 ± 0.02	6.25 ± 0.02	6.25 ± 0.02	3.125 ± 0.01	3.125 ± 0.01	3.125 ± 0.01	6.25 ± 0.02	3.125 ± 0.01	3.125 ± 0.01	156 ± 0.0005

Imip/Nyst-imipenem/Nystatin; he1: hydroethanolic extract of *T. avicenioids*; he2: hydroethanolic extract of *T. macroptera;* he3: hydroethanolic extract of *T. laxiflora;* et1: ethanolic extract of *T. avicenioids;* et2: ethanolic extract of *T. macroptera;* et3: ethanolic extract of *T. laxiflora*; hx1: hexanic extract of *T. avicenioids*; hx2: hexanic extract of *T. macroptera;* hx3: hexanic extract of *T. laxiflora*.

Table 9
Antioxidant activities of the *Terminalia* species extract.

Plant species	Extracts	ABTS (µg TE/100 mg)	FRAP (µg AAE/100 mg)	DPPH (µg AAE/100 mg)	DPPH-IC50 % (µg/mL)
T. avicennioides	he1	35.48 ± 0.29^{bc}	23.56 ± 0.15^{cd}	53.49 ± 0.25^{b}	14.45
	et1	56.72 ± 0.49^{a}	32.59 ± 0.46^{b}	84.74 ± 1.04^{a}	80.57
	hx1	9.25 ± 0.02^{f}	4.23 ± 0.02^{f}	15.84 ± 0.23^{e}	665.23
T. macroptera	he2	46.57 ± 0.61^{b}	47.38 ± 0.41^{a}	49.58 ± 0.34^{b}	22.90
	et2	37.69 ± 1.02^{bc}	19.54 ± 0.53^{d}	53.52 ± 0.23^{b}	44.12
	hx2	23.47 ± 0.25^{d}	7.35 ± 0.01^{e}	15.35 ± 0.08^{e}	732.10
T. laxiflora	he3	32.69 ± 0.15^{c}	20.45 ± 0.23^{d}	46.67 ± 0.84^{c}	21.46
	et3	47.89 ± 0.28^{b}	25.47 ± 0.61^{c}	51.35 ± 0.24^{bc}	80.17
	hx3	17.48 ± 0.25^{e}	4.58 ± 0.02^{f}	19.56 ± 0.43^{d}	461.45

Antioxidant activity was estimated using the ABTS, FRAP, and DPPH assays. Values with the same superscript letters are considered statistically identical. Statistical significance is set at $p < 0.05$.
he1: hydroethanolic extract of *T. avicenioids*; he2: hydroethanolic extract of *T. macroptera;* he3: hydroethanolic extract of *T. laxiflora;* et1: ethanolic extract of *T. avicenioids;* et2: ethanolic extract of *T. macroptera;* et3: ethanolic extract of *T. laxiflora*; hx1: hexanic extract of *T. avicenioids*; hx2: hexanic extract of *T. macroptera;* hx3: hexanic extract of *T. laxiflora*.

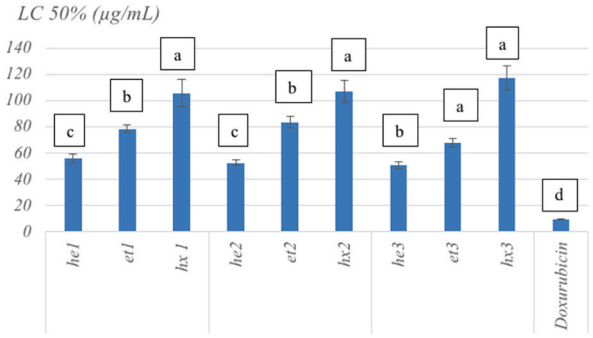


Fig. 2. Cytotoxicity (LC50 %) of *Terminalia* species extracts.
Values with the same superscript letters are considered statistically identical. Statistical significance is set at $p < 0.05$.
he1: hydroethanolic extract of *T. avicenioids*; he2: hydroethanolic extract of *T. macroptera;* he3: hydroethanolic extract of *T. laxiflora;* et1: ethanolic extract of *T. avicenioids;* et2: ethanolic extract of *T. macroptera;* et3: ethanolic extract of *T. laxiflora*; hx1: hexanic extract of *T. avicenioids*; hx2: hexanic extract of *T. macroptera;* hx3: hexanic extract of *T. laxiflora*.
LC50 % values (µg/mL) represent the mean ± standard error (n = 3). Extracts with LC50 % < 20 µg/mL are considered toxic (according to the established safety standard for cytotoxicity). The positive control used was Doxorubicin.

3.7. Data analysis ACP and CAH

3.7.1. Analysis of variables

This graph demonstrates the selection of axes F1 (55.57 %) and F2 (13.23 %). Different tests carried out with the plant extracts highlighted two major groups: the first group comprised *C. tropicalis* (Ct), *C. krusei* (Ck), *K. pneumonia* (Kp), coumarins (Co), total flavonoids (Tf), flavonols (Fl), total phenolics (Tp), DPPH, FRAP, and ABTS; the second group included *C. glabrata* (Cg), *C. albicans* (Ca), *S. aureus* (Sa), *P. aeruginosa* (Pa), and D-IC50 %.

The different vectors revealed considerable variable intensities, with the highest recorded at the level of the antioxidant tests (DPPH and ABTS) and the lowest at the level of the MIC of fungi, particularly Cg and Ck (Fig. 3).

3.7.2. Behaviour of the different extracts on the axes

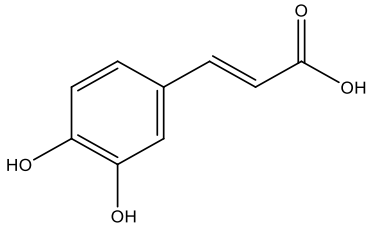
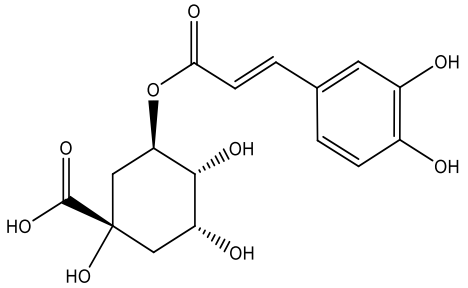
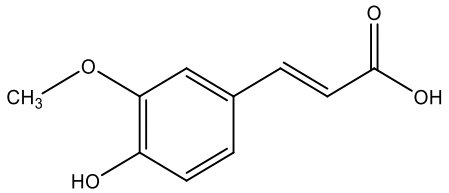
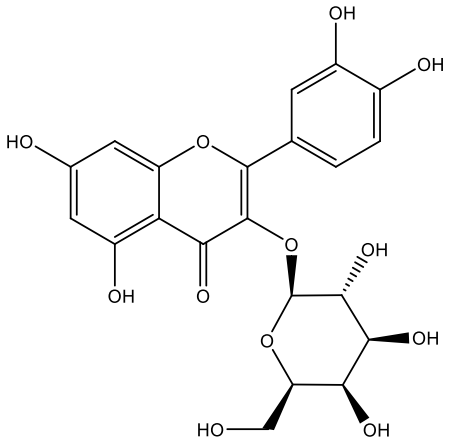
This graph revealed two forms of groupings along the first axis F1, of which the part recorded in the positive zone consisted of *T. laxiflora* hydroethanolic extract (he3), *T. avicennioides* hydroethanolic extract (he1), *T. avicennioides* ethanolic extract (et1), and *T. avicennioides* hexane extract (hx1), while the negative zone was essentially comprised of *T. laxiflora* ethanolic extract (et3), *T. macroptera* ethanolic extract (et2), *T. macroptera* hydroethanolic extract (he2), *T. laxiflora* hexane extract (hx3), and *T. macroptera* hexane extract (hx2).

The right side of the F2 axis had *T. laxiflora* hydroethanolic extract (he3), *T. avicennioides* hydroethanolic extract (he1), *T. avicennioides* ethanolic extract (et1), *T. laxiflora* ethanolic extract (et3), *T. macroptera* hydroethanolic extract (he2), and *T. macroptera* ethanolic extract (et2). The left part primarily contained the hexane extracts (hx1, hx2, and hx3) (Fig. 4).

3.7.3. Biplot analysis

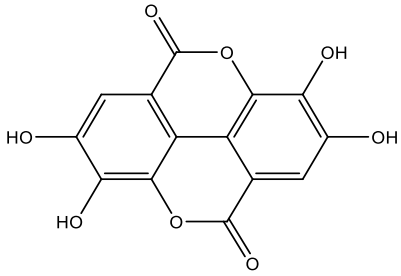
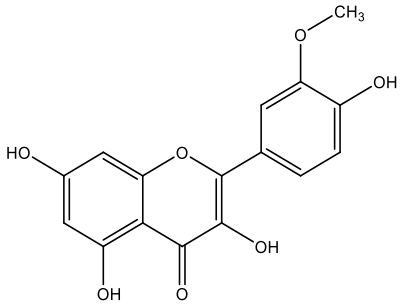
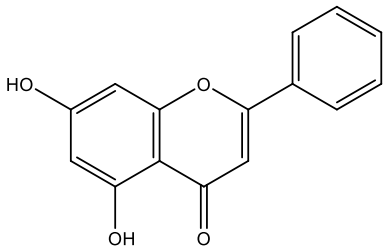
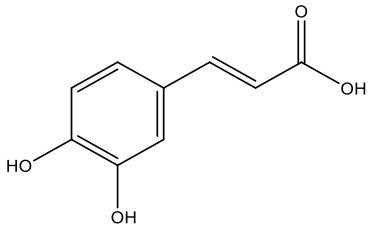
The biplot illustrates the correlation between the ethanolic and hydroethanolic extracts of different plant species and a majority of

Table 10
Summary of the properties of bioactive polyphenols in the ethanolic extracts of three *Terminalia* species.

Polyphenol compounds	Structure	Properties
Gallic acid	 Caffeic acid	Antihyperlipidemic, treatment for respiratory diseases, cardioprotective [34], antioxidant, anti-inflammatory [33], antineoplastic properties [35].
Chlorogenic acid	 Chlorogenic acid	Treatment for hepatic steatosis, anti-carcinogenic [36], preventing neurological diseases [37], anti-inflammatory [38].
Ferulic Acid	 Ferulic acid	Treatment for skin and respiratory diseases [39], anti-diabetic, anti-hypertensive, anti-hyperlipidemic [40].
Hyperoside	 Hyperoside	Anti-carcinogenic, neuroprotective effect [41].

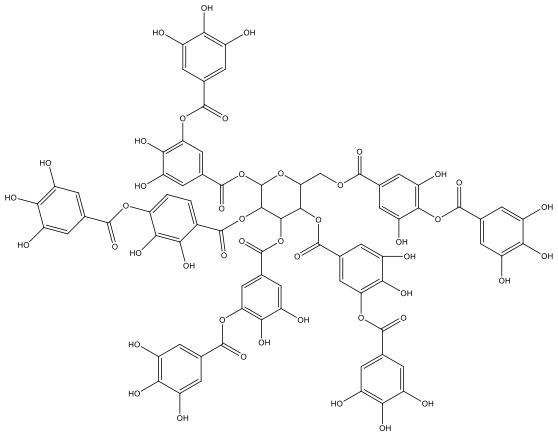
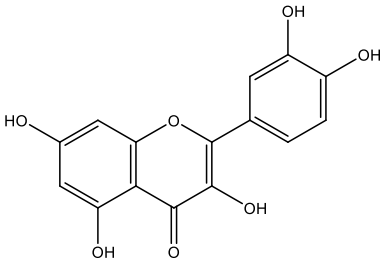
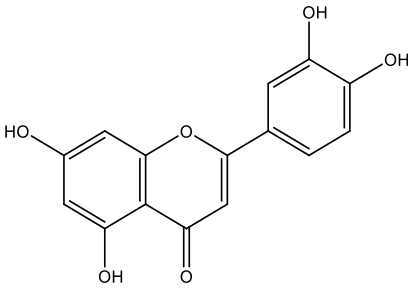
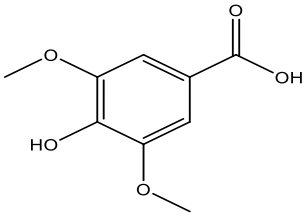
(*continued on next page*)

Table 10 (*continued*)

Polyphenol compounds	Structure	Properties
Ellagic acid	 Ellagic acid	Beneficial for respiratory diseases, antioxidant, anti-inflammatory [42].
Isorhamnetin	 Isorhamnetin	Antimicrobial, chemo preventive agent [43].
Chrysine	 Chrysine	Antidepressant effects, prevention of neuroinflammation, improves blood circulation, antioxidant [44].
Caffeic acid	 Caffeic acid	Anti-carcinogenic, prevents premature aging, prevents neurodegenerative diseases like Parkinson's disease, reduce inflammation [37,39].

(*continued on next page*)

Table 10 (*continued*)

Polyphenol compounds	Structure	Properties
Tannic acid	 Tannic acid	Antiviral, anti-bacterial, anti-fungal [45,46].
Quercetin	 Quercetin	Anti-carcinogenic, reduces the risk of heart diseases, prevents neurological diseases, anti-inflammatory, antiviral activities [47].
Luteolin	 Luteolin	Anti-diabetic, anti-allergic, anti-inflammatory, hepato- and renal-protective agent, anti-inflammatory, anticancer [48].
Syringic acid	 Syringic acid	Anti-inflammatory, anti-microbial [49], anti-cancer, antioxidant [39].

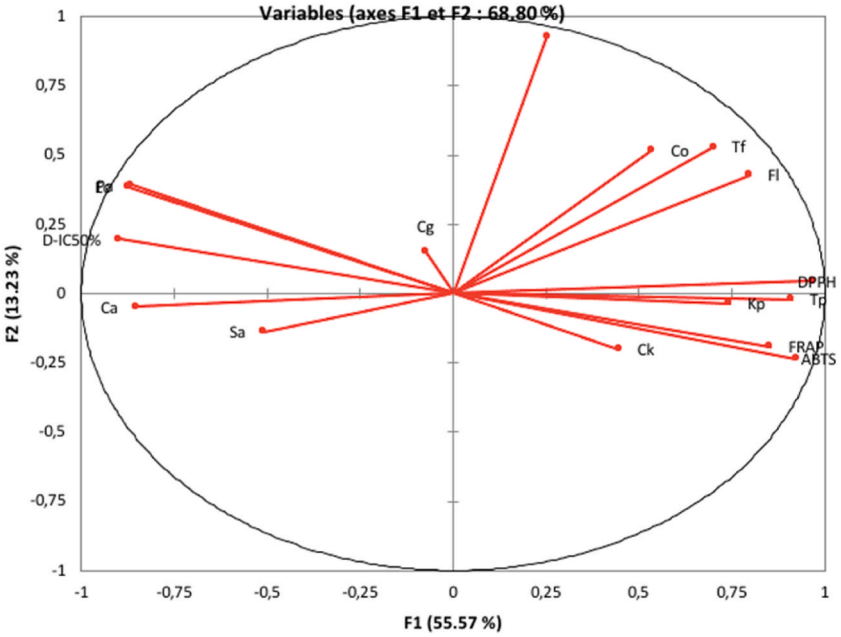


Fig. 3. Graph of the variables.
Tp: Total phenolics; Tf: Total flavonoids; Fl: Flavonols; Co: coumarins; Ec: *E. coli*; Kp: *K.a pneumonia*; Pa: *P. aeruginosa*; Sa: *S. aureus*; Ca: *C. albicans*; Ck: *C. krusei*; Cg: *C. glabrata*; Ct: *C. tropicalis*.

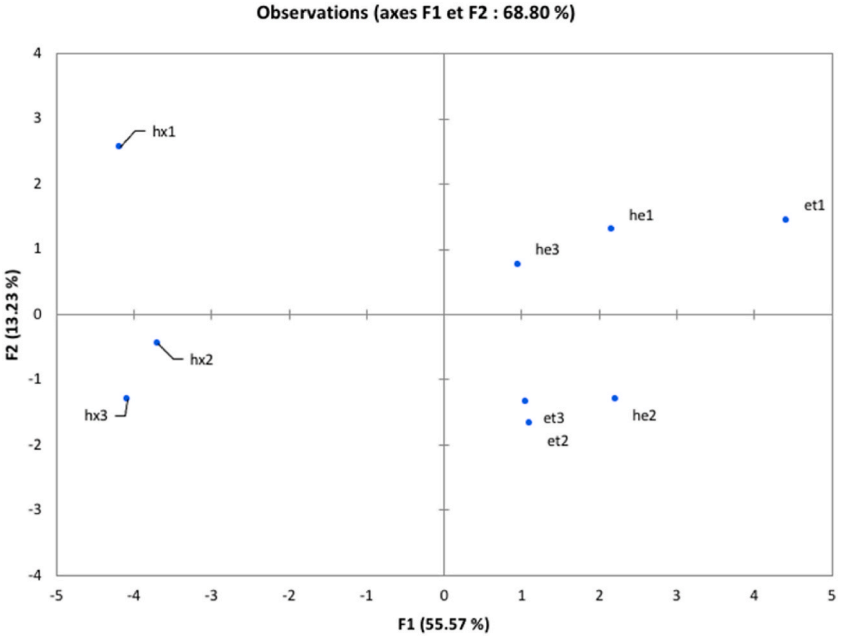


Fig. 4. Behaviour of the *Terminalia* species extracts on the axes.

the tests conducted, including antioxidant tests (DPPH, ABTS, and FRAAP) on the one hand, and antimicrobial activities on the other (Ct, Ck, Kp).

The hexane extracts exhibited a relationship with DPPH antioxidant activity (D-IC50 %) and antimicrobial activities (Pa, Sa, Ca, and Cg) (Fig. 5).

3.7.4. Correlation matrix

The correlation matrix showed strong positive correlations between certain antioxidant activities and the contents of secondary

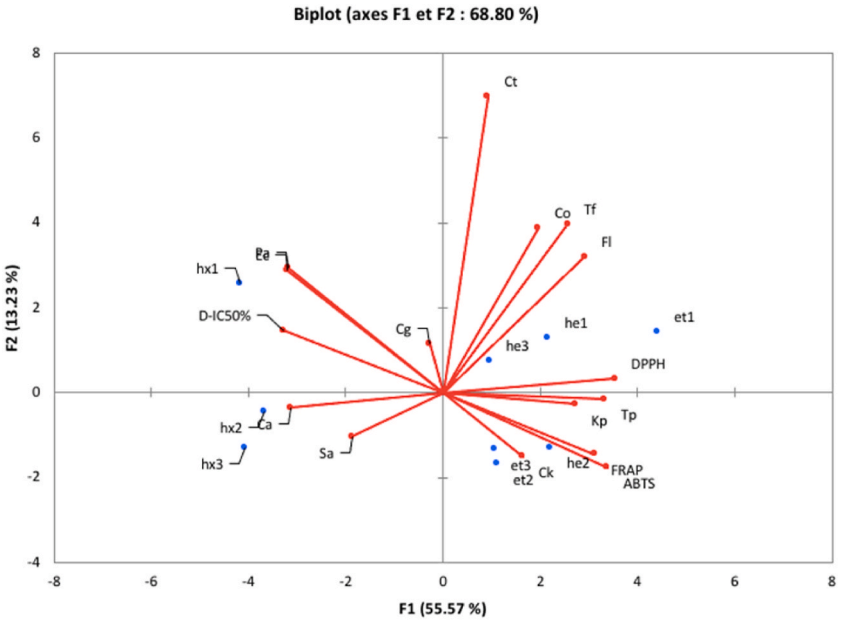


Fig. 5. Biplot of *Terminalia* species extracts.
he1: hydroethanolic extract of *T. avicenioids*; he2: hydroethanolic extract of *T. macroptera;* he3: hydroethanolic extract of *T. laxiflora;* et1: ethanolic extract of *T. avicenioids;* et2: ethanolic extract of *T. macroptera;* et3: ethanolic extract of *T. laxiflora*; hx1: hexanic extract of *T. avicenioids*; hx2: hexanic extract of *T. macroptera;* hx3: hexanic extract of *T. laxiflora;*.
Tp: Total phenolics; Tf: Total flavonoids; Fl: Flavonols, Co: coumarins; Ec: *E. coli*; Kp: *K. pneumonia*; Pa: *P. aeruginosa*; Sa: *S. aureus*; Ca: *C. albicans*; Ck: *C. krusei*; Cg: *C. glabrata*; Ct: *C. tropicalis*.

metabolites, such as ABTS and Tp, DPPH and Fl, and DPPH and Tp (0.8 correlation coefficient).

The antimicrobial activity tests revealed good correlation coefficients (0.8) between the total phenolic content and the MICs of the extracts against *P. aeruginosa*.

Negative correlation coefficients were obtained between the contents of certain secondary metabolites and DPPH antioxidant activity (IC50 %) (Table 11).

3.7.5. Dendrogram analysis

The dendrogram identified three distinct classes. The first class had the largest number of extracts (et1, et2, et3, he1, he2, and he3). The second class contained only the hexane extract of *T. laxiflora* (hx3), and the third class was composed of the hexane extracts of *T. avicennioides* (hx1) and *T. macroptera* (hx2) (Fig. 6).

4. Discussion

4.1. Phytochemical study

The extraction yields varied from the bark varied among the three Terminalia species and differed depending on the solvent used for each species. The highest yields were obtained using hydroethanolic extraction for each species. These findings demonstrate the superior capacity of polar solvents for extraction compared to non-polar solvents such as hexane. Morrison and Twumasi similarly reported that polar solvents, such as the water-methanol mixtures, produced better yields than non-polar solvents when extracting from plant powders [50]. The variability in extraction yields using the same solvent highlights the diverse textural organization and distribution of organic substances within the tissues of various Terminalia species [51].

Phytochemical screening revealed the presence of chemical compounds likely to confer antimicrobial properties in plant extracts. The primary groups identified were alkaloids, phenolics, tannins, flavonoids, and saponins. However, some phytochemicals were absent in certain extracts. Alkaloids and saponins were not found in the ethanolic extract of *T. macroptera*, whereas flavonoids were found in both the bark of *T. macroptera* and the hydroethanolic extract of *T. avicennioides*. Flavonoids, tannins, and polyphenols were detected in all the extracts of the three plant species. Flavonoids are known for their multiple medicinal properties and efficacy against several diseases, including venereal and infectious diseases [52]. The extracts of the studied plants contained different types of tannins, which exhibit therapeutic effects such as astringent, antibacterial, antiviral, antifungal, and antiseptic properties. Tannins are used for their antioxidant and haemostatic activities [53]. Saponins, a heterogeneous group of natural products found in all eight extracts, possess surfactant, antifungal, antibacterial, and antiviral properties and demonstrate protective activities on veins and capillaries, as well as oedematous and hormonal activities. Alkaloids were detected only in two extracts. Alkaloids are highly sought after owing to

Table 11
Correlation matrix (Pearson [n]).

Variables	ABTS	FRAP	DPPH	D-IC50 %	Ec	Kp	Pa	Sa	Ca	Ck	Cg	Ct	Tp	Tf	Fl	Co
ABTS	**1**															
FRAP	0.858	**1**														
DPPH	0.913	0.749	**1**													
D-IC50 %	−0.787	−0.773	−0.831	**1**												
Ec	−0.870	−0.750	−0.808	0.908	**1**											
Kp	0.617	0.685	0.708	−0.623	−0.573	**1**										
Pa	−0.857	−0.756	−0.791	0.905	0.999	−0.586	**1**									
Sa	−0.569	−0.297	−0.579	0.442	0.467	0.158	0.428	**1**								
Ca	−0.761	−0.731	−0.763	0.776	0.737	−0.565	0.726	0.452	**1**							
Ck	0.430	0.413	0.519	−0.475	−0.445	0.465	−0.449	−0.163	0.057	**1**						
Cg	−0.234	0.195	−0.268	0.001	0.075	0.174	0.027	0.528	0.087	0.008	**1**				–	
Ct	−0.037	−0.025	0.285	−0.129	0.081	0.100	0.091	−0.316	−0.238	0.006	0.131	**1**				
Tp	0.802	0.743	0.874	−0.795	−0.766	0.882	−0.762	−0.214	−0.863	0.272	−0.078	0.177	**1**			
Tf	0.600	0.637	0.660	−0.402	−0.349	0.578	−0.347	−0.299	−0.678	0.033	0.177	0.554	0.667	**1**		
Fl	0.714	0.660	0.806	−0.620	−0.478	0.428	−0.456	−0.654	−0.749	0.164	−0.142	0.555	0.654	0.848	**1**	
Co	0.277	0.215	0.588	−0.426	−0.345	0.533	−0.346	−0.219	−0.266	0.545	0.103	0.723	0.484	0.459	0.413	**1**

Tp: Total phenolics, Tf: Total flavonoids, Fl: Flavonols, Co: coumarins, Ec: *E. coli*, Kp: *K. pneumonia*, Pa: *P. aeruginosa*, Sa: *S. aureus*, Ca: *C. albicans*, Ck: *C. krusei*, Cg: *C. glabrata*, Ct: *C. tropicalis*.

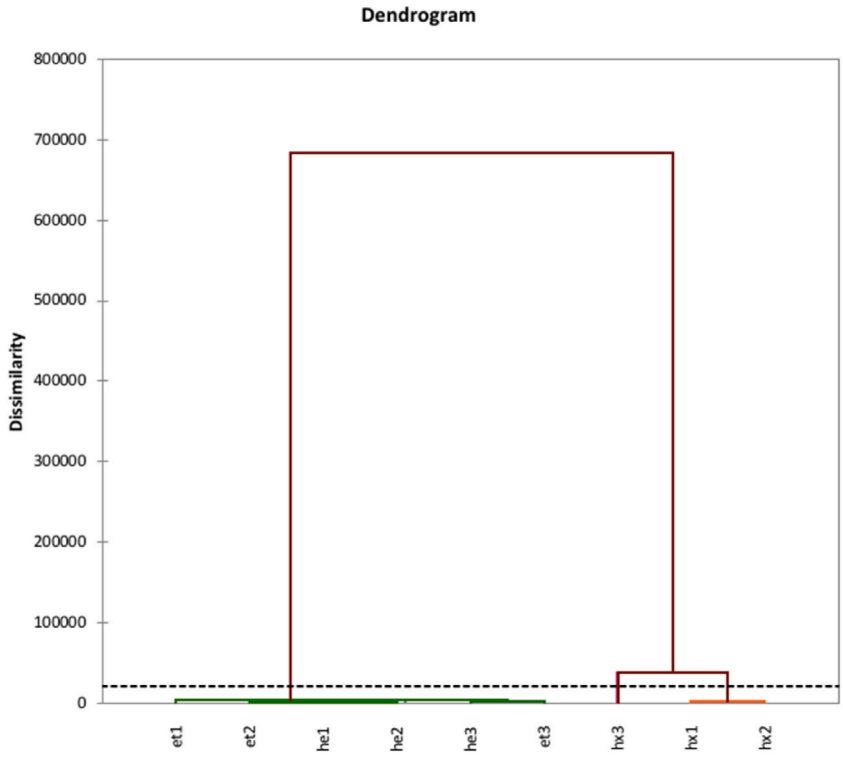


Fig. 6. Dendrogram of *Terminalia* species extracts.
he1: hydroethanolic extract of *T. avicenioids*; he2: hydroethanolic extract of *T. macroptera;* he3: hydroethanolic extract of *T. laxiflora;* et1: ethanolic extract of *T. avicenioids;* et2: ethanolic extract of *T. macroptera;* et3: ethanolic extract of *T. laxiflora*; hx1: hexane extract of *T. avicenioids*; hx2: hexane extract of *T. macroptera;* hx3: hexane extract of *T. laxiflora.*

their broad spectrum of biological activities, including antibiotic, antiparasitic, anaesthetic, antitumour, anticancer, analgesic, and spasmolytic properties [54]. Alkaloids also exert effects on the central nervous system [55].

Secondary metabolites, such as total phenolics, flavonoids, and flavonols, were present in high levels in the extracts obtained using polar solvents. Polar solvents like water and ethanol have a very high capacity to extract the large group of secondary metabolites. Dirar et al. found that the hydroethanolic and ethanolic extracts contained high concentrations of total phenolics and flavonoids [56].

The coumarin contents was relatively high in the ethanolic extracts. Aryati et al. (2020) similarly revealed the presence of coumarins in the ethanolic extract of *Cinnamomum burmanni* trunk bark. This highlights the storage mechanism of this form of metabolite in the barks of plants like those in the *Terminalia* genus [57].

4.2. Antimicrobial activities

The examination of antimicrobial activities revealed that the ethanolic extracts had the lowest MIC values. This broad antimicrobial activity can be attributed to the presence of biomolecules, particularly flavonoids, whose antimicrobial properties have been demonstrated previously [58]. Additionally, extensive research has shown that phenols and their derivatives possess both antifungal and antimicrobial properties. Numerous studies have emphasised the significant role of tannins as effective antibacterial agents. Research has also confirmed that phenols and their derivatives have antifungal and antimicrobial properties [59]. Carpano et al. found lower MIC values for methanolic and aqueous extracts of *Terminalia* genus on microorganisms such as *C. albicans* (MIC = 180 and 250 μg/mL, respectively) and *C. kruzzei* (MIC = 250 and 300 μg/mL, respectively) [60]. Mbwambo et al. revealed MICs for bacteria of interest such as *S. aureus* (14.0 ± 1.1 μg/mL), *E. coli*, *P. aeruginosa* (12.0 ± 1.1 μg/mL), *K. pneumonia* (6.0 ± 1.0 μg/mL), *S. typhi,* and *B. anthracis* (13.0 ± 1.0 μg/mL) [61]. Not all tested extracts demonstrated the same antimicrobial potential in our study. This was probably due to the difference in chemical composition of the three extracts from the three *Terminalia* species. The mechanism of action of bioactive molecules can also explain the variability in the activities obtained [62].

4.3. Antioxidant activity

Antioxidant test revealed a very high capacity for ethanolic extracts compared to other extracts. Antioxidant properties of *Terminalia* species have been demonstrated previously [63,64]. The DPPH radical scavenging assay revealed relatively high antioxidant activity of the methanolic extracts of the *Terminalia* genus (IC50 % = 0.24, 1.02, and 0.25 mg/mL) [65]. Polar solvents, such as ethanol,

have the ability to extract secondary metabolites with high antioxidant potential, including phenolic compounds. Because of its high solubility and diffusion rates, ethanol easily penetrates into the matrix and extracts antioxidant phytomolecules with very high affinity [66]. This finding was confirmed by ABTS, DPPH, and FRAP tests [67]. These extract can be used to absorb free radicals in the body, thus inhibiting the peroxidation of membrane lipids [68]. Consequently, their anti-radical potential suggests a significant preventive role in human and animal health [69].

4.4. Cytotoxicity assay

The results of the cytotoxicity test showed that, compared to the control, none of the extracts were toxic. Das et al. [70] reported that the petroleum ether fraction exhibited maximum cytotoxicity with a lower LC50 % value of 10.94 g/mL. Compared with the positive control (vincristine sulfate), the cytotoxic potential exhibited by the *Terminalia* species extracts was negligible. However, greater safety was observed with hexane extracts compared to hydroethanolic and ethanolic extracts. Polar solvents are generally recognised for their association with cellular toxicity due to their ease of assimilation or permeability at the cellular level [71].

5. Conclusion

This study highlighted the phytochemical, antioxidant, and anti-microbial properties of *T. macroptera*, *T. avicennioides,* and *T. laxiflora*, which traditionally used to treat infectious diseases. This finding identified molecules in ethanolic extracts that may explain their diversity in terms of use in traditional medicine and suggest their potential as alternatives to combat microbial resistance to conventional antibiotics, particularly in the treatment of respiratory tract and skin diseases. The ethanolic extracts exhibited varying degrees of activity depending on the bacteria studied. Phytochemical analysis revealed that the ethanolic extract of *T. avicennioides* contained chemical compounds of interest that confer biological properties. Antioxidant tests showed a significant inhibitory effect on free radicals, demonstrating the notable antioxidant capacity of this extract. These results constitute a scientific basis that confirms the potential of using the trunk barks of the three *Terminalia* species in traditional medicine and extend the pharmacological action of these plant extracts from the traditional medical heritage of Burkina Faso.

CRediT authorship contribution statement

Jotham Yhi-pênè N'do: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dramane Paré:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Loyapin Bondé:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Adama Hilou:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

Data statement

Data used in this article are available for any requests.

Funding

No funding was obtained for this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

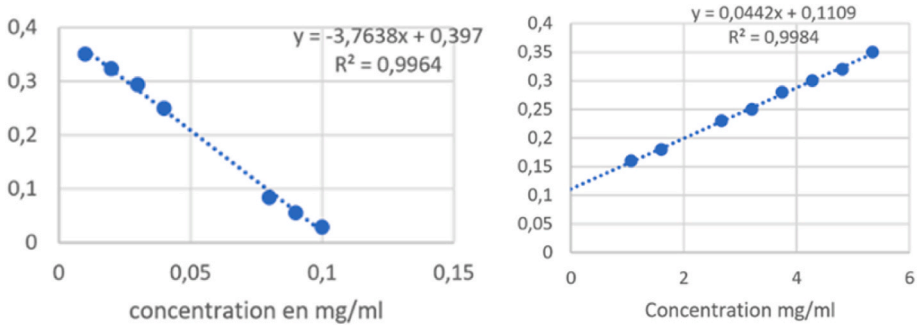
Acknowledgements

These results were obtained thanks to the collaboration of traditional practitioners, herbalists and sellers of traditional pharmacopoeia products from the Mouhoun region. We would also like to thank the biodiversity laboratory which facilitated the identification and authentication of plant species as well as the coding of the herbaria.

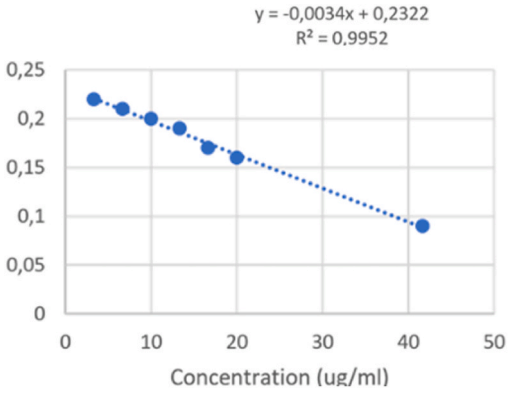
We thank the Laboratory of Biochemistry and Applied Chemistry, and the Laboratory of Microbiology and Microbial Biotechnology for their contributions in obtaining the phytochemical and biological results.

We appreciate the reviewers and editor for helping to improve the quality of the article.

Appendix (A)



A1: ABTS standard curve.A2: FRAP standard curve.



A3: DPPH standard curve.

References

[1] G. Huang, F. Guo, Loss of life expectancy due to respiratory infectious diseases: findings from the global burden of disease study in 195 countries and territories 1990–2017, Springer 39 (2022) 1, https://doi.org/10.1007/s12546-021-09271-3. Netherlands.

[2] C.J.L. Murray, The global burden of disease study at 30 years, Nat. Med. 28 (10) (2022) 2019–2026, https://doi.org/10.1038/s41591-022-01990-1.

[3] P. Eby, et al., Pathogen spillover driven by rapid changes in bat ecology, Nature 613 (7943) (2023) 340–344, https://doi.org/10.1038/s41586-022-05506-2.

[4] E. Aninagyei, et al., Utilization of 18s ribosomal RNA LAMP for detecting Plasmodium falciparum in microscopy and rapid diagnostic test negative patients, PLoS One 17 (10 October) (2022) 1–12, https://doi.org/10.1371/journal.pone.0275052.

[5] W. Rhimi, et al., Antifungal, antioxidant and antibiofilm activities of essential oils of cymbopogon spp, Antibiotics 11 (6) (2022) 1–13, https://doi.org/10.3390/antibiotics11060829.

[6] U. Anand, et al., Ethnodermatological use of medicinal plants in India: from ayurvedic formulations to clinical perspectives – a review, J. Ethnopharmacol. 284 (2022), https://doi.org/10.1016/j.jep.2021.114744.

[7] T. Report, Global, "Global research agenda for antimicrobial resistance in human health Policy brief Background the global research agenda for antimicrobial resistance in human health prioritizes forty research topics for evidence generation to inform policy by 2030,", World Heal. Organ. (2023) 12. June 2023, no. June.

[8] M.A.M. Ahmed, M.F. Ali, N.M. Mohamed, S.A.L. Bayoumi, A.M. Zahran, K.I. Elsayh, Exploring the efficacy of various wheat bran extracts in promoting burn wound healing: a comparative analysis, J. Ethnopharmacol. 319 (P1) (2024) 117174, https://doi.org/10.1016/j.jep.2023.117174.

[9] J.Y. N'do, et al., Indigenous knowledge and uses of medicinal plants in Ouagadougou, Burkina Faso, Ethnobot. Res. Appl. 29 (22) (2024) 15.

[10] M. Fik-Jaskolka, V. Mittova, C. Motsonelidze, M. Vakhania, C. Vicidomini, G.N. Roviello, Antimicrobial metabolites of caucasian medicinal plants as alternatives to antibiotics, Antibiotics 13 (6) (2024) 487, https://doi.org/10.3390/antibiotics13060487.

[11] P. Chaudhary, et al., Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases, Front. Chem. 11 (May) (2023) 1–24, https://doi.org/10.3389/fchem.2023.1158198.

[12] M.U. Akbar, et al., Molecular regulation of antioxidants and secondary metabolites act in conjunction to defend plants against pathogenic infection, South Afr. J. Bot. 161 (2023) 247–257, https://doi.org/10.1016/j.sajb.2023.08.028, 2023.

[13] M.A. Acquavia, et al., Analytical methods for extraction and identification of primary and secondary metabolites of apple (Malus domestica) fruits: a review, Separations 8 (7) (2021) 26, https://doi.org/10.3390/separations8070091.

[14] E.S. Prasedya, et al., Effect of particle size on phytochemical composition and antioxidant properties of Sargassum cristaefolium ethanol extract, Sci. Rep. 11 (1) (2021) 1–9, https://doi.org/10.1038/s41598-021-95769-y.

[15] F.W. Nana, A. Hilou, J.F. Millogo, O.G. Nacoulma, Phytochemical composition, antioxidant and xanthine oxidase inhibitory activities of Amaranthus cruentus L. and Amaranthus hybridus L. Extracts, Pharmaceuticals 5 (6) (2012) 613–628, https://doi.org/10.3390/ph5060613.

[16] S.A. Mahamat, N. Lahat, A.N. Cyrille, C. Mady, D.C. Mar, Phytochemical screening, total phenolic and antioxidant activity of numerous extracts from leaves and bark of *Bauhinia rufescens* lam, Food Nutr. Sci. 12 (6) (2021) 544–556, https://doi.org/10.4236/fns.2021.126041.

[17] V.L. Singleton, R. Orthofer, R. Lamuela, M. Rosa, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, in: P. Lester (Ed.), Methods Enzymol, vol. 299, Acad. Press, 1999, pp. 152–178.

[18] M. Ben Harb, S. Abubshait, N. Etteyeb, M. Kamoun, A. Dhouib, Olive leaf extract as a green corrosion inhibitor of reinforced concrete contaminated with seawater, Arab. J. Chem. 13 (3) (2020) 4846–4856, https://doi.org/10.1016/j.arabjc.2020.01.016.

[19] S. Suresh, et al., Phytochemical analysis and pharmacological evaluation of methanolic leaf extract of Moringa oleifera Lam. in ovalbumin induced allergic asthma, South Afr. J. Bot. 130 (2020) 484–493, https://doi.org/10.1016/j.sajb.2020.01.046.

[20] N. Almaraz-Abarca, M. da Graça Campos, J.A. Ávila-Reyes, N. Naranjo-Jiménez, J. Herrera Corral, L.S. González-Valdez, Antioxidant activity of polyphenolic extract of monofloral honeybee-collected pollen from mesquite (Prosopis juliflora, Leguminosae), J. Food Compos. Anal. 20 (2) (2007) 119–124, https://doi.org/10.1016/j.jfca.2006.08.001.

[21] J. Kocot, M. Kiełczykowska, D. Luchowska-Kocot, J. Kurzepa, I. Musik, Antioxidant potential of propolis, bee pollen, and royal jelly: possible medical application, Oxid. Med. Cell. Longev. 2018 (2018) 30, https://doi.org/10.1155/2018/7074209.

[22] Y. Bansal, G. Bansal, Analytical methods for standardization of Aegle marmelos : a review, JournL Pharm. Educ. Res. 2 (2) (2011) 2–9.

[23] M. Loncar, et al., Coumarins in food and methods of their determination 9 (5) (2020), https://doi.org/10.3390/foods9050645.

[24] M.M. Rahman, et al., Relative evaluation of in-vitro antioxidant potential and phenolic constituents by HPLC-DAD of Brassica vegetables extracted in different solvents, Heliyon 8 (10) (2022) e10838, https://doi.org/10.1016/j.heliyon.2022.e10838.

[25] S. Ahmed, A. Jubair, M.A. Hossain, M.M. Hossain, M.S. Azam, M. Biswas, Free radical-scavenging capacity and HPLC-DAD screening of phenolic compounds from pulp and seed of Syzygium claviflorum fruit, J. Agric. Food Res. 6 (2021) (2021) 100203, https://doi.org/10.1016/j.jafr.2021.100203.

[26] M.H. Dehghani, et al., Recent trends in the applications of sonochemical reactors as an advanced oxidation process for the remediation of microbial hazards associated with water and wastewater: a critical review, Ultrason. Sonochem. 94 (2023) 106302, https://doi.org/10.1016/j.ultsonch.2023.106302. October 2022.

[27] Z. Saddiqe, I. Naeem, C. Hellio, A.V. Patel, G. Abbas, Phytochemical profile, antioxidant and antibacterial activity of four Hypericum species from the UK, South Afr. J. Bot. 133 (2020) 45–53, https://doi.org/10.1016/j.sajb.2020.05.018.

[28] W.A. Negm, et al., Promising Antifungal Activity of Encephalartos laurentianus de Wild against Candida albicans Clinical Isolates: In Vitro and In Vivo Effects on Renal Cortex of Adult Albino Rats, J. Fungi 8 (5) (2022) 1–24, https://doi.org/10.3390/jof8050426.

[29] H.S. Elshafie, L. Caputo, L. De Martino, S.H. Sakr, V. De Feo, I. Camele, Study of bio-pharmaceutical and antimicrobial properties of pomegranate (Punica granatum l.) leathery exocarp extract, Plants 10 (1) (2021) 1–9, https://doi.org/10.3390/plants10010153.

[30] İ. Gulcin, S.H. Alwasel, DPPH radical scavenging assay, Processes 11 (8) (2023) 20, https://doi.org/10.3390/pr11082248.

[31] F.T. Pastor, D.M. Šegan, S. Gorjanović, A.M. Kalušević, D. Sužnjević, Development of voltammetric methods for antioxidant activity determination based on Fe (III) reduction, Microchem. J. 155 (January) (2020) 104721, https://doi.org/10.1016/j.microc.2020.104721.

[32] Y.W. Kang, N.M. Joo, Comparative analysis on phytochemical properties, anti-oxidative, and anti-inflammatory activities of the different organs of the common ice plant Mesembryanthemum crystallinum L, Appl. Sci. 13 (4) (2023), https://doi.org/10.3390/app13042527.

[33] P.A.S.R. Yolin Angel, et al., Topical antifungal keratitis therapeutic potential of Clitoria ternatea Linn. flower extract: phytochemical profiling, in silico modelling, and in vitro biological activity assessment, Front. Microbiol. 15 (January) (2024) 18, https://doi.org/10.3389/fmicb.2024.1343988.

[34] S.B. Aa Zanwar, S.I. Badole, P.S. Shende, M.V. Edge, Role of gallic acid in cardiovascular disorders, in: Polyphenols Hum. Heal. Dis., 2013, pp. 1045–1047.

[35] C. Aneklaphakij, et al., Diversity of chemical structures and biosynthesis of polyphenols in nut-bearing species, Front. Plant Sci. 12 (April) (2021) 1–15, https://doi.org/10.3389/fpls.2021.642581.

[36] J. Kochman, K. Jakubczyk, J. Antoniewicz, H. Mruk, K. Janda, Health benefits and chemical composition of matcha green tea: a review, Molecules 26 (1) (2020) 11, https://doi.org/10.3390/molecules26010085.

[37] K. Socała, A. Szopa, A. Serefko, E. Poleszak, P. Wlaź, Neuroprotective effects of coffee bioactive compounds: a review, Int. J. Mol. Sci. 22 (1) (2021) 1–64, https://doi.org/10.3390/ijms22010107.

[38] M. Samtiya, R.E. Aluko, T. Dhewa, J.M. Moreno-Rojas, Potential health benefits of plant food-derived bioactive components: an overview, Foods 10 (4) (2021) 1–25, https://doi.org/10.3390/foods10040839.

[39] M. Alam, et al., Therapeutic implications of caffeic acid in cancer and neurological diseases, Front. Oncol. 12 (March) (2022) 1–18, https://doi.org/10.3389/fonc.2022.860508.

[40] L. Ye, et al., Protective effects of ferulic acid on metabolic syndrome: a comprehensive review, Molecules 28 (1) (2023) 1–18, https://doi.org/10.3390/molecules28010281.

[41] K. Sun, et al., Hyperoside ameliorates the progression of osteoarthritis: an in vitro and in vivo study, Phytomedicine 80 (2021) (2021) 153387, https://doi.org/10.1016/j.phymed.2020.153387.

[42] S. Saud, A.M. Salamatullah, Relationship between the chemical composition and the biological functions of coffee, Molecules 26 (24) (2021) 1–14, https://doi.org/10.3390/molecules26247634.

[43] M. El Oirdi, Harnessing the power of polyphenols: a new frontier in disease prevention and therapy, Pharmaceuticals 17 (6) (2024) 692, https://doi.org/10.3390/ph17060692.

[44] J. Alhage, et al., Isolation of bioactive compounds from calicotome villosa stems, Molecules 23 (4) (2018) 20, https://doi.org/10.3390/molecules23040851.

[45] B. Kaczmarek, Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials-A minireview, Materials 13 (14) (2020) 13, https://doi.org/10.3390/ma13143224.

[46] F. Castellana, et al., Trends in coffee and tea consumption during the covid-19 pandemic, Foods 10 (10) (2021) 1–10, https://doi.org/10.3390/foods10102458.

[47] M. Li, M. Qian, Q. Jiang, B. Tan, Y. Yin, X. Han, Evidence of flavonoids on disease prevention, Antioxidants 12 (2) (2023), https://doi.org/10.3390/antiox12020527.

[48] M. Imran, et al., Luteolin, a flavonoid, as an anticancer agent: a review, Biomed. Pharmacother. 112 (January) (2019) 10, https://doi.org/10.1016/j.biopha.2019.108612.

[49] C. Srinivasulu, M. Ramgopal, G. Ramanjaneyulu, C.M. Anuradha, C. Suresh Kumar, Syringic acid (SA) – A review of its occurrence, biosynthesis, pharmacological and industrial importance, Biomed. Pharmacother. 108 (August) (2018) 547–557, https://doi.org/10.1016/j.biopha.2018.09.069.

[50] J.F. Morrison, S.K. Twumasi, Comparative studies on the in vitro antioxidant properties of methanolic leafy extracts from six edible leafy vegetables of India, Asian J. Pharmaceut. Clin. Res. 6 (3) (2013) 96–99.

[51] J. Gerke, The central role of soil organic matter in soil fertility and carbon storage, Soil Syst 6 (2) (2022) 14, https://doi.org/10.3390/soilsystems6020033.

[52] H.U. Ugboko, O.C. Nwinyi, S.U. Oranusi, T.H. Fatoki, C.A. Omonhinmin, Antimicrobial importance of medicinal plants in Nigeria, Sci. World J. 2020 (2020), https://doi.org/10.1155/2020/7059323.

[53] M. Fraga-Corral, et al., Traditional applications of tannin rich extracts supported by scientific data: chemical composition, bioavailability and bioaccessibility, Foods 10 (2) (2021), https://doi.org/10.3390/foods10020251.

[54] S. Bhambhani, K.R. Kondhare, A.P. Giri, Diversity in chemical structures and biological properties of plant alkaloids, Molecules 26 (11) (2021) 29, https://doi.org/10.3390/molecules26113374.

[55] A. Rajput, R. Sharma, R. Bharti, Pharmacological activities and toxicities of alkaloids on human health, Mater. Today Proc. 48 (2021) 1407–1415, https://doi.org/10.1016/j.matpr.2021.09.189.

[56] A.I. Dirar, D.H.M. Alsaadi, M. Wada, M.A. Mohamed, T. Watanabe, H.P. Devkota, Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants, South Afr. J. Bot. 120 (2019) 261–267, https://doi.org/10.1016/j.sajb.2018.07.003, 2019.

[57] W.D. Aryati, A. Nadhira, D. Febianli, F. Fransisca, A. Mun'im, Natural deep eutectic solvents ultrasound-assisted extraction (Nades-uae) of trans-cinnamaldehyde and coumarin from cinnamon bark [cinnamomum burmannii (nees & t. nees) blume], J. Res. Pharm. 24 (3) (2020) 389–398, https://doi.org/10.35333/jrp.2020.161.

[58] F.J. Álvarez-Martínez, E. Barrajón-Catalán, J.A. Encinar, J.C. Rodríguez-Díaz, V. Micol, Antimicrobial capacity of plant polyphenols against gram-positive bacteria: a comprehensive review, Curr. Med. Chem. 27 (15) (2018) 2576–2606, https://doi.org/10.2174/0929867325666181008115650.

[59] A.O. Adeluola, K.U. Nwaeze, A.E. Akindele, O. Okusami, Phytochemical study and antibacterial activity of ethanolic extracts of Azadirachta indica leaves on previously profiled multidrug resistant aerobic bacteria, Afr. J. Biomed. Res. 26 (September) (2023) 439–445.

[60] S.M. Carpano, E.D. Spegazzini, J.S. Rossi, M.T. Castro, Antigungal activity of Terminalia australis, Fitoterapia 74 (3) (2023) 294–297.

[61] Z.H. Mbwambo, M.J. Moshi, P.J. Masimba, M.C. Kapingu, R.S.O. Nondo, Antimicrobial activity and brine shrimp toxicity of extracts of Terminalia brownii roots and stem, BMC Compl. Alternative Med. 7 (2007) 5–9, https://doi.org/10.1186/1472-6882-7-9.
[62] M.A.M. Vis, K. Ito, S. Hofmann, Impact of culture medium on cellular interactions in in vitro Co-culture systems, Front. Bioeng. Biotechnol. 8 (August) (2020) 1–8, https://doi.org/10.3389/fbioe.2020.00911.
[63] Y. Kim, S.B. Lee, M. Cho, S. Choe, M. Jang, Indian almond (Terminalia catappa linn.) leaf extract extends lifespan by improving lipid metabolism and antioxidant activity dependent on AMPK signaling pathway in Caenorhabditis elegans under high-glucose-diet conditions, Antioxidants 13 (1) (2024), https://doi.org/10.3390/antiox13010014.
[64] M.P.J. Dharmaratne, et al., Terminalia bellirica fruit extracts: in-vitro antibacterial activity against selected multidrug-resistant bacteria, radical scavenging activity and cytotoxicity study on BHK-21 cells, BMC Compl. Alternative Med. 18 (1) (2018) 1–12, https://doi.org/10.1186/s12906-018-2382-7.
[65] X.R. Zhang, J.S. Kaunda, H.T. Zhu, D. Wang, C.R. Yang, Y.J. Zhang, The genus Terminalia (combretaceae): an ethnopharmacological, phytochemical and pharmacological review, Springer 9 (6) (2019), https://doi.org/10.1007/s13659-019-00222-3. Singapore.
[66] L. Shi, W. Zhao, Z. Yang, V. Subbiah, H.A.R. Suleria, Extraction and characterization of phenolic compounds and their potential antioxidant activities, Environ. Sci. Pollut. Res. 29 (54) (2022) 81112–81129, https://doi.org/10.1007/s11356-022-23337-6.
[67] N.V. Kupaeva, E.A. Kotenkova, Current view on the assessment of antioxidant and antiradical activities: a mini review, IOP Conf. Ser. Earth Environ. Sci. 854 (1) (2021) 8, https://doi.org/10.1088/1755-1315/854/1/012048.
[68] B. Akbari, N. Baghaei-Yazdi, M. Bahmaie, F. Mahdavi Abhari, The role of plant-derived natural antioxidants in reduction of oxidative stress, Biofactors 48 (3) (2022) 611–633, https://doi.org/10.1002/biof.1831.
[69] A.M. Pisoschia, F.I. Aneta Popa, L. Stancaa, G. Predoia, A.I. Serban, European journal of medicinal Chemistry, Eur. J. Med. Chem. 56 (2020), https://doi.org/10.1016/j.polymertesting.2020.106972. September.
[70] N. Das, M. Sharif Hasan, Z. Al Mahmud, S. Zahir Raihan, D. Goshwami, Evaluation of antioxidant, antimicrobial and cytotoxic activities of Terminalia citrina Leaves, J. Pharm. Res. 10 (1) (2016) 8–15 [Online]. Available: www.jprsolutions.info.
[71] A. Molino, et al., Recent developments in supercritical fluid extraction of bioactive compounds from microalgae: role of key parameters, technological achievements and challenges, J. CO2 Util. 36 (September 2019) (2020) 196–209, https://doi.org/10.1016/j.jcou.2019.11.014.