ECLÉTICA QUÍMICA

Eclet. Quim. 51 | e-1558, 2026

Original Article

https://doi.org/10.26850/1678-4618.eq.v51.2026.e1558

Biochemical analysis of anti-pathogenic bacteria activity of bioactive components from *Sclerocarya birrea* stem bark via experimental and *in-silico* approaches

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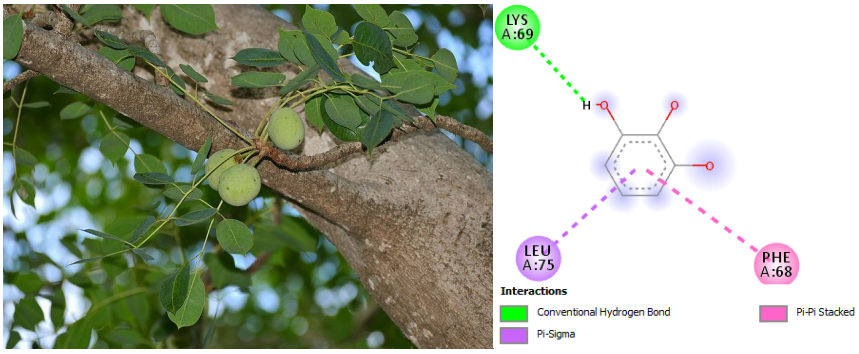
Abstract

In this study, two compounds derived from the bioactive components of *Sclerocarya birrea* (*S. birrea*), a plant native to Nigeria, were examined for their biochemical composition, antibacterial effectiveness, and molecular structure. Pyrogallol (94.72 and 46.77%) and hydroxyquinol, which was exclusively identified in the hexane extract, were the main bioactive components extracted from the study plant *S. birrea* (52.71%). A polyphenolic chemical known as pyrogallol has been shown to have extensive antibacterial activity. The ethanol and hexane bark extracts from *S. birrea* demonstrated significant antibacterial potential against the entire panel of bacteria tested in this study, indicating that the plant's extracts have a wide range of activity compared to the bacteria of interest most frequently responsible for urinary tract infections. Due to the high phenolic concentration in the plant's bark extract, the antiradical results also showed that *S. birrea* had a very significant antioxidant capacity. It was found from the molecular docking that pyrogallol could inhibit 80% of the studied receptors.



Article History

Received	April 28, 2024
Accepted	September 20, 2025
Published	March 02, 2026

Keywords

1. pyrogallol;
2. *Sclerocarya birrea*;
3. molecular docking;
4. urinary tract infections.

Section Editors

Mário Antônio Alves da **Cunha**

Highlights

- Biochemical components from *Sclerocarya birrea* stem bark were examined.
- The biochemical components were studied for their antibacterial effectiveness.
- An induced fit docking study examined the potential inhibiting activities.
- The nonbonding interactions involved between the studied complexes were observed.

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1. Introduction

One of the most prevalent bacterial illnesses worldwide is urinary tract infections (UTIs). Although other pathogenic organisms may also impact the urinary system, uropathogenic *E. coli* accounts for some UTIs. Gram-negative bacteria such as *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae* are implicated in UTIs (Ait-Mimoune *et al.*, 2022). Although UTIs can also affect children and men, UTIs are significantly more common in women (Zeng *et al.*, 2022). After respiratory infections, it is the most prevalent communicable disease (Mohammed *et al.*, 2022). There is an urgent need to evaluate alternative treatment options employing medicinal herbs because recurrent UTIs and antibiotic resistance are both significantly rising (Reid, 2003). There are several claims regarding the beneficial effects of dietary supplements and products made from natural herbs in the prevention of UTIs (Gauba and Rahman, 2023; Shmuely *et al.*, 2012). Medicinal plants are common in traditional medicine worldwide because they are considered less poisonous and have fewer adverse effects than synthetic medications (Dubey *et al.*, 2004). Assessments into bioactive components obtained from plants as a source of alternative medicine to synthetic drugs have received much awareness and attention (Abisoye, 2021). One of the historically necessary native fruits of Western, Eastern, Southern, and Central Africa is *S. birrea* (**Fig. 1**), often known as Marula. Its geographic dissemination extends from Nigeria in West Africa, especially in the northern region of Nigeria, including Katsina, Kaduna, Kebbi, Zamfara, Sokoto, and Adamawa, via the Gambia, up to Sudan and Ethiopia, both in East Africa, and typically stretches to South Africa (Kamatou *et al.*, 2008; Mashau *et al.*, 2022). Due to its delicious fruit and medicinal properties, the *S. birrea* tree is highly prized in northern Nigeria. The enormous, deciduous, and dioecious tree is typically found there. The tree, which belongs to the Anacardiacea family, is a medium-sized, clearly defined, upright, and long-lasting tree that grows to a height of 15 meters (Kamatou *et al.*, 2008). Young stems have smooth (greyish) bark, while adult stems have a greyish color and a blistered and jagged texture. Extensive research has been done on the anti-diabetes, anti-snake, anti-diarrhea, anti-dysentery, anti-ulcer, anti-inflammation, antimicrobial, anti-arthritis, and anti-hypertensive potentials of *S. birrea extracts*, including leaves, bark stem, fruit, and root (Daniel *et al.*, 2022; Dorothy *et al.*, 2022; Kagambega *et al.*, 2022; Mabasa *et al.*, 2022; Maharaj *et al.*, 2022). Njume *et al.* (2011) studied the volatile components of *S. birrea* stem bark and found that the main components that distinguished the stem bark were 4-terpinenol (35.83%), pyrrolidine (32.15%), sesquiterpenoids aromadendrene (13.63%), and α-gurjunene (8.77%), whereas Viljoen *et al.* (2008) found that the main components that distinguished the fruit extract of *S. birrea* are primarily composed of hydrocarbons and esters, with the main ingredients being heptadecene (16.1%), pentanoic acid, 4-methyl phenyl methyl ester (8.8%), cis-3-decen-1-ol (8.4%), butanoic acid, phenylmethyl ester (6.7%), cis-13-octadecenal (6.2%), and decylcyclopentadecane. Although certain bacteria resist known antibiotic medications, antibiotics are still an effective treatment for bacterial illnesses. As a result, many natural product experts are searching for novel therapeutic chemicals in plants to replace some well-known drugs. Phytochemicals called polyphenols are commonly referred to as secondary metabolites in plants. They are generally generated as a reaction to reduce stress in plants (Citarasu, 2012; Daglia, 2012). Additionally, some polyphenols have antibacterial actions on both gram-positive and gram-negative bacteria. It has been demonstrated to hunt free radicals and prevent oxidation (Bravo, 2009; Taguri *et al.*, 2004).

Figure 1. Image of the bark of *Sclerocarya birrea.*
Source: Elaborated by the authors

2. Material and methods

2.1. Chemicals and reagents

The following are the chemicals and reagents utilized in this experiment: The following materials were purchased: Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2-azinobis-(3-ethylbenzothiazolin-6-sulfonic acid) diammonium, potassium persulfate (PPS), and Mueller-Hinton agar from Oxford Ltd. (Hampshire, England). Ethanol and dimethyl sulfoxide (DMSO) (Fluka Chemicals) analytical-grade chemicals and reagents were utilized exclusively.

2.2. Plant material collection

The stem bark of *S. birrea* was collected from Dutse in Dutse LGA of Jigawa State in Northern Nigeria. A qualified taxonomist carried out the sample identification and certification. A voucher number (Zafiya-001) was assigned to the plant housed in the Biological Science herbarium at Federal University Dutse. (Coordinates: **11°42′04″ N 9°20′31″ E**)

2.3. Extraction of hexane and ethanol extracts from the stem bark of *S. birrea*

The stem barks were mechanically chopped into tiny particles after being air-dried at room temperature (Polymix, PX-MFC 90D). The biochemical constituents of the stem bark were extracted using the cold maceration process. It was pulverized, soaked in 1.5 L of ethanol and hexane, and then agitated with an orbital shaker for 48 h. Separate filter papers were used to separate the resultant mixes (Whatman no. 1). The filtrates were concentrated under reduced pressure by a rotating evaporator at 40 °C and stored in two different vials in the refrigerator at around 4 °C overnight until the extracts were needed for analysis.

2.4. Instrumental evaluation

The separation and identification of the bioactive ingredients used a 30 m x 250 mm x 0.25 mm HP-5MS column and a 5973 mass selective detector linked to an Agilent gas chromatograph (GC) (6890N). The ion source and chamber temperatures were set to 230 and 150 °C, respectively, while the flow rate of the carrier gas, helium, was programmed to 1 mL/min, moderate velocity, and a beginning pressure of 27 cm/sec and 13 psi. The attained scan mass varied between 50 and 500 atomic

mass units (amu). The initial temperature was set to range from 35 ºC/min to 350 ºC and was initially held at 70 °C for 2.5 min, yielding a total run time of 49.77 min. Separately, reconstituted in hexane (1 µL), the ethanol and hexane crude extracts of *S. birrea* were injected by splitless mode into the GCMS at 252 °C with a 51 mL/min purge flow.

2.5. Component identification

The GC-MS chromatogram was used to identify the *S. birrea* extract's constituent parts by contrasting the retention times (RT) of the extracts with the homologous series of n-alkanes found in the NIST library of 2014. The distinctness of the mass fragmentation arrays and intended retention times of all compounds were checked and matched with the existing ones in the databases (Adeyemi *et al.*, 2021; Larayetan *et al.*, 2018).

2.6. Phytochemical screening

Using established techniques as described by Yadav *et al.* (2014), the qualitative phytochemical screening of ethanol and hexane extracts of *S. birrea* stem bark was verified to identify the presence of primary bioactive ingredients like tannins, saponins, flavonoids, and alkaloids. The presence of alkaloids was determined using Mayer's and Wagner's reagents, while saponins were determined using the form test, tannins were determined using the ferric chloride test, and flavonoids were determined using the lead acetate test.

2.7. Quantitative analysis of stem bark extracts of *S. birrea*

2.7.1. Determination of overall phenolic contents

The phenolic content in the two extracts was measured using a spectrophotometric approach that modified the method described by Kim *et al.* (2003). To do this, 1 mL of the Folin-Ciocalteu mixture and 12.5 mL of deionized water were added separately to 1 mL of the stem bark extracts. After allowing the mixture to stay for about 8 min, 12 mL of a solution containing 7% sodium trioxocarbonate(IV) was distinctly added. The resulting collection was vortexed for roughly 45 s and then placed in a dark cabinet for 30 min to develop its color. Separate measurements of absorbance were made at 750 nm. It was carried out three times, and the findings were expressed in milligrams of gallic acid equivalent per 100 g (mg GAE/100 g) using a standard graduation curve and line **Eq. 1** with the notation:

$$y = 0.009x + 0.012 \ (R^2 = 0.999) \quad (1)$$

where x is the concentration, and y is the gallic acid equivalent.

2.7.2. Determination of overall flavonoid content

The total flavonoid content of the two *S. birrea* extracts was ascertained using the method of Ordonez *et al.* (2006), with a minor modification. One milliliter (1 mL) of each extract was added to 0.6 mL of a 5% aluminum chloride solution in ethanol, which was then allowed to stand at 25 °C for 2 h in a shaded area while the absorbance was measured at 420 nm. The flavonoids present were conveyed as mg RE/100 g of rutin via the linear **Eq. 2** given as:

$$y = 0.023x + 0.022 \ (R^2 = 0.982) \quad (2)$$

where x is defined as the concentration and y as the rutin equivalent.

2.7.3. Determination of total tannin contents

With very minor modifications, the method described by Van Buren and Robinson (1969) was used to determine the total tannin content (tannic acid). The extracts of both stem barks of *S. birrea* (20 mg) were separately blended with 35 mL of methanol. Distilled water (12 mL) was introduced into 2 mL of each ensuing methanol extract above in a vial. The vial enclosing each combination was again blended with 3 mL (10-fold dilution equivalent) of 0.1 mol/L iron(III) chloride, which was later added to 0.1 mol/L hydrochloric acid solution and tetrapotassium ferrocyanide solution (0.008 mol/L). The resulting combinations produced above were thoroughly swirled for about 2 min and permitted to stand for 5 min before recording the reading of their absorbance at 605 nm together with the control solution. These gradations were assessed on a 7-point curve of tannic acid standard graduation (20, 40, 60, 80, 100, 140, and 200 mg/L) in distilled water. Total tannin content was conveyed as equivalents of tannic acid (TAEs).

2.7.4. Saponin content estimation

The approach of Obadoni and Ochuko (2002) was used to estimate the total saponin content of both *S. birrea* extracts with some modification. About 0.6 g of stem bark extract was added separately to about 90 mL of 85% ethanol, which was then heated continuously for about 4 h. The resulting solution was filtered and extracted again with an additional 90 mL of 85% ethanol. The amount of the successive mixtures decreased to 45 mL using a rotavapor (Buchi Model R153 Rotavapor). Diethyl ether (90 mL) was packed into a separate funnel, giving two separate diethyl ether and the hexane portion strata, eliminating any fat content from the blends. By gradually decanting off the organic layer, separating the diethyl ether layer from the hexane layer (diethyl ether) was possible. A discrete addition of 50 mL of n-butanol and 10 mL of 5% hexane sodium chloride was made for additional cleaning. The resulting liquids were then evaporated to dryness in several beakers. The total saponin content was then calculated and expressed as a percentage.

2.8. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Both stem bark extracts from *S. birrea* were evaluated for their potential to scavenge free radicals and their antioxidant properties in conjunction with the free radical DPPH. DMSO mixed with DPPH was incubated with varying concentrations (25–125 µg/mL) of both extracts and the positive control, Vitamin C, for roughly 35 min at room temperature (37 °C in a shady place). The individual solutions were meticulously combined using a vortex machine, and the absorbance of the mixtures was measured at 517 nm. The following **Eq. 3** was used to evaluate the stem bark extracts' capacity to scavenge DPPH free radicals in the various assortments, as demonstrated by Larayetan *et al.* (2019).

$$\% \text{ Inhibition} = [(\text{Acontrol-Avo}) / \text{Acontrol}] \times 100 \quad (3)$$

where Acontrol is the absorbance of DPPH + DMSO; Avo is the absorbance of DPPH + extracts, or the commercial antioxidant.

2.9. 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The efficiency of the two stem bark extracts of *S. birrea* was assessed using the updated Nantitanon (2007) technique with a few minor modifications. The working mixture was created by

oxidizing the ABTS-concentrated solution (7 mmol/L) with 2.4 mmol/L potassium peroxydisulfate in equal proportions. The mixture was then left to blend for 10 h at room temperature. After mixing an aliquot of the resulting mixture (0.5 mL) with 50 mL of methanol for 7 min, the absorbance measurement at 734 nm (0.706 ± 0.001) was recorded using a UV spectrophotometer. Concisely, the different extract concentrations (vacillating between 0.025 and 0.4 mg/mL) were mixed with the methanol solution of ABTS for 6 min at room temperature in a dark cupboard. An assessment of the absorbance was carried out spectrophotometrically at 760 nm, and the % inhibition of ABTS of the extracts alongside the positive control (vitamin C) was articulated through the equation depicted for the DPPH test above.

2.10. Antibacterial activity assays

2.10.1. Bacterial sensitivity to ethanol and hexane extracts of *S. birrea* stem bark

We used the Collins technique, also known as agar, well diffusion (Collins *et al.*, 2004), to evaluate the antibacterial potential of *S. birrea* extracts. The bacterial cultures were grown for 24 h at 37 ± 0.1 °C after being injected into nutrient broth (Oxoid). Using a clean swab stick that had been moistened with the bacterial suspension, test bacteria cultures that had been standardized to the McFarland standard of 0.5 were spread over solidified congealed sterile Mueller Hilton agar (Oxoid) medium. To create 6 mm diameter wells in the uniformly distributed congealed Mueller Hilton agar (Oxoid) spread in Petri plates that have been dosed with the standardized inoculum, a cork borer (disinfected) was used. Reconstituted in 5% dimethyl sulfoxide (DMSO) at 30 mg/mL, ethanol and hexane bark extracts were used as a stock concentrate. Each well received exactly 50 µL of each stem bark extract, and the wells were properly labeled. The comparable negative control used was a 5% DMSO solution. To ascertain the sensitivity of the test bacterium to streptomycin, the disk diffusion method was applied, and a streptomycin (10 µg) disk (MAST-Group, United Kingdom) was utilized as a positive control. The inhibition zones were measured millimeters using a vernier caliper after incubating the plates at 37 ºC for 24 h.

2.10.2. Determination of MIC and MBC of the ethanol and hexane stem bark extracts of *S. birrea*

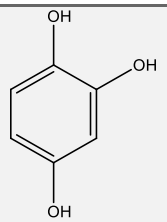
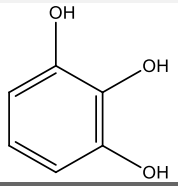
The extracts' MIC was determined using the agar dilution procedure reported in Balouiri *et al.* (2016) and Akinpelu *et al.* (2015). An agar dilution procedure determined the extracts' minimum inhibitory concentration (MIC). Mueller Hilton Agar was prepared in McCartney bottles, extracts were mixed with the extract solution, and the mixture was poured into Petri dishes. Bacterial inoculums were streaked on the plates and incubated at 37 °C. The plate with the minimum concentration of the extract inhibiting visible growth was considered the MIC.

The study aimed to determine an extract's minimum bactericidal concentration (MBC) against sensitive organisms using streaking and incubation. Samples were streaked on antimicrobial-free nutrient agar plates, and the MBC was determined after 72 h, with streptomycin as a positive control and 5% DMSO as a negative control.

2.11. Computational details

Two molecular compounds (**Table 1**) from *S. birrea* stem bark with the highest percentage yield were optimized using 6-31G* via Spartan 14 software (Oyebamiji *et al.*, 2022) and docked against five gram-positive bacteria (*Bacillus steorothermophilus* (PDB ID:1h2f), *Clostridium sporogenes* (PDB ID:4q0y), *Bacillus subtilis (PDB* ID:6koc), *Bacillus cereus (PDB* ID:1ah7), *Staphylococcus aureus* (PDB ID:6qx2), and four gram-negative bacteria (*Proteus vulgaris (PDB* ID:1hzo), *Klebsiella pneumonia* (PDB ID:6rd3), *Escherichia coli* (PDB ID:4or1), and *Pseudomonas aeruginosa (PDB* ID:6p8s). The potential of each compound to inhibit the studied receptors was calculated and reported in **Table 1**. The names of the compounds studied were benzene-1, 2, 4-triol (**A**) and benzene-1, 2, 3-triol (**B**). The software involved in the studied docking method was EduPyMOL-v1.7.4.4-Win32, Autodock Tool 1.5.6, Autodock Vina 1.1.2, and Discovery Studio.

Table 1. 2D Structure of the studied compounds obtained from *Sclerocarya birrea* stem bark.

Compound	Chemical Structures
A	
B	

Source: Elaborated by the authors.

2.12. Statistics evaluation

All the sensitivity experiments were carried out in triplicate, and the data was expressed as an arithmetic mean ± SD (standard deviation). The one-way ANOVA test was carried out on the zones of inhibition (ZI) to determine the significant difference between the ZI exhibited against the test isolates by the different antibacterial agents used in this study with and without considering the Gram status of the test isolate. The significant differences in ZI were ascertained via SPSS version 23. It was also used for the mean comparison, and the P-value was set at 0.05.

3. Results and discussion

Table 2 revealed the phytochemical investigations of ethanol and hexane extracts of *S. birrea* stem bark, and signs used were interpreted below **Table 2**.

Table 2. Qualitative phytochemical analysis of ethanol and hexane extracts of *S. birrea* stem bark.

Phytochemical components	Ethanol extract	Hexane extract
Tannin (Braymer's test)	+	+
Saponins (Froth's test)	+	+
Flavonoids (Lead acetate test)	+	+
Steroids (Salkowaski's test)	–	–
Phenols (Ferric chloride)	+	+
Terpenoids (Salkowaski's test)	–	+

Note: **(+)** Present, **(–)** Absent.
Source: Elaborated by the authors.

3.1. Phytochemical constituents of *S. birrea* stem extracts

S. birrea stem bark extracts in ethanol and hexane forms underwent qualitative phytochemical screening. According to the results of the phytochemical screening, the two plant extracts contain important bioactive substances such as tannins, saponins, flavonoids, steroids, phenols, and terpenoids, which are noted in **Table 2**. Similar research was conducted by Belemnaba *et al.* (2024) on *S. birrea*, and similar phytochemicals, such as flavonoids, tannins, and saponins, were also detected in the trunk bark of the plant, corroborating the same phytochemicals in this present study. According to research by Doughari *et al.* (2009), phytochemical components deposited in various plant parts exhibit antibacterial and biological potential, making them good candidates for therapeutic agents to manage infectious diseases in people. Furthermore, these plant bioactive components have bactericidal or bacteriostatic properties against multidrug-resistant bacteria and can be used as precursors to produce antibiotics for managing infectious agents, primarily bacteria that cause urinary tract infections (Gadisa *et al.*, 2019; Khameneh *et al.*, 2019; Rossiter *et al.*, 2017).

3.2. Constituents of the ethanol and hexane stem bark extract of S. birrea

In this work, we observed that ethanol extracts of *S. birrea* contained twelve components, while hexane bark extracts of *S. birrea* are made up of four components, and this shows that the total ethanol and hexane bark extracts of *S. birrea* were 99.9% and 99.98% of the total bioactive components. **Table 3** lists the molecular weights, molecular formulas, and percentage components. The major constituents included in both extracts differ in quality and quantity. In both the ethanol and hexane stem bark of *S. birrea*, pyrogallol, also known as pyrogallic acid or benzene, 1, 2, 3-triol, an organic phenol component that occurs naturally in a wide variety of plants, was discovered to be one of the main phenolic constituents (94.72 and 46.77%). The organic chemical pyrogallol has the molecular formula $C_6H_3(OH)_3$. According to Selvaraj *et al.* (2018), it was first identified as a phenol family member and found in a natural extract of *Abrus precatorius* seeds. Hydroxyquinol is an organic molecule with the chemical formula $C_6H_3(OH)_3$. It was discovered as the major constituent in the hexane extract of the same stem bark of *S. birrea* (52.71%) but was absent in the ethanol extract of the same plant, which may be due to the different extraction solvents (ethanol and hexane) employed in removing the Phyto-constituents. The polarity of solvents has a substantial effect on the type of bioactive components to be extracted. It is one of three isomeric benzenetriols that typically occur in nature as a product of biodegradation of a known component, catechin, found in plants (Hopper and Mahadevan, 1997). In the same stem bark of *S. birrea*, hydroxyquinol was discovered to be the main component in the hexane extract (52.71%), but it was absent in the ethanol extract. This difference may have resulted from using two different extraction solvents (ethanol and hexane) to remove the phytochemicals from the *S. birrea* bark extract. Varied solvents are thought to have different affinities for certain secondary (Mahato and Sen, 1997). Njume *et al.* (2011) identify terpinen-4-ol (35.83%) as the predominant component of the stem bark of *S. birrea,* followed by pyrrolidine (32.15%), aromadendrene (13.63%), and α-gurjunene (8.77%).

Table 3. GC-MS analysis of the ethanol and hexane stem bark extract of *S. birrea*.

Sr. No.	RT (min)	Phytochemicals	EE	HE	MW	MF	Class
1	5.835	Catechol	0.11	0.40	256	$C_6H_6O_2$	Phenols
2	7.258	Propargylamine	0.17	-	284	C_3H_5N	Amine
3	8.823	Pyrogallol	94.72	46.77	126	$C_6H_6O_3$	Phenols
4	10.245	Hydroxyquinol	-	52.71	126	$C_6H_6O_3$	Phenols
5	15.509	3- Isopropyl-1-methyl-4-methylamino-pyrrole-2, 5-dione	0.85	-	182	$C_9H_{14}N_2O_2$	Others
6	26.251	2-butyl-2-octenal	0.89	-	182	$C_{12}H_{22}O$	Aldehydec
7	27.531	Trans-9-octadecene	1.30	-	252	$C_{18}H_{36}$	Hydrocarbon
8	33.756	Trans-2-Dodecen-1-ol,trifluoroacetate	0.29	-	280	$C_{24}H_{23}F_3O_2$	Ester
9	35.214	10-Undecenehydroxamic acid	1.03	-	182	$C_{11}H_{18}O_2$	Amides
10	35.890	Vinyl 10-undecenoate	0.24	-	210	$C_{13}H_{22}O_2$	Ester
11	37.775	5-oxo-2-propylhexanoic acid	-	0.10	172	$C_9H_{16}O_3$	Fatty acid
12	39.233	9-oxononanoic acid	0.39	-	172	$C_9H_{16}O_3$	Fatty acid
		Total	**99.99%**	**99.98%**			

***Note*: RT:** Retention time; **EE:** Ethanol extract (% area); **HE:** Hexane extract (% area); **MW:** Molecular weight; **MF:** Molecular formula.
Source: Elaborated by the authors.

It is crucial to remember that geographical location, extraction techniques, and portion of the plant being examined can all affect the phytochemical components of plants (Mariod *et al.*, 2008). The differences in bioactive components from this present study could be attributed to differences in geographical location between South Africa and Nigeria; solvents used for extraction of the components could also affect the bioactive constituents. A rich composition of bioactive components, including various phenolic compounds, has been shown by the phytochemical profile of *S. birrea*, also referred to as marula. Pyrogallol (1, 2, 3-trihydroxybenzene) is a prominent component. One research study that examined the chemical makeup of *S. birrea* leaves, for example, found that pyrogallol was one of the main phenolic chemicals found in the leaves (Braca *et al.*, 2003). However, there is a lack of information in the literature on the occurrence of benzene-1, 2, 4-triol, sometimes referred to as hydroxyquinol, in *S. birrea*. Many phenolic compounds exist in *S. birrea*; however, previous research has not specifically identified hydroxyquinol. Hydroxyquinol was reported for the first time as a bioactive constituent of *S. birrea* in this present study. Further minor components in the stem bark of *S. birrea* are trans-9-octadecene (1.30%), 10-undecenehydroxamic acid (1.03%), 2-butyl-2-octenal (0.89), and 3-isopropyl-1-methyl-4-methylamino-pyrrole-2, 5-dione (0.85) (**Fig. 2**).

https://doi.org/10.26850/1678-4618.eq.v51.2026.e1558

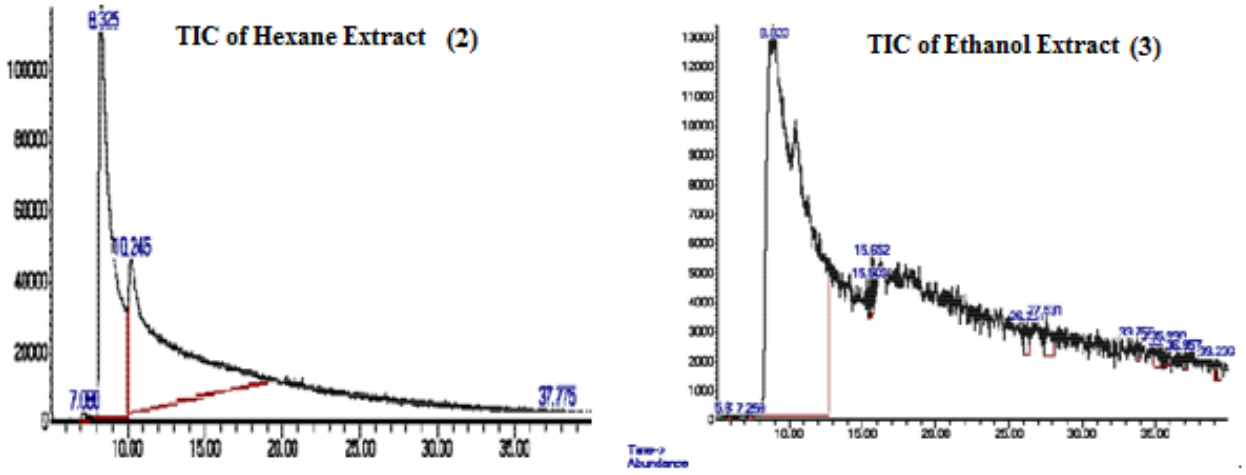


Figure 2. Total ion chromatograms (TIC) of hexane and ethanol extract of *S. birrea.*
Source: Elaborated by the authors.

3.3. Antibacterial properties of *S. birrea* ethanol and hexane stem bark extracts

This study evaluated the *in-vitro* antibacterial potencies of *S. birrea's* stem bark extract in ethanol and hexane solutions against four important gram-negative and five important gram-positive bacteria strains. Streptomycin, ethanol extract, and hexane extract all produced zones of inhibition (ZI) that ranged from 0.0 mm to 21 mm, 16.0 mm to 24.0 mm, and 14.0 mm to 19.0 mm, respectively (**Table 4**). None of the test isolates were responsive to the negative control (5%) DMSO. Streptomycin, ethanol, and hexane extracts all showed antibacterial activity based on the mean ZI against all the tested bacteria, regardless of the Gram status, and this difference was significantly different at F (2, 51) = 5.970, P = 0.05. Similarly, the effectiveness of streptomycin, ethanol extract, and hexane fraction against the tested bacteria based on their Gram status is significantly different at F (5, 48) = 5.253, P = 0.01. The minimum bactericidal concentrations (MBCs) and the minimum inhibitory concentrations (MICs) were established. The MIC of the ethanol extracts against the test bacteria ranged from 0.69 ± 0.02 mg/mL to 15.00 ± 0.01 mg/mL, whereas the MIC of the hexane solution ranged from 1.38 ± 0.00 mg/mL to 15.00 ± 0.02 mg/mL, and the MIC of streptomycin ranged from 1.25 ± 0.00 g/mL to 10.02 ± 0.02 g/mL (**Table 5**). However, the MBC measured against the test bacterial isolates ranged from 3.75 ± 0.01 mg/mL to 30.00 ± 0.02 mg/mL for ethanol extract and from 7.50 ± 0.02 mg/mL to 30.00 ± 0.03 mg/mL for hexane extracts (**Table 5**). For streptomycin, the positive control, the MBC ranged from 5.00 ± 0.00 mg/mL to >10 mg/mL. *S. birrea's* ethanol and hexane bark extracts demonstrated notable antibacterial activity against all the test panels of bacteria, including those that weren't responsive to the positive control drug streptomycin. This demonstrates that *S. birrea* stem bark extract has many actions. The zone of inhibition results for antibacterial activity indicate that ethanol extract is more active than hexane extract. Except for one test isolate (*Pseudomonas aeruginosa*), the ZI displayed by the ethanol was higher than that attained by the hexane fraction, and the statistical analysis validated this finding. Since more hexane extract was required to achieve the MIC and MBC for most of the studied bacteria, the MIC and MBC analyses further revealed that the ethanol extract of *S. birrea* bark demonstrated better activity than hexane extracts (**Table 5**). Tanih and Ndip (2012) found that ethanol had greater antibacterial activity in their investigation than hexane extracts of mature *S. birrea* stem bark. According to this finding, Ethanol is a superior solvent for removing the bioactive components from *S. birrea* bark. Pyrogallol was found to be the most prevalent bio-compound in the bark of *S. birrea* by GS-MS analysis, and curiously, ethanol extracted more pyrogallol from the bark of *S. birrea* than hexane. A polyphenolic substance called pyrogallol displays broad-spectrum antibacterial activity. Previous research has demonstrated that pyrogallol has antibacterial action against human pathogenic bacteria that can cause infections such as urinary tract infections, including methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and others (Chew *et al.*, 2020; Cynthia *et al.*, 2018; Lin *et al.*, 2022; Tinh *et al.*, 2016). In their work on *Quercus infectoria* gall extract, Nur Saeida *et al.* (2014) demonstrated that plants with high pyrogallol content may be helpful as an antibacterial agent for treating and preventing urinary tract infections. Natural antibacterial agents have reportedly been found to be reasonably harmless compared to synthetic antibacterial agents. Thus, after investigating the pyrogallol content of the *Q. infectoria* gall extract published by Nur Saeida *et al.* (2014) and the *S. birrea* bark reported in this present study, this active constituent would be a suitable replacement for synthetic pyrogallol.

3.4. Antioxidant activities of *S. birrea* stem bark extracts

The DPPH• antioxidant assay is based on the idea that any substance that can give away an electron or a hydrogen atom qualifies as an antioxidant. The sample under investigation turns purple to yellow due to creating a neutral DPPH-H molecule following the reception of a hydrogen atom from the antioxidant species (Guerrini *et al.*, 2009). It has been noted that using more than one approach is preferable while conducting a test on antioxidant activity. The antioxidant capacity of the ethanol and hexane extracts of the stem bark of *S. birrea* were evaluated *in vitro* using two radical prototypes (DPPH and ABTS), and the antioxidant capacity of the two crude extracts was assessed based on their effective IC50 concentration, or the concentration at which the extracts could lower the initial DPPH• absorbance by 50%. In the DPPH experiment, the hexane extract had a lower IC50 (1.92 mg/mL) than the ethanol extract (0.97 mg/mL), but both extracts had less activity than the positive controls (vitamin C and gallic acid), which had IC50s of 0.007 and 0.006 mg/mL, respectively (**Table 6**).

Table 4. Antibacterial sensitivity test of the *S. birrea* stem bark extracts against the experimental organisms.

Test Organism	Positive Control	Zone of Inhibition (mm)	
	Streptomycin (10 µg) mm	Ethanol stem bark extract (30 mg/mL) mm	Hexane bark extract (30mg/mL) mm
Gram-Positive			
Bacillus steorothermophilus (NCIB 8222)	21.0 ± 0.3 (S)	16.0 ± 0.7	15.0 ± 0.5
Clostridium sporogenes (NCIB 532)	20.0 ± 0.7 (S)	16.0 ± 0.7	14.0 ± 0.7
Bacillus subtilis (NCIB 3610)	0.0 ± 0.0 (R)	24 ± 1	18.0 ± 0.9
Bacillus cereus (NCIB 6349)	18.0 ± 0.3 (S)	19 ± 1	17.0 ± 2.0
Staphylococcus aureus (NCIB 8588)	20.0 ± 0.8 (S)	19.0 ± 0.0	17 ± 0.3
Gram-Negative			
Proteus vulgaris (NCIB 67)	14.0 ± 0.6 (I)	16.0 ± 0.7	17 ± 0.8
Klebsiella pneumonia (NCIB 418)	0.0 ± 0.0 (R)	17 ± 1	20 ± 0.7
Escherichia coli (NCIB 86)	17.0 ± 0.2 (S)	19.0 ± 0.0	18.0 ± 0.0
Pseudomonas aeruginosa (NCIB 950)	0.0 ± 0.0 (R)	17 ± 1	19.0 ± 0.9

***Note*: NCIB:** Means National Collection of Industrial Bacteria; **R:** Resistance; **I:** Intermediate.
Source: Elaborated by the authors.

Table 5. Antibacterial agents displaying minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations.

Bacteria strains	Ethanol stem bark extract		Hexane stem bark extract		Streptomycin		DMSO
	MIC (mg mL^{-1})	MBC (mg mL^{-1})	MIC (mg mL^{-1})	MBC (mg mL^{-1})	MIC (µg mL^{-1})	MBC (µg mL^{-1})	0.4 mL
Bacillus steorothermophilus (NCIB 8222)	15.00 ± 0.01	30.00 ± 0.02	15.00 ± 0.02	30.00 ± 0.03	5.02 ± 0.01	10.00 ± 0.02	VG
Clostridium sporogenes (NCIB 532)	1.38 ± 0.03	3.75 ± 0.01	3.75 ± 0.02	7.50 ± 0.03	10.01 ± 0.02	>10	VG
Bacillus subtilis (NCIB 3610)	3.75 ± 0.00	7.50 ± 0.00	3.75 ± 0.00	7.50 ± 0.02	ND	ND	VG
Bacillus cereus (NCIB 6349)	1.38 ± 0.01	15.00 ± 0.02	3.75 ± 0.02	15.00 ± 0.02	5.00 ± 0.00	5.00 ± 0.00	VG
Staphylococcus aureus (NCIB 8588)	1.38 ± 0.02	7.50 ± 0.02	1.38 ± 0.01	15.00 ± 0.01	5.00 ± 0.01	10.02± 0.01	VG
Proteus vulgaris (NCIB 67)	0.69 ± 0.02	30.00 ± 0.02	1.38 ± 0.00	15.00 ± 0.00	7.50 ± 0.01	>10	VG
Klebsiella pneumonia (NCIB 418)	1.38 ± 0.01	30.00 ± 0.02	2.76 ± 0.01	15.00 ± 0.01	5.00 ± 0.00	>10	VG
Escherichia coli (NCIB 86)	0.69 ± 0.00	30.00 ± 0.02	1.38 ± 0.00	30.00 ± 0.00	1.25 ± 0.00	5.00 ± 0.02	VG
Pseudomonas aeruginosa (NCIB 950)	1.38 ± 0.00	7.50 ± 0.02	3.75 ± 0.00	15.00± 0.00	ND	ND	VG

***Note*: ND:** Not determined; **VG:** Visible growth; **NVG:** No Visible Growth.
Source: Elaborated by the authors.

Table 6. Quantitative phyto-constituents of hexane and ethanol *S. birrea* stem bark extracts.

Extract/ Standard drug	Overall Phenolic mg/100 g	Overall Flavonoid mg/100 g	Overall Tannins mg/100 g	Overall Saponins mg/100 g	DPPH IC50 (mg/mL)	ABTS IC50 (mg/mL)
Ethanol	2520 ± 35	1700 ± 8	360 ± 5	189.6 ± 0.9	0.97	1.88
Hexane	2313 ± 25	1201 ± 2	401 ± 2	789.6 ± 0.4	1.92	4.45
Vitamin C					0.007	0.005
Gallic acid					0.006	0.004

Source: Elaborated by the authors.

Regarding the ABTS experiment, it was discovered that the hexane extract, with an IC50 of 4.45 mg/mL, hunted the radicals less effectively than the ethanol extract, with an IC50 of 1.88 mg/mL. Like the DPPH assay, the IC50 values for both extracts under ABTS examination were lower than those for the positive controls (vitamin C and gallic acid), at 0.005 and 0.004 mg/mL, respectively. According to the IC50 results for both investigations, the DPPH test had a better and more productive result (**Table 4**). The anti-radical results from this study showed that the study plant had a very low antioxidant potential in the two antioxidant models used (DPPH and ABTS). Antioxidant potencies have been categorized into four groups according to their IC50: very strong (IC50 < 50 µg/mL), strong (IC50 50-100 µg/mL), moderate (IC50 101-150 µg/mL), and weak (IC50 250-500 µg/mL). The slight antiradical activity recorded in the different extracts may be due to the extremely high levels of phenolic components, especially pyrogallol. Phenolic components were present in both ethanol and hexane extract of the *S. birrea* plant (94.72 and 46.77%), and hydroxyquinol, which was only present in the hexane extract (52.71%), as demonstrated by the GC-MS analysis of the plant extracts. Pyrogallol can neutralize free radicals, have antiradical properties, and stop the production of metabolites that cause cancer (Chen *et al.*, 2007; Defoirdt *et al.*, 2013; Elzaawely *et al.*, 2005; Park *et al.*, 2007).

3.4.1. Overall phenolic content

Most phenolic components are biological substances derived from plants: tannins, flavonoids, and phenolic acids. The phenolic contents (OPC) of *S. birrea* bark extracts in hexane and ethanol were determined using the Folin-Ciocalteu method and a spectrophotometer. Both *S. birrea* bark extracts have total phenolic contents of 2520 ± 35 and 2313 ± 25 mg/100 g, respectively (**Table 6**). Flavonoids and tannins are powerful natural antioxidants that scavenge free radicals and prevent degenerative illnesses, including cardiovascular disease. Furthermore, they also have antibacterial, anti-inflammatory, and antiallergenic qualities and are engaged in cell cycle regulation, inducing apoptosis, inhibiting platelet aggregation, and hindering the development of cancerous cells (Russon *et al.*, 2018). Besides, they also have antibacterial, anti-inflammatory, and antiallergenic qualities and are engaged in cell cycle regulation, inducing apoptosis, inhibiting platelet aggregation, and inhibiting the development of cancerous cells (Russon *et al.*, 2018).

3.4.2. Overall flavonoid content

Flavonoids and polyphenolic compounds that protect plants from harm are examples of bioactive compounds found in plants. According to the Folin-Ciocalteu method, the total flavonoids in the hexane and the ethanol extracts of *S. birrea* were determined. The results were recorded as mg RE/100 g of rutin using the mathematical equation $y = 0.023\ x + 0.022$ ($R^2 = 0.982$). The total flavonoid content per 100 g was 1700 ± 8 and 1201 ± 2 mg/100 g (Table 6). As seen, ethanol extract had a higher total flavonoid concentration than hexane extract, which could be because different extraction solvents (ethanol and hexane) have varying affinities for different bioactive components. According to Louis *et al.* (2018), flavonoids were more concentrated in the stems of plants than in the leaves. This difference in concentration may be due to the extraction solvent or the age of the plant.

3.4.3. Overall tannin content

Polyphenolic compounds called bioactive tannins are also found in different plant parts (Salazar-Orbea *et al.*, 2023). It has been noted that bioactive tannin has anti-inflammatory, anti-bacterial, and anti-radical properties (Okwu, D. and Okwu, M., 2004). Foods high in tannin can have a significant therapeutic and beneficial impact on a person. Although the amount of bioactive tannin content in the hexane extract of the research plant was 401 ± 2 mg/100 g, the OTC in the ethanol extract was 360 ± 5 mg/100 g. Table 6 demonstrates that the hexane extract had a higher overall bioactive tannin concentration than the ethanol extract. Louis *et al.* (2018) posited that tannin content was considerably low in both the leaves and stem bark of *S. birrea.* However, the tannin content was higher in the stem bark than in the leaves.

3.4.4. Overall saponin content

Saponin can be precipitated and coagulated by red blood cells. Additionally, it has cholesterol-binding properties and acts as a natural antibiotic to aid the body in the battle against infections and microbial invasion. The stem of *S. birrea* had more saponin than the leaves of the same plant (Louis *et al.*, 2018). The total saponin content of the stem bark extracts of *S. birrea* in ethanol and hexane extracts was 189.6 ± 0.9 and 789.6 ± 0.4 mg/100 g, respectively (Table 6). It was established that the hexane extract had a considerably higher concentration of saponins than the ethanol extract.

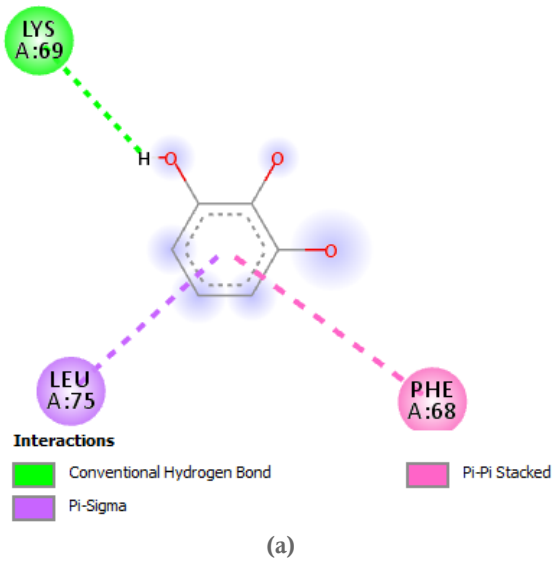
3.5. Molecular docking investigation

Twelve (12) compounds were isolated from the stem bark of *S. birrea*, and two molecular compounds were chosen from among them based on the area % composition of these compounds, as shown in Table 7. The computed binding affinity listed in Table 7 was derived from the docked complexes. *C. sporogenes* (NCIB 532), *B. subtilis* (NCIB 3610), *B. cereus* (NCIB 6349), *S. aureus* (NCIB 8588), *P. vulgaris* (NCIB 67), and *E. coli* (NCIB 86) were found to be more susceptible to inhibition by benzene-1,2,3-triol (B), whereas *K. pneumonia* (NCIB 418) and *P. aeruginosa* were proved to be inhibited bybenzene-1, 2, 4-triol (Fig. 3a–h). The presence of the hydroxyl-OH group on position 3 of benzene may have contributed to the compound's capacity to block 80% of the examined receptor. As a result, it was shown that the influence of the OH group on position 3 of the benzene compound significantly increased the inhibitory ability of benzene-1,2,3-triol (B).

Table 7. Binding affinity of the two targeted compounds from *S. birrea.*

	Compounds of Target		A	B
Binding Affinity (kJ/mol)	Gram Positive	*B. steorothermophilus* (NCIB 8222)	−23.84	−23.84
		C. sporogenes (NCIB 532)	−20.92	−21.33
		B. subtilis (NCIB 3610)	−22.59	−23.01
		B. cereus (NCIB 6349)	−25.94	−26.35
		S. aureus (NCIB 8588)	−13.38	−14.64
	Gram Negative	*P. vulgaris* (NCIB 67)	−20.92	−22.17
		K. pneumonia (NCIB 418)	−21.33	−20.50
		E. coli (NCIB 86)	−17.99	−18.82
		P. aeruginosa (NCIB 950)	−20.08	−19.66

Source: Elaborated by the authors.



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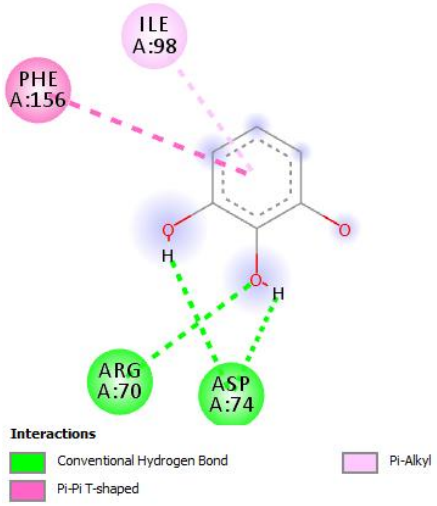

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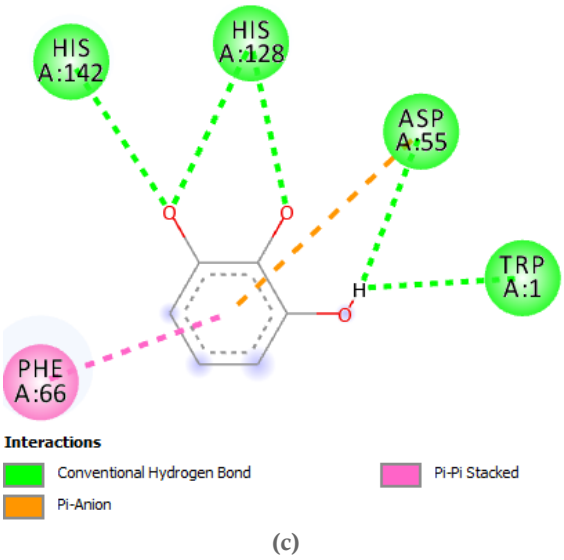
https://doi.org/10.26850/1678-4618.eq.v51.2026.e1558



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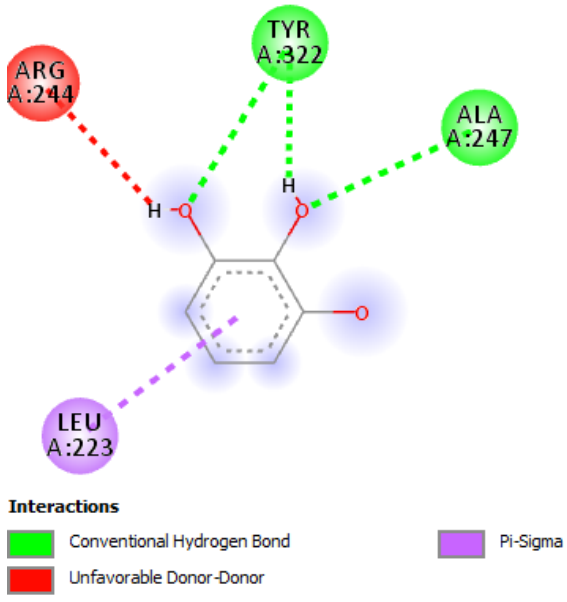

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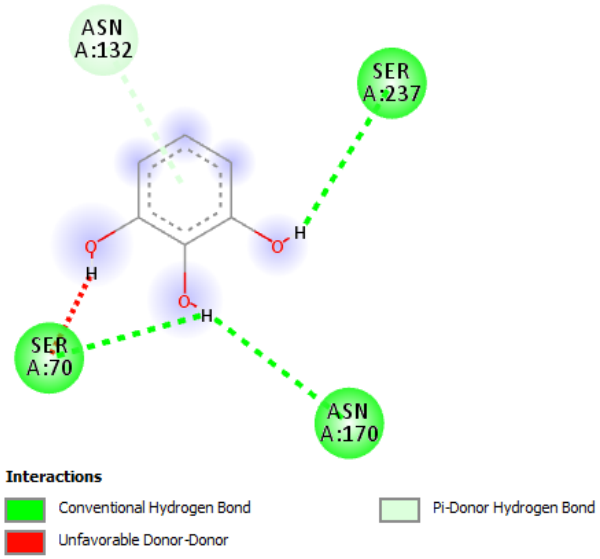

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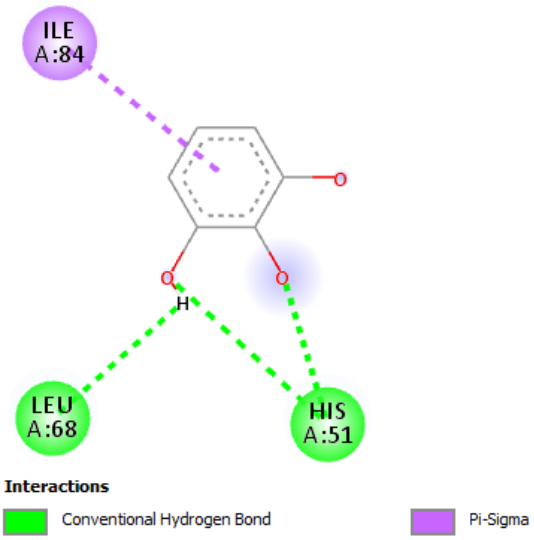

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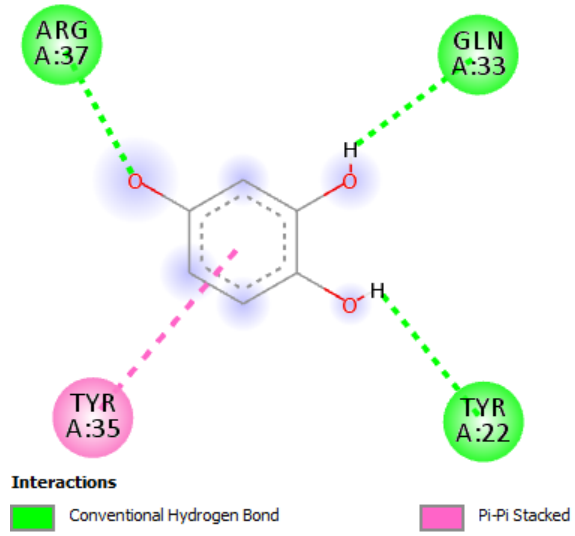

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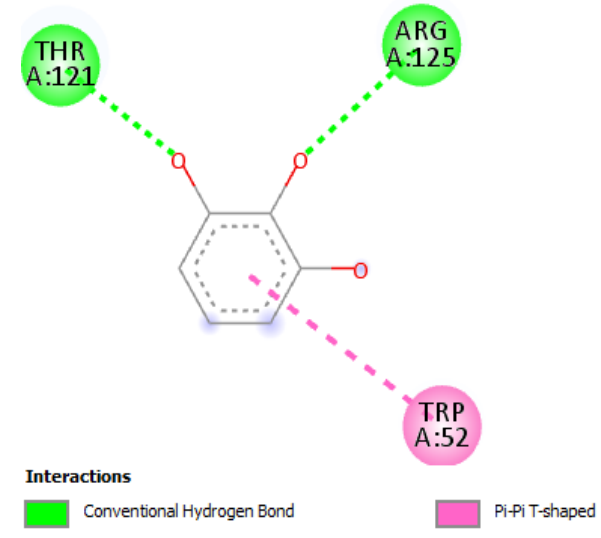

(h)

Figure 3. 2D structure of compound B against **(a)** *Clostridium sporogenes*; **(b)** *Bacillus subtilis*; **(c)** *Bacillus cereus*; **(d)** *Staphylococcus aureus*; **(e)** *Proteus vulgaris*; **(f)** *Escherichia coli*; **(g)** *Klebsiella pneumonia*; **(h)** *Pseudomonas aeruginosa*.
Source: Elaborated by the authors.

4. Conclusions

Scleorocarya birrea obtained from Nigeria contained high phenolic components of pyrogallol; it also demonstrated antimicrobial potencies with varying MIC and MBC values for the array of gram-positive and gram-negative multi-drug-resistant bacteria. From the foregoing, extract from the stem bark of *S. birrea* could serve as a good alternative to synthetic pyrrogallol and be used as a substitute source for developing a formulation that can be used to treat urinary tract infections.

Authors' contribution

Conceptualization: Larayetan Rotimi Abisoye; Abel Kolawole Oyebamiji; **Data curation:** Balogun Sadiya Ufelli; **Formal Analysis:** Larayetan Rotimi Abisoye; **Funding acquisition:** Oluwatayo Emmanuel Abioye; **Investigation:** Larayetan Rotimi Abisoye; **Methodology:** Balogun Joshua; Yahaya Abdulraraq; **Project administration:** Balogun Sadiya Ufelli; **Resources:** Oluwatayo Emmanuel Abioye; **Software:** Abel Kolawole Oyebamiji; Oluwatayo Emmanuel Abioye; **Supervision:** Abel Kolawole Oyebamiji; **Validation:** Ayeni Gideon; Yahaya Abdulraraq; **Visualization:** Yahaya Abdulraraq; **Writing – original draft:** Balogun Sadiya Ufelli; Larayetan Rotimi Abisoye; Abel Kolawole Oyebamiji; **Writing – review & editing:** Larayetan Rotimi Abisoye; Abel Kolawole Oyebamiji.

Conflict of interest

The authors declare that there is no conflict of interest.

Data availability statement

All data sets were generated or analyzed in the current study.

Artificial Intelligence usage statement

The authors declare that they did not use Artificial Intelligence tools at any stage of the preparation, correction, or evaluation of this work.

Funding

Not applicable.

Acknowledgments

Not applicable.

References

Abisoye, L. R. Antimalarial, Antitrypanosomal, Antimicrobial Activities and Volatile Oil Profile of Xylopia aethiopica (Dunal) Rich (Annonaceae). *Lett. Appl. NanoBio Sci.* **2021**, *11*, 3897–3908. https://doi.org/10.33263/LIANBS113.38973908

Adeyemi, S.; Larayetan, R.; Onoja, A. D.; Ajayi, A.; Yahaya, A.; Ogunmola, O. O.; Adeyi, A. O.; Chijioke, O. Anti-Hemorrhagic Activity of Ethanol Extract of Moringa Oleifera Leaf on Envenomed Albino Rats. *Sci. Afr.* **2021**, *12*, e00742. https://doi.org/10.1016/j.sciaf.2021.e00742

Ait-Mimoune, N.; Hassaine, H.; Boulanoir, M. Bacteriological Profile of Urinary Tract Infections and Antibiotic Susceptibility of Escherichia Coli in Algeria. *Iran. J. Microbiol.* **2022**, *14* (2), 156–160. https://doi.org/10.18502/ijm.v14i2.9180

Akinpelu, D. A.; Abioye, E. O.; Aiyegoro, O. A.; Akinpelu, O. F.; Okoh, A. I. Evaluation of Antibacterial and Antifungal Properties OfAlchornea Laxiflora(Benth.) Pax. & Hoffman. *Evid. Based Complement. Alternat. Med.* **2015**, *2015* (1), 684839. https://doi.org/10.1155/2015/684839

Balouiri, M.; Sadiki, M.; Ibnsouda, S. K. Methods for *in Vitro* Evaluating Antimicrobial Activity: A Review. *J. Pharm. Anal.* **2016**, *6* (2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Belemnaba, L.; Sawadogo, B.; Ouedraogo, W. R. C.; Nitiéma, M.; Kaboré, B.; Sawadogo, B.; Koala, M.; Ouedraogo, M.; Ouedraogo, S. "Acute Toxicity, Antioxidant and Vasodilatory Properties of Sclerocarya Birrea (A. Rich.) Hochst (Anacardiaceae) Trunk Bark's Aqueous Decoction". *J. Adv. Med. Pharm. Sci.* **2024**, *26* (8), 1–19. https://doi.org/10.9734/jamps/2024/v26i8704

Braca, A.; Politi, M.; Sanogo, R.; Sanou, H.; Morelli, I.; Pizza, C.; De Tommasi, N. Chemical composition and antioxidant activity of phenolic compounds from wild and cultivated *Sclerocarya birrea* (Anacardiaceae) leaves. *J. Agric. Food Chem.* **2003**, *51* (23), 6689–6695. https://doi.org/10.1021/jf030374m

Bravo, L. Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance. *Nutr. Rev.* **2009**, *56* (11), 317–333. https://doi.org/10.1111/j.1753-4887.1998.tb01670.x

Chen, C.-H.; Liu, T.-Z.; Chen, C.-H.; Wong, C. H.; Chen, C.-H.; Lu, F.-J.; Chen, S. C. The Efficacy of Protective Effects of Tannic Acid, Gallic Acid, Ellagic Acid, and Propyl Gallate against Hydrogen Peroxide-Induced Oxidative Stress and DNA Damages in IMR-90 Cells. *Mol. Nutr. Food Res.* **2007**, *51* (8), 962–968. https://doi.org/10.1002/mnfr.200600230

Chew, Y.-L.; Goh, J.-K.; Arasi, C. Investigation on Antibacterial Activity of Pyrogallol in Methicillin Resistant Staphylococcus Aureus. *Curr. Trends Biotechnol. Pharm.* **2020**, *14* (5), 176–180. https://doi.org/10.5530/ctbp.2020.4s.20

Citarasu T. Natural antimicrobial compounds for use in aquaculture. In: Austin B. (ed.) *Infectious Disease in Aquaculture.* Woodhead Publishing; 2012, p. 419–456.

Collins, C. H.; Lyne, P. M.; Grange, J. M.; Falkinham III, J. O. *Collins and Lyne's Microbiological Methods.* Arnolds London UK; 2004.

Cynthia, I. F.; Hery, S.; Akhmad, D. Antibacterial and antioxidant activities of pyrogallol and synthetic pyrogallol dimer. *Res. J. Chem. Environ*. **2018**, *22*, 39–48.

Daglia, M. Polyphenols as Antimicrobial Agents. *Curr. Opin. Biotechnol.* **2012**, *23* (2), 174–181. https://doi.org/10.1016/j.copbio.2011.08.007

Daniel, J. A.; Mathiyazhagan, J.; Anbazhagan, M.; Jayaraj, R.; Gothandam, K. M. Phytochemistry and Pharmacology of Sclerocarya birrea (A. Rich.) Hochst.: A Review. *Bioact. Pharmacol. Med. Plants.* **2022**, 139–147. https://doi.org/10.1201/9781003281658-10

Defoirdt, T.; Pande, G. S. J.; Baruah, K.; Bossier, P. The Apparent Quorum-Sensing Inhibitory Activity of Pyrogallol Is a Side Effect of Peroxide Production. *Antimicrob. Agents. Chemother.* **2013**, *57* (6), 2870–2873. https://doi.org/10.1128/AAC.00401-13

Dorothy, V.; Lucy, H.; Sunday, O. O.; Mary, O. U. The Anti-Inflammatory Activities of Twelve Nigerian Medicinal Plants: Inhibition of NfkB, Activation of Nrf2, and Antioxidant Content. *Afr. J. Biochem. Res.* **2022**, *16* (1), 1–10. https://doi.org/10.5897/ajbr2021.1134

Doughari, J. H.; Human, I. S.; Benadé, A. J.; Ndakidemi, P. A. Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. *J. Med. Plants Res.* **2009**, *3* (11), 839–848. http://hdl.handle.net/11189/2637s

Dubey, N. K.; Kumar, R.; Tripathi, P. Global promotion of herbal medicine: India's opportunity. *Curr. Sci.* **2004**, *86* (1), 37–41.

Elzaawely, A. A.; Xuan, T. D.; Tawata, S. Antioxidant and Antibacterial Activities of Rumex Japonicus HOUTT. Aerial Parts. *Biol. Pharm. Bull.* **2005**, *28* (12), 2225–2230. https://doi.org/10.1248/bpb.28.2225

Gadisa, E.; Weldearegay, G.; Desta, K.; Tsegaye, G.; Hailu, S.; Jote, K.; Takele, A. Combined Antibacterial Effect of Essential Oils from Three Most Commonly Used Ethiopian Traditional Medicinal Plants on

Multidrug Resistant Bacteria. *BMC Complement. Altern. Med.* **2019**, *19*, 24. https://doi.org/10.1186/s12906-019-2429-4

Gauba, A.; Rahman, K. M. Evaluation of Antibiotic Resistance Mechanisms in Gram-Negative Bacteria. *Antibiotics*. **2023**, *12*, 1590. https://doi.org/10.3390/antibiotics12111590

Guerrini, A.; Sacchetti, G.; Rossi, D.; Paganetto, G.; Muzzoli, M.; Andreotti, E.; Tognolini, M.; Maldonado, M. E.; Bruni, R. Bioactivities of *Piper Aduncum* L. and *Piper Obliquum* Ruiz & Pavon (Piperaceae) essential oils from Eastern Ecuador. *Environ. Toxicol. Pharmacol.* **2009**, *27* (1), 39–48. https://doi.org/10.1016/j.etap.2008.08.002

Hopper, W.; Mahadevan, A. Degradation of catechin by Bradyrhizobium japonicum. *Biodegradation.* **1997**, *8* (3), 159–165. https://doi.org/10.1016/j.etap.2008.08.002

Kagambega, W.; Belem, H.; Meda, R. N.-T.; Koama, B. K.; Drabo, A.-F.; Kabore, J.; Traore, A.; Ouédraogo, G. A.; Benedec, D.; Hanganu, D.; Vlase, L.; Vlase, A.-M.; Voştinaru, O.; Mogoşan, C.; Oniga, I. Polyphenolic Profile, Anti-Inflammatory and Anti-Nociceptive Activities of Some African Medicinal Plants. *Plants.* **2022**, *11* (10), 1377. https://doi.org/10.3390/plants11101377

Kamatou, G. P. P.; Viljoen, A. M.; Başer, K. H. C. Head-Space Volatiles of Marula (Scelerocarya Birrea, Subspecies Caffra). *S. Afr. J. Bot.* **2008**, *74* (2), 325–326. https://doi.org/10.1016/j.sajb.2007.10.005

Khameneh, B.; Iranshahy, M.; Soheili, V.; Fazly Bazzaz, B. S. Review on Plant Antimicrobials: A Mechanistic Viewpoint. *Antimicrob. Resist. Infect. Control*. **2019**, *8*, 118. https://doi.org/10.1186/s13756-019-0559-6

Kim, D.-O.; Jeong, S. W.; Lee, C. Y. Antioxidant Capacity of Phenolic Phytochemicals from Various Cultivars of Plums. *Food Chem.* **2003**, *81* (3), 321–326. https://doi.org/10.1016/s0308-8146(02)00423-5

Larayetan, R.; Osanekwu, S.; Sokwo, M. Phytochemical Components of Methanolic Fruit Extract of *Dennettia tripetala*. *J. Funct. Mater. Biomol.* **2018**, *2*, 48–52.

Larayetan, R.; Ololade, Z. S.; Ogunmola, O. O.; Ladokun, A. Phytochemical Constituents, Antioxidant, Cytotoxicity, Antimicrobial, Antitrypanosomal, and Antimalarial Potentials of the Crude Extracts of *Callistemon Citrinus*. *Evid. Based Complement. Alternat. Med.* **2019**, *2019* (1), 5410923. https://doi.org/10.1155/2019/5410923

Lin, T.-H.; Wu, C.-C.; Tseng, C.-Y.; Fang, J.-H.; Lin, C.-T. Effects of Gallic Acid on Capsular Polysaccharide Biosynthesis in Klebsiella Pneumoniae. *J. Microbiol. Immunol. Infect.* **2022**, *55* (6 Pt 2), 1255–1262. https://doi.org/10.1016/j.jmii.2021.07.002

Louis, H.; Akakuru, O. U.; Linus, M. N.; Innocent, J.; Amos, P. I. Qualitative and Quantitative Phytochemical Analyses of *Sclerocarya birrea* and *Sterculia setigera* in Kem and Yola, Adamawa State, Nigeria. *Am. J. Biomed. Res.* **2018**, *6* (1), 1–10. https://doi.org/10.12691/ajbr-6-1-1

Mabasa, L.; Kotze, A.; Shabalala, S.; Kimani, C.; Gabuza, K.; Johnson, R.; Sangweni, N. F.; Maharaj, V.; Muller, C. J. F. Sclerocarya Birrea (Marula) Extract Inhibits Hepatic Steatosis in Db/Db Mice. *Int. J. Environ. Res. Public Health.* **2022**, *19* (7), 3782. https://doi.org/10.3390/ijerph19073782

Maharaj, V.; Ezeofor, C. C.; Naidoo Maharaj, D.; Muller, C. J. F.; Obonye, N. J. Identification of Antidiabetic Compounds from the Hexane Extract of Sclerocarya Birrea Leaves. *Molecules.* **2022**, *27* (22), 8095. https://doi.org/10.3390/molecules27228095

Mahato, S. B.; Sen, S. Advances in Triterpenoid Research, 1990–1994. *Phytochemistry*. **1997**, *44* (7), 1185–1236. https://doi.org/10.1016/s0031-9422 (96)00639-5

Mariod, A. A.; Matthäus, B.; Hussein, I. H. Antioxidant properties of methanolic extracts from different parts of *Sclerocarya birrea*. *Int. J. Food Sci. Technol.* **2008**, *43* (5), 921–926. https://doi.org/10.1111/j.1365-2621.2007.01543.x

Mashau, M. E.; Kgatla, T. E.; Makhado, M. V.; Mikasi, M. S.; Ramashia, S. E. Nutritional Composition, Polyphenolic Compounds and Biological Activities of Marula Fruit (*Sclerocarya Birrea*) with Its Potential Food Applications: A Review. *Int. J. Food Prop.* **2022**, *25* (1), 1549–1575. https://doi.org/10.1080/10942912.2022.2064491

Mohammed, A. A.; Samad, A.; Omar, O. A. Escherichia Coli Spp, Staph Albus and Klebseilla Spp Were Affected by Some Antibiotics for Urinary Tract Infections in Bani Waleed City. *Brill. Res. Artif. Intell.* **2022**, *2* (2), 66–70. https://doi.org/10.47709/brilliance.v2i2.1564

Nantitanon, W.; Chowwanapoonpohn, S.; Okonogi, S. Antioxidant and Antimicrobial Activities of Hyptis Suaveolens Essential Oil. *Sci. Pharm.* **2007**, *75* (1), 35–54. https://doi.org/10.3797/scipharm.2007.75.35

Njume, C.; Afolayan, A. J.; Green, E.; Ndip, R. N. Volatile Compounds in the Stem Bark of Sclerocarya Birrea (Anacardiaceae) Possess Antimicrobial Activity against Drug-Resistant Strains of Helicobacter Pylori. *Int. J. Antimicrob. Agents.* **2011**, *38* (4), 319–324. https://doi.org/10.1016/j.ijantimicag.2011.05.002

Nur Saeida, B.; Hasmah, A.; Wan Nor Amilah, W. A. W.; Kerian, K. Potential use of Quercus infectoria gall extracts against urinary tract pathogenic bacteria. *Int. J. Res. Pharmacol. Pharmacother.* **2014**, *3* (3), 184–191.

Obadoni, B. O.; Ochuko, P. O. Phytochemical Studies and Comparative Efficacy of the Crude Extracts of Some Haemostatic Plants in Edo and Delta States of Nigeria. *Glob. J. Pure Appl. Sci.* **2002**, *8* (2), 203–208. https://doi.org/10.4314/gjpas.v8i2.16033

Okwu, D. E.; Okwu, M. E. Chemical Composition of Spondias Mombin Linn Plant Parts. *J. Sustain. Agric. Environ.* **2004**, *6* (2), 140–147.

Ordonez, A.; Gomez, J.; Vattuone, M.; Lsla, M. Antioxidant Activities of Sechium Edule (Jacq.) Swartz Extracts. *Food Chem.* **2006**, *97* (3), 452–458. https://doi.org/10.1016/j.foodchem.2005.05.024

Oyebamiji, A. K.; Soetan, E. A.; Akintelu, S. A.; Ayeleso, A. O.; Mukwevho, E. Alpha-Glucosidase Activity of Phytochemicals from Phyllanthus Amarus Leaves via *In-Silico* Approaches. *Pharmacol. Res. - Mod. Chin. Med.* **2022**, *2*, 100054. https://doi.org/10.1016/j.prmcm.2022.100054

Park, W. H.; Han, Y. W.; Kim, S. H.; Kim, S. Z. A Superoxide Anion Generator, Pyrogallol Induces Apoptosis in As4.1 Cells through the Depletion of Intracellular GSH Content. *Mutat. Res. Fundam. Mol. Mech. Mutagen.* **2007**, *619* (1-2), 81–92. https://doi.org/10.1016/j.mrfmmm.2007.02.004

Reid, G. Urogenital Infections in Women: Can Probiotics Help? *Postgrad. Med. J.* **2003**, *79* (934), 428–432. https://doi.org/10.1136/pmj.79.934.428

Rossiter, S. E.; Fletcher, M. H.; Wuest, W. M. Natural Products as Platforms to Overcome Antibiotic Resistance. *Chem. Rev.* **2017**, *117* (19), 12415–12474. https://doi.org/10.1021/acs.chemrev.7b00283

Russon, D.; Miglionico, R.; Carmosino, M.; Bisaccia, F.; Andrade, P.; Valentão, P.; Milella, L.; Armentano, M. A Comparative Study on Phytochemical Profiles and Biological Activities of Sclerocarya Birrea (A.Rich.) Hochst Leaf and Bark Extracts. *Int. J. Mol. Sci.* **2018**, *19* (1), 186. https://doi.org/10.3390/ijms19010186

Salazar-Orbea, G. L.; García-Villalba, R.; Bernal, M. J.; Hernández, A.; Tomás-Barberán, F. A.; Sánchez-Siles, L. M. Stability of phenolic compounds in apple and strawberry: Effect of different processing techniques in industrial set up. *Food Chem.* **2023**, *401*, 134099. https://doi.org/10.1016/j.foodchem.2022.134099

Selvaraj, S.; Rajkumar, P.; Thirunavukkarasu, K.; Gunasekaran, S.; Kumaresan, S. Vibrational (FT-IR and FT-Raman), Electronic (UV–Vis) and Quantum Chemical Investigations on Pyrogallol: A Study on Benzenetriol Dimers. *Vib. Spectrosc.* **2018**, *95*, 16–22. https://doi.org/10.1016/j.vibspec.2018.01.003

Shmuely, H.; Ofek, I.; Weiss, E. I.; Rones, Z.; Houri-Haddad, Y. Cranberry Components for the Therapy of Infectious Disease. *Curr. Opin. Biotechnol.* **2012**, *23* (2), 148–152. https://doi.org/10.1016/j.copbio.2011.10.009

Taguri, T.; Tanaka, T.; Kouno, I. Antimicrobial Activity of 10 Different Plant Polyphenols against Bacteria Causing Food-Borne Disease. *Biol. Pharm. Bull.* **2004**, *27* (12), 1965–1969. https://doi.org/10.1248/bpb.27.1965

Tanih, N. F.; Ndip, R. N. Evaluation of the Ethanol and Hexane Extracts of Mature Stem Bark of *Sclerocarya Birrea* for Antioxidant and Antimicrobial Properties. *Evid. Based Complement. Alternat. Med.* **2012**, *2012*, 834156. https://doi.org/10.1155/2012/834156

Tinh, T. H.; Nuidate, T.; Vuddhakul, V.; Rodkhum, C. Antibacterial Activity of Pyrogallol, a Polyphenol Compound against Vibrio Parahaemolyticus Isolated from the Central Region of Thailand. *Procedia Chem.* **2016**, *18*, 162–168. https://doi.org/10.1016/j.proche.2016.01.025

Van Buren, J. P.; Robinson, W. B. Formation of Complexes between Protein and Tannic Acid. *J. Agric. Food Chem.* **1969**, *17* (4), 772–777. https://doi.org/10.1021/jf60164a003

Viljoen, A. M.; Guy, P. P.; Kamatou, K.; Başer, K. H. C. Head-space volatiles of marula (*Sclerocarya birrea* subsp. *caffra*). *S. Afr. J. Bot.* **2008**, *74* (2), 325–326. https://doi.org/10.1016/j.sajb.2007.10.005

Yadav, M.; Chatterji, S.; Gupta, S. K.; Watal, G. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. *Int. J. Pharm. Pharm. Sci.* **2014**, *6* (5), 539–542.

Zeng, Z.; Zhan, J.; Zhang, K.; Chen, H.; Cheng, S. Global, Regional, and National Burden of Urinary Tract Infections from 1990 to 2019: An Analysis of the Global Burden of Disease Study 2019. *World J. Urol.* **2022**, *40* (3), 755–763. https://doi.org/10.1007/s00345-021-03913-0