GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Antimicrobial activity of ethyl acetate sub-fractions of African Yam Bean (AYB) (*Sphenostylis stenocarpa*) on Multidrug-resistant *Staphylococcus aureus* and *Escherichia coli*

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GSC Biological and Pharmaceutical Sciences, 2025, 32(03), 186-193

Publication history: Received on 05August 2025; revised on 14 September 2025; accepted on 17 September 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.32.3.0360

Abstract

The diverse ethno-medicinal and-pharmacological potentials of *Sphenostylis stenocarpa* commonly known as the African yam bean (AYB) puts this plant in the spotlight for extensive research as a reliable source for bioactive compounds for drug development against both hospital- and community-acquired infections caused by multidrug resistant pathogens. This study evaluated the antibacterial activity of sub-fractions of *Sphenostylis stenocarpa* on some multidrug resistant strains of *Staphylococcus aureus* and *Escherichia coli.* The ethyl acetate fraction was subjected to Vacuum liquid chromatograpic (VLC) technique and the antibacterial potentials of the sub-fractions were tested against multidrug resistant strains of *Staphylococcus aurues, and Escherichia coli* using the agar well diffusion assay technique. The sub-fractions of the ethyl acetate fraction showed broad-spectrum antimicrobial activities except n-Hexane:Ethyl acetate: NH:ETH (3:7) sub-fraction. The active sub-fractions also produced a concentration-dependent microbial growth inhibition, suggesting the potential of the bioactive compounds to attain the highest possible antimicrobial concentration at the site of infection. Previous Phytochemical analysis identified the presence of antibacterial compounds such as alkaloids, tannins, flavonoids, saponins in the crude extract of *Sphenostylis stenocarpa* L. In conclusion, *Sphenostylis stenocarpa* L could serve as a promising potential source of antimicrobial compounds against multidrug resistant pathogens.

Keywords: *Sphenostylis stenocarpa;* Multidrug resistant pathogens; Vacuum liquid chromatography; Broad-spectrum; Sub-fraction

1. Introduction

The ethno-medicinal use of *Sphenostylis stenocarpa*, as well as the reported pharmacological properties such as antimicrobial [1, 2]; antioxidant [1, 2]; anti-diabetic activity [3]; anti-inflammatory, antidiabetics, antioxidant, hepato-renal and hematological activities [4], is an indication of the intrinsic pharmaceutical potentials of this plant and how this properties can be harnessed for the development of therapeutic agents against several clinical pathogens, especially multidrug-resistant microorganisms. *Sphenostylis stenocarpa* is an important leguminous medicinal plant known as African yam bean (AYB), belonging to the family Fabaceae and native to Africa [5, 6]. *Sphenostylis stenocarpa* L. is widely propagated for its health benefits. The established pharmacological properties of legumes, for example, antimicrobial,

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antidiabetic, and anticancer activities, have been attributed to the bioactive compounds present in them [7]. Bacterial resistance to antibiotics has metamorphosed from being previously susceptible to expressing multidrug resistance thus presenting an urgent global health threat. Bacterial infections caused by this group of bacteria have been linked to over 13 million deaths worldwide each year [8]. In response, scientific focus is now on the search for new bioactive agents.

The antimicrobial properties of *Sphenostylis stenocarpa* L. (AYB) crude extracts against some microorganisms show the potential of this plant as a source of bioactive compounds that can be developed into therapeutic agents [2]. Therefore, this paper reports the antimicrobial potentials of the ethyl acetate sub-fractions of *Sphenostylis stenocarpa* L. (AYB) against multidrug resistant *Staphylococcus aureus* and *Escherichia coli* strains.

2. Materials and Methods

2.1. Extraction and preparation of Plant material

Fresh powdered seeds of *Sphenostylis sternocarpa* (African yam bean) weighing 500g were extracted with MeOH in a Soxhlet apparatus [9]. The methanolic crude obtained was partitioned with ethyl acetate, butanol, n-hexane, and water fractions [2]. The ethyl acetate (EtoAc) fraction was subjected to Vacuum liquid chromatography (VLC) separation technique.

2.2. Vacuum liquid chromatography (VLC)

A sintered glass funnel is used as the column with the length of 30cm was clamped using retort stand, and silica gel for column chromatography (200-400 mesh size) was added/packed into the column up to 15cm.

The sample was weighed and dissolved in 5ml of methanol and was added in 20g of silica gel which was tritulated using mortar and pestle. The sample was carefully introduced onto the top of the packed column, ensuring it's evenly distributed, and small amount of silica gel was added in the column to cover up the phase of the sample and cotton wool finally placed on it for gentle and smooth penetration of solvents.

The column is then subjected to vacuum to initiate solvent flow.

A stepwise gradient was used, increasing solvent polarity to elute the compounds.

n-hexane	Ethyl acetate
500mL (10)	(0)0Ml
450mL (9)	(1)50 mL
400 mL (8)	(2) 100 m
350 mL (7)	(3) 150 mL
300 mL (6)	(4) 200 mL
250 mL (5)	(5) 250 mL
200 mL (4)	(6) 300 mL
150 mL (3)	(7) 350 mL
100 mL (2)	(8) 400 mL
50 mL (1)	(9) 450 mL
0 mL (0)	(10) 500 mL

Dichloromethane	METHANOL
450ml (9)	(1) 50ml
350ml (7)	(3) 150ml
250ml (5)	(5) 250ml

The fractions of the eluent were collected as they elute from the column accordingly with round bottom flask and transferred into a beaker for drying.

2.3. Bioassay

2.3.1. Antimicrobial assay

The antimicrobial assay for the VLC sub-fractions was carried out using the agar well diffusion assay as described by [10]. A standardized 0.5 McFarland suspension of each test organism was done using sterile water and inoculated onto previously sterilized Mueller-Hinton Agar plates (diameter: 90 mm). A stock concentration of 50 mg/mL of each of the sub-fractions was made by weighing 100 mg of the crude and each fraction and reconstituting in 2 mL DMSO. Thereafter, a two-fold serial dilution was made from each of the stock concentrations to get graded concentrations (50, 25, 12.5, and 6.23 mg/mL) for each of the extracts. Thereafter, a maximum volume of 80 µl of each dilution (sub-fractions) was transferred into it corresponding well made with a cork borer having a diameter of 5mm. DMSO served as the negative control against the organisms. The cultures were incubated at 37°C for 24 h. The antimicrobial potential for each sub-fraction was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). The experiment was done in triplicate against each organism.

2.4. Statistical analysis

Measurements were done in triplicate (n = 3) and the results expressed as mean ± standard deviation. One way analysis of variance (ANOVA) and SPSS (version 20) was used as the statistical program.

3. Results and Discussion

Table 1 Antimicrobial activities of EtoAc sub-fraction 1 [NH: ETH (10:0)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	3±0	0±0	6±0	6±0	6±0	0±0	7±0	0±0	8±0	0±0
25	0±0	0±0	4±0	4±0	0±0	0±0	6±0.7	0±0	6±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	4±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: *Staphylococcus aureus*; E: Escherichia coli; NH: n-hexane; ETH: Ethyl acetate

Table 2 Antimicrobial activities of EtoAc sub-fraction 2 [NH:ETH (9:1)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	5±0	0±0	4±0	5±0	6±0	4±0	6±0	6±0	4±0	8±0
25	0±0	0±0	4±0.7	5±0.7	6±0.7	4±0.7	0±0	4±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	4±0	0±0	0±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	3±0	0±0	0±0	0±0	0±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: *Staphylococcus aureus*; E: *Escherichia coli*; NH: n-hexane; ETH: Ethyl acetate

Table 3 Antimicrobial activities of EtoAc sub-fraction 3 [DCM:ME (9:1)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	6±0	0±0	8±0	4±0	0±0	7±0	6±0	4±0	5±0	0±0
25	2±0	0±0	6±0	4±0.7	0±0	6±0	2±0	4±0.7	4±0	0±0
12.5	2±0.7	0±0	4±0	0±0	0±0	0±0	0±0	0±0	3±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: *Staphylococcus aureus*; E: *Escherichia coli;* DCM: Dichlroro methane; ME: Methanol

Table 4 Antimicrobial activities of EtoAc sub-fraction 4 [NH:ETH (8:2)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	6±0	0±0	0±0	5±0	5±0	0±0	8±0	4±0	4±0	0±0
25	5±0	0±0	0±0	4±0	4±0.7	0±0	0±0	3±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	4±0.7	0±0	0±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	3±0	0±0	0±0	0±0	0±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: *Staphylococcus aureus*; E: *Escherichia coli*; NH: n-hexane; ETH: Ethyl acetate

Table 5 Antimicrobial activities of EtoAc sub-fraction 5 [NH:ETH (7:3)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	3±0	0±0	4±0	3±0	0±0	5±0	6±0	0±0	5±0	0±0
25	0±0	0±0	4±0	3±0.7	0±0	4±0	4±0	0±0	3±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: Staphylococcus aureus; E: Escherichia coli; NH: n-hexane; ETH: Ethyl acetate

Table 6 Antimicrobial activities of EtoAc sub-fraction 6 [NH:ETH (6:4)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	0±0	0±0	0±0	5±0	0±0	5±0	3±0	0±0	0±0	0±0
25	0±0	0±0	0±0	0±0	0±0	4±0	0±0	0±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: *Staphylococcus aureus*; E: *Escherichia coli*; NH: n-hexane; ETH: Ethyl acetate

Table 7 Antimicrobial activities of EtoAc sub-fraction 7 [DCM:ME (5:5)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	0±0	5±0	0±0	6±0	6±0	0±0	0±0	0±0	5	0±0
25	0±0	0±0	0±0	5±0	3±0	0±0	0±0	0±0	0±0	0±0
12.5	0±0	0±0	0±0	4±0	0±0	0±0	0±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: *Staphylococcus aureus*; E: *Escherichia coli*; DCM: Dichlroro methane; ME: Methanol

Table 8 Antimicrobial activities of EtoAc sub-fraction 8 [NH:ETH (1:9)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	0±0	4±0	0±0	5±0	0±0	4±0	6±0	0±0	3±0	0±0
25	0±0	0±0	0±0	4±0.7	0±0	3±0	5±0	0±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0	0±0	3±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: *Staphylococcus aureus*; E: *Escherichia coli*; NH: n-hexane; ETH: Ethyl acetate

Table 9 Antimicrobial activities of EtoAc sub-fraction 9 [NH:ETH (2:8)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	0±0	0±0	4±0	6±0	4±0	6±0	4±0	0±0	6±0	0±0
25	0±0	0±0	3±0	6±0.7	4±0.7	0±0	0±0	0±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	3±0	0±0	0±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: *Staphylococcus aureus*; E: *Escherichia coli*; NH: n-hexane; ETH: Ethyl acetate

Table 10 Antimicrobial activities of EtoAc sub-fraction 10 [NH:ETH (3:7)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	0±0	0±0	0±0	0±0	0±0	5±0	6±0	0±0	0±0	0±0
25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: *Staphylococcus aureus*; E: *Escherichia coli*; NH: n-hexane; ETH: Ethyl acetate

Table 11 Antimicrobial activities of EtoAc sub-fraction 11 [NH:ETH (4:6)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	3±0	0±0	5±0	5±0	0±0	6±0	6±0	6±0	4±0	0±0
25	0±0	0±0	0±0	4±0	0±0	0±0	4±0	0±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: *Staphylococcus aureus*; E: *Escherichia coli*; NH: n-hexane; ETH: Ethyl acetate

Table 12 Antimicrobial activities of EtoAc sub-fraction 12 [NH:ETH (5:5)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	0±0	0±0	0±0	5±0	4±0	0±0	5±0	9±0	10±0	0±0
25	0±0	0±0	0±0	4±0	3±0	0±0	4±0	6±0	5±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	5±0.7	0±0
6.25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	4±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: *Staphylococcus aureus*; E: *Escherichia coli*; NH: n-hexane; ETH: Ethyl acetate

Table 13 Antimicrobial activities of EtoAc sub-fraction 13 [DCM:ME (7:3)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	4±0	0±0	0±0	0±0	4±0	6±0	5±0	0±0	0±0	0±0
25	0±0	0±0	0±0	0±0	0±0	5±0	0±0	0±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0	5±0.6	0±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: *Staphylococcus aureus*; E: *Escherichia coli*; DCM: Dichlroro methane; ME: Methanol

Table 14 Antimicrobial activities of EtoAc sub-fraction 14 [NH:ETH (0:10)] against the MDR-isolates

Concentration (mg/mL)	Test organisms / inhibition zone diameter (mm)									
	S2	S3	S4	S23	S38	E3	E4	E5	E9	E12
50	0±0	0±0	0±0	4±0	0±0	5±0	5±0	0±0	0±0	0±0
25	0±0	0±0	0±0	4±0.7	0±0	3±0	4±0	0±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0	3±0.7	0±0	0±0	0±0	0±0
6.25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
3.13	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

S: *Staphylococcus aureus*; E: *Escherichia coli*; NH: n-hexane; ETH: Ethyl acetate

MDR-*Escherichia coli* and –*Staphylococcus aureus* are both commonly implicated clinical pathogens, associated with some hospital- and community-acquired infections. Also, *S. aureus* and *E. coli* are common opportunistic pathogens frequently linked to respiratory tract infections [11]. According to Barua *et al*., [11], all isolates of S. *aureus* and *E. coli* expressed resistance, some resistant to ampicillin, amoxicillin, and penicillin. Moreover, MDR resistance has been observed to be common among *S. aureus* and *E. coli.*

Phytochemicals such as alkaloids, tannins, flavonoids, saponins, and steroids have been identified to be biologically active compounds with different mechanisms of antimicrobial activities linked to some of the ethnopharmacological effects of plants, hence their use in traditional medicine [12]. Some of these bioactive plant metabolites have been identified in the genus *Sphenostylis stenocarpa* L. [2].

The antimicrobial potential of African Yam Bean (*Sphenostylis stenocarpa* L) crude extract and fractions has been previously studied, demonstrating effect against both Gram-positive and Gram-negative bacteria, such as *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Salmonella spp* [2]. The bacteria growth inhibitory mechanisms of *Sphenostylis stenocarpa* L are diverse, affecting the cell wall integrity, inhibiting key metabolic enzymes and processes, and enhancing the host response mechanism. The antimicrobial potentials of Sphenostylis *stenocarpa* L highlight a reliable source of bioactive compounds for the development of novel therapeutic agents against MDR-isolates.

In this study, the antimicrobial evaluation of the sub-fractions of the ethyl acetate crude of *Sphenostylis stenocarpa* L showed that all the sub-fractions except NH:ETH (3:7) sub-fraction produced broad-spectrum antimicrobial activities. The antimicrobial activities were observed to be concentration-dependent. The results showed that the Gram negative bacteria were the most susceptible to the growth inhibitory actions of the sub-fractions. The bacteria growth inhibition was observed to range from 2 to 10 mm, with a minimum inhibitory concentration that ranged from 6.25 to 50 mg/mL. All the sub-fractions showed antimicrobial activities against at least one test bacteria. In comparison with previous findings by Onwughalu *et al*., [2], the antimicrobial activities of the sub-fractions tested showed lower MIC values than the crude extract.

The antibacterial properties of these ethyl acetate sub-fractions are primarily attributed to the combined effects of the abundant phytochemicals associated with the plant, particularly alkaloids, tannins, and flavonoids previously reported by Onwughalu *et al.,* [2]. These phytochemicals have been shown to have diverse biological properties, including antibacterial activities [13]. Antibacterial activities of flavonoids against *S. aureus* and *E. coli* occurs via different inhibitory mechanisms, such as altering the membrane integrity of *S. aureus* through the inhibition of iron and inhibition of the enzymatic activities of the DNA gyrase enzyme in *E. coli*, resulting in a compromised cellular process. Also, Anlas *et al.,* [14] observed that the presence of tannins and flavonoid in the extracts of *Quercus coccifera* may be responsible for the observed antibacterial effects against *P. aeruginosa* and *E. coli.* Both flavonoids and Tannins execute their antibacterial effects through disruption of the synthesis of bacteria cell wall and protein [15].

4. Conclusion

The potency of the sub-fractions against the MDR-strains recorded in this study may have been influenced by the phytochemicals, including tannins and flavonoids, which interfere with microbial processes, leading to a compromise in cell membrane integrity and enzyme function. The results of the present study validates the folkloric usage of decoctions of *Sphenostylis stenocarpa L* and suggests that the antibacterial compounds present in each of the fractions may be isolated as potential bioactive agents for the development of therapeutics active against resistant bacterial strains.

Compliance with ethical standards

Acknowledgments

The authors are sincerely grateful to Onwughalu Izuchukwu Camilus for defraying the publication fee.

Disclosure of conflict of interest

Authors declare no competing financial interest.

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