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Nutrient Compositions, Antioxidant Capacity and Biological Activities of Five-finger Tree (*Averrhoa carambola*) Stem bark, Root, Leaf and Fruit

Godwin Ndarake Enin, Anthony Ayodeji Adegoke, Kate Onyeje Igoche, Kooffreh Kooffreh, Abraham Uduak Ekpe, Ndifreke Ime Asuquo, and Ubong Okon Jeremiah
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Abstract: *Averrhoa carambola (star fruit) is widely used in traditional medicine, yet comparative studies on the nutrient composition and bioactivities of its different parts remain limited. This study investigated the nutrient composition, antioxidant capacity, and antibacterial activity of A. carambola fruit, leaves, stem bark, and roots. Proximate and mineral analyses were conducted using standard methods. Total phenolic (TPC) and flavonoid (TFC) contents, DPPH and FRAP antioxidant activities, were determined spectrophotometrically. Antibacterial activity of the stem bark crude ethanol extract, butanol fraction (BF), and aqueous fraction (AF) was evaluated against clinical isolates using agar well diffusion, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determined by broth dilution. Results showed higher ash content in roots (6.52%) and leaves (6.04%), while crude fat was highest in roots (6.84%). TPC was 38.21 mg GAE/g (stem bark) and 28.18 mg GAE/g (leaves); TFC was 639.00 mg QE/mg (stem bark). Stem bark extract exhibited superior DPPH (IC_{50} = 20.25 µg/mL) and FRAP (38.21 µg/mL) activities compared to ascorbic acid. Mineral analysis revealed high levels of K, Ca, and Mg across all parts, though elevated Cr, Pb, and Cd concentrations raise safety concerns. The BF and crude extract demonstrated potent antibacterial activity (MIC = 25 mg/mL; MBC = 50 mg/mL), surpassing imipenem against several isolates. GC-MS analysis identified stigmasta-3,5-diene, catechol, and fatty acids as major compounds. These findings suggest that A. carambola stem bark possesses promising antioxidant and antibacterial properties, supporting its therapeutic potential, though heavy metal contamination warrants caution about consumption.*

Keywords: *Averrhoa carambola, star fruit, nutrient composition, antioxidant activity, antibacterial activity, phenolic compounds, flavonoids, GC-MS analysis, minimum inhibitory concentration (MIC), heavy metals*

Godwin Ndarake Enin*
[1]Department of Chemistry, Faculty of Physical Sciences, University of Uyo, Uyo, Nigeria
[2] Centre for Wetlands and Waste Management Studies, University of Uyo, Uyo, Nigeria
Email: gowinenin@uniuyo.edu.ng
https://orcid.org/0000-0001-7611-722X

Anthony Ayodeji Adegoke
Department of Microbiology, Faculty of Biological Sciences, University of Uyo, Nigeria
[4] Faculty of Health Science, Durban University of Technology, South Africa
Email: anthonyadegoke@uniuyo.edu.ng

Kate Onyeje Igoche
Department of Microbiology, Faculty of Biological Sciences, University of Uyo, Nigeria
Email: kateigoche@uniuyo.edu.ng

Kooffreh Kooffreh
Department of Microbiology, Faculty of Biological Sciences, University of Uyo, Nigeria
Email: Kooffrehkooffreh@gmail.com

Abraham Uduak Ekpe
Department of Chemistry, Faculty of Physical Sciences, University of Uyo, Nigeria
Email: rixabraham9@gmail.com

Ndifreke Ime Asuquo
Department of Chemistry, Faculty of Physical Sciences, University of Uyo, Uyo, Nigeria
Email: ndifrekeasuquo009@gmail.com

Ubong Okon Jeremiah
Department of Chemistry, Faculty of Physical Sciences, University of Uyo, Nigeria
Email: ubongokon024@gmail.com

1.0 Introduction

Natural product chemistry has played a pivotal role in drug discovery and therapeutic development for centuries, since ancient civilizations such as the Mesopotamians and Egyptians, who discovered the therapeutic potential of plants and their derivatives. These natural substances were processed into preparations such as spices and used for the management of infections, inflammatory disorders, and febrile illnesses. (Newman *et al*., 2000; Newman and Cragg, 2009; Xie *et al*., 2025).

Ancient Egyptian, Chinese, and Ayurvedic medicinal systems further advanced the therapeutic application of medicinal plants with extensive efforts to formulate portions, decoctions and oils for local users. The development of traditional Chinese traditional herbal medicine, Shennong and Tang herbals, and the emergence of the Indian Ayurvedic system significantly stimulated and shaped research in this field (Newman *et al*. 2009; Bernardini *et al*., 2018).

Medicinal plants constitute important sources of nutrients, bioactive compounds, and pharmaceutical agents, with their bioactive compounds forming the backbone of both traditional remedies and modern pharmaceuticals. The therapeutic value of medicinal plants resides mainly in their secondary metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds (Dewick, 2009; Hussein and El-Anssary, 2019).

Unlike primary metabolites, which are vital for growth and reproduction, secondary metabolites evolved as defense molecules against herbivores, pathogens, and environmental stress. These metabolites exhibit biological activities such as antioxidant, antimicrobial, anti-inflammatory, and anticancer properties, thereby making medicinal plants promising candidates for drug discovery (Bocso & Butnariu, 2022; Chinou, 2008).

The emergence of antimicrobial resistance (AMR) has become one of the major global public health challenges, which has encouraged renewed interest in natural products (Demain, 2009; Abdallah *et al.,* 2023). Traditional medicines, principally those derived from plants, have been broadly acknowledged for their potential to afford substitute resolutions to drug-resistant pathogens. One such medicinal plant that has gained considerable scientific attention is *Averrhoa carambola* (star fruit).

Averrhoa carambola (*Oxalidaceae*) is a small to medium-sized tree, which typically grows to a height of 10–12 m. It has sleek, pinnate leaves, and the flowers are usually small, pink, or white, which produce characteristic star-shaped fruits upon ripening. Traditionally, the plant has been used in the management of diabetes, arthralgia, vomiting, cough, chickenpox, and ringworm. (Lakmal *et al*., 2021; Luan *et al*., 2021). Different parts of the plant, including the leaves, fruits, and stem bark, are utilized in ethnomedicine. for the treatment of fever, and skin infections (Albuquerque *et al*., 2015). Phytochemical investigations of the stem bark have revealed the presence of flavonoids, saponins, tannins, and alkaloids (Mia *et al*., 2007; Enin *et al*., 2025).

The fruits have been reported to be rich sources of minerals and antioxidants (Khoo *et al*., 2017; Zainudin *et al*., 2014; Vargas-Madriz *et*

al., 2021). Decoctions prepared from the leaves and fruits are reportedly used to treat fevers, aphthous stomatitis, angina, high blood pressure, eczema, diarrhea, and kidney dysfunction (Gowrishankar *et al*., 2018; Yang *et al*., 2020). Extracts of the leaves and fruits have demonstrated antiulcer, antidiabetic, anti-inflammatory, antihypertensive, anti-obesity, antimicrobial, hepatoprotective, neuroprotective, and anticancer activities (Manda *et al*., 2012; Luan *et al*., 2021; Beas-Guzman *et al*., 2024). Methanolic leaf extracts have been reported to exhibit dose-dependent toxicity in experimental rats (Saghir *et al*., 2022). The leaf fractions also showed anti-hyperlipidemic effects in a poloxamer-407-induced rat model (Abduh *et al*., 2023). Recent studies have also reported antioxidant and antidiabetic activities of the stem bark extract in Wistar rats (Enin *et al* 2025).

Although previous studies have largely focused on the fruits and leaves of *A. carambola*, comparative investigations involving the stem bark and roots remain limited. Furthermore, comprehensive comparative information regarding the proximate composition, mineral profile, antioxidant potential, and antibacterial activities of the various plant parts remains insufficient in the scientific literature. Understanding the nutritional composition and biological activities of different parts of *A. carambola* may contribute to the development of functional foods, natural antioxidants, and alternative antimicrobial agents for therapeutic applications. Therefore, this study was designed to comparatively evaluate the nutrient element composition and antioxidant activity of *Averrhoa carambola* "fruit, leaves, stem bark, and roots, as well as the antibacterial activities of the stem bark extract and its fractions against selected clinical bacterial isolates, thereby providing scientific evidence for their nutraceutical, pharmaceutical, and ethnomedicinal applications.

2.0 Materials and methods

2.1 Plant collection and identification

Fresh fruits, leaves, stem bark, and roots of Averrhoa carambola were collected from a farmland located at Nung Ukim Ikono, Uyo Local Government Area, Akwa Ibom State, Nigeria. The plant material was identified and authenticated by Professor (Mrs.) Margarette Bassey of the Botany and Ecological Study Department, University of Uyo, Nigeria. A voucher specimen (UUH4541) was deposited at the Herbarium of the Faculty of Biological Sciences.

2.2 Preparation of plant material

The fruit, leaf, stem bark and root of *A. carambola* were washed in clean water, air-dried, pulverized to powder form, stored in air-tight bottles labeled as AV-F, AV-L, AV-S and AV-R, and used for the proximate and mineral element analyses. Another portion of the pulverized stem bark was macerated in 70% ethanol for 72 h at room temperature, filtered, and the filtrate was concentrated under reduced pressure using a rotary evaporator to obtain the stem bark crude ethanol extract. The extract was partitioned successively with hexane, ethylacetate, butanol and water. The crude, butanol and aqueous extracts were stored in sample bottles and used for the biological assay.

2.3 Proximate and mineral elements analysis

The proximate analysis for crude fibre, ash, and protein contents (g/100 g) was evaluated based on the established protocol of the Association of Official Analytical Chemists (AOAC, 2003). For the mineral analysis, dried stem bark (AV-S), roots (AV-L), leaves (AV-L) and fruits (AV-F) of *A. carambola* were digested with mineral acids and the filtrates were utilized for the mineral composition study using atomic spectrometric method (Oyeyinka *et al*., 2019).

2.4 Total Phenolic, Flavonoid Content and Antioxidant Activities

Total phenolic content (TPC) and total flavonoid content (TFC) were determined spectrophotometrically according to standard

methods (Kim *et al*., 2003). The phenolic content was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/ g) while the flavonoid content was expressed in milligrams of quercetin equivalent per gram of dry weight (mg QE/ g). Antioxidant activities were conducted by the DPPH radical scavenging activity assay (Khalaf *et al*., 2008), while the FRAP assay was performed after a previous method (Hamid *et al*., 2020). All determinations were done in triplicates and data were subjected to the analysis of variance.

2.5 GC-MS analysis

GC–MS analysis of the stem bark crude ethanol extract was performed using an Agilent 7890A gas chromatograph coupled to an Agilent 5977B inert mass spectrometer with an electron impact source (Agilent Technologies). The stationary phase of separation of the compounds was carried out on RTX1ms capillary column coated with 5% of Phenyl Methyl Siloxane (15m length × 0.25 mm diameter × 0.25 µm film thickness). Helium was the carrier gas used at a constant flow rate of 1.3 mL/min, an initial nominal pressure of 9.7853 psi and at an average velocity of 39.923 cm/sec. One µL of the samples was injected in splitless mode at an injection temperature of 300 °C. The oven was initially programmed at 50 °C (1 min), then ramped at 15 °C/min to 300 °C (10 min). Run time was 18.231 minutes with a 3 min solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C and transfer line temperature of 280 °C. Scanning of possible compounds was from m/z 50 to 550 amu at 2.62s/scan rate and were identified by comparing measured mass spectral data with those in NIST 14 Mass Spectral Library (Kadhim *et al*., 2016).

2.6 Identification and confirmatory test on test organisms

Test organisms used for this study were *Pseudomonas aeruginosa* (PA-1 and PA-2), *Bacillus sp.*, *Klebsiella* sp. and *Escherichia coli* (EC-1 and EC-2). These isolates were obtained from the postgraduate laboratory of the Department of Microbiology. A well-isolated colony of the bacteria was picked using a sterile inoculating wire-loop and transferred into nutrient agar and incubated at 37 ºC for 24 hours before the susceptibility test. Identification and confirmatory tests were carried out on the organisms using appropriate biochemical tests like Gram staining, catalase, oxidase, citrate, urease, motility, indole reaction and sugar fermentation.

2.7 Determination of antibacterial activity

The agar well method was used to determine the activity index of the plant extracts. A cork borer of 4 mm was used to create the agar well on the Mueller-Hinton agar, then different concentrations of the plant extract and fractions (12.5 mg/mL, 25 mg/mL, 50 mg/mL and 100 mg/mL) were dropped into the well using a sterile pipette. They were used together with a conventional antibiotic (Imipenem (10 µg)) and incubated in an incubator for 18 hours at 37 ^{o}C. The zone of inhibition was measured using a transparent ruler, and the diameter of the cork borer was subtracted from the result (Gberikon *et al*., 2015).

The crude extract and fractions of *Averrhoa carambola* were subjected to further (quantitative) tests to determine their minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC).

2.7.1 Determination of minimum inhibitory concentration (MIC)

The crude extract and fractions of *Averrhoa carambola* were further tested to determine the minimum inhibitory concentration (MIC) for the bacterial samples. The MICs of these extracts and fractions were determined by the broth (tube) dilution method. The stock solution was serially diluted with methanol, in sterile test tubes labelled and

arranged from the highest to lowest concentration of extract desired (100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5 mg/mL). Test tubes that did not show turbidity were further analysed.

2.7.2 Determination of minimum bacteriocidal concentration (MBC)

Test tubes which did not show turbidity were plated in a petri dish to determine the minimum bacteriocidal concentration (MBC). Petri plates with growth were recorded as positive, while the plates without growth were recorded as negative.

2.8 Statistical analysis

All determinations were done in triplicate or duplicate as indicated. The data generated were computed into the computer and analysed using Excel. Means and standard deviation of results obtained from the chemical analysis were calculated.

3.0 Results and Discussion

3.1 Results

3.1.1 Mineral elements

The mineral elemental composition of the stem bark (AV-S), leaves (AV-L), fruits (AV-F), and roots (AV-R) of *Averrhoa carambola* is presented in Table 1. The results revealed the presence of both macro elements and trace elements in varying concentrations across the different plant parts. Major mineral elements detected in all the samples included sodium (Na), magnesium (Mg), calcium (Ca), potassium (K), phosphorus (P), iron (Fe), and aluminium (Al), indicating that the plant may serve as a valuable source of essential dietary minerals.

Potassium (K) was the most abundant mineral element detected in all the samples, with concentrations ranging from 899.504 ± 0.41 mg/100 g in the stem bark to 1270.998 ± 0.74 mg/100 g in the leaves. The high potassium content observed particularly in the leaves and roots suggests that these plant parts may contribute significantly to electrolyte balance, neuromuscular coordination, and cardiovascular health. The root also contained appreciable potassium content (1171.46 ± 0.69 mg/100 g), while the fruit contained 920.523 ± 0.57 mg/100 g.

Calcium (Ca) content varied markedly among the samples. The highest concentration was detected in the root (694.514 ± 0.45 mg/100 g), closely followed by the leaf (660.483 ± 0.51 mg/100 g), whereas comparatively lower concentrations were observed in the fruit (87.246 ± 0.08 mg/100 g) and stem bark (24.733 ± 0.00 mg/100 g). The elevated calcium content in the root and leaf indicates that these plant parts may possess strong nutritional importance in bone mineralization, teeth development, muscle contraction, and blood pressure regulation (Wardlaw *et al*., 2004).

Magnesium (Mg), another important macroelement involved in enzyme activation and metabolic regulation, was highest in the stem bark (323.963 ± 0.12 mg/100 g), followed by the leaves (265.075 ± 0.22 mg/100 g), roots (244.698 ± 0.05 mg/100 g), and fruits (120.268 ± 0.07 mg/100 g). The comparatively high magnesium levels observed in the stem bark may contribute to its reported therapeutic properties (Muir, 2000).

Sodium (Na) concentrations were relatively lower compared to potassium and calcium. The root sample recorded the highest sodium content (131.167 ± 0.10 mg/100 g), whereas similar lower concentrations were observed in the stem bark, leaf, and fruit samples (17.734 ± 0.05 mg/100 g). Phosphorus (P) was also detected in all plant parts, with the highest concentration recorded in the root (171.952 ± 0.00 mg/100 g), followed by the stem bark (114.272 ± 0.01 mg/100 g), fruit (101.941 ± 0.00 mg/100 g), and leaf (72.488 ± 0.13 mg/100 g).

Iron (Fe), which is essential for haemoglobin synthesis and oxygen transport (Geissler *et al*., 2011), was present in appreciable quantities in all the samples. The root showed the highest iron concentration (11.811 ± 0.00 mg/100 g),

followed by the fruit (8.870 ± 0.01 mg/100 g), stem bark (8.268 ± 0.01 mg/100 g), and leaves (6.258 ± 0.01 mg/100 g). Aluminium (Al) was detected in all the plant parts, with the highest value observed in the root (17.061 ± 0.02 mg/100 g), while the leaves recorded the lowest concentration (1.788 ± 0.01 mg/100 g).

Trace elements such as chromium (Cr), manganese (Mn), zinc (Zn), copper (Cu), nickel (Ni), cobalt (Co), selenium (Se), vanadium (V), arsenic (As), lead (Pb), and cadmium (Cd) were also detected in varying concentrations. Manganese was particularly abundant in the stem bark (28.336 ± 0.01 mg/100 g) and leaves (24.799 ± 0.22 mg/100 g), whereas zinc concentrations were relatively high in the stem bark (24.157 ± 0.01 mg/100 g) and leaves (20.036 ± 0.01 mg/100 g). Selenium was detected only in the stem bark and root samples, while cobalt and arsenic were absent in the stem bark and leaf but present in the fruit and root samples. Lead was detected only in the leaf sample (0.947 ± 0.01 mg/100 g), whereas cadmium was present in all samples at very low concentrations ranging from 0.038 ± 0.00 mg/100 g to 0.057 ± 0.00 mg/100 g.

Overall, the results demonstrated that different parts of *A. carambola* possess distinct mineral distribution patterns, with the roots and leaves generally exhibiting higher concentrations of essential mineral nutrients. These findings suggest that the plant may possess important nutritional and pharmacological value due to its rich mineral composition.

Tabel 1. Nutrient elements and trace metals composition in *A. carambola* (mg/100 g)

Element	AVC-S	AVC-L	AVC-F	AVC-R
Na	17.734±0.05	17.734±0.05	17.734±0.05	131.167±0.10
Mg	323.963±0.12	265.075±0.22	120.268±0.07	244.698±0.05
Ca	24.733±0.00	660.483±0.51	87.246±0.08	694.514±0.45
K	899.504±0.41	1270.998±0.74	920.523±0.57	1171.46±0.69
V	ND	0.053±0.01	0.052±0.01	0.042±0.02
Cr	0.087±0.003	0.091±0.00	0.168±0.02	0.135±0.01
Mn	28.336±0.01	24.799±0.22	2.083±00.00	4.874±0.00
Al	3.164±0.01	1.788±0.01	4.797±0.05	17.061±0.02
Fe	8.268±0.01	6.258±0.01	8.870±0.01	11.811±0.00
Co	ND	ND	0.102±0.00	0.147±0.00
Ni	0.463±0.01	ND	0.087±0.01	0.702±0.10
As	ND	ND	0.209±0.00	0.765±0.020
Se	0.141±0.02	ND	ND	0.468±0.02
P	114.272±0.01	72.488±0.13	101.941±0.00	171.952±0.00
Zn	24.157±0.01	20.036±0.01	4.338±0.04	19.348±0.02
Cu	1.199±0.01	0.458±0.01	0.647±0.01	0.956±0.02
Pb	ND	0.947±0.01	0.459±0.02	ND
Cd	0.0520±0.00	0.057±0.00	0.040±0.00	0.038±0.00

****Values are the average of three replicate determinations. ND = Not detected.**

3.1.2 Heavy Metal Composition

The concentrations of selected heavy metals and trace elements in the stem bark (AV-S), leaves (AV-L), fruits (AV-F), and roots (AV-R) of *Averrhoa carambola* are presented in Table 1. The analysed elements included lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), vanadium (V), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), and selenium (Se). The results revealed variations in the distribution and accumulation of these metals among the different plant parts,

indicating differential uptake, translocation, and storage mechanisms within the plant tissues.

Among the trace metals analysed, manganese (Mn) and zinc (Zn) were present in relatively higher concentrations compared to the other heavy metals. Manganese concentrations ranged from 2.083 ± 0.00 mg/100 g in the fruit to 28.336 ± 0.01 mg/100 g in the stem bark. The leaves also contained appreciable manganese content (24.799 ± 0.22 mg/100 g), whereas the roots contained 4.874 ± 0.00 mg/100 g. The comparatively high manganese levels in the stem bark and leaves suggest active involvement of this element in plant metabolic processes such as enzyme activation, photosynthesis, and antioxidant defense systems (Soumya and Nair, 2016).

Zinc concentrations were also relatively high across the samples, with the stem bark recording the highest concentration (24.157 ± 0.01 mg/100 g), followed by the leaves (20.036 ± 0.01 mg/100 g), roots (19.348 ± 0.02 mg/100 g), and fruits (4.338 ± 0.04 mg/100 g). Zinc is an essential micronutrient required for immune function, protein synthesis, and enzymatic activities (Costa *et al*., 2023). The appreciable levels detected in the plant samples indicate that *A. carambola* may contribute beneficial trace nutrients when consumed in moderate quantities.

Copper (Cu), another essential trace element, was detected in all plant parts at relatively low concentrations ranging from 0.458 ± 0.01 mg/100 g in the leaves to 1.199 ± 0.01 mg/100 g in the stem bark. Chromium (Cr) was also present in all samples, with the highest concentration observed in the fruit (0.168 ± 0.02 mg/100 g), followed by the root (0.135 ± 0.01 mg/100 g), leaf (0.091 ± 0.00 mg/100 g), and stem bark (0.087 ± 0.003 mg/100 g). Chromium is known to play a role in glucose metabolism and insulin regulation when present in trace quantities (Jaishankar *et al*., 2014).

Vanadium (V) concentrations were very low and were not detected in the stem bark sample, while the leaves, fruits, and roots contained 0.053 ± 0.01 mg/100 g, 0.052 ± 0.01 mg/100 g, and 0.042 ± 0.02 mg/100 g, respectively. Similarly, cobalt (Co) was absent in the stem bark and leaf samples but detected in the fruit (0.102 ± 0.00 mg/100 g) and root (0.147 ± 0.00 mg/100 g). Nickel (Ni) was detected in the stem bark, fruit, and root samples, with the highest concentration recorded in the root (0.702 ± 0.10 mg/100 g), while it was not detected in the leaf sample. Arsenic (As), a potentially toxic metalloid, was absent in the stem bark and leaf samples but detected in the fruit (0.209 ± 0.00 mg/100 g) and root (0.765 ± 0.020 mg/100 g). The relatively higher arsenic concentration in the root may be attributed to direct absorption from the soil and limited translocation to aerial plant tissues. Selenium (Se), an important antioxidant trace element, was detected only in the stem bark (0.141 ± 0.02 mg/100 g) and root (0.468 ± 0.02 mg/100 g), while it was absent in the fruit and leaf samples.

Lead (Pb) and cadmium (Cd), which are among the most toxic heavy metals due to their cumulative toxicological effects, were detected at relatively low concentrations. Lead was detected only in the leaf sample (0.947 ± 0.01 mg/100 g), whereas cadmium was present in all plant parts within the range of 0.038 ± 0.00 mg/100 g to 0.057 ± 0.00 mg/100 g. The comparatively low cadmium levels suggest minimal contamination; however, the presence of lead in the leaves may indicate environmental exposure from anthropogenic activities such as vehicular emissions, agricultural inputs, or soil contamination.

Generally, the distribution pattern of heavy metals varied among the plant parts. The root sample exhibited relatively higher accumulation of potentially toxic elements such as arsenic, nickel, cobalt, and selenium, which may be associated with the root's direct contact with contaminated soil matrices. In contrast, the stem bark contained

comparatively higher concentrations of manganese, zinc, and copper, while the leaves accumulated more lead and vanadium. These variations may be influenced by differences in metal mobility, plant physiology, soil composition, and translocation efficiency within the plant system.

The heavy metal concentrations obtained in this study were generally within tolerable limits for medicinal plants reported by international regulatory bodies, although certain elements such as Pb and As warrant careful monitoring due to their toxicological implications at elevated concentrations. Previous studies on *A. carambola* and related medicinal plants have similarly reported the presence of trace heavy metals, particularly manganese, zinc, copper, chromium, and cobalt, in varying concentrations depending on geographical location and environmental conditions. The observed differences in heavy metal concentrations compared to previous reports may be attributed to differences in soil chemistry, climatic conditions, agricultural practices, and environmental pollution levels.

From a nutritional and pharmacological perspective, trace metals such as Zn, Cu, Mn, Cr, and Se are essential cofactors involved in antioxidant defense, immune modulation, enzyme activation, and metabolic regulation. However, excessive accumulation of toxic metals such as Pb, Cd, and As may pose health risks including neurotoxicity, nephrotoxicity, carcinogenicity, and cardiovascular disorders following prolonged exposure (Jaishankar *et al*., 2014; Jomova *et al*., 2025). Therefore, while the results support the medicinal and nutritional potential of *A. carambola*, continuous monitoring of heavy metal contamination is necessary to ensure its safety for therapeutic and dietary applications.

3.1.3 Proximate Analysis

The proximate composition of the stem bark, root, leaf, and fruit of *Averrhoa carambola* is presented in Table 2. The results revealed noticeable variations in the nutritional composition among the different plant parts, indicating differential accumulation of nutrients and bioactive constituents within the plant tissues. The evaluated parameters included ash content, crude fat, crude protein, moisture content, crude fibre, carbohydrate, and related nutritional indices.

Table 2. Nutritional Analysis of different parts of *A. carambola* (g/100 g)

Parameters	AVC-S	AVC-L	AVC-F	AVC-R	AA
Crude Protein	2.24±0.00	ND	ND	ND	-
Crude Fat	1.66±0.00	3.45±0.00	3.84±0.00	6.84±0.00	-
Crude ash	5.17±0.00	6.04±0.00	1.93±0.00	6.52±0.00	-
TPC	38.21 mg GAE/g	28.18 mg GAE/g	ND	ND	-
TFC	639.00 mg QE/mg	376.71 mg GAE/g	ND	ND	-
DPPH	20.25 µg/mL	28.89 µg/mL	ND	ND	28.57 µg/mL
FRAP	38.21 µg/mL	77.50 µg/mL	ND	ND	56.800 µg/mL

****Values are average of three replicates determinations. ND = Not determined.**

The ash content, which reflects the total mineral composition of the plant material, was highest in the root, followed by the leaf and stem bark, while the fruit recorded the lowest ash value (Table 2). Specifically, the root exhibited an ash content of approximately 6.52%, whereas the fruit contained about 1.93%. The comparatively higher ash content observed in the root and leaf suggests that these plant parts are richer in inorganic mineral

elements and may serve as important reservoirs of nutritionally valuable minerals (Iniaghe *et al*., 2009; Kathpalia and Bhatla, 2018). This observation is consistent with the mineral elemental analysis presented in Table 1, where elevated concentrations of calcium, potassium, magnesium, and phosphorus were detected in the root and leaf samples. Similar findings have been reported for medicinal plants where higher ash values are commonly associated with increased mineral bioavailability and nutritional importance (Monti *et al*., 2008).

The crude fat content followed the order: root > fruit > leaf > stem bark (Table 2). The root possessed the highest lipid content, indicating that it may contribute more significantly to caloric and energy value than the other plant parts. In contrast, the stem bark contained the lowest crude fat content, suggesting a lower lipid accumulation within the bark tissues. The relatively low-fat content observed across all plant parts is nutritionally advantageous because excessive dietary fat intake has been associated with obesity, cardiovascular disorders, and metabolic diseases (Antia *et al*., 2006). Therefore, the moderate lipid composition of *A. carambola* suggests that the plant could be suitable for dietary and therapeutic applications with minimal risk of excessive fat consumption.

An appreciable amount of crude protein was detected in the stem bark sample (Table 2), whereas the protein content in the other plant parts was either very low or below detectable limits under the analytical conditions employed. The relatively higher protein content of the stem bark may indicate the presence of nitrogen-containing metabolites, enzymes, structural proteins, or bioactive peptides. Protein-rich medicinal plant materials are often associated with improved nutritional quality and enhanced biological activity because proteins participate in tissue repair, enzyme synthesis, immune responses, and cellular metabolism (Eming *et al*., 2017).

The proximate composition obtained in this study compares favourably with previous investigations on *Averrhoa carambola* and related medicinal plants. Edem et al. reported comparable ash and crude fat values for unripe *A. carambola* fruit, although slight differences may arise from environmental conditions, soil composition, plant maturity, climatic variation, and extraction methods (Edem *et al*., 2008). Variations in proximate composition among plant parts may also be attributed to differences in physiological function and nutrient translocation within the plant system.

Overall, the results presented in Table 2 demonstrate that *A. carambola* possesses appreciable nutritional potential, particularly in terms of mineral content, moderate lipid composition, and beneficial dietary constituents. The higher ash content in the root and leaf supports their possible use as mineral-rich nutraceutical materials, while the moderate fat content suggests potential health benefits associated with dietary consumption and medicinal application.

3.1.4 Total Phenolic, Total Flavonoid Content and Antioxidant Activities

The total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities of the stem bark and leaf extracts of *Averrhoa carambola* are presented in Table 2. The results revealed that the stem bark extract contained higher concentrations of phenolic and flavonoid compounds than the leaf extract, indicating that the stem bark may represent a richer source of antioxidant phytochemicals.

The total phenolic content of the stem bark extract was 38.21 mg GAE/g, whereas the leaf extract contained 28.18 mg GAE/g (Table 2). Similarly, the flavonoid content was markedly higher in the stem bark extract (639.00 mg QE/mg) compared to the leaf extract (376.71 mg QE/mg). These results suggest that the stem bark accumulates greater quantities of secondary metabolites with reducing and free radical scavenging capacities. Phenolic compounds and flavonoids are widely

recognized as important phytochemicals that contribute significantly to the antioxidant properties of medicinal plants through their ability to donate hydrogen atoms or electrons to neutralize reactive oxygen species.

The antioxidant activities of the extracts were evaluated using DPPH radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays, and the results are presented in Table 2. The stem bark extract exhibited a DPPH IC_{50} value of 20.25 µg/mL, which was lower than those of the leaf extract (28.89 µg/mL) and the standard antioxidant, ascorbic acid (28.57 µg/mL). Since lower IC_{50} values indicate stronger antioxidant activity, the stem bark extract demonstrated superior free radical scavenging ability relative to both the leaf extract and ascorbic acid.Similarly, the FRAP assay showed that the stem bark extract possessed stronger ferric reducing power with an IC_{50} value of 38.21 µg/mL compared to the leaf extract (77.50 µg/mL) and ascorbic acid (56.80 µg/mL). The enhanced antioxidant activity of the stem bark correlates strongly with its higher phenolic and flavonoid contents, indicating that these phytochemicals are likely responsible for the observed antioxidant potential.The strong antioxidant activities observed in the stem bark extract may be attributed to the synergistic effects of polyphenolic compounds identified in the GC–MS analysis (Table 3 and Figure 1), including catechol, phenolic derivatives, benzenediol compounds, and unsaturated fatty acids. Phenolic compounds such as catechol and methoxyphenols are known to possess strong hydrogen-donating properties capable of neutralizing oxidative radicals and inhibiting oxidative stress.

Oxidative stress has been implicated in the pathogenesis of several chronic diseases, including cancer, cardiovascular diseases, diabetes mellitus, neurodegenerative disorders, and inflammatory conditions. Therefore, the potent antioxidant activity exhibited by the stem bark extract suggests that *A. carambola* may possess considerable therapeutic value as a natural antioxidant source. Previous studies have similarly reported that medicinal plants rich in phenolics and flavonoids exhibit strong antioxidant, anti-inflammatory, antimicrobial, and anticancer activities (Bellik *et al*., 2012; Sobhani *et al*., 2021; Rakha *et al*., 2022).

The findings of this study compare favourably with earlier reports on *A. carambola* and other medicinal plants. Variations in phenolic and flavonoid contents between studies may arise from differences in geographical origin, extraction solvent, environmental conditions, harvesting season, and analytical procedures. Nevertheless, the consistently high antioxidant performance observed in the stem bark supports its ethnomedicinal use and highlights its potential application in pharmaceutical, nutraceutical, and functional food formulations.

Generally, the results presented in Table 2 demonstrate a strong positive relationship between phenolic/flavonoid composition and antioxidant activity in *A. carambola*. The stem bark extract, in particular, exhibited superior antioxidant properties, indicating its potential usefulness as a natural source of antioxidant compounds capable of combating oxidative stress–related diseases.

3.1.5 GC–MS Spectroscopy Analysis

The GC–MS chromatographic profile of the ethanol crude extract of *Averrhoa carambola* stem bark is presented in Figure 1, while the identified phytochemical constituents, retention times, peak areas, and molecular weights are summarized in Table 3. The GC–MS analysis revealed the presence of numerous bioactive organic compounds, indicating the chemically diverse nature of the stem bark extract.

A total of thirty-one phytochemical compounds were identified from the chromatogram (Figure 1 and Table 3). The major constituents detected based on peak area percentages included stigmasta-3,5-diene (26.01%), catechol

(11.49%), 9-octadecenoic acid, (E)- (9.23%), n-hexadecanoic acid (8.88%), naphthalene, 1,1'-(1,10-decanediyl)bis- (5.35%), 9,12-octadecadienoic acid (Z,Z)- (4.65%), 1,3-benzenediol, 4-propyl- (4.11%), octadecanoic acid (2.49%), 2-ethenylphenol (2.34%), toloxatone (1.94%), and several oxadiazole derivatives (Table 3

Table 3. GC-MS analysis of stem bark ethanol extract of *A. carambola*

Peak	RT (min)	Compound	Peak Area (%)	Mol Weight ($mgmol^{-1}$)
1	2.465	Phenol, 2-methoxy-	1.23	124
2	3.226	2-Aminomethyl-5-methylamino-1,3,4-oxadiazole	1.19	128
3	3.495	Catechol	11.49	110
4	3.615	2-Ethenylphenol	2.34	120
5	4.353	2-Methoxy-4-vinylphenol	0.95	150
6	4.628	Phenol, 2,6-dimethoxy-	1.04	154
7	5.389	trans-Cinnamic acid	0.83	148
8	5.858	Cyclododecane	0.72	168
9	6.362	Cycloheptasiloxane, tetradecamethyl-	1.84	518
10	6.425	Aniline, N-tert-butyldimethylsilyl-	1.11	207
11	6.510	Cyclopropane, 1,1-dimethyl-2-(1-methylethoxy)-3-(3-methyl-1-pentynyl)-	1.03	208
12	6.739	1,3-Benzenediol, 4-propyl-	4.11	152
13	6.974	1,3,5-Benzenetriol	0.99	126
14	7.437	Pentane, 1,1,2,3,4,5-hexachloro-1,2,3,4,5,5-hexafluoro-	1.57	383
15	7.683	N-(Trifluoroacetyl)-N,O,O',O''-tetrakis(trimethylsilyl)norepinephrine	1.67	553
16	8.135	4H-1-Benzothiopyran-4-one, 2,3-dihydro-	1.12	164
17	8.828	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	0.75	444
18	9.4111	n-Hexadecanoic acid	8.88	256
19	9.612	1H-Pyrazole, 4,5-dihydro-3-phenyl-	0.88	146
20	9.846	Hexasiloxane, tetradecamethyl-	0.55	458
21	10.418	9,12-Octadecadienoic acid (Z,Z)-	4.65	280
22	10.476	9-Octadecenoic acid, (E)-	9.23	282
23	10.636	Octadecanoic acid	2.49	284
24	14.664	2-Ethylacridine	1.19	207
25	14.807	Carbonic acid, monoamide, N-(2-ethylphenyl)-, propyl ester	0.91	207
26	14.956	Toloxatone	1.94	207
27	15.076	1,2,5-Oxadiazol-3-amine, 4-(4-methoxyphenoxy)-	1.85	207

28	15.122	Naphthalene, 1,1'-(1,10-decanediyl)bis-	5.35	394
29	15.219	Stigmasta-3,5-diene	17.76	396
31	16.255	Stigmasta-3,5-diene	8.25	396
32	17.027	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)-	1.57	207

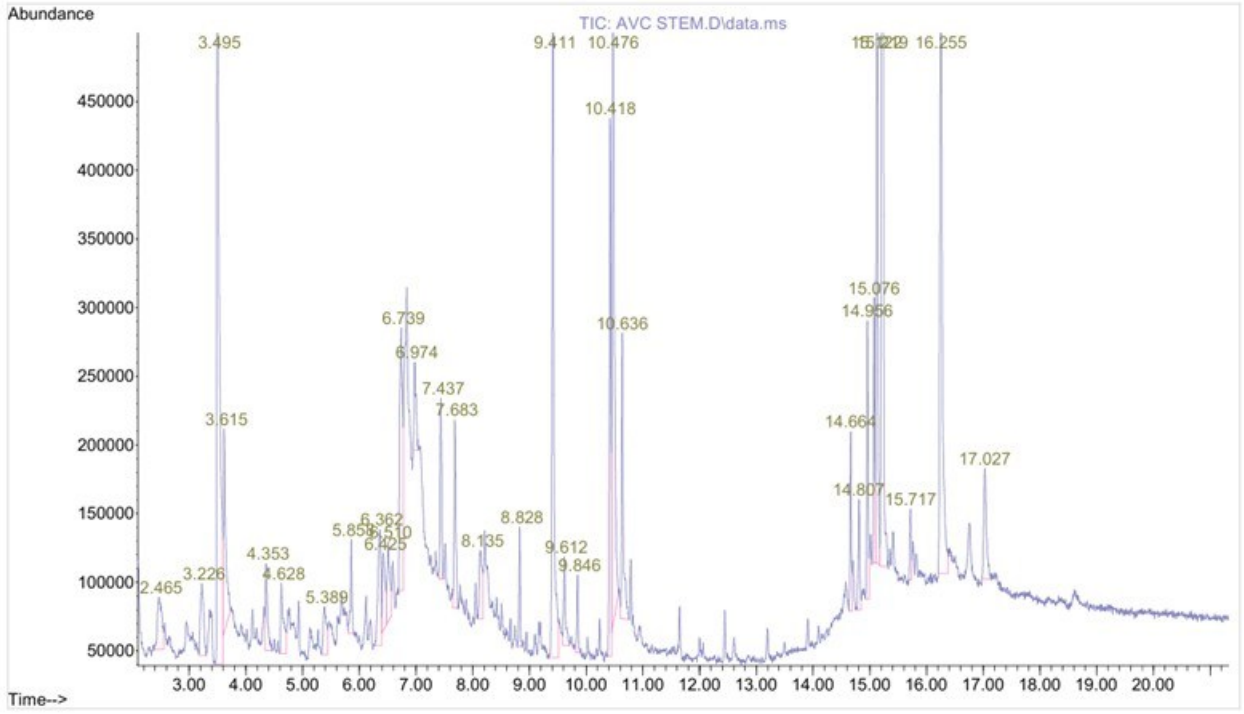

Fig. 1. GC-MS Chromatograph of stem bark ethanol extract of *A. carambola*

Among the identified compounds, stigmasta-3,5-diene was the most abundant constituent, accounting for approximately 26% of the total detected compounds. Steroidal compounds such as stigmasta derivatives are known to possess anti-inflammatory, antimicrobial, antioxidant, and membrane-stabilizing properties (Jeong *et al*., 2009; Krishnaveni *et al*., 2014). Their abundance in the stem bark extract may therefore contribute significantly to the biological activities observed in this study.

Catechol, which represented 11.49% of the total composition, is a well-known phenolic compound with strong antioxidant and antimicrobial properties. The presence of catechol supports the strong antioxidant activities observed in the DPPH and FRAP assays (Table 2). Catechol and related phenolic compounds have been reported to inhibit microbial growth through membrane disruption, enzyme inhibition, and oxidative stress induction within microbial cells (Abubakar and Majinda 2016; Ousman *et al*., 2025).

Fatty acid derivatives identified in the extract, including n-hexadecanoic acid, 9-octadecenoic acid, octadecanoic acid, and 9,12-octadecadienoic acid (Z,Z)-, are compounds frequently associated with antibacterial, anti-inflammatory, antioxidant, and hypo-cholesterolemic activities (Jeong *et al*., 2009; Krishnaveni *et al*., 2014). The relatively high abundance of these fatty acids suggests that they may contribute synergistically to the antibacterial activity exhibited by the extract

against Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa, and Bacillus spp. as presented in Tables 5–7.

The identification of aromatic phenolic compounds such as 2-ethenylphenol, phenol derivatives, and benzenediol compounds further confirms the phytochemical richness of the stem bark extract. These compounds are known for their reducing abilities and antimicrobial activities. Similarly, the oxadiazole derivatives detected in the extract are pharmacologically important heterocyclic compounds previously reported to possess antibacterial, antifungal, anti-inflammatory, and antioxidant properties (Mai *et al*., 2003; Alfalahi *et al*., 2024; Ferrazzano *et al*., 2016 & Olu *et al*., 2022).

The chromatogram shown in Figure 1 demonstrates well-resolved peaks, indicating successful separation and detection of phytochemical constituents within the extract. The presence of multiple peaks with varying retention times reflects the complexity of the stem bark phytochemical composition and suggests the coexistence of compounds with different polarities, volatilities, and molecular structures.

The GC–MS profile obtained in this study compares favourably with previous reports on medicinal plants rich in phenolic compounds, fatty acids, and sterol derivatives. Several of the identified compounds, including catechol, n-hexadecanoic acid, and octadecadienoic acid derivatives, have previously been associated with potent antimicrobial and antioxidant activities in medicinal plant extracts.

The antibacterial activities observed against the tested clinical isolates (Tables 5–7) may therefore be attributed to the synergistic interaction of these phytochemical constituents. The strong activity exhibited by the butanol fraction and crude extract suggests that semi-polar bioactive compounds present in the stem bark are largely responsible for the antimicrobial effects observed in this study.

Overall, the GC–MS results presented in Table 3 and Figure 1 provide important chemical evidence supporting the antioxidant and antibacterial activities of *A. carambola* stem bark extract. The presence of numerous biologically active compounds highlights the pharmaceutical and therapeutic potential of the plant and justifies its traditional medicinal applications.

3.1.6 Biochemical Test

The cultural, morphological, and biochemical characteristics of the bacterial isolates used in this study are presented in Table 4. The results obtained from Gram staining, sugar fermentation profiles, enzymatic reactions, motility, spore formation, and other biochemical assays enabled the identification and characterization of the bacterial isolates as Escherichia coli (EC-1 and EC-2), Klebsiella sp., Bacillus sp., and Pseudomonas aeruginosa (PA-1 and PA-2).

The Gram staining reaction showed that five of the isolates were Gram-negative rods, while one isolate (Bacillus sp.) was Gram-positive (Table 4). The Gram-negative isolates included *Escherichia coli*, *Klebsiella sp*., and *Pseudomonas aeruginosa*, which is consistent with the known taxonomic classification of these organisms. The Gram-positive nature of *Bacillus sp*. further corroborated its identification because members of the genus Bacillus are characteristically Gram-positive, rod-shaped, and spore-forming bacteria.

The isolates identified as Escherichia coli (EC-1 and EC-2) demonstrated positive methyl-red and indole reactions but negative Voges–Proskauer and oxidase tests (Table 4). These biochemical characteristics are typical diagnostic features of E. coli and confirm its enteric bacterial nature. The isolates also fermented glucose, sucrose, maltose, and lactose with acid and gas production, which further supports their identification as Escherichia coli. Similar biochemical profiles for E. coli have been widely reported in

microbiological studies involving clinical and environmental isolates.

The Klebsiella sp. isolate exhibited positive Voges–Proskauer, citrate utilization, catalase, and urease reactions but was non-motile (Table 4). The organism also fermented glucose, sucrose, maltose, and lactose with acid and gas production. These characteristics are consistent with previously established biochemical properties of *Klebsiella species*. The absence of motility is particularly important because Klebsiella spp. are generally non-motile enteric bacteria.

The *Bacillus sp*. isolate showed positive catalase, oxidase, citrate utilization, methyl-red, motility, and spore formation tests (Table 4). The presence of endospore formation is a distinguishing characteristic of *Bacillus species* and serves as a critical taxonomic marker for the genus. The Gram-positive reaction and rod-shaped morphology observed in this study further confirm the identity of the isolate.

Table 4. **Cultural, Morphological and Biochemical Test Results for the Isolates**

Parameter	AB	JA	WS-6	SS-45	3091	3062
Gram Stain	–	–	–	+	–	–
Cell Morphology	Rod	Rod	Short rod	Rod	Rod	Rod
Voges–Proskauer	–	–	+	–	–	–
Methyl-red	+	+	–	+	–	–
Indole reaction	+	+	–	–	–	–
Citrate Utilization	+	+	+	+	+	+
Catalase Test	+	–	+	+	+	+
Oxidase Test	–	–	–	+	+	+
Haemolysin	β	β	β	γ	β	β
Urease Reaction	–	–	+	–	+	–
Motility Test	+	+	–	+	+	–
Spore formation	–	–	–	+	–	–
GLU	AG	AG	AG	AG	AG	AG
MAN	–	–	–	–	–	–
SUC	AG	AG	AG	AG	A	A
MAL	AG	AG	AG	A	A	A
LAC	AG	AG	AG	A	AG	AG
Probable Organism (Organism code)	*Escherichia coli* (EC-1)	*Escherichia coli* (EC-2)	*Klebsiella* sp.	*Bacillus* sp.	*Pseudomonas aeruginosa* (PA-1)	*Pseudomonas aeruginosa* (PA-2)

Key: GLU = Glucose; MAN = Mannitol; SUC = Sucrose; MAL = Maltose; LAC = Lactose; AG = Acid and Gas; A = Acid; + =

The two *Pseudomonas aeruginosa* isolates (PA-1 and PA-2) exhibited positive oxidase and citrate utilization reactions, which are characteristic biochemical markers of *Pseudomonas species* (Table 4). The isolates were Gram-negative rods and demonstrated

aerobic metabolic characteristics consistent with the genus. The oxidase-positive nature of the isolates is particularly important because it differentiates *Pseudomonas aeruginosa* from many Enterobacteriaceae members, including *Escherichia coli* and *Klebsiella spp*., which are oxidase-negative.

The biochemical characterization performed in this study is consistent with standard microbiological identification procedures. The identified organisms are known opportunistic and pathogenic bacteria frequently associated with urinary tract infections, gastrointestinal infections, respiratory tract infections, wound infections, and nosocomial diseases. Their use in the present antibacterial investigation is therefore appropriate for evaluating the therapeutic potential of *Averrhoa carambola* stem bark extracts against clinically relevant pathogens.

Table 5. Antibacterial activity of Averrhoa carambola extract and activity indices with conventional antibiotic (imipenem)

Organism	Extract / Parameter	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	IMI (10 µg)
Escherichia coli (EC-1)	BF (M.Z ± S.D / A.I)	53 ± 1.5 / 1.96	37 ± 1.2 / 1.37	22 ± 1.0 / 0.82	14 ± 0.8 / 0.52	27 ± 1.0
	CE (M.Z ± S.D / A.I)	47 ± 1.3 / 1.68	31 ± 1.1 / 1.11	20 ± 0.9 / 0.71	15 ± 0.7 / 0.54	28 ± 1.1
	AF (M.Z ± S.D / A.I)	25 ± 1.1 / 1.00	20 ± 0.9 / 0.80	15 ± 0.8 / 0.60	8 ± 0.6 / 0.32	25 ± 1.0
Escherichia coli (EC-2)	BF (M.Z ± S.D / A.I)	44 ± 1.4 / 1.00	35 ± 1.1 / 0.80	25 ± 1.0 / 0.57	17 ± 0.8 / 0.39	44 ± 1.3
	CE (M.Z ± S.D / A.I)	42 ± 1.3 / 0.98	35 ± 1.0 / 0.81	22 ± 0.9 / 0.51	16 ± 0.7 / 0.37	43 ± 1.2
	AF (M.Z ± S.D / A.I)	30 ± 1.1 / 0.67	21 ± 0.9 / 0.47	15 ± 0.8 / 0.33	9 ± 0.5 / 0.20	45 ± 1.4
Klebsiella sp.	BF (M.Z ± S.D / A.I)	55 ± 1.6 / 1.83	39 ± 1.2 / 1.30	25 ± 1.0 / 0.83	17 ± 0.8 / 0.57	30 ± 1.2
	CE (M.Z ± S.D / A.I)	59 ± 1.7 / 1.90	42 ± 1.3 / 1.36	25 ± 1.0 / 0.81	19 ± 0.9 / 0.61	31 ± 1.3
	AF (M.Z ± S.D / A.I)	20 ± 0.9 / 0.57	15 ± 0.8 / 0.43	9 ± 0.6 / 0.26	3 ± 0.4 / 0.09	35 ± 1.1
Bacillus sp.	BF (M.Z ± S.D / A.I)	45 ± 1.3 / 1.13	30 ± 1.0 / 0.75	21 ± 0.8 / 0.53	15 ± 0.7 / 0.38	40 ± 1.2
	CE (M.Z ± S.D / A.I)	43 ± 1.2 / 0.98	33 ± 1.0 / 0.75	21 ± 0.8 / 0.48	14 ± 0.7 / 0.32	44 ± 1.3
	AF (M.Z ± S.D / A.I)	30 ± 1.1 / 0.65	22 ± 0.9 / 0.48	17 ± 0.8 / 0.37	11 ± 0.6 / 0.24	46 ± 1.3
Pseudomonas aeruginosa (PA-1)	BF (M.Z ± S.D / A.I)	44 ± 1.3 / 1.83	30 ± 1.0 / 1.25	22 ± 0.9 / 0.92	13 ± 0.7 / 0.54	24 ± 1.0

	CE (M.Z ± S.D / A.I)	47 ± 1.4 / 1.57	32 ± 1.1 / 1.07	25 ± 0.9 / 0.83	17 ± 0.8 / 0.57	30 ± 1.2
	AF (M.Z ± S.D / A.I)	25 ± 1.0 / 0.64	14 ± 0.7 / 0.36	9 ± 0.5 / 0.23	5 ± 0.4 / 0.13	39 ± 1.4
Pseudomonas aeruginosa (PA-2)	BF (M.Z ± S.D / A.I)	47 ± 1.4 / 1.74	36 ± 1.1 / 1.33	27 ± 0.9 / 1.00	19 ± 0.8 / 0.70	27 ± 1.1
	CE (M.Z ± S.D / A.I)	45 ± 1.3 / 1.80	32 ± 1.0 / 1.28	27 ± 0.9 / 1.08	17 ± 0.8 / 0.68	25 ± 1.1
	AF (M.Z ± S.D / A.I)	20 ± 0.9 / 1.00	12 ± 0.6 / 0.60	9 ± 0.5 / 0.45	5 ± 0.4 / 0.25	20 ± 1.0

Key: BF = Butanol Fraction; CE = Crude Extract; AF= Aqueous Fraction; MZ = Mean Zone; SD = Standard Deviation; A.I = Activity Index; IMI = Imipenem (10µg)

Table 6. Minimum Inhibitory Concentration for *A. carambola* extracts

Organism	Plant extract	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	MIC
Escherichia coli (EC-1)	BF	-	-	-	+	25
	CE	-	-	-	+	25
	AF	-	-	+	+	50
Escherichia coli (EC-2)	BF	-	-	-	+	25
	CE	-	-	-	+	25
	AF	-	-	+	+	50
Klebsiella sp.	BF	-	-	-	+	25
	CE	-	-	-	+	25
	AF	-	-	+	+	50
Bacillus sp.	BF	-	-	-	+	25
	CE	-	-	-	+	25
	AF	-	-	-	+	25
Pseudomonas aeruginosa (PA-1)	BF	-	-	-	+	25
	CE	-	-	-	+	25
	AF	-	-	+	+	50
Pseudomonas aeruginosa (PA-2)	BF	-	-	-	+	25
	CE	-	-	-	+	25
	AF	-	-	+	+	50

Key: - = No growth; + = Growth; BF = Butanol Fraction; CE = Crude Extract; AF= Aqueous Fraction

The results presented in Table 4 confirmed the successful isolation and identification of medically important bacterial species suitable for antimicrobial susceptibility studies. The biochemical profiles obtained further validate the reliability of the microbial characterization procedures employed in this study.

3.1.7 Antibacterial Activities of the Crude Extract (CE), Butanol Fraction (BF), and Aqueous Fraction (AF) of Averrhoa carambola

The antibacterial activities of the crude extract (CE), butanol fraction (BF), and aqueous fraction (AF) of *Averrhoa carambola* stem

bark against *Escherichia coli* (EC-1 and EC-2), *Klebsiella sp*., *Bacillus sp*., and *Pseudomonas aeruginosa* (PA-1 and PA-2) are presented in Table 5. The antibacterial activity was evaluated using the agar well diffusion method and expressed as Mean Zone of Inhibition (M.Z ± SD, mm) together with the Activity Index (A.I) relative to the standard antibiotic imipenem (IMI, 10 µg).

The results revealed that all extracts exhibited antibacterial activity against the tested bacterial isolates, although the degree of inhibition varied depending on the extract type, bacterial species, and concentration employed (Table 5). In general, the antibacterial activity increased with increasing extract concentration, demonstrating a concentration-dependent inhibitory effect. Higher inhibition zones were consistently observed at 100 mg/mL compared to 50, 25, and 12.5 mg/mL, indicating that the antibacterial potency of the extracts is directly related to the concentration of bioactive constituents present in the samples.

Table 7. Minimum Bacteriocidal Concentration of *A. carambola* stem bark extracts

Organism	Plant extract	100 (mg/m)	50 (mg/mL)	25 (mg/mL)	MBC
Escherichia coli (EC-1)	BF	-	-	+	50
	CE	-	-	+	50
	AF	-	+	0	100
Escherichia coli (EC-2)	BF	-	-	+	50
	CE	-	-	+	50
	AF	-	+	0	100
Klebsiella sp.	BF	-	-	50	50
	CE	-	-	50	50
	AF	-	+	0	100
Bacillus sp.	BF	-	-	+	50
	CE	-	-	+	50
	AF	-	-	+	50
Pseudomonas aeruginosa (PA-1)	BF	-	-	+	50
	CE	-	-	+	50
	AF	-	+	0	100
Pseudomonas aeruginosa (PA-2)	BF	-	-	+	50
	CE	-	-	+	50
	AF	-	+	0	100

Key: - = No growth; + = Growth; 0 = Not plated; BF = Butanol Fraction; CE = Crude Extract; AF= Aqueous Fraction

Among the tested fractions, the butanol fraction (BF) exhibited the strongest antibacterial activity against most of the bacterial isolates. At 100 mg/mL, the BF produced inhibition zones of 53 ± 1.5 mm against *Escherichia coli* (EC-1), 55 ± 1.6 mm against *Klebsiella sp*., 45 ± 1.3 mm against *Bacillus sp*., 44 ± 1.3 mm against *Pseudomonas aeruginosa* (PA-1), and 47 ± 1.4 mm against *Pseudomonas aeruginosa* (PA-2) (Table 5). These values were considerably higher than those produced by the aqueous fraction and, in several cases, exceeded the inhibitory activity of the standard antibiotic imipenem.

The crude extract (CE) also demonstrated pronounced antibacterial activity against all tested organisms. Interestingly, the crude

extract exhibited the highest inhibition zone of 59 ± 1.7 mm against *Klebsiella sp*. at 100 mg/mL, surpassing both the butanol fraction and the standard antibiotic (Table 5). This observation suggests possible synergistic interactions among phytochemical constituents present in the crude extract before fractionation. The synergistic activity of multiple phytochemicals within crude plant extracts has been widely reported in medicinal plant research and is often associated with enhanced antimicrobial efficacy (Amenu, 2014).

In contrast, the aqueous fraction (AF) consistently showed lower antibacterial activity compared to the butanol fraction and crude extract. The inhibition zones produced by the AF ranged from 3 ± 0.4 mm to 30 ± 1.1 mm depending on the bacterial isolate and concentration (Table 5). The comparatively lower activity of the aqueous fraction may be attributed to the poor solubility of many antimicrobial phytochemicals in water. Most phenolic compounds, flavonoids, fatty acid derivatives, and sterol compounds identified in the GC–MS analysis (Table 3 and Figure 1) are more readily extracted into semi-polar organic solvents such as butanol and ethanol than into aqueous media.

Among the tested organisms, *Klebsiella sp*. exhibited the highest susceptibility to the plant extracts, followed closely by *Escherichia coli* isolates. Conversely, *Pseudomonas aeruginosa* isolates showed relatively lower susceptibility, particularly toward the aqueous fraction. This reduced sensitivity may be associated with the intrinsic resistance mechanisms of *Pseudomonas aeruginosa*, including low outer membrane permeability, biofilm formation, multidrug efflux pumps, and enzymatic detoxification systems (Hancock & Speert, 2000). Nevertheless, both the butanol fraction and crude extract demonstrated appreciable inhibitory effects against *Pseudomonas aeruginosa*, suggesting the presence of potent antibacterial constituents capable of overcoming some of these resistance mechanisms.

The Activity Index (A.I) values presented in Table 5 further demonstrate the strong antibacterial efficacy of the extracts. Several A.I values exceeded 1.00, particularly for the butanol fraction and crude extract at higher concentrations. An Activity Index greater than 1 indicates that the plant extract exhibited stronger antibacterial activity than the standard antibiotic imipenem. For instance, the butanol fraction showed an A.I value of 1.96 against *Escherichia coli* (EC-1), while the crude extract exhibited an A.I value of 1.90 against *Klebsiella sp*. These findings strongly suggest that the stem bark extract of *A. carambola* possesses significant antibacterial potential.

The antibacterial activity observed in this study may be attributed to the phytochemical constituents identified in the GC–MS analysis (Table 3 and Figure 1), including catechol, stigmasta-3,5-diene, n-hexadecanoic acid, 9-octadecenoic acid, phenolic compounds, and oxadiazole derivatives. Phenolic compounds are known to disrupt bacterial cell walls and membranes, interfere with enzyme activity, and induce oxidative stress within microbial cells (Othman *et al*., 2019; Takó *et al*., 2020). Fatty acid derivatives may also contribute to membrane destabilization and inhibition of microbial metabolic processes (Krishnaveni *et al*., 2014).

3.1.8 Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) values of the crude extract, butanol fraction, and aqueous fraction of *Averrhoa carambola* stem bark against the tested bacterial isolates are presented in Table 6. The MIC represents the lowest concentration of the extract capable of inhibiting visible bacterial growth and serves as an important indicator of antimicrobial potency.

The results showed that the butanol fraction (BF) and crude extract (CE) exhibited strong inhibitory activity against all tested bacterial

isolates, with MIC values of 25 mg/mL (Table 6). This indicates that relatively low concentrations of these extracts were sufficient to suppress bacterial growth. The strong inhibitory activity observed for the BF and CE supports the antibacterial results presented in Table 5 and further confirms the presence of potent antimicrobial constituents within the semi-polar and crude fractions of the plant extract.

In contrast, the aqueous fraction (AF) demonstrated comparatively weaker inhibitory activity, with MIC values ranging from 25 to 50 mg/mL depending on the bacterial isolate (Table 6). The AF exhibited an MIC value of 50 mg/mL against *Escherichia coli* (EC-1 and EC-2), *Klebsiella sp*., and *Pseudomonas aeruginosa* isolates, whereas a lower MIC value of 25 mg/mL was observed against *Bacillus sp*. This suggests that Gram-positive *Bacillus sp*. was more susceptible to the aqueous extract than the Gram-negative organisms.

The lower MIC values observed for the butanol fraction and crude extract indicate greater antimicrobial potency because lower concentrations were required to inhibit bacterial growth. These findings are consistent with previous reports that alcohol-soluble and semi-polar plant fractions often exhibit stronger antimicrobial activity than aqueous extracts due to improved extraction of phenolics, flavonoids, terpenoids, and fatty acid derivatives.

3.1.9 Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) values of the *Averrhoa carambola* stem bark extracts against the tested bacterial isolates are presented in Table 7. The MBC represents the lowest concentration of the extract capable of completely killing the bacterial cells.

The results showed that both the butanol fraction (BF) and crude extract (CE) exhibited bactericidal activity at 50 mg/mL against all tested bacterial isolates (Table 7). This demonstrates the strong killing potential of the extracts and suggests that the antibacterial activity is not merely inhibitory but bactericidal in nature at moderate concentrations.

The aqueous fraction (AF), however, exhibited weaker bactericidal activity. The AF showed MBC values of 100 mg/mL against *Escherichia coli*, *Klebsiella sp*., and *Pseudomonas aeruginosa*, while a lower MBC value of 50 mg/mL was recorded against *Bacillus sp*. (Table 7). These findings further confirm the comparatively lower antibacterial potency of the aqueous fraction and support the MIC observations presented in Table 6.

The relationship between MIC and MBC values observed in this study indicates that the butanol fraction and crude extract possess strong bactericidal properties, particularly against clinically important Gram-negative pathogens. The potent activity observed may be associated with the synergistic effects of the bioactive compounds identified in the GC–MS analysis, including phenolics, fatty acid derivatives, sterols, and heterocyclic compounds.

The results presented in Tables 5–7 demonstrate that the stem bark extract of *Averrhoa carambola*, particularly the butanol fraction and crude ethanol extract, possesses significant antibacterial activity against important pathogenic bacteria. The strong inhibitory and bactericidal effects observed in this study support the traditional medicinal use of *A. carambola* and highlight its potential as a natural source of antimicrobial agents for pharmaceutical and therapeutic applications..

4.0 Conclusion

Averrhoa carambola plant, as reported in this study, contains appreciable amounts of nutrient elements, phenolic compounds and flavonoids which make it a useful antioxidant agent. Reasonable amounts of mineral elements such as Ca, K, Mg, and Fe were detected in all parts of the plants, making it a potential source of

essential nutrients. However, concentrations of trace metals like Cr, Co, Pb, and Cd were high and, in some cases, exceeded than admissible limits, thereby challenging its suitability for consumption. The results of the antibacterial assay suggest that the stem bark extract contains therapeutically relevant molecules which could be further harnessed for pharmaceutical applications.

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5.0 References

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Declaration

Consent for publication

Not applicable

Availability of data

Data shall be made available on demand.

Competing interests

The authors declared no conflict-of-interest

Ethical Consideration

Not applicable

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Authors' contributions

Godwin Ndarake Enin conceived and supervised the study, interpreted data, and drafted the initial manuscript. Anthony Ayodeji Adegoke designed the microbiological study and interpreted data. Kate Onyeje Igoche, and Kooffreh Kooffreh supervised the microbiological and antibacterial analyses. Abraham Uduak Ekpe, Ndifreke Ime Asuquo, and Ubong Okon Jeremiah performed the eperiments. All authors reviewed and approved the final manuscript.