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Original Research Article

Phytochemical Profiling and *In-vitro* Antibacterial Activity of Methanol, Acetone and n-hexane Extracts of *Artocarpus heterophyllus* Fruit Peel Against Multidrug Resistant Gram -Negative Bacteria.

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ABSTRACT

Multidrug-resistant (MDR) Gram-negative bacteria pose a persistent threat to global healthcare, necessitating continuous exploration of alternative antimicrobial agents. This study profiled the phytochemical constituents and evaluated the antibacterial activity of methanol, acetone and n-hexane extracts of *Artocarpus heterophyllus* fruit peel (MAH, AAH and HAH, respectively) against MDR Gram-negative clinical isolates. Cold maceration gave yields of 4.5%, 3.4% and 3.3% for MAH, AAH and HAH, respectively. Qualitative screening (Harborne's method) detected phenols, tannins, flavonoids, terpenoids, saponins, cardiac glycosides and coumarins in all extracts; alkaloids were present only in HAH and AAH, while steroids and anthraquinones were absent. In agar well diffusion assays, MAH was the most active extract, producing concentration-dependent inhibition zones (11.5–22.0 mm) against *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter hormaechei*; at 500 mg/mL its zones against *E. coli* (20.5 ± 2.12 mm) and *A. baumannii* (22.0 ± 1.41 mm) were statistically comparable to norfloxacin ($p > 0.05$). *Pseudomonas aeruginosa* was inhibited only weakly (6.0 ± 1.41 mm; no MIC within 50 mg/mL). The MICs of MAH were 6.25 mg/mL for *E. coli*, *A. baumannii* and *E. hormaechei*, and 12.5 mg/mL for *K. pneumoniae*. HAH inhibited only *K. pneumoniae* (12.0–14.0 mm); AAH inhibited *K. pneumoniae* only at 500 mg/mL (6.0 mm). *A. heterophyllus* fruit peel, particularly the methanolic extract, is a promising source of bioactive agents against clinically relevant MDR Gram-negative pathogens. Further studies are warranted to isolate the major active constituents and elucidate their mechanisms of action.

Keywords: Antibacterial activity, *Artocarpus heterophyllus*, Bioactive compounds, Fruit peels, Multidrug -resistance

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Introduction

The overreliance on and misuse of conventional antibiotics for treating infectious diseases has, in recent years, driven the emergence of multidrug-resistant (MDR) bacteria, now recognised as one of the most serious threats to global healthcare. MDR organisms are broadly defined as those non-susceptible to at least one agent in three or more antimicrobial categories.[1] Left unchecked, this resistance crisis threatens to return modern medicine to a pre-antibiotic era, in which routine infections and procedures once again carry life-threatening risk.

Medicinal plants remain a valuable source of antimicrobial agents, and their extracts continue to underpin many potent therapeutic drugs used worldwide.[2] *Artocarpus heterophyllus* Lam. (jackfruit; family Moraceae) originates from the Western Ghats of India and is now cultivated widely across the tropics.[3]

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Its rich phytochemical composition and potential for food-product development have drawn growing pharmacological interest.[4], yet 70-80% of the jackfruit biomass, principally the peel is discarded as waste and remains largely underutilised globally.[5, 6] Such peel fractions are known to be rich in phenolics, flavonoids, and other bioactive metabolites, with demonstrated antioxidant, anti-inflammatory, antidiabetic, and antimicrobial properties.[6, 7]

Valorising fruit peel waste as a source of bioactive compounds has gained considerable momentum in the natural product sciences, since such compounds have been shown to possess hypoglycaemic, antimicrobial, and anti-inflammatory properties.[8] For example, polyphenolic extracts from apple peel are active against a range of Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [8, 9] Earlier studies of *A. heterophyllus* peel identified phenols, tannins, flavonoids, terpenoids, alkaloids, and glycosides as its predominant secondary metabolite classes.[10] yet a comprehensive chromatographic characterisation of peel extracts in relation to MDR bacterial activity has, until now, remained limited.

Among MDR Gram-negative bacteria, members of the ESKAPEE group are particularly problematic nosocomial pathogens, combining broad-spectrum resistance with diverse virulence mechanisms, which underscores the pressing need for plant-derived agents with demonstrable activity against them. The present study, therefore aimed to characterise the bioactive compounds present in methanol, acetone, and n-hexane extracts of *A. heterophyllus* fruit peel using HPLC and GC-MS, and to evaluate the in vitro antibacterial activity of these

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crude extracts against MDR Gram-negative bacteria isolated from immunocompromised patients.

Materials and Methods

Test organisms

The test bacteria specie used in the study were pure culture of multidrug resistant Gram- negative bacteria isolated from immunocompromised patients and were obtained from the Microbiology Laboratory of the Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. Isolates were sub-cultured on nutrient agar to confirm purity. Multidrug resistance was confirmed by subjecting the bacteria specie to an antibiotic susceptibility test using the modified Kirby Bauer disc diffusion method in accordance with the Clinical and Laboratory Standard Institute (CLSI).[10], using the following antibiotics (MEM 10µg= Meropenem, ETP (10 µg) = Ertapenem, CAZ (10µg) = Ceftazidime, CRO (30 µg) = Ceftriaxone, CTX (10 µg) =. Cefotaxime, GM (30 µg) = Gentamicin, CIP (5 µg) = Ciprofloxacin, NOR (10 µg) = Norfloxacin). 0.1ml of the 24- hour bacterial culture was inoculated on Mueller Hinton agar and a sterile swab was used to spread the inoculum across the entire surface of the plate using spread plating. The inoculum was allowed to diffuse into the agar for 10-20 minutes after which the antibiotic discs were placed with equidistant to each other with the aid of a sterile forceps. The plates were incubated at 37^{o}C for 24 hours. After the incubation period, the diameter of the clear zones around each disc were measured in millimetre. The diameter of each zone was interpreted as sensitive, intermediate and resistant using the guidelines of CLSI.[11] MDR bacteria were categorized as those that showed resistance to at least one agent in three or more antimicrobial categories.[1]

Collection, Authentication and Preparation of Plant Material

Fresh ripe jackfruit (*Artocarpus heterophyllus* Lam.; family Moraceae) was obtained in December, 2023 from a private farm in Enugu State, Nigeria (coordinates: 6.2677°N, 7.3550°E; WGS84). The plant was authenticated by a qualified plant taxonomist at the Herbarium, Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Oyo State, Nigeria, and a voucher specimen was deposited under accession number UIH-23480.

The fruit was rinsed thoroughly with running tap water, followed by distilled water. The peel was manually separated from the pulp and core using a clean stainless-steel knife, cut into small pieces, and shade-dried at ambient temperature (25–30°C) with adequate ventilation for four weeks until a constant weight was attained, thereby minimising photochemical and thermal degradation of heat-sensitive secondary metabolites. The dried peel was ground to a coarse powder using a porcelain mortar and pestle, then further reduced to a uniform fine powder using an electric laboratory blender.

Extraction of Plant Material

Cold maceration extraction was performed by soaking 200 g of the fine powder in 1,000 mL of analytical-grade methanol, acetone and n-hexane (BDH Chemicals, Poole, UK) in a sealed amber glass vessel at room temperature (25°C) for 72 hours with intermittent manual agitation every 12 hours. The macerates were filtered sequentially through muslin cloth and Whatman No. 1 filter paper (Whatman, Maidstone, UK). The filtrates were concentrated to a semi-solid residue under reduced pressure at 30°C using a rotary evaporator (Büchi R-215; Büchi Labortechnik AG, Flawil, Switzerland), and residual solvents were completely removed in a water bath at 60°C. The crude extracts, designated MAH, AAH and HAH (methanol, acetone and N-hexane extract of *A. heterophyllus* respectively), were weighed, transferred to a sealed amber glass vial, and stored at −25°C until use. The percentage yield was calculated.

$$PY = \frac{WE}{WC} \times 100\%$$

PY= Percentage yield, WC= Weight of dried plant, WE= Weight of crude extracts

Phytochemical screening of methanol, acetone and n-hexane crude extract of A. heterophyllus fruit peel

Following the procedures outlined by Harborne.[12], the following secondary metabolite classes were tested for in the MAH crude extract using standard qualitative colorimetric and precipitation methods: alkaloids, cardiac glycosides, phenols, tannins, saponins, coumarins, steroids, anthraquinone, and terpenoids. For each phytochemical class, results were recorded as either positive (+) or negative (−).

HPLC screening of methanol, acetone and n-hexane crude extract of A. heterophyllus fruit peel

The extracts were subjected to HPLC analysis using an Agilent 1260 Infinity II series, which was equipped with a poroshell column 120 EC-C 18 4µm (4.6x150 mm), PN 693970-902 (T), SN USHKB12136, LN B18447, Quat Pump VL G711A (DEAEY01907), auto Sampler G7129A (DEAEQ22974), Column Oven of G7130 (DEAEQ22974), and DAD WR G71115A (DEAC606992). An electron ionization system using DAD UV light ionization energy was run in electron impact mode for HPLC detection. Detection was at 210 nm and the column was maintained at ambient temperature. External calibration was performed using the following standards: ferulic acid, caffeic acid, tannic acid, saponin, cholesterol, p-coumaric acid, rutin hydrate, kaempferol, gallic acid, maleic acid, salicylic acid, naringin, and quercetin. Compound concentrations were quantified in µg/mL.

GC-MS analysis of crude extracts of A. heterophyllus fruit peel

A gas chromatography (GC) system from Agilent Technologies (5977B GC/MSD) coupled with an Agilent 8860 auto-sampler was used to analyse the crude extracts of fruit peel of *Artocarpus heterophyllus*. The GC-MS was a gas chromatograph interfaced to a mass spectrometer equipped with an Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused-silica capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system operating in electron impact mode with an ionization energy of 70 eV was used. Helium gas (99.999%) was used as a carrier gas, with a constant flow rate of 1 millilitre per minute. A 10:1 split ratio and a 1µl injection volume were employed. Starting at 110 °C (isothermal for two minutes), the oven temperature was programmed to increase at 10 °C/min to 200 °C, then at 5 °C/min to 280 °C, with a final 9-minute hold at 280 °C.

The injector temperature was kept at 250 °C, the ion-source temperature was 200 °C. Mass spectra were recorded at 70 eV over a scan range of 45–450 m/z, with a scanning interval of 0.5 s. There was a 0–3 minutes solvent delay. Major constituents were identified using a computer-driven algorithm referenced against the National Institute of Standards and Technology (NIST) database, which contains more than 62,000 spectral patterns. The spectrum of each unknown component was compared with the spectra of known compounds stored in the NIST library.

Antibacterial activity of the crude extracts of A. heterophyllus fruit peel

The antibacterial activity of the crude extracts was evaluated by agar well diffusion assay, following the method of Assefa *et al*. [13], with minor modifications. Analytical-grade dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) was used to generate stock solutions of MAH, HAH and AAH at 500 mg/mL. Subsequent serial dilution was made to obtain 200 mg/mL. Twenty-four-hour cultures of each test organism, adjusted to a turbidity equivalent to 0.5 McFarland standard (approximately 1.5 × 10^8 CFU/ml), were inoculated onto Mueller-Hinton agar (Oxoid, UK) by flood inoculation. Wells of 6 mm diameter were bored using a sterile cork-borer, and 100 µL of each extract at concentrations of 200 and 500 mg/ml was introduced into designated wells. Norfloxacin (10 µg/disc) was used as the positive control, and dimethyl sulfoxide (DMSO; 10% v/v) served as the negative control. Plates were left at room temperature for 30 min to allow pre-diffusion of extracts, then incubated at 37°C for 24 hours. Zones of inhibition (ZOI) were measured to the nearest millimetre. All experiments were performed in triplicate (n = 3) and results expressed

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as mean ± standard deviation (SD). The MIC (Minimum Inhibitory Concentration) was performed using the double microdilution assay following the NCCLS broth dilution procedure. 0.5g of the methanol extract of *Artocarpus heterophyllus* fruit peel was dissolved in 10mls of DMSO to give final concentration of 50 mg/ml which was serially diluted to give a concentration of 25, 12.5, 6,25 and 3.125 mg/ml. A 24- hour culture of each tested bacteria was standardized to 0.5 McFarland standard and 1ml was inoculated inside test-tubes containing 1ml of various concentration of the extract. 10% DMSO was used as the negative control. It was incubated at 37°C for 24-hours, the test tubes with the lowest concentration that showed no growth (turbidity) after the incubation period was recorded as the MIC.

Statistical analysis

Data are expressed as mean ± SD. Statistical comparisons of zones of inhibition were performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison post-hoc, using IBM SPSS Statistics version 26.0. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Confirmation of multidrug resistance in test isolates

All five clinical isolates were confirmed as multidrug-resistant by the modified Kirby–Bauer disc diffusion method (CLSI).[11], being resistant to at least one agent in ≥3 antimicrobial categories. Table 1 shows the susceptibility profiles: each isolate was resistant to 4–6 of the 8 antibiotics tested, with resistance spanning carbapenem (meropenem, ertapenem), cephalosporins (cefotaxime, ceftriaxone, ceftazidime), an aminoglycoside (gentamicin) and fluoroquinolones (ciprofloxacin, norfloxacin). Table 2 summarises the resistance patterns; notably *P. aeruginosa* was resistant to five agents including both carbapenems (MEM, ETP) and all the tested cephalosporins. The VITEK® 2 system was used for the identification of the test isolates. The VITEK® 2 system correctly identified all five bacterial isolates, corroborating reports of its high accuracy for species-level identification. [29, 30, 31] The susceptibility data confirmed all to be multidrug resistant with each isolate resistant to at least four of the eight antibiotics across carbapenem, third generation cephalosporins, aminoglycoside and fluoroquinolone classes. This is epidemiologically significant, as these organisms, particularly *K. pneumoniae* and *A. baumannii* are prominent ESKAPEE pathogens implicated in nosocomial infections of immunocompromised patients, for which therapeutic options are increasingly exhausted. [4]

Table 1: Antibiotic susceptibility profiles of Gram-negative bacteria.

Bacteria	**Antibiotics/ Zones of inhibition (µg/mL)**							
	MEM (10)	**ETP (10)**	**NOR (10)**	**CTX (30)**	**GM (30)**	**CRO (30)**	**CAZ (10)**	**CIP (5)**
Pseudomonas aeruginosa	11 (R)	10 (R)	24 (S)	10 (R)	10 (S)	10 (R)	8 (R)	29 (S)
Escherichia coli	20 (I)	19 (I)	21 (S)	13 (R)	15 (R)	16(R)	10 (R)	16 (R)
Acinetobacter baumannii	24 (S)	25 (S)	24 (S)	16 (R)	15 (R)	17 (R)	16 (R)	21 (R)
Klebsiella pneumoniae	21 (I)	25 (S)	22 (S)	20 (R)	13 (R)	22(I)	17 (R)	24 (R)
Enterobacter hormaechei	11 (R)	15 (R)	23 (I)	19 (R)	12 (R)	20 (I)	12 (R)	18 (R)

KEYS: S= Sensitive, I= Intermediate, R= Resistance, MEM= Meropenem, ETP = Ertapenem, CAZ = Ceftazidime, CRO = Ceftriaxone, CTX =. Cefotaxime, GM= Gentamicin, CIP = Ciprofloxacin, NOR = Norfloxacin

Table 2: Multidrug resistance pattern of Gram-negative bacteria

Bacteria	Resistance pattern
Pseudomonas aeruginosa	MEM, ETP, CTX, CRO, CAZ, GM
Escherichia coli	CTX, GM, CRO, CAZ, CIP
Acinetobacter baumannii	CTX, GM, CRO, CAZ, CIP
Klebsiella pneumoniae	CTX, GM, CAZ, CIP
Enterobacter hormaechei	MEM, ETP, CTX, GM, CAZ, CIP

KEYS: MEM= Meropenem, ETP = Ertapenem, CAZ = Ceftazidime, CRO = Ceftriaxone, CTX = Cefotaxime, GM= Gentamicin, CIP = Ciprofloxacin, NOR = Norfloxacin

Extraction yield

Cold maceration of 200 g of powdered Artocarpus heterophyllus fruit peel in 1,000 mL of solvent for 72 h at 25 °C gave extract yields that varied with solvent polarity. The methanol extract (MAH) gave the highest yield, followed by acetone (AAH) and n-hexane (HAH), respectively (Table 3). The extraction data showed a clear solvent effect with methanol having the highest yield (4.5%). This polarity-dependence is well established as polar solvents solubilise phenolic and flavonoid glycosides more efficiently than non-polar hydrocarbons. Comparable observations have been reported for *A. heterophyllus* leaf extracts, where methanol extracts carried the highest flavonoid content, and for other unutilised jackfruit parts (peel, fibre, core), where polar extracts likewise outperformed non-polar ones in phenolic recovery and antimicrobial screening. [1-3]

Preliminary phytochemical screening

The results of qualitative phytochemical screening of all three *A. heterophyllus* fruit peel extracts are presented in Table 4. Phenols, tannins, saponins, flavonoids, terpenoids, cardiac glycosides, and coumarins were detected in all three extracts. Alkaloids were present in HAH and AAH but absent in MAH. Anthraquinones and steroids were absent from all extracts. The qualitative phytochemical profile of *A. heterophyllus* fruit peel observed in this study is consistent with that reported by Agu *et al*. [10] and Ajiboye *et al*. [32], who identified phenols, tannins, saponins, flavonoids, terpenoids, alkaloids, and glycosides in

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fruit peel extracts of the same species. The absence of steroids and anthraquinones across all three extracts in the present study likewise concurs with Ajiboye *et al*. [32] The differential detection of alkaloids that is present in HAH and AAH but absent in MAH is consistent with known solubility patterns: alkaloids are generally more soluble in non-polar to moderately polar solvents and may not have been extracted by methanol in amounts detectable by the qualitative test employed. Collectively, the presence of phenols, flavonoids, tannins, terpenoids, saponins, cardiac glycosides, and coumarins across all extracts is pharmacologically relevant, as these compound classes are associated with antimicrobial, antioxidant, anti-inflammatory, and antidiabetic activities. [33, 34.]

Table 3: Percentage yield of extracts of *Artocarpus heterophyllus* fruit peel

Plant extract	Nature	Weight (g)	Yield (g)	Percentage yield (%)
AAH	Dark brown and sticky	200	6.9	3.4
HAH	Yellowish and oily	200	6.6	3.3
MAH	Light brown and sticky	200	8.98	4.5

Table 4: Qualitative phytochemicals composition of fruit peel extracts of *Artocarpus heterophyllus*

Phytochemical test	HAH	MAH	AAH
Phenol	+	+	+
Tannin	+	+	+
Saponin	+	+	+
Anthraquinone	-	-	-
Flavonoids	+	+	+
Terpenoids	+	+	+
Cardiac glycosides	+	+	+
Coumarins	+	+	+
Steroids	-	-	-
Alkaloid	+	-	+

Keys: MAH= Methanol extract of *A. heterophyllus*, HAH= n-Hexane extract of *A. heterophyllus*, AAH= Acetone extract of *A. heterophyllus*, += presence of phytochemicals, - = absence of phytochemicals

HPLC profiling of phenolic compounds

HPLC analysis identified 10, 12, and 11 phenolic compounds in HAH, MAH, and AAH, respectively (Tables 5, 6 and 7). MAH had the highest total identified content (13.99 µg/mL), dominated by p-coumaric acid (5.90 µg/mL), followed by kaempferol (2.74 µg/mL), naringin (2.53 µg/mL), and quercetin (1.74 µg/mL). By contrast, gallic acid was the most abundant compound in both HAH (0.047 µg/mL) and AAH (0.12 µg/mL), although the overall phenolic content of these two extracts was substantially lower (0.10 and 2.61 µg/mL, respectively). Salicylic acid, rutin hydrate, and cholesterol were undetectable in HAH, while naringin and cholesterol were absent from AAH. HPLC profiling revealed a clear solvent-dependent pattern in phenolic content, with MAH yielding the highest total concentration (13.99 µg/mL), followed by AAH (2.61 µg/mL) and HAH (0.10 µg/mL). Methanol's superior extraction efficiency is attributable to its high polarity index and protic nature, which enable it to solubilise a broader spectrum of phenolic and flavonoid compounds than acetone (similar polarity index but aprotic) or n-hexane (non-polar). [35; 36] p-Coumaric acid was the dominant compound in MAH (5.90 µg/mL), whereas gallic acid predominated in both HAH and AAH which is a pattern that partially diverges from Jiang *et al*. [37], who reported gallic acid, chlorogenic acid, and catechin as the three dominant phenolics in jackfruit peel. This discrepancy may reflect differences in extraction methodology, cultivar, and geographical origin of the plant material. [38] Phenolics are well-established as the principal natural antioxidants in plant matrices [9], and compounds such as p-coumaric acid, kaempferol, quercetin, and naringin are known to possess direct antimicrobial activity.

Table 5: HPLC profile of n-hexane extract of *Artocarpus heterophyllus* fruit peel

Peak no	**Retention time**	**Phenolic/Flavonoid compound**	**Quantity (µg/mL)**
1	1.752	Caffeic acid	0.0027
2	1.831	Ferulic acid	0.028
3	2.178	Maleic acid	0.0012
4	2.366	Salicylic acid	ND
5	4.418	Gallic acid	0.047
6	5.252	Tannic acid	0.0029
7	5.872	Saponins	0.0039
8	6.156	Rutin hydrate	ND
9	7.472	Naringin	0.0013
10	7.692	Kaempferol	0.0058
11	8.458	Quercetin	0.0014
12	11.485	P-coumaric acid	0.013
13	14.525	Cholesterol	ND

Keys: ND = Not detected

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Table 6: HPLC of methanol extract of *Artocarpus heterophyllus* fruit peel

Peak no	Retention time	Phenolic/Flavonoid compound	Quantity (µg/mL)
1	1.740	Caffeic acid	0.0095
2	1.827	Ferulic acid	0.039
3	2.153	Maleic acid	0.0045
4	2.396	Salicylic acid	0.018
5	4.313	Gallic acid	0.0018
6	5.393	Tannic acid	0.033
7	5.933	Saponins	0.91
8	6.140	Rutin hydrate	0.12
9	7.493	Naringin	2.53
10	7.667	Kaempferol	2.74
11	8.4827	Quercetin	1.74
12	11.300	P-coumaric acid	5.9
13	14.525	Cholesterol	ND

Key: ND = Not detected

Table 7: HPLC of acetone extract of *Artocarpus heterophyllus* fruit peel

Peak no	Retention time	Phenolic/Flavonoid compound	Quantity (µg/mL)
1	1.745	Caffeic acid	0.0013
2	1.878	Ferulic acid	0.022
3	2.191	Maleic acid	0.0011
4	2.511	Salicylic acid	0.046
5	4.391	Gallic acid	0.12
6	5.348	Tannic acid	0.012
7	5.925	Saponins	0.038
8	6.018	Rutin hydrate	0.012
9	7.492	Naringin	ND
10	7.751	Kaempferol	0.0039
11	8.479	Quercetin	0.00095
12	11.99	P-coumaric acid	0.0016
13	14.525	Cholesterol	ND

Key: ND = Not detected

GC-MS profiling

GC-MS analysis identified 24, 25, and 25 bioactive compounds in MAH, HAH, and AAH, respectively (Figures 1–3). Benzoic acid methyl ester was the predominant compound in MAH (22.37%), while pentadecanoic acid 14-methyl ester (15.92%) and 9,19-cyclolanost-24-en-3-ol (3β-) (19.30%) predominated in HAH and AAH, respectively. The pharmacological activities reported for the principal bioactive compounds identified across all three extracts are summarised in Table 8. GC-MS analysis revealed qualitatively compound profiles across the three extracts, reflecting differences in solvent polarity and selectivity. Benzoic acid methyl ester (methyl benzoate), the predominant compound in MAH (22.37%) and HAH (22.53%), and abundant in AAH (18.98%) is a volatile benzoate ester with reported antioxidant, antimicrobial, and cytotoxic activities.[39, 14] Fatty acid-derived constituents, including pentadecanoic acid 14-methyl ester, n-hexadecanoic acid, methyl stearate, and 9-octadecenoic acid methyl ester, were present across multiple extracts and collectively contribute significant antimicrobial and antioxidant potential [16, 18, 22] Squalene, identified exclusively in AAH, is a triterpenoid with established antioxidant, anti-inflammatory, and antimicrobial properties [24], while the triterpenoids 9,19-cyclolanost-24-en-3-ol and its congener, also unique to AAH, have been associated with antibacterial, anticancer, antioxidant, and anti-inflammatory activities. [28]

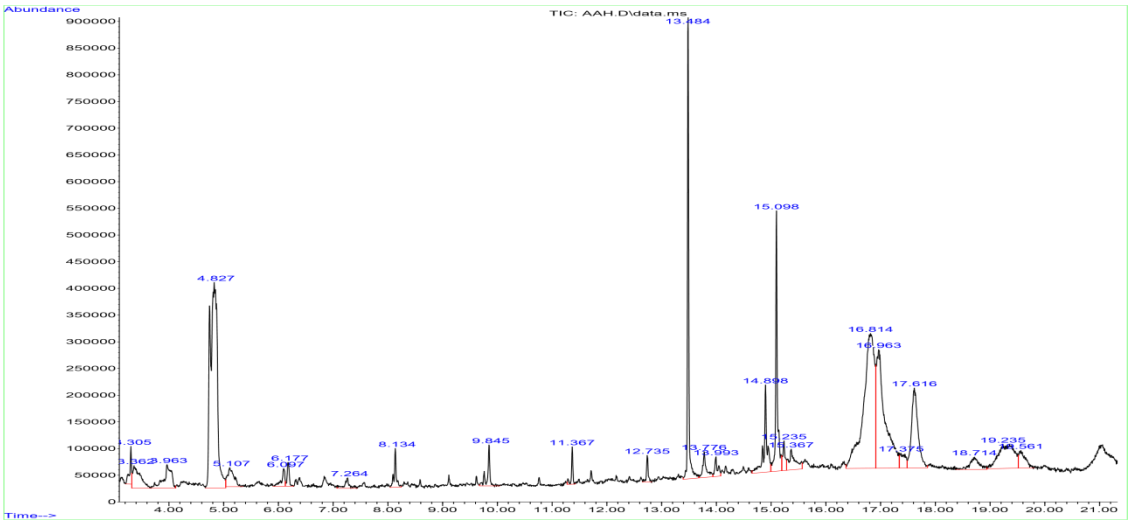


Figure 1: GCMS chromatogram of acetone extract of *Artocarpus heterophyllus* fruit peel

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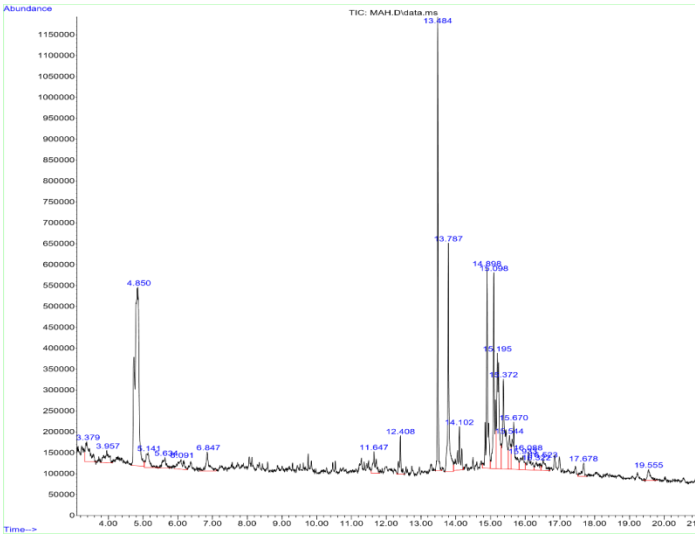


Figure 2: GCMS chromatogram of methanol extract of *Artocarpus heterophyllus* fruit peel

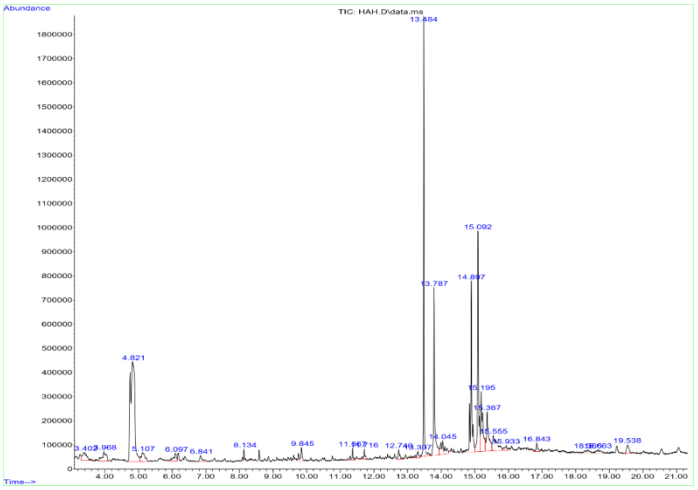


Figure 3: GCMS chromatogram of n-hexane extract of *Artocarpus heterophyllus* fruit peel

Antibacterial activities of fruit peel extracts of Artocarpus heterophyllus

The antibacterial activity of MAH, AAH and HAH (200 and 500 mg/mL) against the five isolates, with 10% DMSO as the negative control and norfloxacin (10 µg) as the reference standard, is presented in Table 9. The negative control produced no inhibition (0.0 mm) against any isolate, confirming that inhibition was not attributable to the solvent.

Table 8: Pharmacological activities of principal phytoconstituents in crude extracts of *Artocarpus heterophyllus* fruit peel

Name of compound	Percentage abundance %			Activity	Reference
	MAH	AAH	HAH		
Benzoic acid, methyl ester	22.37	18.98	22.53	Antimicrobial	[14]
				Antidiabetic	[15]
Pentadecanoic acid, 14-methyl-, methyl ester	10.13	7.85	15.92	Antimicrobial, antioxidant	[16, 17]
n-Hexadecanoic acid	7.86	NP	8.57	Antimicrobial, anticancer, antioxidant, anti-inflammatory	[18, 19]
				Antidiabetic	[20]
9-Octadecenoic acid, methyl ester	7.79	3.27	10.17	Antioxidant, lower HDL cholesterol	[21]
Methyl stearate	7.88	5.73	10.91	Antimicrobial	[22]
				Anti-inflammatory	[23]
Squalene	NP	6.52	NP	Antioxidant, anticancer Antimicrobial	[21]
				Antidiabetic	[24]
9-Octadecenoic acid, (E)-	7.72	NP	NP	Antidiabetic, antibacterial	[25, 26]
Octadecanoic acid	6.80	NP	4.35	Antimicrobial, antioxidant, anti-inflammatory	[27]

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9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	NP	19.30	NP	Antibacterial, anticancer, antioxidant, antidiabetic anti-inflammatory	[28]
9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, (3.beta.,4.alpha.,5.alpha.)-	NP	13.0	NP	Antioxidant	[28]
cis-Vaccenic acid	NP	NP	5.58	Antioxidant, hypolipodemic, antibacterial	[24]

KEY: NP= Not present

Table 9: Zones of inhibition (mm) of fruit peel extracts of *Artocarpus heterophyllus* against multidrug- resistant Gram-negative bacteria.

Bacteria	**Zones of inhibition (mm)**							
	MAH (mg/ml)		**AAH (mg/ml)**		**HAH (mg/ml)**		**10% DMSO**	**Norfloxacin (10µg)**
	200	**500**	**200**	**500**	**200**	**500**		
Acinetobacter baumannii	13.5±0.71^{b}	22.0±1.41^{c}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	24.0±0.0^{c}
Escherichia coli	12.5±2.12^{b}	20.5±2.12^{c}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	21.0±1.41^{c}
Pseudomonas aeruginosa	0.0±0.0^{a}	6.0±1.41^{c}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	21.5±0.71^{d}
Klebsiella pneumoniae	11.5±0.71^{c}	16.0±1.41^{d}	0.0±0.0^{a}	6.0±1.41^{b}	12.0±1.41^{c}	14.0±1.41^{cd}	0.0±0.0^{a}	23.5±2.12^{e}
Enterobacter hormaechei	13.5±0.71^{b}	21.5±2.12^{c}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	23.5±0.71^{d}

Zones of inhibition expressed as mean ± SD (mm); n = 3. Different superscript letters within a row indicate statistically significant differences (Duncan's multiple comparison post-hoc test, $p < 0.05$). Concentration in mg/ml. MAH = methanol extract; HAH = n-hexane extract; AAH = acetone extract; DMSO = dimethyl sulfoxide (negative control).

MAH was the most active extract, inhibiting all five isolates at 500 mg/mL in a concentration-dependent manner (zones at 200 → 500 mg/mL: *A. baumannii* 13.5 → 22.0 mm; *E. coli* 12.5 → 20.5 mm; *K. pneumoniae* 11.5 → 16.0 mm; *E. hormaechei* 13.5 → 21.5 mm). At 500 mg/mL, the zones produced by MAH against E. coli (20.5 ± 2.12 mm) and *A. baumannii* (22.0 ± 1.41 mm) were statistically comparable to those of norfloxacin ($p > 0.05$). In contrast, MAH produced only minimal inhibition of *P. aeruginosa* (6.0 ± 1.41 mm at 500 mg/mL) and none at 200 mg/mL. The acetone extract exhibited negligible activity, inhibiting only *K. pneumoniae* at 500 mg/mL (6.0 ± 1.41 mm), while the n-hexane extract inhibited only *K. pneumoniae*, at both concentrations (12.0 ± 1.41 and 14.0 ± 1.41 mm at 200 and 500 mg/mL, respectively), although significantly less than norfloxacin. Methanol extract of *A. heterophyllus* fruit peel exerted the broadest and most potent antibacterial activity, inhibiting all five MDR isolates tested. MAH exerted broad, concentration-dependent activity against all five MDR isolates and matched norfloxacin against *E. coli* (20.5 ± 2.12 mm) and *A. baumannii* (22.0 ± 1.41 mm) at 500 mg/mL ($p > 0.05$). This is consistent with finding of Ajinwo *et al.* [40], who likewise reported antibacterial activity of *A. heterophyllus* extracts against *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, and *E. coli*. The seed ethanolic extracts of *A. heterophyllus* have also been reported to produce zones of 19.83–31.42 mm against *Salmonella typhi*.[4] GC-MS identified benzoic acid methyl ester, a phenolic ester with documented antimicrobial properties as the dominant constituent in all three extracts (18.98–22.53%), while HPLC quantified p-coumaric acid (5.9 µg/mL) and kaempferol (2.74 µg/mL) in MAH. The superior potency of MAH is therefore best explained mechanistically by its high phenolic/flavonoid content acting through membrane disruption, impairment of protein synthesis and interference with quorum sensing mechanisms that are partially effective even against isolates that have shown resistance to multiple antibiotics, which is consistent with the observed parity with norfloxacin against *E. coli* and *A. baumannii*. The recalcitrance of *P. aeruginosa* with a zone of only 6.0 mm at 500 mg/mL and no MIC within 50 mg/mL, deserves specific comment. The isolate in the present study was resistant to five of the eight antibiotics, including both carbapenems, third generation cephalosporins and gentamicin, and the intrinsic physiology of *P. aeruginosa*, low outer-membrane permeability, constitutive efflux pumps and inducible β-lactamases is known to limit the penetration of crude botanical extracts. Reduced susceptibility of this species to plant-derived antibacterials is therefore expected and does not diminish the promise of MAH against the other four clinically urgent ESKAPEE pathogens.

The activity of HAH and AAH, limited to *K. pneumoniae*, may reflect both their lower total phenolic content and the intrinsic resistance mechanisms of the other test organisms including efflux pumps, porin loss, and carbapenemase production which are common among *A. baumannii*, *E. coli*, and *E. hormaechei* isolates from immunocompromised patients. The restricted activity of the non-polar extracts of HAH inhibiting only *K. pneumoniae* (12.0–14.0 mm) and AAH inhibiting it only at 500 mg/mL (6.0 mm), correlates directly with their very low phenolic loads (0.10 and 2.61 µg/mL, respectively). Notably, alkaloids were confined to HAH and AAH and yet these extracts were the least active, suggesting that the phenolic/flavonoid fraction, not the alkaloid fraction, drives the antibacterial effect in this matrix. The disproportionate susceptibility of *K. pneumoniae* to the weaker extracts is intriguing yet not unprecedented as anti-*Klebsiella* activity of methanol extracts of dietary plants, including *A. heterophyllus* bark and leaves, has been documented against MDR phenotypes.[10]

Minimum inhibitory concentration (MIC)

The MIC of the methanol extract (MAH) was determined by the double broth microdilution method (NCCLS), with growth scored visually after 24 h at 37 °C (Table 10; + = no growth, − = turbid growth). MAH inhibited *E. coli*, *A. baumannii* and *E. hormaechei* at a

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MIC of 6.25 mg/mL, and *K. pneumoniae* at 12.5 mg/mL. No MIC was recorded for *P. aeruginosa* within the tested concentration range (up to 50 mg/mL), corroborating the weak zone observed in the diffusion assay.

Table 10: MIC values of methanol extracts of *Artocarpus heterophyllus* fruit peel against multidrug- resistant Gram-negative bacteria.

Bacteria	Concentrations of MAH (mg\ml)						DMSO
	50	25	12.5	6.25	3.125	1.562	10% v/v
Pseudomonas aeruginosa	-	-	-	-	-	-	-
Escherichia coli	+	+	+	+	-	-	-
Acinetobacter baumannii	+	+	+	+	-	-	-
Klebsiella pneumoniae	+	+	+	-	-	-	-
Enterobacter hormaechei	+	+	+	+	-	-	-

+ = No growth (inhibition); − = turbid growth (growth). DMSO served as the solvent control.

The MIC values obtained in the study (6.25 mg/mL for *E. coli*, *A. baumannii* and *E. hormaechei*; 12.5 mg/mL for *K. pneumoniae*) are markedly lower than the MIC of 31.25 mg/mL reported for ethanolic seed extracts of *A. heterophyllus* against diarrhoea-causing superbugs,[8] suggesting the methanolic peel extract is comparatively potent, a meaningful advance given that those seed extracts were already cited as promising candidates against MDR *Pseudomonas aeruginosa* and MRSA. The activity of *Artocarpus* seed extracts against MDR *E. coli* from urinary tract infections reported in separate studies further supports the genus-level potential.[9] Assays were performed with crude extracts whose active compound is unknown.; MBC, time–kill kinetics, synergy with conventional antibiotics and in vivo safety/efficacy were not assessed; and the weak but reproducible activity against *K. pneumoniae* by non-polar extracts warrants mechanistic follow-up. Despite these constraints, the convergence of yield, phytochemical and bioactivity data makes a compelling case for the methanolic peel extract of *A. heterophyllus* as a candidate source of antibacterial lead compounds.

Conclusion

This study demonstrates that *Artocarpus heterophyllus* fruit peel is a rich source of pharmacologically relevant phytochemicals, including phenolics, flavonoids and bioactive fatty-acid derivatives, as characterised by HPLC and GC-MS. The methanol extract exhibited the highest yield (4.5%), the highest phenolic content (13.99 µg/mL) and the broadest, most potent antibacterial activity against MDR Gram-negative bacteria, achieving zones of inhibition comparable to norfloxacin ($p > 0.05$) against *E. coli* and *A. baumannii* at 500 mg/mL, with MICs of 6.25–12.5 mg/mL. The superiority of the methanol extract confirms the critical influence of solvent polarity on the recovery of bioactive compounds. These findings support the valorisation of jackfruit peel waste as a candidate source of agents against clinically relevant MDR pathogen. The study recommends future investigations to focus on isolation and structural elucidation of the major active constituents, MBC determination, elucidation of mechanisms of antibacterial action, and *in vivo* validation of efficacy and safety. Future work should also address time-kill kinetics and synergy testing with conventional antibiotics.

Conflict of interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by authors.

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