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Research Article

ANTIBACTERIAL POTENTIAL OF *ARTOCARPUS HIRSUTUS* LAM. EXTRACT AGAINST NOSOCOMIAL PATHOGENS

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ABSTRACT

This study investigates the antimicrobial potential of *Artocarpus hirsutus* leaf extracts against antibiotic-resistant clinical isolates of human pathogens. Nosocomial infection is a growing global health concern, necessitating the exploration of alternative treatments. Antibiotics are used to treat bacterial infections but in recent years, clinical strains of bacteria show antibiotic resistance. *A. hirsutus*, traditionally used for treating infections, was evaluated for its antibacterial properties. Leaf extracts were prepared using hexane, ethyl acetate, and ethanol, tested against pathogens like *Staphylococcus aureus*, *Enterococcus* sp, *Klebsiella* sp, *Escherichia coli, Pseudomonas* sp, *and Enterobacter* sp. The ethanol extract demonstrated significant broad-spectrum antimicrobial activity, with inhibition zones ranging from 9.33±1.15 mm against *S. aureus* to 16.33±1.15 mm against *E. coli.* Phytochemical screening and GC-MS analysis identified various bioactive compounds, including alkaloids, flavonoids, and terpenoids, which likely contribute to the observed antimicrobial effects. FTIR analysis further confirmed the presence of functional groups associated with antimicrobial activity. Toxicity studies using Zebra fish indicated a favourable safety profile for the extracts, with LC^{50} values of 438.25 ppm for ethyl acetate and 426.53 ppm for ethanol extracts. These findings suggest that *A. hirsutus* could serve as a promising source for developing new antimicrobial agents to combat resistant bacterial strains. Future research focused on isolating active compounds and exploring synergistic effects with conventional antibiotics.

Keywords: Antibiotic resistance, *Artocarpus hirsutus*, Antimicrobial activity, Phytochemical screening, Toxicity.

INTRODUCTION

A nosocomial infection, also known as a hospital-acquired infection (HAI), is an infection that is acquired in a hospital or other healthcare facility. Bacteria, viruses, fungi, or other pathogens can cause these infections. Nosocomial infections can have serious consequences, including increased morbidity, mortality, and healthcare costs. Preventing these infections is essential to ensuring patient safety and improving healthcare outcomes. Antibiotic resistance has evolved into a critical emergency in public health in the 21st century. (Aslam *et al.,* 2021). The excessive and inappropriate use of antibiotics in healthcare has accelerated the development of resistant bacterial populations and undermined standard treatment protocols (Friedman *et al.,* 2016). This global health challenge is even more challenging due to a major slowdown in antibiotic innovation without the emergence of a critical new class of antibiotics for over 30 years (Lubrano *et al.* (2025). Health organisations around the world, including WHO, have highlighted antibiotic resistance as a fundamental threat, which could lead to an era where daily infectious diseases are not treated. (Munita and Arias 2016). Traditional healing systems have long used the antibacterial properties of different plants for the treatment of infectious diseases for centuries. *Artocarpus hirsutus* (Wild Jack) has developed as a particularly promising medicine. (Meenu *et al.,* 2022). *Artocarpus hirsutus* was prominently found in the Western Ghats of India, the plant has traditionally been used to treat conditions such as skin infections, ulcers and joint pain (Suma, 2021). Modern scientific research is beginning to underpin these historical applications. It shows that *A. hirsutus* extracts have strong antibacterial effects against many bacterial tribes, including those resistant to traditional antibiotics. This test assesses

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the efficacy of traditional antibiotics and *Artocarpus hirsutus.* The study will examine jack fruit as a potential alternative treatment. (Meenu *et al.,* 2022). Through a combination of clinical data and laboratory analysis, this study examines the effectiveness of *A. hirsutus* extracts against resistant microorganisms, contributing to the development of innovative clinical treatment approaches. (Meenu *et al.,* 2021). This result has a significant impact on strategies and clinical protocols for public health, and could provide a practical solution to the escalating challenges of antibiotic resistance to infection acquired in municipalities.

MATERIALS AND METHODS

Sample collection

A total of 150 samples were collected from patients, healthcare environments, and healthcare workers during the rainy season in Tirunelveli district health care and hospitals. The samples included ear swabs, sputum, stool, urine, wound pus, and from surfaces, equipment, or air in healthcare settings to ensure comprehensive microbiological surveillance. All samples were processed within 30 minutes to ensure sample integrity. Sterile containers were maintained to prevent contamination, crucial for accurate microbiological analysis. (Leal *et al.,* 2016). Pathogens isolated include *Pseudomonas* sp, *Klebsiella* sp, *Escherichia coli*, *Enterobacter* sp, *Enterococcus* sp, and *Staphylococcus aureus*.

Media Preparation and Culturing

Sterilization was performed using an autoclave at 121°C for 120 minutes to ensure the elimination of all microorganisms. Pathogens such as *Klebsiella* sp, and others were cultured by streaking them on solid agar and incubating them, resulting in the observation of isolated colonies. This critical step facilitated colony isolation and subculturing on Blood and MacConkey agar, which were incubated at 37°C for 24 hours. The spread plate method was used for bacterial isolation, a technique crucial for accurate sample analysis in microbiological research (Karimi-Maleh *et al.,* 2021).

Isolation and Screening

Bacterial strains from isolation samples were inoculated onto Blood and MacConkey agars and incubated at 37°C for 24–48 hours. Colony counts exceeding 10^5 CFU/mL indicated bacteria. Gram staining and biochemical tests, including catalase and indole tests, were used to identify the isolates, which were stored in an icebox for further analysis. Species-level identification was based on morphological, biochemical, and cultural characteristics (Kato *et al.,* 2018).

Gram Staining and Biochemical Test

Gram staining and biochemical tests identified bacterial isolates after 24-hour incubation at 37°C. Pure cultures were stored for further analysis (Moyes *et al*., 2009).

Antibiotic susceptibility test

Antibiotic susceptibility tests were conducted following standardized protocols, utilizing the Kirby-Bauer disk diffusion method to assess the efficacy of specified commercial antibiotics. These included Streptomycin (10 mcg), Ampicillin AMP (10µg), Tetracycline (10 mcg), Chloramphenicol (10 mcg), Kanamycin (30 mcg), Gentamycin (10 mcg), Neomycin (30 mcg), Nalidixic Acid NA (30 mcg). The tests were performed on Mueller-Hinton agar plates inoculated with isolated pathogens. Post-incubation, plates were incubated at 37°C for 24 hours to determine resistance patterns. (Patel *et al*., 2019)

Plant Collection and Extract Preparation

Artocarpus hirsutus leaves were collected in Padmanabhapuram, Latitude 8.239125 and Longitude 77.337635, Kaniyakumari district, Tamil Nadu, India. The plant was identified by the facility at Xavier Research Foundation, Palayamkottai, Tamil Nadu. The leaves were washed with sterile water, stored and dried at 25-30°C until brittle. They were ground into a fine powder, sieved, and stored in airtight containers. For analysis, 50 grams of powder were extracted using hexane, ethyl acetate, and ethanol in a Soxhlet extraction for 72 hours. Extracts were filtered and solvents evaporated to yield concentrated extracts, suitable for phytochemical and other studies. (Patel *et al*., 2016).

Qualitative phytochemical screening

Phytochemical analysis was undertaken to detect bioactive compounds in the plant extracts. Tests were performed for alkaloids, flavonoids, tannins, saponins, and other therapeutic secondary metabolites (Dubale *et al.,* 2023).

Spectrometry analysis (GC–MS)

GC-MS analysis identified and quantified plant extract compounds, revealing bioactive constituents associated with antibacterial activity (Nabi *et al.,* 2022).

Anti-bacterial activity

The antimicrobial activity of *Artocarpus hirsutus* leaf extract such as Hexane, ethyl acetate, ethanol was carried out using disc diffusion method (Kamble *et al.,* 2022). Bacterial suspension (20 µL, 1×10^7 CFU/mL) was inoculated on MHA agar, followed by placement of 6 mm sterile paper disks loaded with 100 µL of extract (5mg/mL). Disks were placed in wells prepared in agar plates that had been inoculated with bacterial strains. The plates were incubated at 35 ± 2 ◦C for 24 h, and the entire process was conducted in triplicate.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FT-IR spectroscopy was performed using a Spectrum-100 PerkinElmer spectroscope on KBr-mixed formulation extracts, scanning 400-4000 cm^{-1} at 4 cm resolution to

identify functional groups and chemical bonds (Kavitha *et al.,* 2020).

Toxicity analysis

Zebra fish lethality assay was performed to evaluate the toxicity of plant extracts following the method described by (JV *et al.,* 2021) with slight modifications. The Zebra fish toxicity assay was conducted using plant extracts (ethyl acetate and ethanol) dissolved in DMSO at concentrations of 1, 10, 100, 500 and 1000ppm, with 30 Zebra fish fingerlings per concentration tested in triplicate, using potassium dichromate as a positive control and recording mortality after 24 hours. LC50 values were determined.

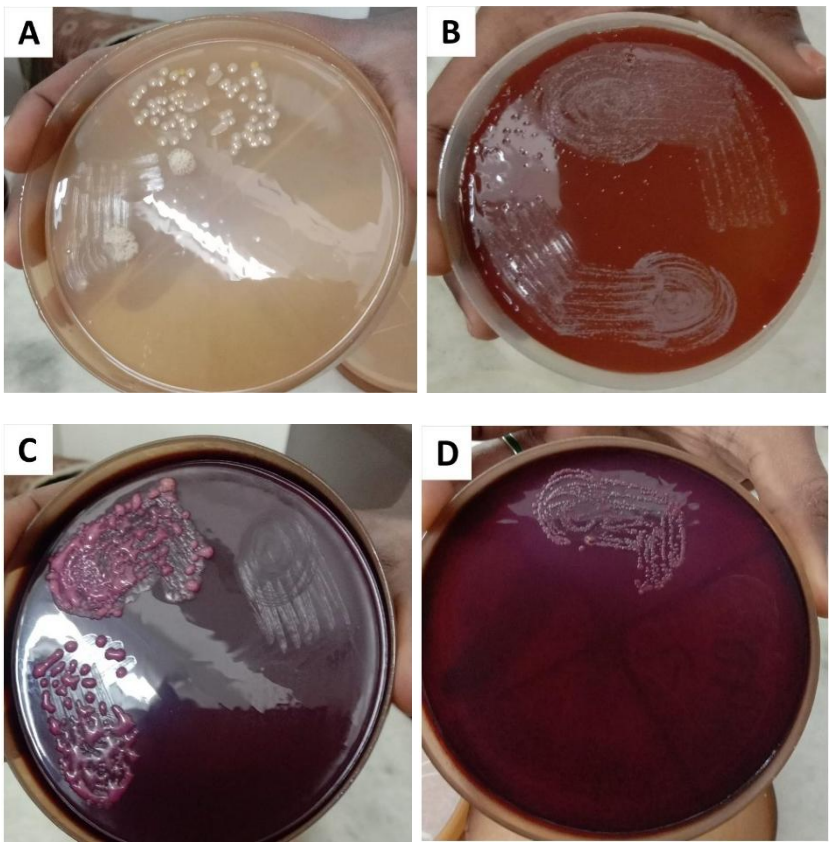
$$\text{Mortality (\%)} = \frac{N0.of\ dead\ animal}{Total\ No.of\ animal} \times 100$$

Statistical analysis

Statistical analysis was done using Microsoft Excel 2007 and SPSS 12 for one-way ANOVA at a 95% confidence level. P-values below 0.05 indicated significance. LC50 values calculated via linear regression in R software.

RESULTS AND DISCUSSION

Six bacterial strains were identified from samples collected at healthcare facilities in Tirunelveli city using Blood Agar and MacConkey Agar media. *Staphylococcus aureus* exhibited beta-hemolysis on Blood Agar with no MacConkey growth due to its Gram-positive nature. *Enterococcus* sp showed gamma-hemolysis on Blood Agar and no MacConkey growth. *Klebsiella* sp displayed mucoid, gamma-hemolytic colonies on Blood Agar and pink lactose-fermenting colonies on MacConkey Agar. *Escherichia coli* demonstrated beta-hemolysis on Blood Agar and lactose fermentation on MacConkey Agar. *Pseudomonas* sp showed beta-hemolysis on Blood Agar without lactose fermentation on MacConkey Agar. *Enterobacter* sp formed gamma-hemolytic colonies on Blood Agar and fermented lactose on MacConkey Agar (Figure 1). These results highlight distinct colony characteristics and growth patterns for each bacterium. Both Gram-positive and Gram-negative bacteria exhibited increased antibiotic resistance, with *Klebsiella* sp being particularly virulent due to various colonization factors. The successful isolation of these bacterial pathogens (*S. aureus*, *Klebsiella* sp) from patient samples underscores their significance in respiratory and urinary tract infections. The distinctive growth patterns observed on Blood Agar and MacConkey Agar align with established microbiological characteristics of these organisms Kuznetsova *et al.* (2025). For instance, the beta-hemolysis exhibited by *S. aureus* on Blood Agar and its inability to grow on MacConkey Agar confirms its morphology, Gram-positive nature and hemolytic properties, consistent with findings by Al-Byti *et al.* (2022).



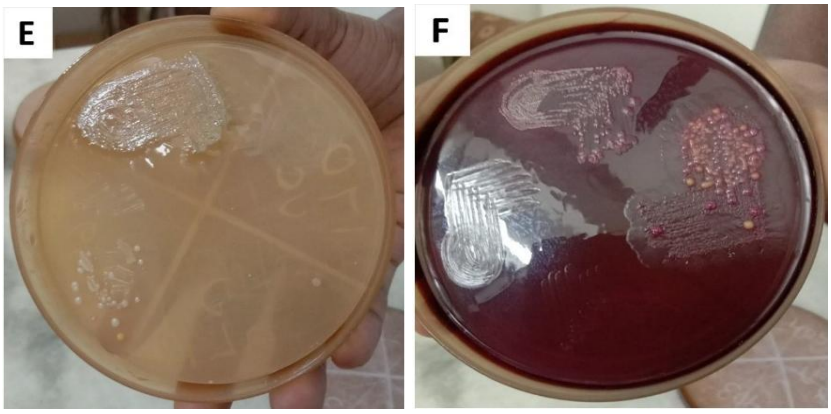


Figure 1. Clinically isolated of A- *S. aureus* in wound, B- *Enterococcus* sp in urine, C-*E. coli* in motion, D-*Klebsiella* sp in sputum, E- *Pseudomonas* sp in ear swap, F-*Enterobacter* sp in urine sample on selective media.

The multi-drug resistance displayed by *Klebsiella pneumoniae* and *P. aeruginosa* is particularly concerning and reflects global trends in antimicrobial resistance (Taha *et al*., 2024). These organisms represent critical priorities for new antimicrobial development. Gram staining and biochemical assays identified bacterial isolates. Optical microscopy analysed cell morphology. Tests included catalase, oxidase, indole, and sugar fermentation to characterize clinically isolated pathogens. Our investigation utilizing Gram staining and biochemical assays identified *Klebsiella* sp and *Escherichia coli* as predominant isolates, both exhibiting rod-shaped morphology. These findings align with contemporary research by Işıl *et al.* (2025), who employed dark-field microscopy with deep learning for virtual Gram staining. Our results corroborate the seminal work of researchers who applied similar techniques to mastitis diagnosis and similarly reported high prevalence of these rod-shaped pathogens *Suzuki et al.* (2025). Antibiotic susceptibility testing revealed varying resistance patterns among isolated pathogens. *E. coli* showed resistance to all antibiotics except gentamycin. *Enterococcus* sp was susceptible to chloramphenicol and gentamycin. *Klebsiella* sp was resistant to all antibiotics except gentamycin and neomycin. *E. coli* exhibited susceptibility only to gentamycin. *Pseudomonas* sp was resistant to all the antibiotics tested and susceptible to streptomycin, kanamycin, neomycin. *Enterobacter* sp displayed especially resistance to tetracycline, kanamycin, and chloramphenicol but susceptibility to gentamycin and nalidixic Acid NA.

Table 1. Antibiotic resistance pattern of isolated pathogens in different antibiotic disc.

S. No	Antibiotics disc	*S. aureus*	*Enterococcus* sp	*Klebsiella* sp	*E. coli*	*Pseudomonas* sp	*Enterobacter* sp
1.	Streptomycin	R	R	R	R	S	R
2.	Ampicillin AMP	R	R	R	R	R	R
3.	Tetracycline	S	R	R	R	R	R
4.	Chloramphenicol	R	S	R	R	R	R
5	Kanamycin	S	R	R	R	R	R
6.	Gentamycin	S	S	S	S	S	S
7.	Neomycin	S	R	S	R	S	S
8.	Nalidixic Acid NA	R	R	R	R	R	S

R – Resistance; S – Susceptible

Our study's findings on antibiotic susceptibility align with those of Ersoy *et al.* (2025) noting that environmental factors impact resistance levels, using antibiotics like tetracycline, streptomycin, and gentamicin. Lubrano *et al*. (2025) identified *E. coli* mutations that diminish susceptibility to these antibiotics. Yakobi *et al*. (2025) assessed microbiological methods for testing antibiotic susceptibility, focusing on doxycycline, ampicillin, and ciprofloxacin. Phytochemical analysis of *Artocarpus hirsutus* leaf extracts using hexane, ethyl acetate, and ethanol identified various bioactive compounds. Ethanol was an effective solvent, extracting a broader range of compounds, like alkaloids, saponins, flavonoids, terpenoids, and phenols. Shanmugapriya *et al.* (2017), who

documented the presence of various secondary metabolites of alkaloids, saponins, flavonoids, terpenoids, and phenols in the *Artocarpus hirsutus* ethanolic extract, suggest potential antimicrobial properties (Vinay Suvarna *et al.,* 2014).

Table 2. Phytochemical analysis of *Atrocarpus hirsutus* in hexane, ethyl acetate and ethanol extracts.

S.No.	Phytochemical tests	Leaf extracts		
		Hexane	Ethyl Acetate	Ethanol
1.	Test for Alkaloids	+	+	+
2.	Test for steroids	-	-	+
3	Test for tannins	-	-	+
4	Test for saponins	+	+	+
5	Test for flavonoids	++	+	++
6	Test for carotenoids	-	+	++
8	Test for cardiac glycosides	-	-	+
9	Test for terpenoids	++	+	++
10	Test for phenols	+	++	++

'+' presence '++' strong presence '-' absence

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of *Artocarpus hirsutus* leaf extracts, specifically the ethyl acetate and ethanol extracts, revealed a diverse range of bioactive compounds. Notably, Neophytadiene was identified as a dominant compound, known for its potential biological activities. The ethyl acetate extract showed a modest presence of 3,7,11,15-Tetramethylhexadec-2-ene, n-Hexadecanoic acid, Hexadecanoic acid ethyl ester, and Phytol. The ethanol extract revealed a similar chemical profile, with compounds like butanal 3-methyl, benzene, and 1,3-dimethyl contributing to its chemical diversity. Additionally, compounds like 2-methyl-3-propyloxirane and 2,4-di-tert-butylphenol serve as intermediates in polymer and pharmaceutical synthesis.

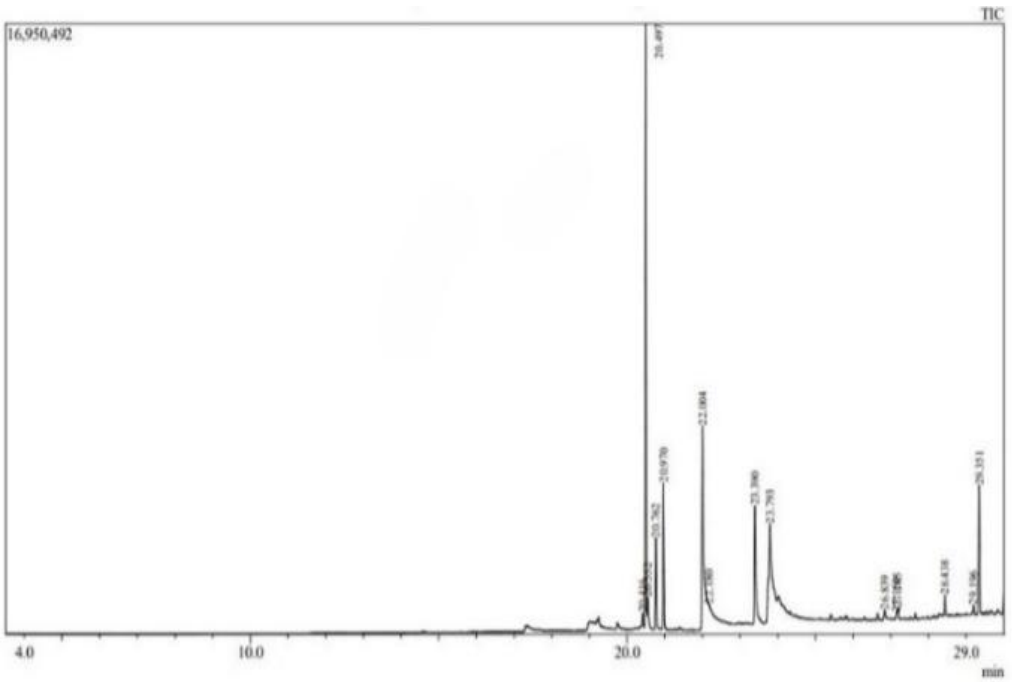


Figure 2. GC-MS chromatogram of *Artocarpus hirsutus* leaf ethyl acetate extract.

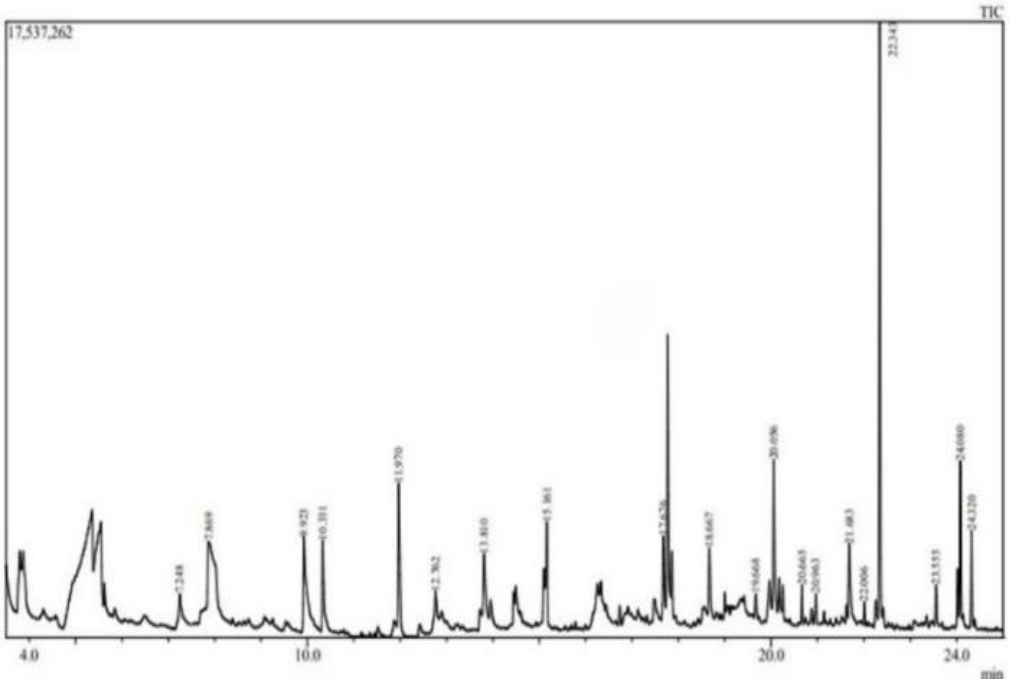


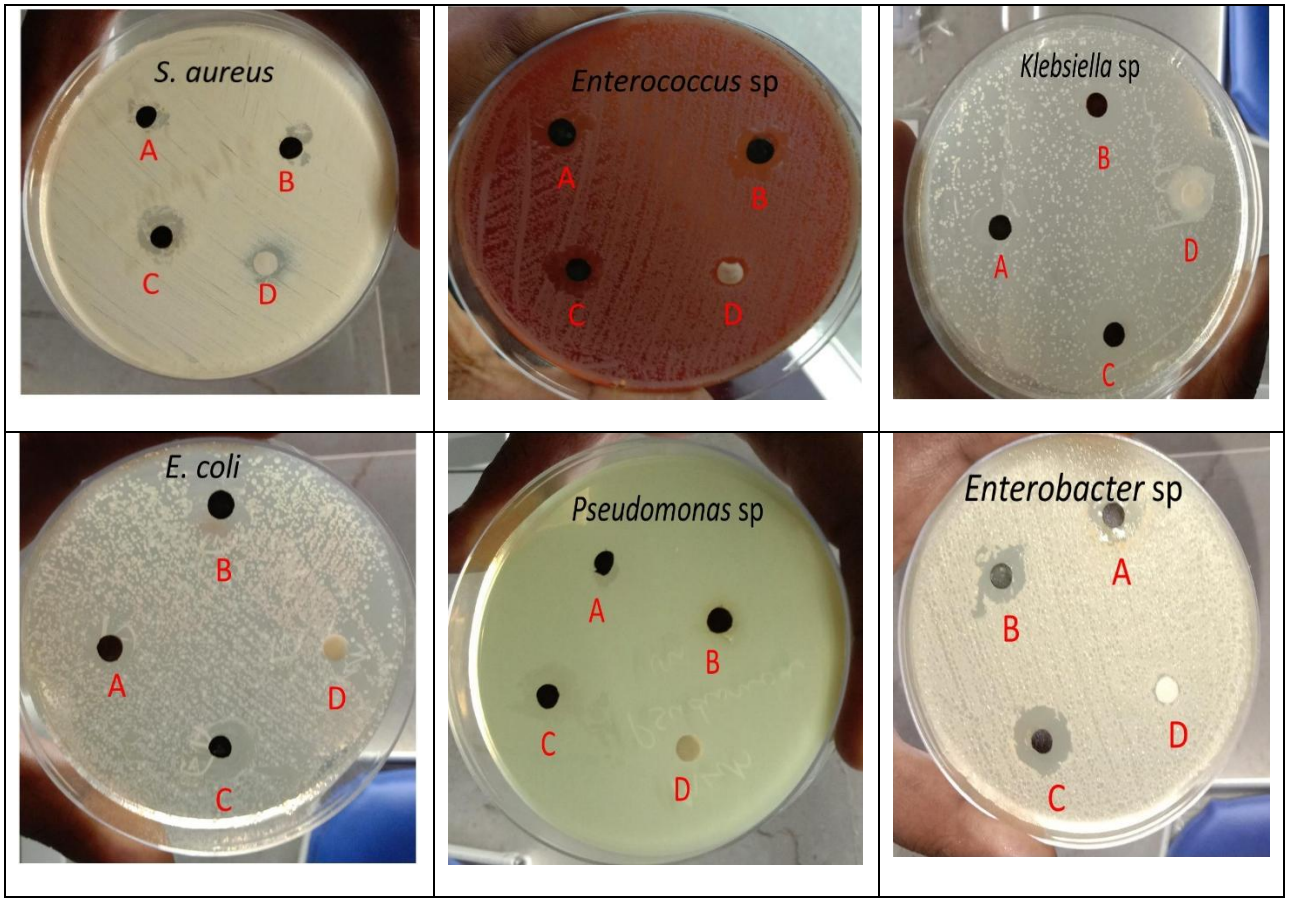
Figure 3. GC-MS chromatogram of of *Artocarpus hirsutus* leaf ethanol extract.

The GC-MS analysis provided deeper insights into the specific compounds present in *A. hirsutus* leaf extracts. The identification of Neophytadiene as a dominant compound is significant, as it has been reported to possess antimicrobial and anti-inflammatory properties Vinothini *et al.,* 2024. Similarly, the presence of n-Hexadecanoic acid and Phytol aligns with findings by Mainasara *et al*. (2019), who documented these compounds in *Artocarpus altilis* with antimicrobial properties. The study of *Artocarpus hirsutus* leaf extract's antibacterial activity against clinically isolated pathogens demonstrated significant efficacy, particularly with ethanol extracts. These extracts showed broad-spectrum activity against both Gram-positive and Gram-negative bacteria, with inhibition zones ranging from 9.33±1.15 mm against *S. aureus* to 16.33±1.15 mm against *E. coli*. Ethanol consistently yielded superior antimicrobial activity across all strains, suggesting the extraction of polar bioactive compounds.

Table 3. Antibacterial activities of *Artocarpus hirsutus* leaf extract.

Clinically isolated pathogens	Zone of Inhibition (mm)		
	Hexane	Ethyl acetate	Ethanol
S. aureus	6.66 ± 1.15^{a}	9.33 ± 1.15^{b}	15.66 ± 0.57^{c}
Enterococcus sp	8.0 ± 2.0^{a}	12.33 ± 1.15^{b}	15.66 ± 1.52^{c}
E. coli	7.1 ± 1^{a}	14 ± 1^{b}	16.33 ± 1.15^{c}
Klebsiella sp	6.6 ± 1.1^{a}	11.66 ± 1.52^{b}	13.33 ± 2.08^{c}
Pseudomonas sp	7.33 ± 0.57^{a}	10 ± 2.0^{b}	13 ± 1.0^{b}
Enterobacter sp	9 ± 1^{a}	12.66 ± 3.2^{b}	15 ± 0.57^{b}

Values are means ± SD; The same superscript within the same row indicates no significant difference (p>0.05).



A- Hexane extract B- Ethyl acetate extract C- Ethanol extract D- Negative Control (DMSO)

Figure 4. Antibacterial activity of *A. hirsutus* against clinically isolated pathogens.

The findings align with traditional medicinal use of *A. hirsutus* and highlight its potential in developing new antimicrobial agents to combat resistance. The significant antibacterial activity demonstrated by *A. hirsutus* leaf ethanol extracts, against clinically isolated pathogens underscores its potential as a source of novel antimicrobial agents to findings by Shanmugapriya *et al.* (2017). The broad-spectrum activity against both Gram-positive and Gram-negative bacteria is particularly significant given the different cell wall structures and inherent resistance mechanisms in these bacterial groups. As noted by Early *et al.* (2018) the outer membrane of Gram-negative bacteria typically presents a formidable barrier to many antimicrobial agents.

The ability of *A. hirsutus* extracts to effectively inhibit both bacterial groups suggests multiple mechanisms of action or the presence of compounds capable of penetrating the complex cell wall structure of Gram-negative bacteria. Interestingly, compounds isolated from *Artocarpus hirsutus* bark, such as Cudraflavone C and Artocarpin, play a crucial role in the antimicrobial activity, superior to conventional antibiotics, with remarkable resistance to bacterial adaptation (Meenu *et al.,* 2021 & 2022). The efficacy against multi-drug-resistant strains such as *S. aureus* and *Pseudomonas* sp is especially promising in the context of the global antimicrobial resistance crisis. These findings align with research by Pearce *et al.* (2021) who demonstrated the potential of plant-derived compounds to overcome conventional antibiotic resistance mechanisms. The Fourier Transform Infrared (FTIR) spectroscopic analysis of *Artocarpus hirsutus* leaf extract in ethanol revealed a rich chemical composition Figure 5. The spectrum exhibited key absorption bands associated with various functional groups, including aldehydes, alcohols, esters, and phenols. Notably, a prominent C=O stretching at 1903.09 cm^{-1} indicated the presence of aldehydes, and 1233.10 cm^{-1} corresponded to ester and carboxylic acid groups, respectively.

The C-C stretching observed at 1631.93 cm^{-1} and 1512.14 cm^{-1} suggested the presence of alkenes, and the C-H bending vibrations reflected alkane and alkene characteristics.

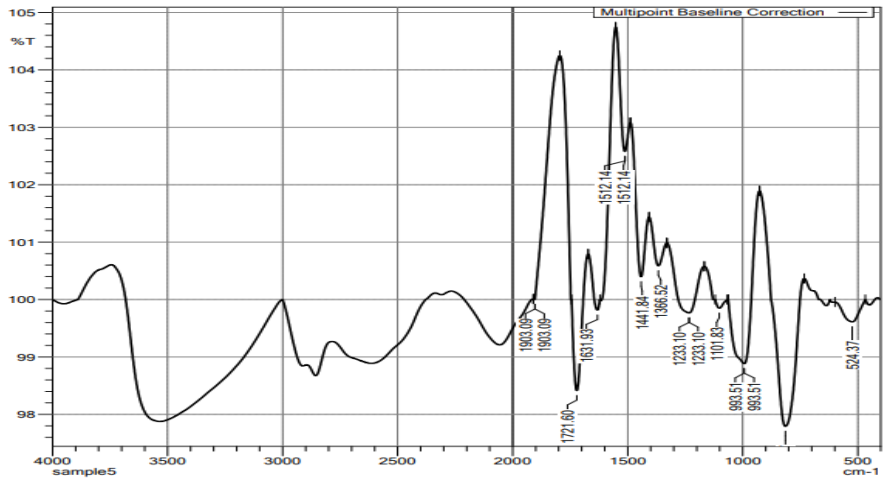


Figure 5. FTIR analysis of *Artocarpus hirsutus* leaf ethanol extract.

The presence of C-N bending and C-H bending further confirmed the presence of amines and amides in the sample. The FTIR spectroscopic analysis further corroborated the chemical diversity of *A. hirsutus* extracts, identifying functional groups associated with phenols, aldehydes, alcohols, and esters. These findings align with previous spectroscopic studies on Artocarpus species by Anjeela *et al.* (2024), who identified similar functional groups in *Artocarpus altilis* extracts with documented antimicrobial activity. The presence of phenolic compounds, indicated by characteristic FTIR peaks, is particularly relevant for antimicrobial activity, as phenolics are known to disrupt bacterial cell membranes and inhibit bacterial enzymes (Lobiuc *et al.,* 2023).

In the *Artocarpus hirsutus* toxicity study using Zebra fish, the results indicated that at the lethal concentration of ethyl acetate extract (LC50: 438.25 ppm at 24 hours), ethanol extract (LC50: 426.53ppm) at 24 hours. At the highest concentration of 1000 ppm, mortality rates were 22.2% for ethyl acetate and 100% for ethanol. Potassium dichromate served as the positive control. The LC50 value for the positive control at 24 hours was 15.23 ppm (Table 4). The toxicity assessment of *A. hirsutus* extracts using Zebra fish revealed differential toxicity profiles, with leaf extracts lethal concentration categorized as low toxic compared to Potassium dichromate after 24-hour exposure

Table 4. *Artocarpus hirsutus* extract lethal concentration on Zebra fish.

Sample	LC50 (ppm)	LC90 (ppm)	LC95 (ppm)
Ethyl acetate	438.25	892.64	967.76
Ethanol	426.53	885.53	1000.00
Potassium dichromate	15.23	26. 87	32.46

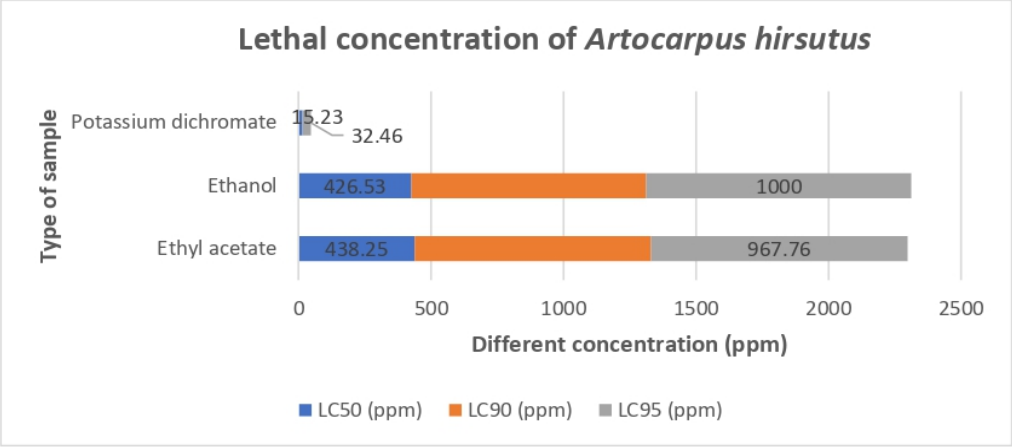


Figure 6. Lethal concentrations values of *Artocarpus hirsutus* leaf extracts.

The toxicity evaluation using Zebra fish provided crucial insights into the safety profile of *A. hirsutus* extracts. The relatively high LC_{50} values of ethanol extracts compared to the positive control suggest a favourable safety margin for potential therapeutic applications. The higher toxicity of ethanolic extracts at the maximum concentration (1000 ppm) didn't show any abnormal behavioural changes, mortality, and morbidity in the Zebrafish compared to ethyl acetate extracts likely reflects the greater extraction efficiency and concentration of bioactive compounds in the polar solvent, as suggested by JV *et al.* (2021) in their comparative toxicity studies. The moderate toxicity of ethanol extracts is attributed to alkaloids, flavonoids, terpenes, and phenolic compounds that readily penetrate the cytoplasmic membrane of Zebra fish, with Artocarpin known to possess toxicity effects in *Artocarpus hirsutus* (Thayumanavan *et al.,*2022). In contrast, *Artocarpus heterophyllus* extracts demonstrated significant non-toxic and no developmental defects on zebrafish embryos. (Kusumaningtyas *et al.,*2021). These findings provide a preliminary safety framework for future investigations into the therapeutic potential of *A. hirsutus* extracts.

CONCLUSION

Artocarpus hirsutus leaf extracts demonstrated significant antibacterial activity against multidrug-resistant clinical isolates, with ethanol extracts showing broad-spectrum efficacy. Phytochemical analysis identified bioactive compounds including neophytadiene, alkaloids, and flavonoids, validating traditional medicinal use. Clinical isolates exhibited alarming antibiotic resistance, emphasizing the need for alternative antimicrobials. Zebra fish toxicity studies revealed favourable safety profiles. This study establishes *A. hirsutus* as a promising natural antimicrobial candidate against antibiotic-resistant infections, warranting further clinical evaluation.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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