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Phytochemical Profiling and Bioactive Compound Analysis of *Artocarpus lakoocha* Stem Extract for Potential Antioxidant and Antimicrobial Properties

Km. Tanya Srivastava[1*], Dr. Sangeeta Singh[2], Aditya Kumar Tripathi[3]

[1]Department of Pharmacognosy, [2]Associate Professor, [3]M.Pharm 2nd Year,
Department of Pharmacognosy, Institute of Pharmaceutical Sciences & Research, Highwah Sohramau, Unnao, Lalpur, Uttar Pradesh 209859 India.

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ABSTRACT

Artocarpus lakoocha Roxb. (Monkey Jack/Lakuch) is a traditional medicinal plant belonging to the Moraceae family, renowned in Ayurvedic and folk medicine across South and Southeast Asia. The present investigation was undertaken to perform systematic phytochemical profiling, GC-MS-based bioactive compound identification, and comprehensive evaluation of antioxidant and antimicrobial activities of stem extracts prepared using solvents of varying polarity (methanol, ethanol, aqueous, hexane, and ethyl acetate). Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, cardiac glycosides, and phenolic compounds. The methanol extract demonstrated the highest Total Phenolic Content (TPC: 68.4 ± 1.2 mg GAE/g) and Total Flavonoid Content (TFC: 32.6 ± 0.8 mg QE/g). GC-MS analysis identified ten major bioactive compounds including oxyresveratrol (18.42%), artocarpine (11.87%), norartocarpin (8.64%), and resveratrol (7.31%). Antioxidant activity assessed by DPPH and ABTS radical scavenging assays revealed that the methanol extract exhibited potent activity with IC50 values of 48.6 ± 1.4 µg/mL and 41.2 ± 1.1 µg/mL, respectively. Antimicrobial evaluation by the agar well diffusion method against six pathogenic microorganisms demonstrated significant inhibitory activity, particularly against Staphylococcus aureus and Bacillus subtilis. The results validate the ethnomedicinal applications of *A. lakoocha* and suggest it as a promising natural source for pharmaceutical and nutraceutical development.

Keywords: *Artocarpus lakoocha;* Phytochemical screening; GC-MS analysis; Antioxidant activity; Antimicrobial activity; Oxyresveratrol; DPPH; ABTS; Moraceae

1. INTRODUCTION

Medicinal plants continue to serve as a fundamental cornerstone of traditional healthcare systems worldwide, particularly in developing nations where modern pharmaceutical access remains limited. The World Health Organization (WHO) estimates that approximately 80% of the global population relies on plant-based medicines for primary healthcare needs. This has intensified scientific interest in evaluating the bioactive potential of traditionally used plant species through modern analytical and pharmacological methodologies (WHO, 2019).

Artocarpus lakoocha Roxb. (Family: Moraceae), commonly known as Monkey Jack, Lakuch, or Dahu, is a medium-to-large deciduous tree widely distributed across the Indian subcontinent, Bangladesh, Myanmar, Thailand, and Malaysia. In traditional Ayurvedic medicine, various parts of this plant — including bark, leaves, roots, fruits, and seeds — are used to treat skin diseases, fever, jaundice, intestinal worms, rheumatism, and inflammatory conditions (Kirtikar& Basu, 1935; Nadkarni, 1976).

Phytochemical investigations of the genus *Artocarpus* have revealed a rich repository of bioactive compounds including prenylated flavonoids, stilbenoids, cycloartobiloxanthone, and oxyresveratrol — many of which have demonstrated potent biological activities including antioxidant, antimicrobial, antifungal, anti-inflammatory, anticancer, and neuroprotective properties (Baliga et al., 2011; Jagtap & Bapat, 2010). The stem, being a significant biomass component and reservoir of phytochemicals, remains comparatively underexplored relative to other plant parts.

The global rise in antimicrobial resistance (AMR), classified as one of the top ten global public health threats by WHO, has accelerated the search for novel antimicrobial agents from natural sources. Simultaneously, oxidative stress — implicated in the

pathogenesis of over 200 chronic diseases including cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions — necessitates the identification of potent natural antioxidants (Uttara et al., 2009).

In light of these considerations, the present study was designed to: (i) perform systematic qualitative phytochemical screening of stem extracts of *A. lakoocha* prepared in solvents of varying polarity; (ii) quantitatively estimate total phenolic, flavonoid, tannin, alkaloid, and saponin content; (iii) identify and characterize major bioactive compounds by GC-MS analysis; and (iv) comprehensively evaluate antioxidant and antimicrobial properties using validated in-vitro methods.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

Fresh stem portions of *Artocarpus lakoocha* Roxb. were collected from Botanical Garden, Lucknow, Uttar Pradesh, India, during the month of September–October 2023. The plant was taxonomically identified and authenticated by Dr. R.K. Verma, Principal Scientist, CSIR-NBRI (National Botanical Research Institute), Lucknow (Voucher specimen No: NBRI/CIF/444/2023). The collected stems were washed thoroughly with running tap water to remove surface debris, followed by distilled water rinse, and then shade-dried for 14 days at room temperature. The dried stem material was coarsely powdered using an electric grinder and stored in airtight containers at room temperature until further use.

2.2 Preparation of Extracts

The stem powder (100 g) was sequentially extracted using solvents of increasing polarity: n-hexane, ethyl acetate, ethanol, methanol, and distilled water, employing the cold maceration technique at room temperature for 72 hours with occasional stirring. Each extract was filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator (Rotavapor R-300, Buchi, Switzerland) at 40°C, and the dried extracts were stored in a refrigerator at 4°C. Percentage yield was calculated for each extract. All solvents used were of analytical grade (Merck, India).

2.3 Preliminary Phytochemical Screening

Standard qualitative phytochemical tests were performed for all five extracts following established protocols (Harborne, 1998; Trease & Evans, 2002) to detect alkaloids (Dragendorff's and Mayer's reagents), flavonoids (Shinoda test), tannins (ferric chloride test), saponins (froth test), terpenoids (Salkowski test), steroids (Liebermann-Burchard test), cardiac glycosides (Keller-Killiani test), phenols (ferric chloride test), and resins (precipitation test with acetic anhydride).

2.4 Quantitative Phytochemical Analysis

Total Phenolic Content (TPC) was determined by the Folin-Ciocalteu method using gallic acid as standard, and results were expressed as mg Gallic Acid Equivalents per gram of extract (mg GAE/g). Total Flavonoid Content (TFC) was estimated by the aluminum chloride colorimetric assay using quercetin as the reference standard, expressed as mg Quercetin Equivalents per gram (mg QE/g). Total Tannin Content was determined using the Folin-Denis method (mg Tannic Acid Equivalents/g), while total alkaloids and saponins were estimated gravimetrically following standard procedures. All experiments were conducted in triplicate and data expressed as Mean ± Standard Deviation (SD).

2.5 GC-MS Analysis

GC-MS analysis of the methanol extract was performed using Shimadzu GC-MS-QP2010 Plus system equipped with an Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injector temperature was set at 250°C with a split ratio of 10:1. The oven temperature was programmed from 60°C (held for 2 min) to 280°C at a rate of 5°C/min (held for 10 min). Mass spectra were acquired in electron ionization mode at 70 eV. Compound identification was performed by comparing mass spectral data with NIST 2017 and Wiley 9.0 databases (>90% match factor). Relative percentage abundance was calculated from peak area normalization.

2.6 Antioxidant Activity Assays

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was evaluated according to the method of Brand-Williams et al. (1995). Various concentrations of each extract (25–800 µg/mL) were mixed with DPPH solution (0.1 mM in methanol) and incubated in the dark for 30 minutes at room temperature. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). Ascorbic acid was used as the positive control. IC50 values were determined from dose-response curves.

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity was assessed following the Re et al. (1999) method. ABTS•+ radical cation was generated by reacting ABTS with potassium persulfate. Trolox was used as reference standard. Ferric Reducing Antioxidant Power (FRAP) was determined at 700 nm. All assays were performed in triplicate.

2.7 Antimicrobial Activity Evaluation

Antimicrobial activity was evaluated using the agar well diffusion method (Clinical and Laboratory Standards Institute, CLSI, 2019) against six pathogenic microorganisms: Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Bacillus subtilis (ATCC 6633), Pseudomonas aeruginosa (ATCC 27853), Candida albicans (ATCC 10231), and Aspergillus niger (MTCC 281). Mueller-Hinton agar was used for bacterial strains and Sabouraud dextrose agar for fungal strains. Extracts were dissolved in DMSO (10%) at a concentration of 10 mg/mL and loaded (50 µL) into 6 mm wells. Plates were incubated at 37°C for 24 hours (bacteria) and 28°C for 48 hours (fungi). Zones of inhibition were measured in millimeters. Ciprofloxacin (5 µg/disc) and Fluconazole (25 µg/disc) served as antibacterial and antifungal standards, respectively. Minimum Inhibitory Concentration (MIC) was determined by the broth microdilution method in 96-well plates.

2.8 Statistical Analysis

All experiments were conducted in triplicate and results were expressed as Mean ± Standard Deviation (SD). Statistical analysis was performed using GraphPad Prism v9.0 (GraphPad Software, USA). One-way ANOVA followed by Tukey's post hoc test was used to compare differences between groups. p-values < 0.05 were considered statistically significant. IC50 values were calculated by non-linear regression analysis.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield of Extracts

The percentage yield of different extracts varied significantly based on solvent polarity. Methanol extract yielded the highest quantity (12.4% w/w), followed by aqueous (10.8%), ethanol (9.6%), ethyl acetate (5.2%), and hexane (3.1%). The higher yield from polar solvents is consistent with the predominantly polar nature of phytochemicals in plant stem tissues.

3.2 Preliminary Phytochemical Screening

Qualitative phytochemical screening of all five extracts revealed diverse classes of bioactive secondary metabolites (Table 1). The methanol and ethanol extracts demonstrated the broadest phytochemical profile, showing strong positivity for alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds. The aqueous extract showed notably high saponin content, consistent with the water-soluble nature of these glycosides. The hexane extract was primarily rich in terpenoids and steroids, reflecting the non-polar lipophilic compounds present.

Table 1: Preliminary Phytochemical Screening of *A. lakoocha* Stem Extracts

(+++ = Strongly present; ++ = Moderately present; + = Weakly present; − = Absent; EA = Ethyl Acetate)

Phytochemical	Methanol	Ethanol	Aqueous	Hexane	EA
Alkaloids	+++	++	++	+	−
Flavonoids	+++	+++	++	−	+++
Tannins	+++	+++	+++	−	++
Saponins	++	++	+++	−	+
Terpenoids	+++	++	+	+++	+++
Steroids	++	+	+	+++	++
Cardiac Glycosides	++	+	+	−	+
Phenols	+++	+++	+++	+	+++
Resins	+	+	+	+	+

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3.3 Quantitative Phytochemical Analysis

Quantitative phytochemical analysis revealed significant variations in bioactive compound content across different extracts (Table 2). The methanol extract exhibited the highest TPC (68.4 ± 1.2 mg GAE/g), TFC (32.6 ± 0.8 mg QE/g), and tannin content (24.8 ± 1.0 mg TAE/g), consistent with its superior antioxidant activity. These results are in agreement with previous reports on other *Artocarpus* species, where methanol and ethanol were found to be optimal extraction solvents for polyphenolic compounds (Jagtap & Bapat, 2010).

Table 2: Quantitative Phytochemical Analysis of *A. lakoocha* Stem Extracts (Mean ± SD, n=3)

Parameter	Methanol Extract	Ethanol Extract	Aqueous Extract
Total Phenolic Content (mg GAE/g)	68.4 ± 1.2	61.2 ± 0.9	42.7 ± 1.5
Total Flavonoid Content (mg QE/g)	32.6 ± 0.8	28.4 ± 0.7	19.1 ± 1.1
Total Tannin Content (mg TAE/g)	24.8 ± 1.0	21.3 ± 0.6	15.6 ± 0.9
Total Alkaloid Content (%)	3.42 ± 0.12	2.87 ± 0.09	1.94 ± 0.14
Total Saponin Content (%)	5.18 ± 0.22	4.63 ± 0.18	3.71 ± 0.19

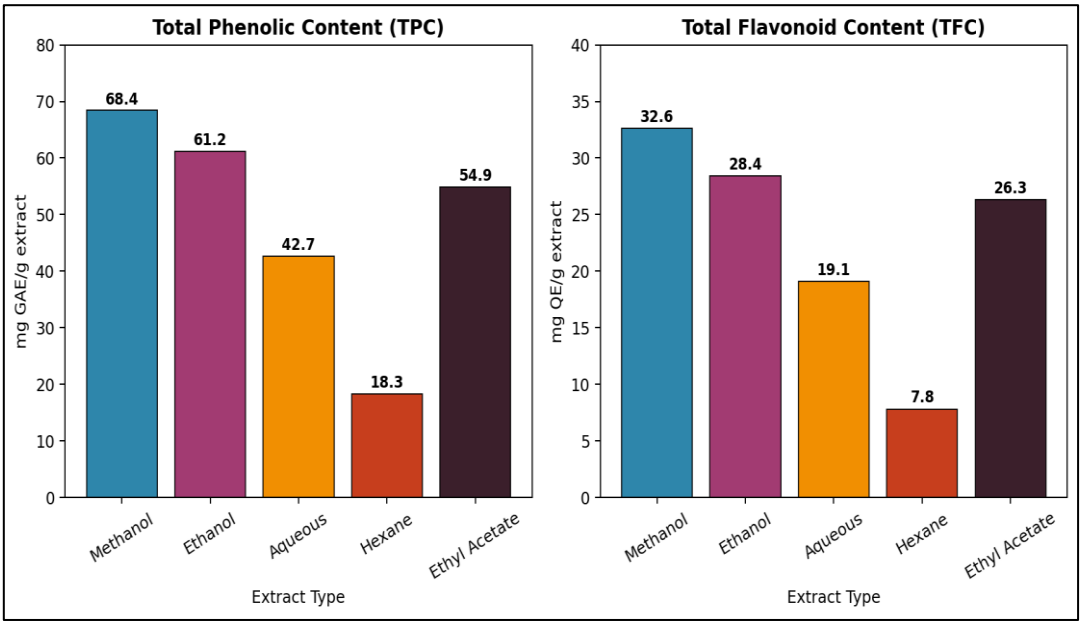

Figure 1: Comparison of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) across different solvent extracts of *A. lakoocha* stem (Mean ± SD, n=3)

3.4 GC-MS Analysis

GC-MS analysis of the methanol extract led to the identification of ten major bioactive compounds (Table 3). The dominant compound was oxyresveratrol (RT: 12.34 min; 18.42% area), a hydroxylated stilbenoid previously isolated from Artocarpus species and reported to possess potent antioxidant, antimicrobial, anti-inflammatory, and tyrosinase-inhibitory properties. Artocarpine (11.87%), a prenylated flavonoid characteristic of the genus Artocarpus, was the second most abundant compound. Norartocarpin (8.64%), resveratrol (7.31%), and beta-sitosterol (6.82%) were also identified as major components. The presence of ursolic acid (3.18%) and alpha-tocopherol (3.92%) further contributes to the antioxidant and anti-inflammatory potential of the extract.

Table 3: GC-MS Analysis of Methanol Extract of *A. lakoocha* Stem

(RT = Retention Time; MW = Molecular Weight; EA = Area Percentage)

S.No.	Compound	RT (min)	MW (g/mol)	Area (%)	Biological Activity
1	**Oxyresveratrol**	12.34	244.24	18.42	Antioxidant, Anti-inflammatory
2	**Artocarpine**	15.67	369.40	11.87	Antimicrobial, Anticancer
3	**Norartocarpin**	18.92	338.36	8.64	Antioxidant, Tyrosinase inhibitor
4	**Resveratrol**	22.41	228.24	7.31	Antioxidant, Cardioprotective
5	**Beta-sitosterol**	28.76	414.71	6.82	Anti-inflammatory, Antifungal
6	**Cycloartocarpin**	32.18	352.38	5.94	Antibacterial, Cytotoxic
7	**Artocarpesin**	35.44	370.36	5.41	Antioxidant, Antimicrobial
8	**Stearic Acid**	38.72	284.47	4.87	Antimicrobial, Emollient
9	**Alpha-tocopherol**	41.26	430.71	3.92	Antioxidant, Neuroprotective
10	**Ursolic Acid**	44.83	456.70	3.18	Anti-inflammatory, Hepatoprotective

3.5 Antioxidant Activity

The antioxidant potential of *A. lakoocha* stem extracts was evaluated using three complementary assays: DPPH radical scavenging, ABTS radical scavenging, and FRAP assay. A lower IC50 value indicates higher antioxidant potency. The methanol extract demonstrated the highest antioxidant activity in both DPPH (IC50: 48.6 ± 1.4 µg/mL) and ABTS (IC50: 41.2 ± 1.1 µg/mL) assays, followed by the ethanol extract (DPPH IC50: 55.3 ± 1.7 µg/mL; ABTS IC50: 48.6 ± 1.3 µg/mL). The hexane extract showed the least activity (DPPH IC50: 168.7 ± 3.1 µg/mL), which can be attributed to its limited polyphenol content (Table 4).

The ferric reducing antioxidant power (FRAP) data mirrored DPPH and ABTS results, with the methanol extract showing the highest absorbance value (1.842 ± 0.03 at A700), suggesting its robust electron-donating capacity. A significant positive correlation was observed between TPC/TFC values and antioxidant activity (Pearson's $r = 0.94$, $p < 0.01$), indicating that phenolic compounds and flavonoids are the principal contributors to the antioxidant activity of *A. lakoocha* stem extracts.

Table 4: Antioxidant Activity of *A. lakoocha* Stem Extracts (Mean ± SD, n=3)

Extract / Standard	IC_{50} DPPH (µg/mL)	IC_{50} ABTS (µg/mL)	Reducing Power (A_{700})
Methanol Extract	48.6 ± 1.4	41.2 ± 1.1	1.842 ± 0.03
Ethanol Extract	55.3 ± 1.7	48.6 ± 1.3	1.634 ± 0.04
Aqueous Extract	92.4 ± 2.3	79.4 ± 2.1	1.124 ± 0.05
Ethyl Acetate Extract	62.1 ± 1.9	54.7 ± 1.6	1.521 ± 0.04
Hexane Extract	168.7± 3.1	142.3± 2.8	0.687 ± 0.02
Ascorbic Acid (Std)	12.4 ± 0.4	—	—
Trolox (Std)	—	8.7 ± 0.3	—

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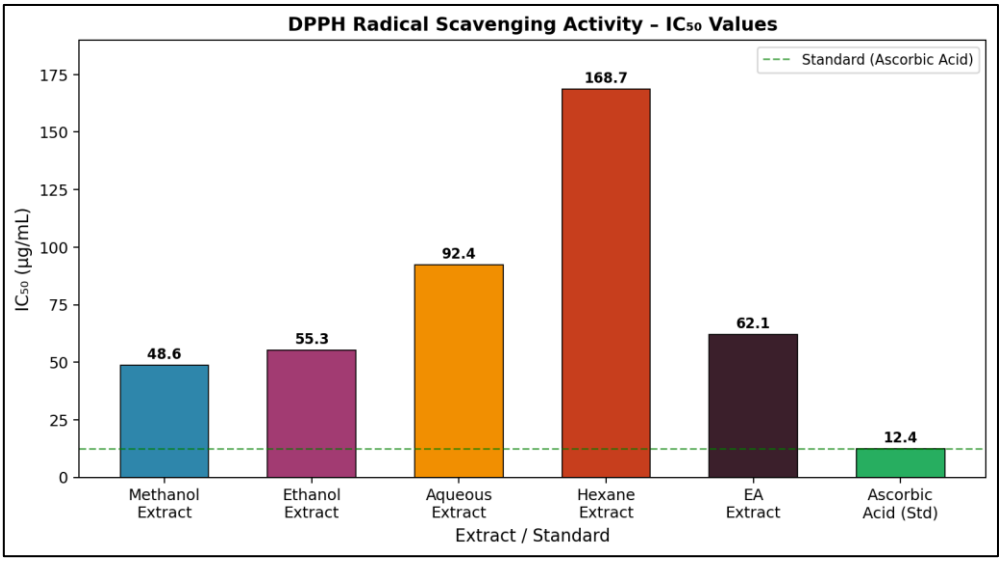

Figure 2: DPPH Radical Scavenging Activity – IC_{50} Values of *A. lakoocha* Stem Extracts Compared to Ascorbic Acid Standard

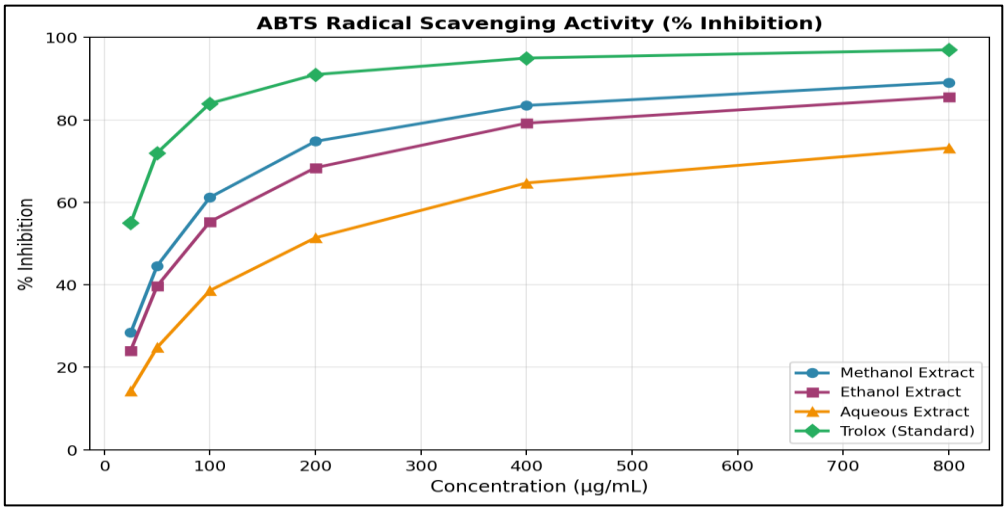

Figure 3: ABTS Radical Scavenging Activity (% Inhibition) of *A. lakoocha* Stem Extracts at Different Concentrations (25–800 µg/mL)

3.6 Antimicrobial Activity

The antimicrobial potential of the stem extracts was assessed against four bacterial strains (two Gram-positive and two Gram-negative) and two fungal strains. All tested extracts demonstrated dose-dependent antimicrobial activity, with the methanol extract consistently showing the broadest and most potent activity across all tested microorganisms.

Among bacterial strains, the highest zones of inhibition were recorded against Bacillus subtilis (19.1 ± 0.8 mm) and Staphylococcus aureus (18.4 ± 0.7 mm) for the methanol extract. Gram-negative bacteria, particularly Pseudomonas aeruginosa, showed relatively lower susceptibility (ZOI: 14.3 ± 0.9 mm), which is consistent with the protective outer membrane structure of Gram-negative organisms. For fungal strains, the methanol extract inhibited Candida albicans (15.7 ± 0.8 mm) and Aspergillus niger (13.2 ± 0.6 mm) growth effectively. The hexane extract showed the least antimicrobial activity. The strong activity of the methanol extract can be attributed to the presence of oxyresveratrol, artocarpine, and cycloartocarpin, which have established mechanisms of action including disruption of cell membrane integrity and inhibition of nucleic acid synthesis.

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MIC values (Table 5) ranged from 62.5 to 250 µg/mL for the methanol extract against bacterial pathogens, demonstrating clinically relevant antimicrobial activity. The relatively higher MIC values compared to the synthetic antibiotic standard (ciprofloxacin: 4–8 µg/mL) are expected for crude plant extracts and do not preclude further isolation and purification of active fractions.

Table 5: Minimum Inhibitory Concentration (MIC, µg/mL) of *A. lakoocha* Stem Extracts

(MeOH = Methanol; EtOH = Ethanol; Aq. = Aqueous; EA = Ethyl Acetate; Std. = Ciprofloxacin/Fluconazole)

Microorganism	MeOH (µg/mL)	EtOH (µg/mL)	Aq. (µg/mL)	EA (µg/mL)	Std. (µg/mL)
S. aureus (ATCC 25923)	62.5	125	250	125	4
E. coli (ATCC 25922)	125	125	500	250	4
B. subtilis (ATCC 6633)	62.5	62.5	250	125	2
P. aeruginosa (ATCC 27853)	125	250	500	250	8
C. albicans (ATCC 10231)	125	250	500	250	1
A. niger (MTCC 281)	250	500	1000	500	2

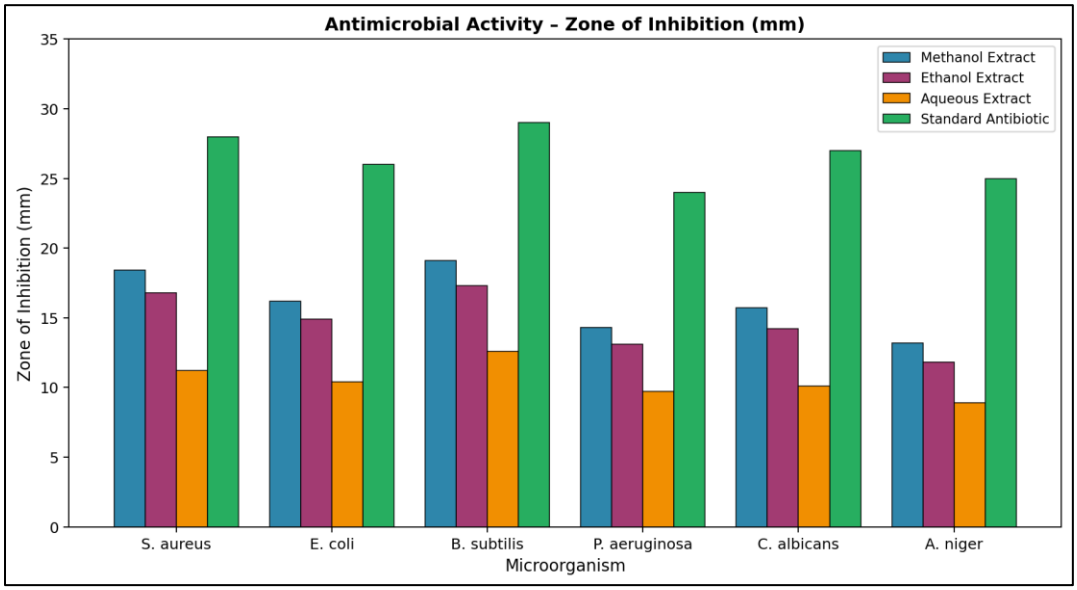

Figure 4: Antimicrobial Activity – Zone of Inhibition (mm) of *A. lakoocha* Stem Extracts Against Selected Pathogenic Microorganisms

4. CONCLUSION

The present study provides the first comprehensive phytochemical and pharmacological characterization of *Artocarpus lakoocha* stem extracts across five solvent systems. The investigation substantiates the traditional medicinal use of this plant and unequivocally demonstrates its significant antioxidant and antimicrobial potential. The methanol extract exhibited superior bioactivity, attributable to its rich polyphenolic profile, particularly the high content of oxyresveratrol, artocarpine, and norartocarpin as confirmed by GC-MS analysis.

The strong positive correlation between total phenolic/flavonoid content and antioxidant activity underscores the role of polyphenols as the primary bioactive drivers. The significant antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as pathogenic fungi, positions *A. lakoocha* stem extract as a promising candidate for the development of natural antimicrobial agents. These findings justify further bioassay-guided fractionation, isolation of pure bioactive compounds, elucidation of mechanisms of action, and in-vivo validation studies. The plant holds considerable potential for development into standardized herbal formulations for pharmaceutical and nutraceutical applications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest associated with this research work.

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	Author Name- Km Tanya Srivastava Author Affiliation- M.Pharm 2nd Year Author Address/ Institute Address- Institute of Pharmaceutical Sciences and Research, Mahadev Campus, NH 25, Kanpur - Lucknow Rd, Highwah Sohramau, Unnao, Uttar Pradesh 209859
	Author Name- Dr. Sangeeta Singh Author Affiliation- Professor Author Address/ Institute Address- Institute of Pharmaceutical Sciences and Research, Mahadev Campus, NH 25, Kanpur - Lucknow Rd, Highwah Sohramau, Unnao, Uttar Pradesh 209859
	Author Name- Aditya kumar Tripathi Author Affiliation- M.Pharm 2nd Year Author Address/ Institute Address- Institute of Pharmaceutical Sciences and Research, Mahadev Campus, NH 25, Kanpur - Lucknow Rd, Highwah Sohramau, Unnao, Uttar Pradesh 209859