

# Exploring Wide Range of Antimicrobial, Antioxidant and Cytotoxicity Profile of *Acacia chundra*. (Roxb) DC (Kheri Plant)

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## ABSTRACT

**Background:** *Acacia chundra* (Roxb.) DC (AC) is a xerophytic habitat plant. It is one of the important medicinal plants found in Asia, India and in the Indian Ocean area. In the present study, phytochemical analysis, antimicrobial, antioxidant and cytotoxicity potential of three solvent extracts of three parts (Leaves, Bark and Flower) of AC were assessed. **Materials and Methods:** The antimicrobial potential was tested against 11 pathogenic bacteria and 4 pathogenic fungi by using REM assay, Antioxidant potential was assessed by DPPH and OH radical scavenging assay while cytotoxicity was tested by using Hemolytic assay. **Results:** Qualitative phytochemical analysis revealed the presence of alkaloids, saponins, tannins, steroids, flavonoids, phenolics and terpenoids in all extracts except ACBC. In disk diffusion assay, all crude extracts samples possess good inhibitory potential against tested bacterial pathogens except ACLC, ACBC and ACFC. While ACBC doesn't exhibited any antifungal potential whereas ACLE and ACFW showed low and remaining have moderate to good antifungal potential. Minimum inhibitory concentration was calculated by using REM assay manifest that ACLE, ACBE, and ACFE showed best MIC ( $3.25 \pm 0.01 \mu\text{g}/\text{mL}$ ) results towards selected panel of bacterial pathogens EC, BM, BS, PA, PAC, EF while all other extracts showed moderate to good ( $6.5 \pm 0.02 \mu\text{g}/\text{mL}$  to  $208.33 \pm 0.72 \mu\text{g}/\text{mL}$ ). On the other side, ACLC, ACBW, ACFE and ACFC showed good MIC ( $3.25 \pm 0.01 \mu\text{g}/\text{mL}$ ) results towards CA, AF, RHS and remaining have moderate to low ( $6.5 \pm 0.02$  to  $416.66 \pm 0.144 \mu\text{g}/\text{mL}$ ) inhibitory potential. The presence of phytochemical diversity was characterized by FTIR and UV spectrum which reveals presence of diverse phytochemicals. **Conclusion:** The tested plant samples showed the best antimicrobial activity and so forth antioxidant and negligible cytotoxicity. These results suggest that phytochemicals present in the plants are effective against a range of bacterial pathogens including both Gram-positive and Gram-negative pathogens and fungal pathogens which causes wide infections to humans including oral, dermal, pulmonary and genital.

**Keywords:** Antimicrobial, *Acacia chundra* (Roxb.) DC, Biological, FTIR, Phytochemical.

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**Received:** 26-12-2025;

**Revised:** 16-02-2026;

**Accepted:** 03-04-2026.

## INTRODUCTION

Plants have played a significant role in the treatment of various diseases for centuries. In fact, more than 90% of prescribed medicines have their origins in plants. However, the overuse of antibiotics has led to a surge in side effects and the emergence of antibiotic-resistant bacterial strains (Salam *et al.*, 2023).

To address this growing concern, researchers have turned to traditional herbal remedies as a potential source for the development of new chemotherapy drugs. These herbal remedies offer a fascinating and largely unexplored avenue for combating resistance and reducing the toxicity associated with commercially available antibiotics (Li *et al.*, 2024). Studies have shown that extracts derived from medicinal plants possess potent antimicrobial properties, making them effective against pathogenic bacteria that affect humans. Furthermore, these extracts exhibit minimal adverse effects on the body. Numerous plants have been extensively investigated for their antimicrobial and antioxidant activities, as well as their potential toxicity. By harnessing the therapeutic potential of these plants, researchers aim to develop safer and more effective treatments for infectious



DOI: 10.5530/pres.20260237

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diseases (Saravanan *et al.*, 2017; Makwana *et al.*, 2015; Hidera *et al.*, 2024; Antonelo *et al.*, 2021).

Acacia species, a fascinating medicinal source, possesses a multitude of therapeutic properties. The bark of *Acacia chundra* (AC) plant exhibits remarkable antioxidant, astringent, anti-inflammatory, anti-bacterial, and antifungal characteristics. Its extract is commonly employed in the treatment of various ailments, including sore throats, diarrhea, high blood pressure, dysentery, colitis, gastric problems, bronchial asthma, cough, and leprosy. Furthermore, it serves as an effective mouthwash for oral and dental infections, gum issues, and sore throats. The heartwood of AC yields a concentrated aqueous extract known as cutch, which displays astringent, cooling, and digestive properties. This extract finds utility in addressing coughs, ulcers, boils, and skin eruptions (Seigler *et al.*, 2003). Additionally, the decoction of the plant's bark is administered internally for the management of leprosy. It is worth noting that *Acacia* spp. produces gum exudates, commonly referred to as gum Arabic or gum Acacia, which are extensively utilized in the food industry as emulsifiers, adhesives, stabilizers, and even in cases of chronic renal failure (Ali *et al.*, 2009).

Phytochemicals, which possess antimicrobial and antioxidant properties, hold immense potential in suppressing both plant and human diseases. An antioxidant is a molecule that donates electrons or inhibits reduction reactions. These compounds, characterized by their small molecular weights, can effectively neutralize free radicals and reactive molecules, thereby preventing degenerative diseases. In addition to antibacterial derived from natural sources like plants, spices, and microorganisms, recent discoveries have unveiled a plethora of antibacterial agents from these natural ingredients (Chinedum *et al.*, 2016). Notably, AC has been found to contain a significant amount of antioxidant components and antimicrobial molecules, which could aid in the prevention of various diseases. Recently, there has been a growing interest in studying and investigating medicinal plants to validate their activities and develop safe alternatives to synthetic drugs. Medicinal plants offer low-cost production processes and pose fewer environmental hazards, side effects, and toxicities compared to synthetic drugs (Tomayo *et al.*, 2024). The primary objective of this study is to conduct a comprehensive assessment of the pharmacognostic screening, phytochemical analysis, antimicrobial activity, and antioxidant potential of the extract derived from AC leaves, bark and flowers collected from Maharashtra, India. By delving into these aspects, we aim to gain deeper insight into the medicinal properties and therapeutic potential of this plant species (Antonelo *et al.*, 2023).

The results of the study revealed that the ethanol extract obtained from AC leaves, bark and flowers exhibited significant antibacterial activity against human pathogenic bacteria. Additionally, the extract demonstrated potent antioxidant

activity, suggesting its potential in preventing degenerative diseases. The pharmacognostic screening and phytochemical analysis unveiled the presence of various bioactive compounds, including flavonoids, alkaloids, and phenolic compounds. These compounds are renowned for their antimicrobial and antioxidant properties, making them promising candidates for combating both plant and human diseases. The findings imply that AC holds promise as a valuable resource for the development of novel chemotherapy drugs, addressing the challenges associated with resistance and toxicity observed in current commercial antibiotics. Furthermore, the low-cost production processes, minimal environmental hazards, and reduced side effects and toxicity of medicinal plants position them as safe alternatives to synthetic drugs.

## MATERIALS AND METHODS

### Collection and authentication of plant samples

In September 2023, fresh leaves, bark, and flowers of *Acacia chundra* (AC) were collected from the Swami Ramanand Teerth Marathwada University campus, Nanded, Maharashtra, India. The plant specimens were authenticated by a taxonomist, and voucher specimens were deposited in the Herbarium, Department of Botany, School of Life Sciences, Swami Ramanand Teerth Marathwada University). The plant materials (leaves, bark and flowers) were meticulously washed with running tap water, followed by sterilized distilled water, to remove contaminants. The samples were then shade-dried at  $25\pm 2^\circ\text{C}$  to preserve bioactive compounds, and the dried materials were finely powdered using a sterilized pestle and mortar. The powdered samples were stored in airtight containers at  $4^\circ\text{C}$  until further use.

### Preparation of plant extract

The powdered plant samples (100 g/500 mL) were extracted successively with Solvents (water, chloroform and ether) using Soxhlet apparatus at  $55\text{--}85^\circ\text{C}$  for 8-10 hr in order to extract the polar and non-polar compound. For each solvent extraction, the powdered pack material was air dried and then used. The solvents of the respective extracts were stored at  $4^\circ\text{C}$  for further use. To analyze *in vitro* antimicrobial and antioxidant activity of plant extract dissolve the dried extracts in dimethyl sulfoxide and made the solution of 10 mg/10 mL (Hidera *et al.*, 2024).

### Material, Chemicals and reagents

To evaluate the antimicrobial, antioxidant, and cytotoxic potential of *Acacia chundra* extracts, the following materials and reagents were used. Dragendorff's reagent,  $\text{FeCl}_3$ , glacial acetic acid, Terpenoids, HCL, Sulphuric acid, DPPH (2,2-Diphenyl-1-Picryl-Hydrazil) (Sigma Aldrich), paper disk (Sterile Susceptibility test disk SD067 Himedia Labs. Pvt. Ltd., Mumbai), Mueller-Hinton agar/ Potato Dextrose agar medium, Mueller-Hinton / Potato Dextrose broth medium, Alamer blue solution (0.01% in sterile

D/W), Antibiotics (Streptomycin, Ethambutol, Ampicillin, Fluconazole) (Himedia Labs. Pvt. Ltd., Mumbai),

**Plant samples:** *Acacia chundra* (AC). Collected from S.R.T. M. University Nanded campus.

### Test microorganisms

All Microbial cultures were procured from the Indian Institute of Microbial Technology Chandigarh India. **Bacterial culture:** *Escherichia coli* MCC 2412, *Bacillus subtilis* MCC 2048, *Bacillus megaterium* MCC 1684, *Staphylococcus aureus* MCC 2408, *Klebsiella* spp., *Shigella* spp., *Pseudomonas aeruginosa* MCC 2081, *Staphylococcus epidermidis* MCC 435, *Propionibacterium acnes* MCC 1951, *Enterococcus faecalis* MCC 2409, *Mycobacterium tuberculosis* MCC 300. **Fungal culture:** *Candida albicans* NIH 3147, *Aspergillus niger* MCC 281, *Aspergillus flavus* MCC 281, *Rhizopus* spp. MCC 262.

### Phytochemical analysis

Preliminary phytochemical screening of the leaves, bark and flower solvent extracts of AC was performed as per standard procedure for identifying secondary metabolites in plants. The presence or absence of Alkaloids, saponins, tannins, steroids, flavonoids, phenolics and terpenoids have been examined by conducting tube test methods (Hidera *et al.*, 2024).

### Fourier transform infrared spectroscopy (FTIR)

Functional group and covalent bonding information were detected using FTIR spectroscopic analysis (Cary 630, Agilent Technologies, Virginia, USA). The built-in Resolution Pro software (2.5.5, Agilent) was used to analyze the data. (Antunes *et al.*, 2024; Yassin *et al.*, 2024).

### UV- spectrum analysis

UV visible absorption studies were performed with Shimadzu spectrophotometer (model UV1900i, Shimadzu Corporation, Japan) using matched quartz cuvettes having path length of 1 cm. The phytoextracts was monitored by using UV-vis spectrum at wavelength 400-800 nm. As per shown in Figure 4.

### Antimicrobial activity

#### Disk diffusion assay

The antimicrobial potential of the plant extract was assessed using the disk diffusion assay (More *et al.*, 2018). The assay was performed for every bacterial species on freshly prepared Muller Hinton solidified agar medium. Here, we have followed the standard guidelines given by Clinical and Laboratory Standards Institute (CLSI). For disk diffusion assay Sterile paper disks (Sterile Susceptibility test disk SD067 Himedia Labs. Pvt. Ltd.) were prepared, each loaded with 50  $\mu$ L of the individual plant extract sample at a concentration of 1 mg/mL. These disks were placed on the surface of sterile Mueller-Hinton agar or Potato

Dextrose agar medium, which had been previously inoculated with bacterial and fungal cultures Streptomycin (for *E. coli*, *B. subtilis*, *B. megaterium*, *S. aureus*, *P. aeruginosa*), Ethambutol for *M. tuberculosis*), Ampicillin (for *Shigella*, *P. acne*, *S. epidermidis*, *E. faecalis*) and fluconazole (for *A. niger*, *C. albicans*, *A. flavous*, *Rhizopus* spp.) were used as standard references at a concentration of 1 mg/mL. After 3 hr of refrigerated diffusion, the plates were transferred to an incubator set at 37°C and 30°C for 24 hr. Following incubation, the zones of inhibition around the paper disks were measured using a zone scale (Himedia Pvt. Ltd., Mumbai). This allowed us to assess the effectiveness of the test compounds against the bacterial and fungal strains.

### Resazurin Microtiter Assay (REM) for MIC evaluation

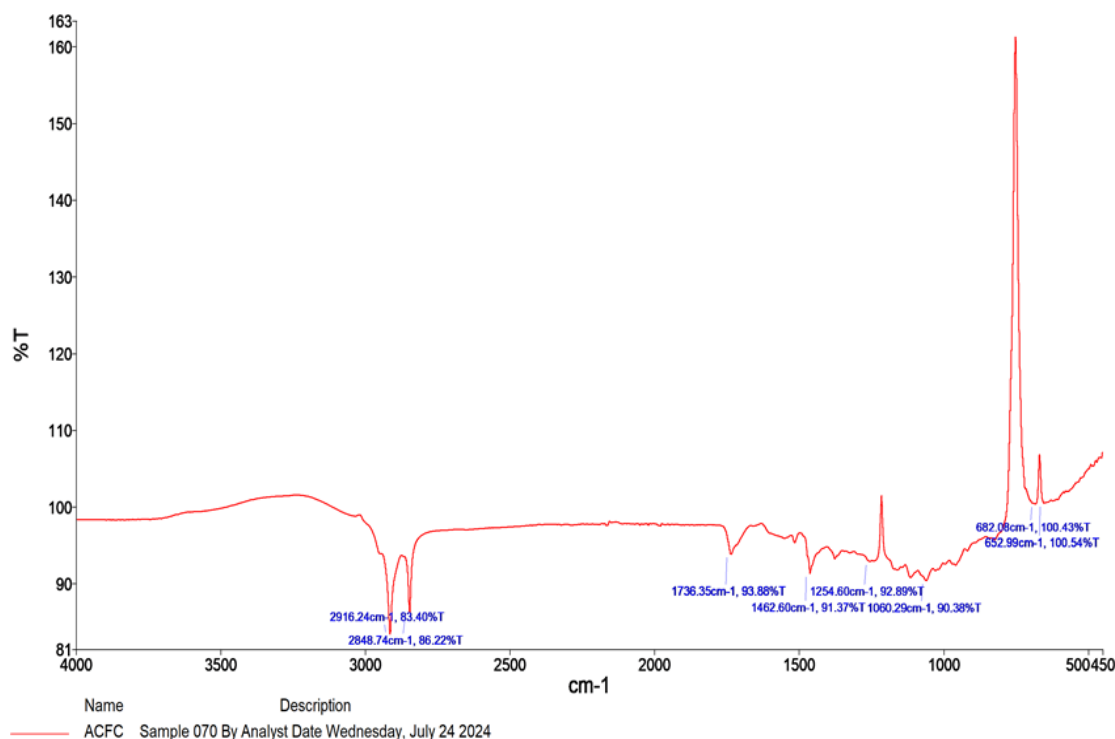
The REMA plate assay was conducted as follows: While performing REM assay and calculating the MIC's CLSI guidelines followed. 100  $\mu$ L of Mueller-Hinton or Potato Dextrose broth medium was aseptically dispensed into each well of a sterile flat-bottom 96-well plate. Serial two-fold dilutions of the test sample were directly prepared in the plate. Subsequently, 100  $\mu$ L of inoculum (0.5 McFarland standards, approximately equal to  $1.5 \times 10^8$  CFU/mL) was added to each well. To prevent evaporation during incubation, sterile cold water was added to the perimeter wells. The plate was then covered with a sterile lid and incubated at 37°C. After 24 hr of incubation, 30  $\mu$ L of alamar blue solution (0.01% in sterile distilled water) from Himedia Labs Pvt. Ltd., was added to each well. For reference standard drugs were used against respective pathogen at concentration of 1 mg/mL. The plate was further incubated for 8 hr. A change in color from blue to pink indicated the growth of bacteria/fungi, and the Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of the compound that prevented this color change. The concentration range tested for the test compounds and standards was 0.97-500  $\mu$ g/mL. for reference standard drugs were used as mentioned in disk diffusion methodology (Rakhe *et al.*, 2024; Shaikh *et al.*, 2024; More *et al.*, 2020).

### Antioxidant Activity

#### DPPH (2, 2-Diphenyl-1-Picrylhydrazyl radical scavenging assay)

The electron donation ability of each compound was assessed by monitoring the decolonization of a DPPH solution (More *et al.*, 2020; Said *et al.*, 2018) DPPH, a stable reagent, was used in this spectrophotometric assay. Briefly, equal volumes of the DPPH solution and the test compound were mixed to obtain a final volume of 3 mL. The mixture was then incubated for 20 min, followed by measuring the absorbance at 517 nm using a UV Spectrophotometer (Shimadzu Corp. Japan). Ascorbic acid (1 mM) served as the standard for comparison. The percent inhibition or radical scavenging activity was calculated using the formula:





**Figure 2:** FTIR spectroscopy: *Acacia chundra* Flower chloroform extract.

and terpenoids) as shown in Table 1. The presence of these phytoconstituents in the three extracts (aqueous, ethanolic, and chloroform), therefore informed the utilization of the extracts for biological investigations. The results of phytochemical screening of different extracts are shown in (Table 1). In which ACLW, ACLE, ACBW, ACFW, and ACFE extracts contained alkaloids, saponins, and tannin. Steroids were absent in all extracts except for ACLE and ACFE. Flavonoids and phenolics were detected in ACLE, ACLC, ACBW, ACFE and ACFC the extracts. Terpenoids were present in ACLW, ACLE, ACFW and ACFC extracts. On the other hand, ACBC extracts did not contain alkaloids, saponins, tannin, steroids, Flavonoids, Phenolics, or Terpenoids.

### Fourier Transform Infrared Spectroscopy (FTIR)

The ACBC, ACFC and ACLC shows good amount of phytoconstituents in the phytochemical analysis hence further it was assessed by FTIR spectra as shown in Figures 1-4. The FTIR spectra of ACBC showed total 1-8 peaks. As mentioned in Table 2 the peaks at 3319 cm represent N-H stretching and 2973 and 2882 -CH methyl stretch in alkane. The peaks at 1379 indicative of methyl group in alkanes and 1328 cm<sup>-1</sup> correspond to the C<sub>6</sub>H<sub>4</sub>C<sub>12</sub>, 1087 represent C-O (C-O-C) in 4,4-diaminodiphenyl ether and 1045 cm<sup>-1</sup> represents C-N bond in polystyrene, 879.62 corresponds to m<sup>2</sup> symmetric deformation of the CO<sub>3</sub> group in a compound. The ACFC FTIR spectra showed 1-8 peaks, among peak at 2976.24 indicate symmetrical and asymmetrical stretching of -CH<sub>2</sub> functional group in fatty acid, peak at 2848.74 corresponds to the symmetric stretching of the -CH<sub>2</sub> group, 1736.35 is the C=O bond in esters. For example, ethyl acetate has

a strong band at 1736 cm<sup>-1</sup> for the C=O bond. 1462.6 is associated with pyridine. Peak at 1254.6 is associated with the Si-CH<sub>2</sub> bond in the GPTMS structure. Peak at 1060.29 is due to CH-O-CH stretching. Peak at 682.08 is characteristic of lead oxide in the FTIR spectrum of the pigment red lead. 652.99 is the compound Polysar Kynol 652 is available on Spectra Base (Marijana *et al.*, 2022).

The sample ACLC showed in total 1-7 peaks. Peak 3235.05 O-H stretching vibration, 2950.98 corresponds to tetrahedral carbon-hydrogen bonds. Peak at 2076.14 reflect a positive peak in the spectrum of 2-diazomethylpyrazine. Peak at 1639.51 indicate stretching vibration in dibenzalacetone. Peak at 1180.47 indicate. The C=S stretching frequency of thiofenchone is around 1180 cm<sup>-1</sup>. Peak at 1066.7 is the strongest peak in the FTIR spectrum of gaseous ethanol. Peak at 907.16 is associated with the Si-H band in hydrosilylation (Table 2).

### Antimicrobial activities

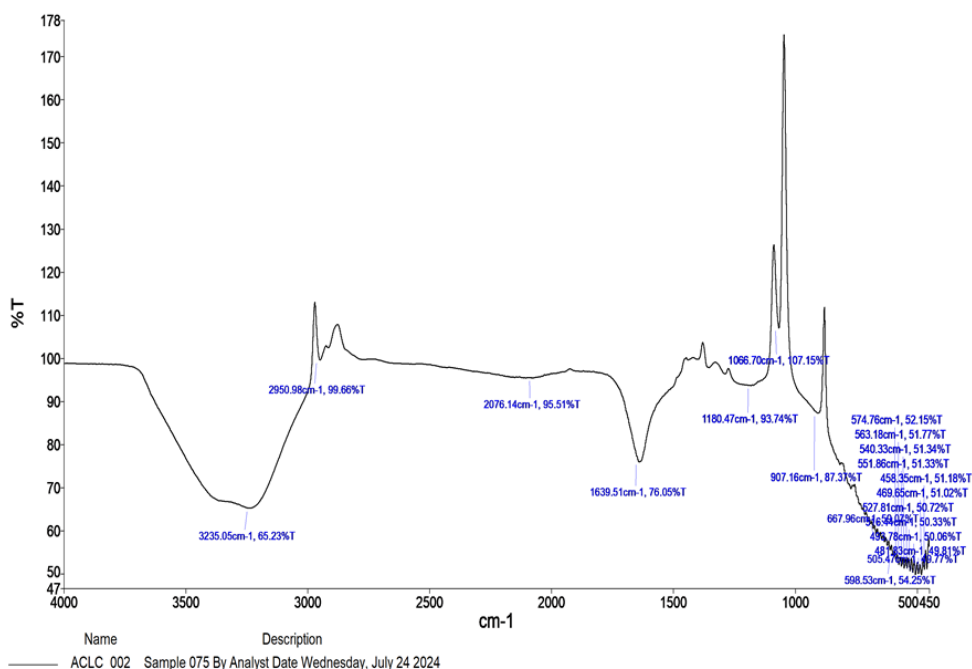
#### Disk diffusion assay

The antimicrobial activity of AC leaves, bark and flower solvent extracts was determined by disc diffusion method and MIC was calculated by REM assay (Rakhe *et al.*, 2024; Shaikh *et al.*, 2024), with slight modification wherever needed. The crude extracts of AC (ethanolic, aqueous and chloroform with respective positive control streptomycin, ethambutol, fluconazole and ampicillin were used as positive control and double deionized water as negative control) were tested against 15 pathogenic microbial species where 11 bacterial and 4 fungal pathogenic species were

**Table 1: Results of phytochemical analysis of different plant parts of AC.**

	Alkaloids	Saponins	Tannin	Steroid	Flavonoids	Phenolics	Terpenoids
Test	Wagner	Foam	FeCl <sub>3</sub>	H <sub>2</sub> SO <sub>4</sub>	Alkaline	FeCl <sub>3</sub>	H <sub>2</sub> SO <sub>4</sub>
ACLW	-	+	+	-	-	-	+
ACLE	+	+	+	-	+	+	+
ACLC	-	-	+	-	+	+	-
ACBW	-	+	+	-	+	+	-
ACBE	+	+	+	-	-	-	-
ACBC	-	-	-	-	-	-	-
ACFW	-	+	+	-	-	-	+
ACFE	+	+	+	-	+	+	+
ACFC	-	-	-	-	+	+	-

- Absence, + Presence

**Figure 3:** FTIR spectroscopy: *Acacia chundra* leaf chloroform extract.

used. The results are shown in Tables 3 and 4. The bacterial species *Escherichia coli* MCC 2412, *Bacillus subtilis* MCC 2048, *Bacillus megaterium* MCC 1684, *Staphylococcus aureus* MCC 2408, *Klebsiella* spp., *Shigella* spp., *Pseudomonas aeruginosa* MCC 2081, *Propionibacterium acnes* MCC 1951, *Staphylococcus epidermidis* MCC 435, *Enterococcus faecalis* MCC 2409, *Mycobacterium tuberculosis* MCC 300 and the fungal species *Aspergillus niger*, *Candida albicans*, *Aspergillus flavous*, *Rhizopus* spp., were used.

The assessment of antibacterial activity of the plant extracts was documented in Table 3, revealing varying levels of efficacy against diverse bacterial strains. Notably, the AC leaf extracts (ACLW, ACLE, ACLC) exhibited substantial antimicrobial activity, with ACLW and ACLE displaying the highest zone of inhibition compared to ACLC. Were standard antibiotics (Streptomycin,

Ampicillin, Ethambutol) used for comparison Similarly, the bark extracts (ACBW, ACBE, ACBC) and flower extracts (ACFW, ACFE, ACFC) demonstrated potent inhibitory effects against the tested microorganisms, with ACBW, ACBE, ACFW, and ACFE exhibiting the most significant zone of inhibition in contrast to the chloroform extracts of bark and flower, which showed minimal inhibitory zones (Table 3). Zone of inhibition of solvent extract of various plant parts of AC against human bacterial pathogens.

### Results are the average mean of three parallel experiments.

EC=*Escherichia coli* MCC 2412, BS=*Bacillus subtilis* MCC 2048, BM=*Bacillus megaterium* MCC 1684, SA=*Staphylococcus aureus* MCC 2408, KS=*Klebsiella* spp., SS=*Shigella* spp., PA=*Pseudomonas aeruginosa* MCC 2081, PAC=*Propionibacterium acnes* MCC

1951, SE=*Staphylococcus epidermidis* MCC 435, EF=*Enterococcus faecalis* MCC 2409, MTB=*Mycobacterium tuberculosis* MCC 300.

Overall, the results from the Disk Diffusion assay suggest that the water and ethanol solvent extracts of AC possess promising potential as natural antimicrobial agents effective against a wide range of human bacterial pathogens as shown in Table 3.

### Results are the average mean of three parallel experiments.

The antifungal efficacy of solvent extracts from various parts of the AC plant was evaluated against human fungal pathogens, with results summarized in Table 4. The extracts exhibited varying degrees of inhibition against *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus* spp., ACLW, ACBW, ACBE, ACFW, and ACFE extracts demonstrated significant antifungal activity, with maximum zones of inhibition against the tested pathogens. ACFC extract showed moderate inhibition, while

ACLC extract exhibited minimal activity. The antifungal potency of the extracts was compared to that of the standard antifungal agent, fluconazole, to assess their relative efficacy.

Overall, the results of the Disk Diffusion assay indicate that the water and ethanol solvents extract of AC have the potential to be used as natural antimicrobial agents against a variety of human fungal pathogens (*Candida albicans* NIH 3147, *Rhizopus* spp. MCC 262, *Aspergillus niger* MCC 281, *Aspergillus flavus* MCC 281).

### REM assay

The Minimum Inhibitory Concentration (MIC) values of the plant extract (ACLW, ACLE, ACLC ACBW, ACBE, ACBC, ACFW, ACFE, ACFC) recorded in Table 5. Against various bacterial pathogens were determined to assess their potency as antimicrobial agents. Table 5 and Figure 5 illustrates the MIC values for specific bacterial strains, highlighting the efficacy of

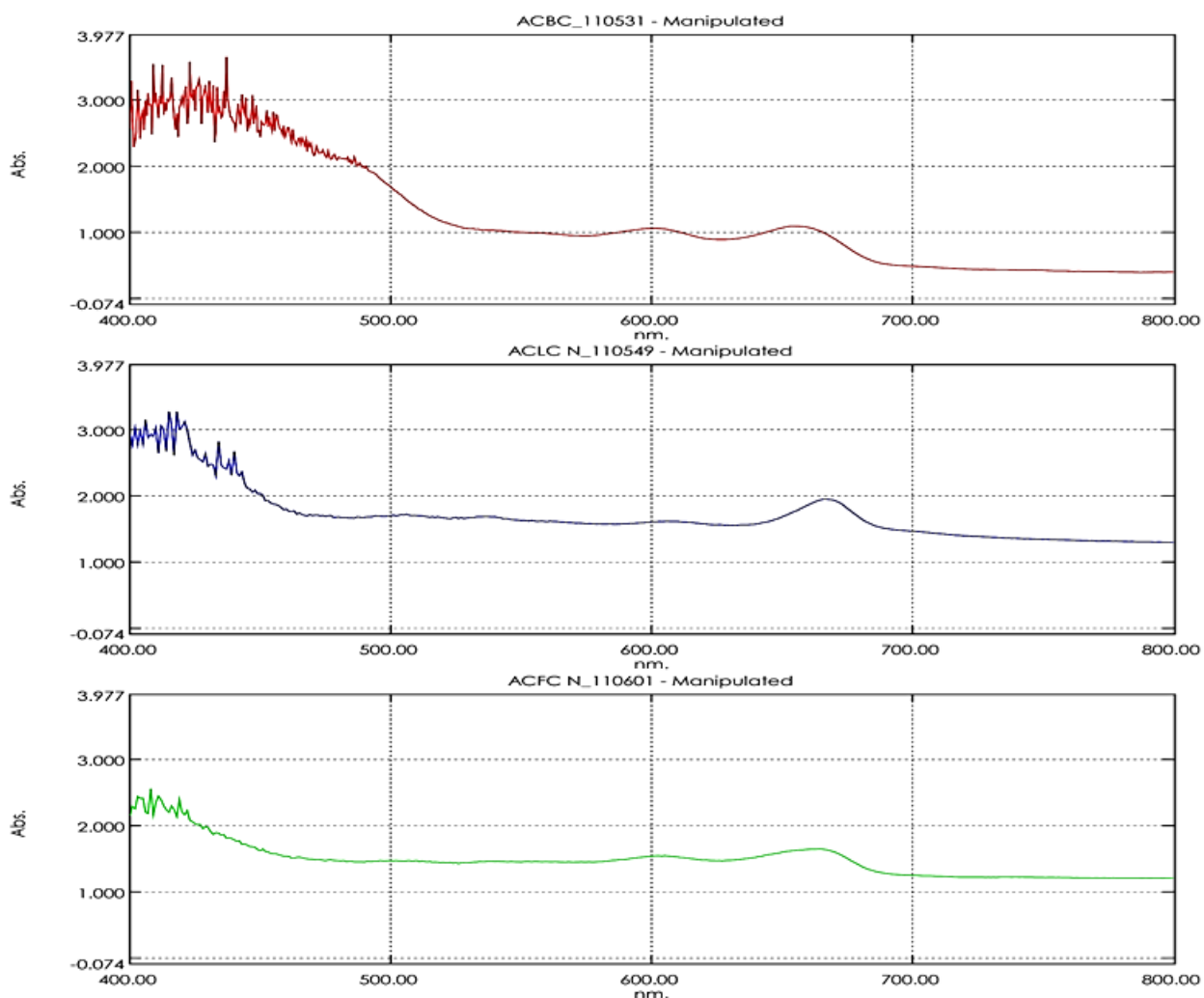
**Table 2: FTIR analysis of different samples of AC.**

Sample	Peak No.	X (cm <sup>-1</sup> )	Y (%T)	Description
ACBC	1	3319.16	93.81	NH- stretching
ACBC	2	2973.5	77.93	C-H stretching in alkane
ACBC	3	2882.87	86.17	C-H stretching in alkane
ACBC	4	1379.87	84.66	Frequency of 1379 cm <sup>-1</sup> is a methyl rock in alkanes
ACBC	5	1328.31	89.51	Associated with C <sub>6</sub> H <sub>4</sub> C <sub>12</sub>
ACBC	6	1087.24	64.59	Is a medium peak in the stretching vibration of C-O (C-O-C) in 4,4-diaminodiphenyl ether
ACBC	7	1045.25	37.73	Is associated with the C-N bond in polystyrene
ACBC	8	879.62	66.54	Corresponds to the m <sup>2</sup> symmetric deformation of the CO <sub>3</sub> group in a compound.
ACFC	1	2916.24	83.4	Symmetrical and asymmetrical stretching of -CH <sub>2</sub> functional group in fatty acid
ACFC	2	2848.74	86.22	Peak at 2848 cm <sup>-1</sup> is due to the symmetric stretching of the -CH <sub>2</sub> group.
ACFC	3	1736.35	93.88	C=O bond in esters. For example, ethyl acetate has a strong band at 1736 cm <sup>-1</sup> for the C=O bond.
ACFC	4	1462.6	91.37	1462 cm <sup>-1</sup> is associated with pyridine
ACFC	5	1254.6	92.89	Frequency is associated with the Si-CH <sub>2</sub> bond in the GPTMS structure.
ACFC	6	1060.29	90.38	Is due to CH-O-CH stretching.
ACFC	7	682.08	100.43	Is characteristic of lead oxide in the FTIR spectrum of the pigment red lead
ACFC	8	652.99	100.54	The compound Polysar Kynol 652 is available on SpectraBase.
ACLC	1	3235.05	65.23	Hydrogen bonded O-H stretching vibration
ACLC	2	2950.98	99.66	Tetrahedral carbon-hydrogen bonds
ACLC	3	2076.14	95.51	Is a positive peak in the spectrum of 2-diazomethylpyrazine.
ACLC	4	1639.51	76.05	Stretching vibration in dibenzalacetone
ACLC	5	1180.47	93.74	The C=S stretching frequency of thiofenchone is around 1180 cm <sup>-1</sup>
ACLC	6	1066.7	107.15	Is the strongest peak in the FTIR spectrum of gaseous ethanol.
ACLC	7	907.16	87.37	Is associated with the Si-H band in hydrosilylation.

**Table 3:** Zone of inhibition of solvent extract of various plant parts of AC against human bacterial pathogens. (1 mg/mL).

Sample/Standard	Zone of inhibition in mm at 1 mg/mL										
	EC	BM	BS	SA	KS	SS	PA	PAC	SE	EF	MTB
ACLW	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
ACLE	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
ACLC	++	+	+	+	++	++	++	++	+	+	+
ACBW	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++
ACBE	+++	+++	+++	+++	+++	+++	+++	+++	NZ	+++	+++
ACBC	++	++	++	++	++	++	++	+	+	+	NZ
ACFW	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
ACFE	+++	+++	++	+++	++	+++	++	+++	++	+++	++
ACFC	++	+++	+++	++	++	++	++	+++	++	++	++
Streptomycin	+++	+++	+++	+++	NA	NA	+++	NA	NA	NA	NA
Ethambutol	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+++
Fluconazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ampicillin	NA	NA	NA	NA	+++	+++	NA	+++	+++	+++	NA

+= < 5 mm, ++= > 5 and < 10 mm, +++= > 10 and < 18 mm, - = No zone, NA = Not applicable.



**Figure 4:** UV Visible spectrum of different crude extracts of *Acacia chundra*.

**Table 4: Zone of inhibition of solvent extract of various plant parts of AC against human fungal pathogens (1 mg/ mL).**

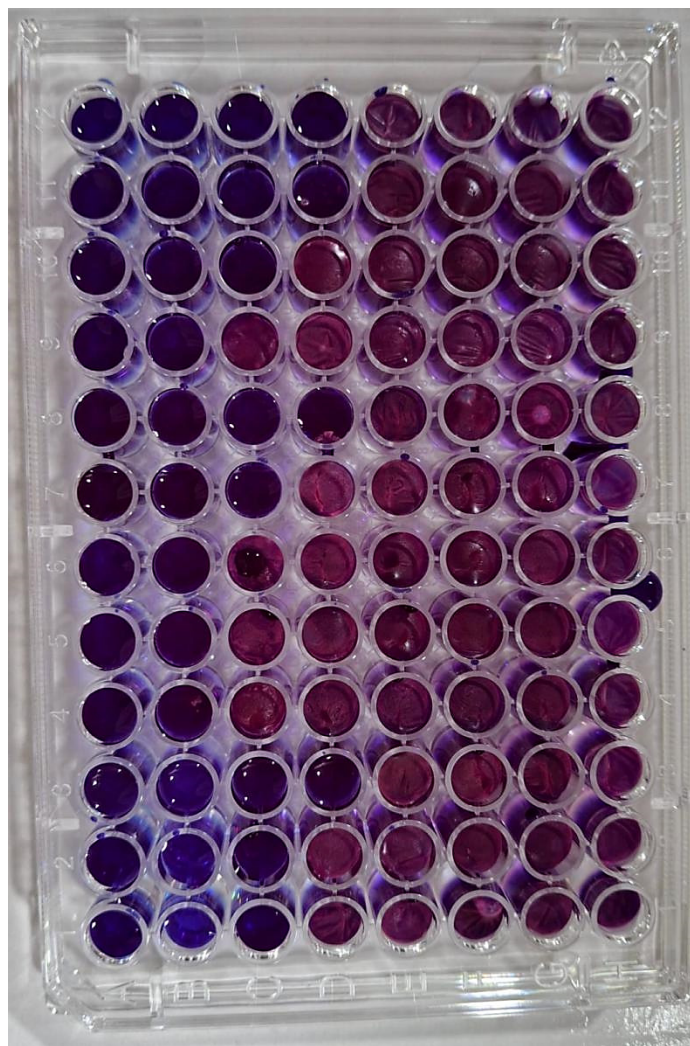
Sample/Standard	Zone of inhibition in mm at 1 mg/mL			
	<i>C. albicans</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus</i> spp.
ACLW	+++	+++	+++	+++
ACLE	NZ	+	NZ	NZ
ACLC	+	+	+	+
ACBW	+++	+++	+++	++
ACBE	+++	+++	+++	NZ
ACBC	NZ	NZ	NZ	NZ
ACFW	+	NZ	+	+
ACFE	++	+++	+++	+++
ACFC	++	++	++	NZ
Fluconazole	+++	+++	+++	+++

+= < 5 mm, ++= > 5 and < 10 mm, +++= > 10 and < 18 mm, - = No zone, NA = Not applicable.

these plant extracts in inhibiting bacterial growth. Notably, ACLE exhibited a MIC of  $3.25 \pm 0.01 \mu\text{g/mL}$  against *Escherichia coli* MCC 2412, *Bacillus subtilis* MCC 2048, and *Bacillus megaterium* MCC 1684, while displaying a slightly higher MIC of  $6.5 \pm 0.02 \mu\text{g/mL}$  for other bacterial species tested, excluding *Klebsiella* spp. This indicates the strong antimicrobial activity of ACLE against these common bacterial pathogens. Similarly, ACBE demonstrated a MIC of  $3.25 \pm 0.01 \mu\text{g/mL}$  for *Escherichia coli* MCC 2412, *Bacillus subtilis* MCC 2048, *Pseudomonas aeruginosa* MCC 2081, and *Propionibacterium acnes* MCC 1951. The MIC for *Enterococcus faecalis* MCC 2409 and *Mycobacterium tuberculosis* MCC 300 was slightly higher at  $6.5 \pm 0.02 \mu\text{g/mL}$ . These results suggest that ACBE is effective against a range of bacterial species, including both Gram-positive and Gram-negative pathogens. Furthermore, ACFE exhibited a MIC of  $3.25 \pm 0.01 \mu\text{g/mL}$  against *Escherichia coli* MCC 2412, *Bacillus subtilis* MCC 2048, *Propionibacterium acnes* MCC 1951, and *Enterococcus faecalis* MCC 2409. This highlights the potent antimicrobial activity of ACFE against these specific bacterial strains.

Overall, the MIC values of ACLE, ACBE, and ACFE indicate their potential as effective natural antimicrobial agents with broad-spectrum activity against a variety of bacterial pathogens, making them promising candidates for further research and development in combating infectious diseases.

The antifungal activity of plant extract demonstrated by their Minimum Inhibitory Concentration (MIC) values, is significant and promising. presents the MIC values for specific fungal species, highlighting the efficacy of these plant extracts in inhibiting fungal growth recorded in Table 6. Specifically, ACLE exhibited a MIC of  $6.5 \pm 0.02 \mu\text{g/mL}$  against *Candida albicans* NIH 3147, *Aspergillus flavus* MCC 281, and *Rhizopus* spp. MCC 262. These results indicate that ACLE has notable antifungal activity against a range of fungal pathogens. On the other hand, ACFC demonstrated a MIC of  $3.25 \pm 0.01 \mu\text{g/mL}$  against *Candida*

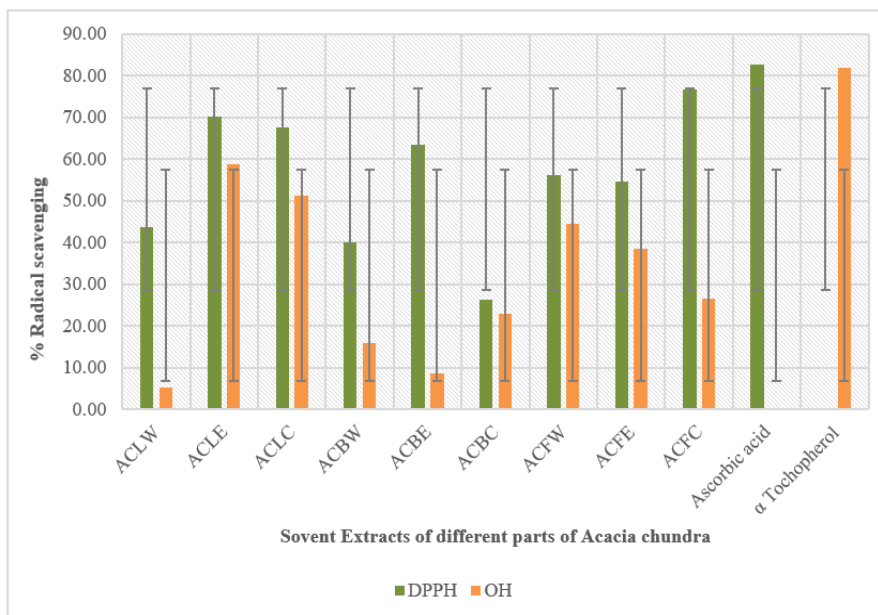
**Figure 5:** REM assay for MIC calculations.

*albicans* NIH 3147 and *Rhizopus* spp. MCC 262. as mentioned in Table 6. This extract showed comparable antifungal activity to the standard antifungal drug fluconazole, suggesting its potential as

**Table 5: MIC of solvent extract of various plant parts of AC against human bacterial pathogens (µg/ mL).**

Sample/ Standard	MIC of plant samples in µg/mL										
	EC	BM	BS	SA	KS	SS	PA	PAC	SE	EF	MTB
ACLW	52.08±0.18	52.08±0.18	41.66±0.18	208.33±0.72	26.01±0.09	208.33±0.72	208.33±0.72	208.33±0.72	208.33±0.72	51.75±0.18	208.33±0.72
ACLE	3.25±0.01	3.25±0.01	3.25±0.01	6.5±0.02	52.08±0.18	6.5±0.02	6.5±0.02	6.5±0.02	3.25±0.01	6.5±0.02	6.5±0.02
ACLC	41.66±0.18	52.08±0.18	208.33±0.72	52.08±0.18	208.33±0.72	52.08±0.18	52.08±0.18	208.33±0.72	208.33±0.72	52.08±0.18	208.33±0.72
ACBW	104.16±0.36	104.16±0.36	104.16±0.36	104.16±0.36	104.16±0.36	104.16667	104.16±0.36	104.16±0.36	208.33±0.72	208.33±0.72	208.33±0.72
ACBE	3.25±0.01	3.25±0.01	26.03±0.09	104.16±0.36	52.08±0.18	13±0.04	3.25±0.01	3.25±0.01	13±0.04	6.5±0.02	6.5±0.02
ACBC	13±0.04	208.33±0.72	208.33±0.72	3.25±0.01	3.25±0.01	13±0.04	3.25±0.01	3.25±0.01	13.1±0.04	3.25±0.01	11.7±0.06
ACFW	208.33±0.72	104.16±0.36	104.16±0.36	208.33±0.72	208.33±0.72	208.33±0.72	208.33±0.72	208.33±0.72	208.33±0.72	208.33±0.72	11.7±0.06
ACFE	3.25±0.01	3.25±0.01	208.33±0.72	104.16±0.36	208.33±0.72	13±0.04	208.33±0.72	3.25±0.01	208.33±0.72	3.25±0.01	52.08±0.18
ACFC	52.16±0.17	104.16±0.36	104.16±0.36	104.16±0.36	104.16±0.36	104.16±0.36	104.16±0.36	104.16±0.36	13±0.04	3.25±0.01	52.08±0.18
Streptomycin	1.6±0.05	3.25±0.01	1.6±0.05	1.6±0.05	NA	NA	1.95	NA	NA	NA	NA
Ethambutol	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3.25±0.01
Ampicillin	NA	NA	NA	NA	1.6±0.05	3.25±0.01	NA	1.6±0.05	3.25±0.01	1.6±0.05	NA

Results are the mean values of three independent experiments  $n=3$ ,  $\pm$ SD.



**Figure 6:** Antioxidant potential of different solvent extracts of AC ( $n=3$ ,  $\pm$ SD).

an effective natural alternative for combating fungal infections. The results suggest that both ACLE and ACFC have promising antifungal properties, with ACFC showing particularly potent activity against *Candida albicans* and *Rhizopus* spp. These findings support further research into the potential use of these plant extracts as natural antifungal agents for the treatment of fungal infections (Li et al., 2024).

### Antioxidant activities

The antioxidant activity of solvent extracts from various plant parts of AC was evaluated using the DPPH scavenging assay, with the results summarized in Figure 6. Revealing distinct levels of efficacy among the tested extracts. Notably, ACLC, ACLE, and ACFC demonstrated outstanding antioxidant potential by exhibiting more than 50% scavenging activity against the

DPPH free radical. In contrast, ACLW, ACBW, ACFW, ACFE, and ACBE showcased significant antioxidant capacity with over 30% scavenging activity, while the remaining extracts displayed moderate scavenging activity.

Moreover, in the hydroxyl radical assay, ACFW, ACLE, and ACLC showcased remarkable antioxidant activity with more than 30% scavenging activity. Conversely, ACBW, ACBC, ACFE, and ACFC demonstrated moderate scavenging activity in this assay. These results underscore the diverse and potent antioxidant capabilities of the solvent extracts derived from different plant parts of AC. The findings highlight the potential of specific extracts as valuable natural antioxidants for a wide range of applications, emphasizing the importance of exploring these natural sources for their beneficial properties.

## Cytotoxicity studies

The cytotoxic effect of plant samples on normal mammalian cells was performed by this assay in which healthy human's fresh RBCs were used. In this study, ACFW, ACLC and ACBC possesses near about 1% cytotoxicity towards human RBC's. All other test samples showed negligible cytotoxicity <1% as compared with the standard Triton X 100 which showed 8.2% cytotoxicity as shown in (Figure 7).

## DISCUSSION

The study evaluated the antimicrobial, antioxidant, and cytotoxic potential of *Acacia chundra* (AC) extracts, revealing promising outcomes. Phytochemical screening indicated the presence of alkaloids, saponins, tannins, flavonoids, phenolics, and terpenoids in most extracts, except ACBC, suggesting these bioactive

compounds contribute to observed activities. Ethanolic extracts (ACLE, ACBE, ACFE) exhibited potent antimicrobial activity, with Minimum Inhibitory Concentrations (MIC) of  $3.25 \pm 0.01$   $\mu\text{g/mL}$  against *Escherichia coli*, *Bacillus megaterium*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, and *Enterococcus faecalis*, comparable to standard antibiotics. ACLE and ACFW showed moderate to good antifungal potential, while ACBC lacked activity, indicating part-specific and solvent-dependent efficacy. FTIR and UV spectral analysis confirmed the presence of diverse phytochemicals, supporting the extracts' broad-spectrum antimicrobial activity. Antioxidant assays (DPPH and OH radical scavenging) revealed significant activity in ACLE, ACLC, and ACFC, attributed to phenolics and flavonoids. Notably, all extracts showed negligible cytotoxicity (<1% hemolysis) towards human RBCs, indicating safety.

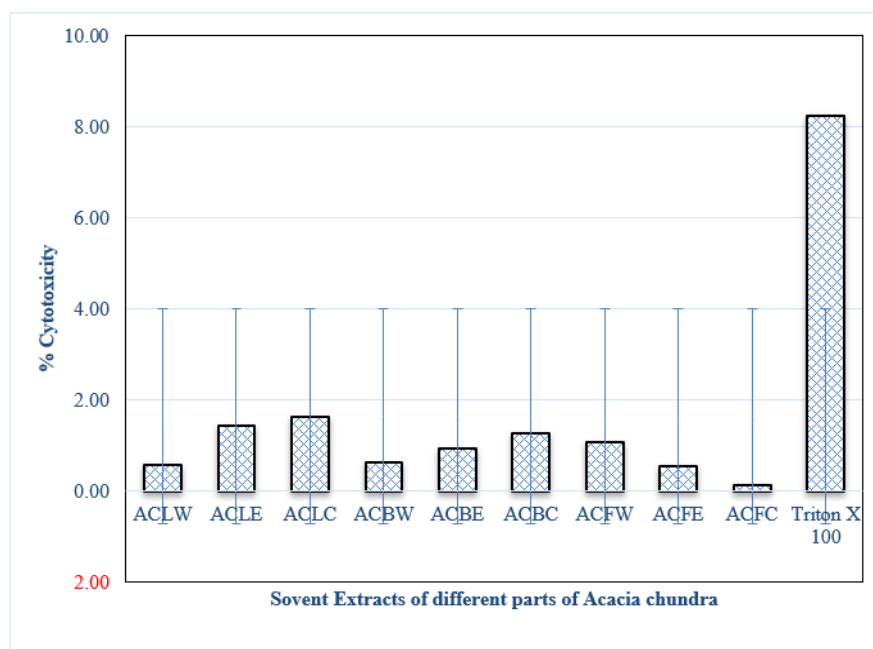


Figure 7: Cytotoxicity potential of different solvent extracts of AC ( $n=3$ ,  $\pm$ SD).

Table 6: MIC of solvent extract of various plant parts of AC against human fungal pathogens. ( $\mu\text{g/ mL}$ ).

Sample/Standard	<i>C. albicans</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus spp.</i>
ACLW	$52.08 \pm 0.18$	$104.16 \pm 0.36$	$104.16 \pm 0.36$	$416.66 \pm 0.144$
ACLE	$6.5 \pm 0.02$	$52.08 \pm 0.18$	$6.5 \pm 0.02$	$6.5 \pm 0.02$
ACLC	$3.25 \pm 0.01$	$208.33 \pm 0.72$	$208.33 \pm 0.72$	$416.66 \pm 0.144$
ACBW	$208.33 \pm 0.72$	$208.33 \pm 0.72$	$3.25 \pm 0.01$	$416.66 \pm 0.144$
ACBE	$13.1 \pm 0.04$	$13 \pm 0.04$	$6.5 \pm 0.02$	$13 \pm 0.04$
ACBC	$208.33 \pm 0.72$	$208.33 \pm 0.72$	$208.33 \pm 0.72$	$416.66 \pm 0.144$
ACFW	$52.08 \pm 0.18$	$104.16 \pm 0.36$	$104.16 \pm 0.36$	$416.66 \pm 0.144$
ACFE	$208.33 \pm 0.72$	$52.08 \pm 0.18$	$208.33 \pm 0.72$	$3.25 \pm 0.01$
ACFC	$3.25 \pm 0.01$	$26.03 \pm 0.09$	$13 \pm 0.04$	$3.25 \pm 0.01$
Fluconazole	$1.6 \pm 0.05$	$1.6 \pm 0.05$	$1.6 \pm 0.05$	$1.6 \pm 0.05$

Results are the mean values of three independent experiments  $\pm$ SD.

## CONCLUSION

*Acacia chundra* extracts, particularly ACLE and ACFE, demonstrate significant antimicrobial and antioxidant activities with minimal cytotoxicity, validating its traditional medicinal use. The broad-spectrum efficacy against bacterial and fungal pathogens suggests potential for treating oral, dermal, pulmonary, and genital infections. Further research is needed to isolate, characterize, and evaluate *in vivo* efficacy and safety of bioactive compounds.

## ACKNOWLEDGEMENT

All authors are thankful to Rastriya Uccharat Shiksha Abhiyan (RUSA) infrastructural grants and DST FIST Phase I for sanctioning grants to Dayanand Science College, Latur.

## ABBREVIATIONS

**CLSI:** Clinical and Laboratory Standards Institute; **RBCs:** Red Blood Cells; **PBS:** Phosphate buffered saline; **EDTA:** Ethylenediaminetetraacetic acid; **D/W:** Distilled water; **T/C:** Treated/Control ratio; **AC:** *Acacia chundra*; **ACLW:** *Acacia chundra* Leaf Water extract; **ACLE:** *Acacia chundra* Leaf Ethanol extract; **ACLC:** *Acacia chundra* Leaf Chloroform extract; **ACBW:** *Acacia chundra* Bark Water extract; **ACBE:** *Acacia chundra* Bark Ethanol extract; **ACBC:** *Acacia chundra* Bark Chloroform extract; **ACFW:** *Acacia chundra* Flower Water extract; **ACFE:** *Acacia chundra* Flower Ethanol extract; **ACFC:** *Acacia chundra* Flower Chloroform extract; **REM:** Resazurin Microtiter Assay; **REMA:** Resazurin Microtiter Assay; **DPPH:** 2,2-Diphenyl-1-Picryl-Hydrazil; **OH:** Hydroxyl radical; **MIC:** Minimum Inhibitory Concentration; **FTIR:** Fourier Transform Infrared Spectroscopy; **UV:** Ultraviolet; **NZ:** No Zone; **NA:** Not Applicable; **EC:** *Escherichia coli* MCC 2412; **BM:** *Bacillus megaterium* MCC 1684; **BS:** *Bacillus subtilis* MCC 2048; **SA:** *Staphylococcus aureus* MCC 2408; **KS:** *Klebsiella* spp. (microorganism code); **SS:** *Shigella* spp. (microorganism code); **PA:** *Pseudomonas aeruginosa* MCC 2081; **PAC:** *Propionibacterium acnes* MCC 1951; **SE:** *Staphylococcus epidermidis* MCC 435; **EF:** *Enterococcus faecalis* MCC 2409; **MTB:** *Mycobacterium tuberculosis* MCC 300; **CA:** *Candida albicans*; **AF:** *Aspergillus flavus*; **RHS:** *Rhizopus* spp.; **+**: Minimum zone of inhibition, **++:** Moderate zone, **+++:** Maximum zone; **±** **SD:** Standard Deviation.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## FUNDING

There is no funding to this research directly but, the work utilizes institutional infrastructure funded by government agencies.

## ETHICAL STATEMENT

There is no any ethical issue related to this research work specifically with human RBC's used in the Haemolytic assay.

## INFORMED CONSENT

Donor have informed and understood and agree to their blood (or RBCs) being used for research.

## AUTHOR CONTRIBUTIONS

Rahul More- conceptualization, methodology, formal analysis, writing-original draft preparation, writing-review and editing, Anuradha Ingle- methodology, formal analysis, investigation, writing-original draft preparation, Shweta Gaikwad- methodology, formal analysis, investigation, Shradda Ghatol- writing-review and editing, supervision, Mahesh Karale- methodology, formal analysis, investigation, writing-original draft preparation, supervision, Nagnath Phartale- formal analysis, investigation, Kailash Sontakke- conceptualization, investigation, Govind Sanap- investigation, supervision, Shreyas Mahurkar- writing-original draft preparation, writing-review and editing, Yuvraj Sarnikar- conceptualization, methodology, Santosh Chobe- investigation, writing-original draft preparation).

## SUMMARY

In this manuscript authors are performed deep investigation of which phytochemical components are responsible for their antimicrobial activity. This investigation is done on the basis that, this plant sample has collected from that region where there is arid conditions majority of the years. Due to the arid conditions plant may synthesize the novel components which can have high potency towards the bacterial pathogens. and the same is observed in the results that the plant sample have considerable to good antimicrobial potential. for the part of quality control, we have performed spectroscopic analysis of plant sample as an additional major to support presence of variety of phytochemicals. this research will definitely helpful for further investigation and formulation of novel drugs against strong human pathogens.

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**Cite this article:** More R, Ingle A, Gaikwad S, Ghatol S, Karale M, Phartale N, et al. Exploring Wide Range of Antimicrobial, Antioxidant and Cytotoxicity Profile of *Acacia chundra*. (Roxb) DC (Kheri Plant). *Pharmacog Res.* 2026;18(3):870-82.