# Screening of Bioactive Components from the *Acacia Chundra* Leaves and In-Vitro Exploration of their Antimicrobial, Antioxidant and Cytotoxic Potentials

**Kakaraparthy Sriramakrishna[1], Sivakumar M[2*]**

[1]Research Scholar, Faculty of Pharmacy, Bharath Institute of Higher Education and Research (DU), Selaiyur, Chennai- 600073, Tamil Nadu, India
[2]Faculty of Pharmacy, Sree Balaji Medical College and Hospital campus, Bharath Institute of Higher Education and Research (DU), Chromepet, Chennai- 600044, Tamil Nadu, India

***Corresponding author Mail Id:**
Dr. Sivakumar M
Professor and Head
Department of Pharmacognosy
Sree Balaji Medical College and Hospital campus,
Bharath Institute of Higher Education and Research (DU),
Chromepet, Chennai- 600044, Tamil Nadu, India
Email: sivakumar.pharm@bharathuniv.ac.in

**ABSTRACT**

**Introduction**: In this Present Study aims to investigates the Bioactive components, antioxidant activity and cytotoxic effect of the *Acacia chundra leaves* towards MCF-7 breast cancer cells.

**Methods:** Antioxidant activity was evaluated using intracellular Reactive Oxygen Species (ROS) detection assay in cultured cells, while antimicrobial activity was assessed through broth micro dilution method for Minimum Inhibitory Concentration (MIC) and biofilm inhibition assay. The extract shows significant reduction in intracellular ROS levels, indicating strong antioxidant potential. Furthermore, it exhibited potent antimicrobial activity with MIC values ranging from 63.5–125 µg/mL and significant inhibition of biofilm formation (>65%). cytotoxic activity with MTT assay.

**Results:** Acacia chundra contained secondary metabolites like Catechin, epicatechin, epigallocatechin (Flavonoids), tannins, Gallic acids. Acacia chundra showed a weak antioxidant activity towards DPPH free radical with IC50 of 215.51 µg/mL and a very active cytotoxic activity on MCF-7cells with IC50 of 29.28 µg/mL and 50.39µg/mL, respectively.

**Conclusion:** These findings suggest that *Acacia chundra* is a promising source of natural antioxidant and antimicrobial and cytotoxic effects.

**Keywords:** Pharmacognosy, Antioxidant, MTT assay, MIC, Biofilm inhibition, *Acacia chundra*

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## INTRODUCTION

Cancer, oxidative stress, and microbial infections are among the major global health challenges affecting human health. Cancer is a complex disease characterized by uncontrolled cell growth, invasion, and metastasis, leading to high morbidity and mortality worldwide. These abnormal cells continue to grow and divide to produce tumors. Cancer cells could spread to adjacent structures and infect through lymph or blood circulation[1]. Breast cancer has the second highest number, behind lung cancer[2]. There are four stages[3] in cancer. Conventional treatments such as chemotherapy, radiotherapy, and surgery are effective to some extent, but they are often associated with severe side effects, toxicity, and drug resistance to the patients[4]. Therefore, there is growing interest in identifying natural products from medicinal plants that possess anticancer potential with fewer adverse effects. Plant-derived Bioactive components such as flavonoids, tannins, alkaloids, and phenolic compounds have shown promising cytotoxic effects against various cancer cell lines. It inhibiting cancer progression by targeting cell signaling pathways. Oxidative stress[5] is caused by an imbalance between free radical production and the body's antioxidant defense mechanisms. Excess reactive oxygen species (ROS) can damage lipids, proteins, and DNA, causing aging, inflammation, diabetes, cardiovascular disease, and cancer. Antioxidants are vital for neutralizing free radicals and protecting cells from oxidative damage. Natural antioxidants derived from medicinal plants are gaining popularity because manufactured antioxidants may have negative effects. As a result, screening plant extracts for antioxidant activity has become a critical[6] aspect of pharmacological research.

Particularly in light of the growing prevalence of antibiotic resistance, microbial illnesses brought on by bacteria, fungus, and other pathogenic microorganisms[6], remain a major public health concern. Multidrug-resistant bacteria have made many antibiotics less effective, necessitating the development of new, safer antimicrobial medicines. Many plant extracts have shown inhibitory actions against pathogenic germs, and medicinal plants have long been important sources of antimicrobial chemicals. Herbal remedies are thought to be accessible, affordable, and generally safer substitutes.Acacia chundra is an important medicinal plant belonging to the family Fabaceae and is traditionally used in various herbal systems of medicine. It is known to contain bioactive compounds such as tannins, flavonoids, phenolic, glycosides, and other secondary metabolites that may contribute to its therapeutic properties. Traditionally, different parts of the plant have been used for wound healing, inflammation, diarrhea, and infections. Due to its rich bioactive compounds profile, it has attracted scientific interest for evaluating multiple biological activities. Several studies suggest that Acacia chundra exhibits significant antioxidant activity by scavenging free radicals and reducing oxidative stress. The presence of phenolic compounds and flavonoids[7] is mainly responsible for its antioxidant potential. In addition, plant extracts may show anticancer activity through mechanisms such as induction of apoptosis[8](programmed cell death), inhibition of cell proliferation, and suppression of tumor growth. These properties make the plant a potential candidate for developing novel natural anticancer agents.

## METHODS

### Research Design

This research is an experimental study, which is divided into two processes, analyzing the bioactive components content Acacia chundra *sp*. evaluating the anti-microbial, Antioxidant with DPPH assay and cytotoxic activity with MTT assay. The authentic plant material i.e. leaves of *Acacia chundra* were obtained from Sri Sailam reserve forest, near Sunni Penta, Srisailam, Andhra Pradesh, India.

### Preparation of Extract of Acacia Chundra

Leaves were washed properly, shed dried and ground into a fine powder and stored in sterile containers in a cool dry place till further use. Extraction of the plant material was carried out. Extraction of 1g of dried plant material with various solvents yielded dried plant extracts ranging from approximately 47mg to 233mg (Table 2). The ethanol and the SFE extracts had the highest yields of dried extracted material (233 and 188mg, respectively). Water, ethyl acetate, chloroform extracted lower masses (approximately 130, 63, and 57mg, respectively). The dried extracts were resuspended in 10mL of deionized water (containing 1% DMSO) resulting in the extract concentrations shown in Table 2. Bioactive studies (Table 2) showed that the ethanol, water extracts contained the widest range and largest amount of Bioactive compounds in this study. Each of these contained high levels of total phenolics, flavonoids and moderate to high levels of saponins, triterpenoids and tannins. Similar classes of Bioactive components were detected in the ethyl acetate, chloroform, extracts, albeit generally at lower levels of bioactive chemical Analyses of Acacia Chundra.

Qualitative bioactive chemical tests for the identification of carbohydrates, tannins, saponins, flavonoids, alkaloids, steroids, phenolics and glycosides were carried out for A. chundra as per the methods described by Trease and Evans[9] , Harborne[10] and Sazada et al[11].

**Test for Proteins**: Few drops of nitric acid were added by the sides of the test tube very gently to 1 ml methanol extract. Formation of yellow color indicated the presence of protein in the sample (Xanthoprotein test).

**Test for carbohydrates**: 1 ml each of Fehling A and Fehling B were added in diluted extract and heated for 30 minutes and observed for the formation of brick red color.

**Test for Resins**: Five milliliter of distilled water was added to the methanol extract and observed for turbidity.

**Test for Tannins:**

5 ml of 45% ethanol was added to 2 g of the ground sample and boiled for 5 min. The mixture was cooled and filtered. Then 3 drops of lead sub acetate solution was added to 1 ml of the filtrate. A gelatinous precipitates were observed which indicates the presence of Tannins. Another 1 ml of the filtrate was added to 0.5 ml of bromine water. A pale brown precipitates were observed indicating the presence of Tannins.

**Test for Saponins**: 0.5 g of methanol extract was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a persistent froth. The frothing was mixed with 3 drops of Olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

**Test for Flavonoids:**

0.5 g of the medicinal plant extract of was introduced into 10 ml of ethyl acetate and heated in boiling water for 1 min. The mixture was then

filtered. 4 ml of the filtrate was shaken with 1 ml of 1% aluminum chloride solution and kept. Formation of yellow color in the presence of 1 ml dilute Ammonia solution indicated the presence of flavonoids.

**Test for Alkaloids:**
5 gm of ground material was extracted with 10 ml Ammoniacal Chloroform and 5 ml chloroform. The mixture was filtered and the filtrate was shaken with 10 drops of 0.5 M Sulphuric acid. Creemish white precipitate was observed for the presence of Alkaloids.

**Tests for Steroids**:
2 ml of acetic anhydride was added to 0.5 g of methanol extract and 2 ml of Sulphuric acid was added by the sides of the test tube and observed for the colour change from violet or blue-green.

**Test for Phenolic compounds**:
Ethanol extract was taken in a test tube and mixed with distilled water and warmed. To this 2 ml of Ferric chloride solution was added and observed for the formation of green or blue colour.

**Test for Glycosides**:
About 0.5 ml of methanol extract was taken in a test tube and added 1 ml glacial acetic acid containing traces of ferric chloride. To this solution 1 ml concentration Sulphuric acid was added and observe for the formation of reddish brown colour at the junction of the two layers and the upper layer turned bluish green in the presence of glycosides. Liquid extraction yields and qualitative Bioactive chemical screening Test for microorganisms. Reference strains of Acini- tobacter baylyi (ATCC33304), Klebsiella pneumoniae (ATCC31488), Proteus mirabilis (ATCC21721), Proteus vulgaris (ATCC21719) and Pseudomonas aeruginosa (ATCC39324. All stock cultures were sub cultured and maintained in nutrient broth at 4oC.

**Evaluation of Antimicrobial activity:**
Antimicrobial activity of all plant extracts was determined using a modified disc diffusion method[12, 13]. Briefly, 100µL of the test bacteria were grown in 10mL of fresh nutrient broth until they reached a count of approximately 108 cells/mL (as determined by direct microscopic determination. One hundred microliters of microbial suspension was subsequently spread onto the agar plates. The extracts were tested using 5mm sterilized filter paper discs. Discs were infused with 10µL of the test sample, allowed to dry and placed onto the inoculated plates. The plates were allowed to stand at 4oC for 2 hours before incubation with the test microbial agents. The plates were then incubated at 30oC for 24 hours and the diameters of the inhibition zones were measured in millimeters. All measurements were to the closest whole millimeter. Each antimicrobial assay was performed in at least triplicate and mean values were determined.

Minimum inhibitory concentration (MIC) determination The minimum inhibitory concentration (MIC) of the Acacia chundra extracts were determined by a modified disc diffusion method[14,15]The plant extracts were diluted in deionized water across a concentration range of 5mg/mL to 0.1mg/mL. Discs were infused with 10µL of the test dilutions, allowed to dry and placed onto inoculated plates. The assay was performed as outlined above and graphs of the zone of inhibition versus concentration were plotted for each extract. Linear regression was used to calculate the MIC values.

**Evaluation of cancer cell anti-proliferative activity**
Evaluation of the anti-proliferative activity of the Acacia chundra ex-tracts was as previously described[16,17]Briefly, 1mL of trypsin (Sigma) was added to the culture flasks and incubated at 37oC, 5% CO2 for 15min to dislodge the cancer cells. The cell suspensions were then transferred to a 10mL centrifuge tube and sedimented by centrifugation. The supernatant was discarded and the cells were resuspended in 9mL of fresh media. Aliquots of the resuspended cells (70µL, containing approximately 5000 cells) were added to the wells of a 96 well plate. A volume of 30µL of the test extracts or cell media (for the negative control) was added to individual wells and the plates were incubated at 37oC, 5% CO2 for 12 hours in a humidified atmosphere[18]. A volume of 20µL of Cell Titer 96 Aqueous One solution was subsequently added to each well and the plates were incubated for a further 3hours. Absorbance were recorded at 490nm using a Molecular Devices, Spectra Max M3 plate reader. All tests were performed in at least triplicate and triplicate controls were included on each plate. The antiproliferative[19] activity of each test was calculated as a percentage of the negative control using the following formula.

Proliferation (% untreated control) = (Act/Acc) x 100

$A_{ct}$ is the corrected absorbance for the test extract (calculated by subtracting the absorbance of the test extract in media without cells from the extract cell test combination) and $A_{cc}$ is the corrected untreated control (calculated by subtracting the absorbance of the untreated control in media without cells from the untreated cell media combination).

**DPPH Assay**
DPPH Assay is a method to evaluate the antioxidant activity from the sample's extract. Firstly, Methanol

will be diluting the DPPH within several test tubes that contains different concentration of the extract ranging from 50-250 µg/ml and one control sample without the extract. Subsequently, the test tubes will be shacked and incubated for 30 minutes at temperature of 37$^{O}$C. The incubated samples will be moved into micro plate and measured through spectrophotometer at wavelength of 517 nm. Determining the antioxidant activity is done by the absorbance results. The higher the ant oxidant activity results in lower absorbance. The radical scavenging activity is calculated through the following equation.[20]

$$\text{Inhibition \%} = \frac{\text{Abs Control} - \text{Abs sample}}{\text{Abs Control}} \times 100$$

IC50 defined as the amount of concentration of the sample that is needed to inhibit 50% of the absorbance. The lower the amount of extract that could reach IC50 results in a higher scavenging activity.[20]

**MTT Assay**

MTT is dissolved by PBS (Phosphate Buffer Saline) with pH of 7.4until the concentration reaches5mg/mL. The MTT mixture will be filtered by0.2µM filter and moved in to a sterile and light protected container. The solution will be kept in light protected storage and in a temperature of 4$^{O}$C. The cultured cells are transferred and inoculated for 24 hours into the 96-well plate. Each well is added with different concentration of the extract and incubated for 48 hours[21].To perform this method, the wells that consist of the cell lines and10mg of extract, which has been incubated for 48 hours, is mixed with 25µL of MTT solution and incubated for 4 hours at 37$^{O}$C. Then, solubilized the solution with 100 µL of DMSO. Lastly, read the absorbance with a spectrophotometry at wavelength of 570 nm. The IC50 is calculated from the following equation.[22]

$$\text{Cell cytotoxicity \%} = 100 - \left(\frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100\right)$$

**Data Analysis:**

Data analysis will be done through Microsoft Excels and 20th version of SPSS. The data will comprise of evaluation of Acacia chundra. Bioactive components, scavenging activity, and cytotoxic activity. Bioactive components will be presented as positive or negative values. Microsoft Excels will be used to calculate the linear regression and R-squared of the scavenging activity and cytotoxic activity[23], in theory if the scavenging activity increases, so does the cytotoxic activity. Normality and Kruskal-Wallis test is used to determine if there is a statistic significant among each category through SPSS.

**RESULTS:**

**Table 1: Screening of bioactive compounds**

| **Bioactive components** | **Acacia chundra sp leaves extract** | | | |
|---|---|---|---|---|
| | Ethanolic extract | Chloroform extract | Ethyl acetate extract | Hexane extract |
| Carbohydrates | ++++ + | +++++ + | ++++ ++ | +++ +++ |
| Alkaloids | ---- | ------ | ------- | ------ --- |
| Glycosides | ----- | ------ | ------- | ------ - |
| Tannins | ++++ ++ | +++++ + | ------- | ------ -- |
| Resins | ++++ +++ | ----- | | +++ +++ |
| Terpenoids | ------ | ----- | ----- | ----- |
| Flavonoids | ++++ +++ | | ++++ +++ | ----- |
| Phenolic compounds | ++++ + | ----- | ++++ +++ | ----- |

**Antimicrobial activity:**

Aliquots (10µL) of each extract were tested in the disc diffusion assay against bacterial species associated with the induction; kylosing spondylitis (Klebsiella pneumoniae, multiple sclerosis (Acinito bacterial Figure 2). The Acacia chundra bark extracts were potent inhibitors of reference and clinical strains of P. mirabilis, with zones of inhibition[24] up to approximately 15mm. The Ethanolic extract was particularly potent, with inhibition zones of 13.0 ± 1.0 and 14.7 ± 1.2 mm against the reference and clinical strains, respectively. The aqueous (9.6 ± 0.6 and 8.8 ± 0.4 mm for the reference and clinical strains respectively) and ethyl acetate (8.9 ± 0.6 and 7.3 ± 0.3mm for the reference and clinical strains, respectively) were also good inhibitors of P. mirabilis growth. The hexane extract also inhibited P. mirabilis growth, although lower efficacy (7.3 ± 0.3mm and 6.7± 0.3mm for the reference and clinical strains, respectively) was evident than for the Ethanolic, aqueous and ethyl acetate extracts. In contrast, the chloroform extract was completely devoid of P. mirabilis growth inhibitory activity. The Acacia chundra leaves extracts were similarly potent growth inhibitors against P. vulgaris (Figure 2b). A zone of inhibition of 9.7 ± 0.6mm was recorded for the Ethanolic extract. Similarly, growth

inhibition zones of 7.5 ± 0.5 and 6.7 ± 0.3mm were seen for the aqueous and ethyl acetate extracts, respectively. In contrast, both the chloroform and hexane extracts were completely devoid of *P. vulgaris* growth inhibitory activity. A similar activity profile was evident for *K. pneumoniae* growth inhibition (Figure 1). The Ethanolic extract was the most potent growth inhibitor, with zones of inhibition of 9.3 ± 0.3mm (reference strain) and8.6 ± 0.3mm (clinical strain). This compares favorably with the ampicillin control (10µg) which had 9.2 ± 0.4 (reference strain) and 8.7 ± 0.3mm (clinical strain) zones of inhibition. The aqueous, ethyl acetate Chloroform, Hexane and subcritical extracts also inhibited *K. pneumoniae* growth, albeit with lower efficacy (6.7 to 7.5mm zones of inhibition). As *K. pneumoniae* can trigger ankylosing spondylitis in genetically susceptible individuals[25], these extracts have potential in the prevention and treatment of this disease.

The Acacia chundra bark extracts were also screened for growth inhibitory activity against bacterial triggers of multiple sclerosis (Acinetobacter baylyi, Figure 4a; Pseudomonas aeruginosa[26,27], Figure 4b) The ethyl acetate extract was the most potent Acinetobacter baylyi. growth inhibitor, with zones. Table 2: The mass of dried extracted material, the concentration after resuspension in deionized water and qualitative Bioactive compound screenings of the Acacia chundra leaves extracts. Of inhibition of 12.0 ± 1.0 and 15.7 ± 1.3mm against the reference and clinical isolate strains, respectively. Potent growth inhibition was also determined for the Ethanolic extract (9.0 ± 1.0 and 10.7 ± 1.2mm for the reference and clinical isolate strains, respectively), hexane (8.3 ± 0.3 and 9.0mm for the reference and clinical isolate strains, respectively) and SFE extracts (9.5 ± 0.5 and 10.0 ± 1.0mm for the reference and clinical isolate strains, respectively). These results compare favorably to the ampicillin control (8.6 ± 0.3mm zones of inhibition against both A. baylyi strains). Although smaller inhibition zones (generally 7-8mm) were measured for the aqueous and chloroform extracts, these extracts were still deemed to be good *A. baylyi* growth inhibitors.

The Acacia chundra Ethanolic, extracts also inhibited Streptococcus pyogenes growth (Figure 5), albeit with zones of inhibition that indicate only moderate inhibitory activity. The aqueous leaf extract was the most potent growth inhibitor, with an inhibition zone of 7.3 ± 0.3mm, whilst both the Ethanolic and subcritical extracts gave inhibition zones substantially <7mm. However, this would be considered to be only moderate inhibition and is substantially less potent than the ampicillin control (8.3 ± 0.3mm zones of inhibition). In contrast, the ethyl acetate, chloroform and hexane extracts were completely devoid of inhibitory activity. S. pyogenes has been implicated in a number of diseases including rheumatic fever. Thus, the *Acacia chundra* Ethanolic, aqueous extracts have some potential in the prevention and treatment of these diseases. The relative level of antimicrobial activity was further evaluated by determining the MIC values (Table 3) for each extract against the bacterial species which were shown to be susceptible by disc diffusion assays. Most of the extracts were effective at inhibiting microbial growth at low concentrations, with many MIC values against the bacterial species that they inhibited generally < 1000µg/mL (<10µg impregnated in the disc), indicating the potent antimicrobial activity of these extracts. These MIC values compare favorably with the dosages of the pure standard ampicillin which was tested using 10µg per disc. The ethanol, water and hydro alcoholic extracts were particularly potent with MIC values in the range 160- 500µg/ml against several species. Indeed, MIC values of 185µg/mL (approximately 1.9mg in the disc; P. mirabilis reference strain), 167µg/mL (approximately 1.7mg in the disc; *K. pneumoniae* reference strain) and 212µg/mL (approximately 2.2mg in the disc; A. baylyi clinical strain), were determined for the Ethanolic extract. Similarly potencies were evident for the aqueous extracts. Whilst the ethyl acetate, chloroform had broad spectrum inhibitory activity against many of the bacterial species, they generally had much lower efficacies (with some MIC values >2000µg/ml).

**Antioxidant Activity of *Acacia chundra* sp**.

Antioxidant activity of Acacia chundra sp. is measured by DPPH assay[28] through the radical scavenging activity. The results from test were identify as IC50 value, which is amount of extract concentration to reach 50% inhibition. The radical scavenging activity could be influenced by the Bioactive components of the extracts. Secondary constituents such as flavonoids, tannins, have showed that they have antioxidant biological activities. Only two out of three extract of Acacia chundra. can be analyzed. HE was not included due to having a characteristic of non-polar which is not dissolved in ethanol as a polar solvent for DPPH assay. The positive control has been established by using ascorbic acid, which have very active antioxidant activity as reported by Pehlivan et al. (2017). The antioxidant activity test on DPPH was done three times. The linear regression equation of y = ax +b is obtained by plotting of concentration of the extract in x axis versus percentage of inhibition in y axis. Substitution of y with 50 in linear regression of y = ax +b will give x value which is equivalent with IC50 value. The IC50 value for ascorbic acid, EE, and EAE, were 0.782 µg/mL; >1000 µg/mL; and215.51 µg/mL, respectively (Table 3). The linear regression equation for EE is y = 15,554x - 25,214 with R2 =0.9618. Whereas the

linear regression equation for EAE is y = 25,979x - 10,624 with $R^2$ = 0.9856. Both of the linear regression can be seen in Figure 4.

Antioxidant properties of Acacia chundra sp. extracts are categorized based on its IC50 value as provided by Marjoni et al. (2017)[29], with the following orders that could be seen in Table 4. From the data that has been gathered, EE is categorized as not active (IC50 >1000 µg/ mL), whereas EAE is classified into having a moderate antioxidant activity (IC50: 215.51 µg/mL), compared to the control group of ascorbic acid, which categorized very active (IC50: 0.782 µg/mL). From bioactive compounds screening, it was stated that EE is positive in flavonoid and alkaloid, compared to ethanol extract only positive in flavonoid. This could lead to ethyl acetate extract having a lower IC50 than ethanol extract. Gebreyohannes et al. (2019), used acacia chundra sp. and extracted it with three solvents which are chloroform, 70% of ethanol, and hot water, resulting in chloroform extract, ethanol extract, and hot water extract, respectively[30] The three extracts undergone DPPH assay to determine the scavenging activities of the extracts. Considering the classification by Marjoni16, ethanol extract is categorized as very active (IC50: 40 µg/mL), whereas chloroform (IC50: 50 µg/mL) and hot water (IC50: 60 µg/mL) is categorized as active.[29] The difference results between this research and Gebreyohannese research may be due to the origin of the plant and different methods of extracting.

**Cytotoxicity of Acacia chundra towards breast MCF-7 Cells**

**MTT assay is used to determine the cytotoxicity**

Evaluation of A. chundra sp. extracts towards MCF-7 breast cancer cells. Cytotoxic activities of Acacia chundra sp. extracts against breast MCF-7 cancer cells are presented in IC50 value (Table 5). The results indicate a relatively low overall of IC50 value of all of the extracts, recorded below 100 µg/mL. This indicates that there is cytotoxic activity in all of the extract, with the strongest cytotoxic activity is the EAE (IC50: 0.21 µg/mL), followed by EE (IC50: 29.28 µg/mL), and HE (IC50: 50.39 µg/ mL). Doxorubicin was used as the positive control, had a small IC50 value (IC50: 0.020 µg/mL). The results are compared to an established classification by Atjanasuppat et al. (2009) which is presented in Table6.Based on the cytotoxicity classification by Atjanasuppat et al., EE and HE are classified into the active classification (IC50: 20-100 µg/ mL), while EAE is classified into the very active classification (IC50 < 20 µg/mL). These results could be benefit towards MCF-7 cancer cells by being categorized active and very active cytotoxic activity and needs to be explored extensively. Novaković et al. (2016) reported about the cytotoxic activity of Acacia chundra towards MCF-7 breast cancer cell line with MTT assays. Using two extracts, which are ethanol extract with IC50 of 333.3 µg/mL and water extract with IC50 of 285.7 µg/mL against MCF-7 cells[31], showed that both extracts are categorized into the moderate cytotoxicity on MCF-7 cells (IC50: 100-1000 .µg/mL), according to classification by Atjanasuppat. The EE of Acacia chundra sp. in this research had a lower IC50 value compared to Auricularia auricula-judae. This phenomenon could be caused by the different genus and method of extracting by respective researchers.

| | *P. mirabilis* | | *P. vulgaris* | *K. pneumoniae* | | *A. baylyi* | | *P. aeruginosa* | | *S. pyogenes* | *G. duodenalis* | Carcinoma cells | | Toxicity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Reference | Clinical | Clinical | Reference | Clinical | Reference | Clinical | Reference | Clinical | Clinical | Clinical | $CaCo_2$ | HeLa | *A.* |
| M | 185 | 290 | 288 | 167 | 233 | 608 | 212 | 1218 | 659 | 1437 | 187 | 268 | 155 | 1255 |
| W | 345 | 384 | 524 | 727 | 628 | 1956 | 1620 | 2750 | 2385 | 1200 | CND | CND | CND | 2247 |
| E | 1212 | 1426 | 1493 | 1424 | 1120 | 586 | 323 | 2313 | 1510 | - | CND | - | - | 1886 |
| C | - | - | - | 3350 | 3210 | 2544 | 1975 | 659 | 375 | - | - | - | - | 2250 |
| H | 986 | 1252 | - | 1870 | 2180 | 1774 | 1692 | 1718 | 1674 | - | - | - | - | 2080 |
| SC | 211 | 432 | 446 | 251 | 387 | 670 | 588 | 1337 | 1290 | 1685 | 328 | 470 | 174 | 1360 |
| Met | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT | 18 | NT | NT | NT |
| Cis | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT | 8400 | 15710 | NT |

Numbers indicate the mean MIC, IC50 and LC50 values of triplicate determinations. - indicates no inhibition. CND indicates that an $IC_{50}$ or $LC_{50}$ value could not be determined as inhibition or mortality did not exceed 50% at any concentration tested. NT = not tested; M = ethanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; SC = suberitidae extract; Met. = metronidazole; Cis = ciprofloxacin.

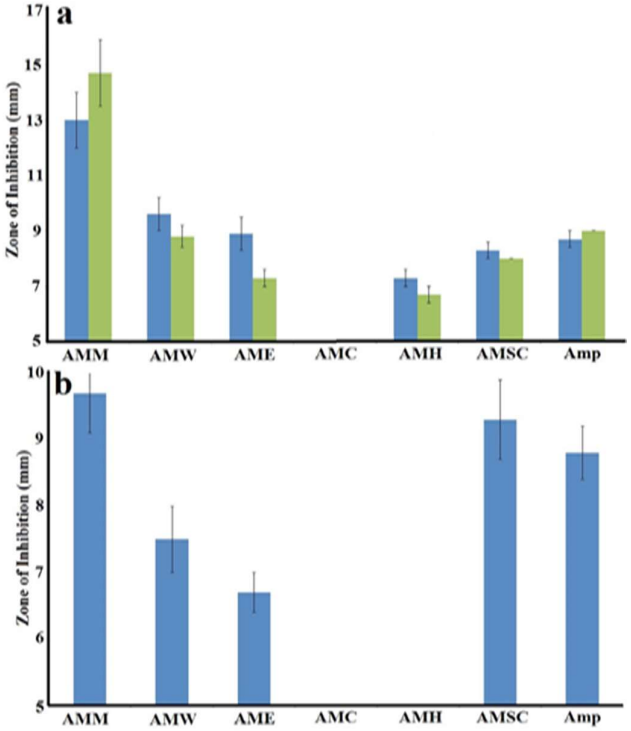


**Figure 1: Antibacterial activity of the Acacia chundra extracts measured as zones of inhibition (mm) against bacterial triggers of rheumatoid arthritis: (a) Proteus mirabilis, (b) Proteus vulgaris. Results are expressed as mean ± SEM of at least triplicate determinations. Blue bars represent inhibition of the refer- ence bacterial strain. Green bars represent inhibition of the clinical bacterial strain.**

AMM = methanolic extract; AMW = aqueous extract; AME = ethyl acetate extract; AMC = chloroform extract; AMSC = SFE extract; Amp = ampicillin control (10µg).

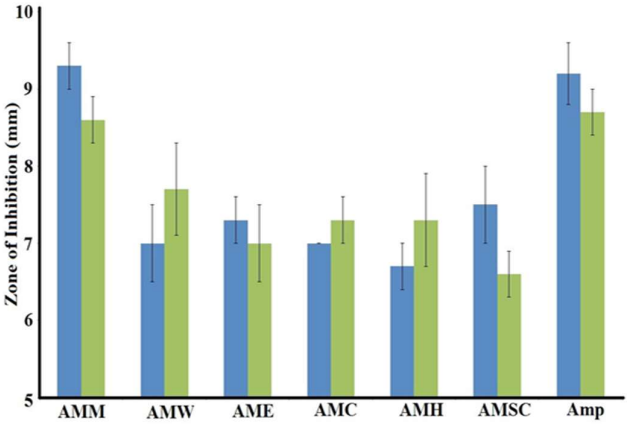


**Figure 2: Antibacterial activity of Acacia chundra extracts measured as zones of inhibition (mm) against a bacterial trigger of ankylosing spondylitis (Kleb- siella pneumoniae). Results are expressed as mean ± SEM of at least triplicate determinations. Blue bars represent inhibition of**

**the reference bacterial strain. Green bars represent inhibition of the clinical bacterial strain.**

AMM= methanolic extract; AMW = aqueous extract; AME = ethyl acetate extract; AMC = chloroform extract; AMH = hexane extract; AMSC = SFE extract; Amp= ampicillin control (10µg).

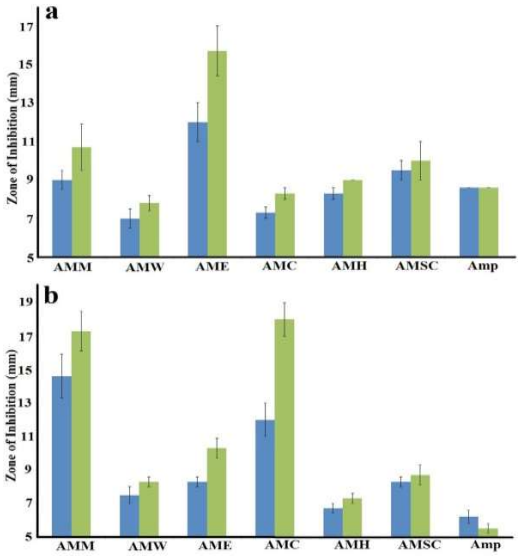


**Figure 3: Antibacterial activity of Acacia chundra extracts measured as zones of inhibition (mm) against a bacterial trigger of multiple sclerosis: (a) Acinito bacter baylyi, (b) Pseudomonas aeruginosa. Results are expressed as mean ± SEM of at least triplicate determinations. Blue bars represent inhibition of the reference bacterial strain. Green bars represent inhibition of the clinical bac- terial strain.**

AMM = methanolic extract; AMW = aqueous extract; AME = ethyl acetate extract; AMC = chloroform extract; AMH = hexane extract; AMSC = SFE extract; Amp = ampicillin control (10µg).

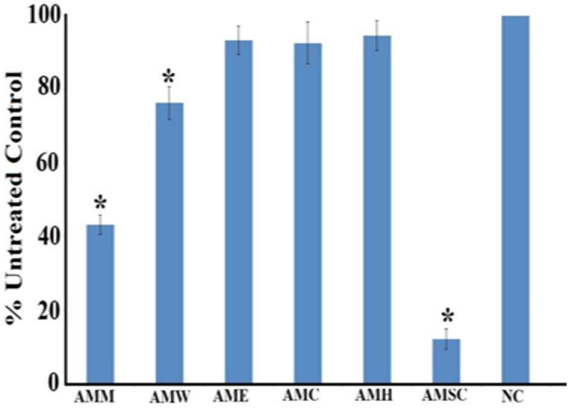


**Figure 4: Antibacterial activity of Acacia chundra extracts measured as zones of inhibition (mm) against a bacterial trigger of multiple sclerosis: (a) Acinetobacter baylyi, (b) Pseudomonas aeruginosa. Results are expressed as mean ± SEM of at least triplicate determinations. Blue bars represent inhibition of the reference bacterial strain. Green bars represent inhibition of the clinical bacterial strain.**

AMM = methanolic extract; AMW = aqueous extract; AME = ethyl acetate extract; AMC = chloroform extract; AMH = hexane extract; AMSC = SFE extract; Amp = ampicillin control (10 µg)

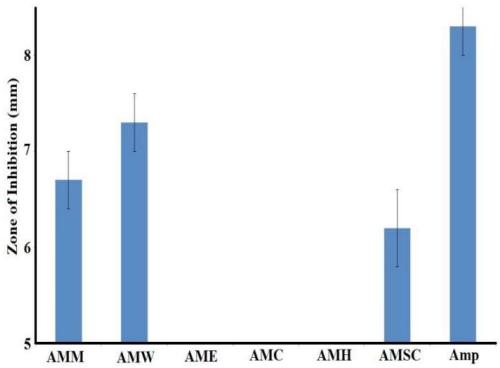


**Figure 5: Antiproliferative activity of Acacia hydaspica extracts and untreated controls against HeLa carcinoma cells, measured as percentages of the untreated control cells. Results are expressed as mean percentages ± SEM of at least triplicate determinations. * indicates results that are significantly different from the untreated control ($p < 0.01$).**

AMM = methanolic extract; AMW = aqueous extract; AME = ethyl acetate extract; AMC = chloroform extract; AMH = hexane extract; AMSC = SFE extract; NC = negative control; Cis = cisplatin control (50 µg/mL); Amp = ampicillin control (10 µg).

**Table3: IC50value of ascorbicacid andAcacia chundra sp.extract onDPPH**

| **Sample** | IC50(ug/ml) on DPPH |
|---|---|
| Ascorbic acid | 0.782 |
| Ethanol extract of *Acacia chundra sp.* (EE)<br>Ethyl acetate extract of *Acacia chundra.* (EAE) | >1000<br>215.51 |

**Table4: Antioxidant Activity Classification**

| IC50 (µg/mL) | **Classification** |
|---|---|
| < 20 (µg/mL) | Very active |
| 20-100 (µg/mL) | Active |
| 100-1000 (µg/mL) | Moderate |
| >1000 (µg/mL) | Not Active |

| **Test sample** | **$IC_{50}$(µg/mL)*** |
|---|---|

| | |
|---|---|
| Doxorubicin (positive control) | 0.022 |
| n-Hexane extract (HE) | 51.29 |
| Ethyl acetate extract (EAE) | 22 |
| Ethanol extract (EA) | 29.18 |

**Table 5: $IC_{50}$ value (µg/mL) of Acacia chundra *sp.* extracts on breast MCF-7 cells**

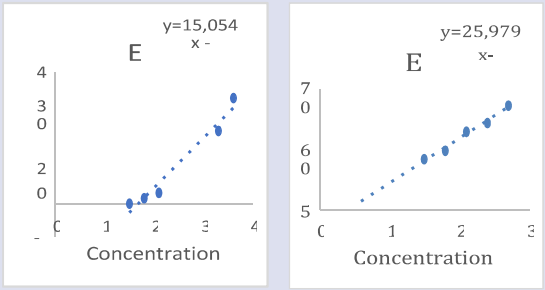


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**Figure 4: Linear graph of EE (4a) and EAE (4b) of Acacia chundra On DPPH**

## DISCUSSION

The present findings indicate that plant-derived flavonoid-rich extracts demonstrate notable biological activity across antioxidant, antimicrobial, and cytotoxic evaluations. Bioactive compounds screening commonly reveals the presence of flavonoids together with phenolics, tannins, alkaloids, and related secondary metabolites, while extraction procedures influence both yield and biological potency. Among these constituents, flavonoids are particularly important because their chemical structure enables free-radical scavenging, metal chelation, modulation of cellular signaling pathways, and interactions with microbial cell components. These combined properties make flavonoid-containing extracts valuable candidates for pharmacognostic and bioactive compounds investigation. In antioxidant studies, assays such as the DPPH assay generally demonstrate concentration-dependent radical scavenging activity, indicating the capacity of plant extracts to donate hydrogen atoms or electrons and neutralize reactive oxygen species. Since oxidative stress contributes to cellular damage, aging, inflammation, and several chronic disorders, antioxidant activity suggests potential therapeutic relevance. The flavonoid fraction often shows stronger activity because hydroxyl-rich structures can stabilize free radicals and interrupt oxidative chain reactions. Thus, antioxidant screening provides an important preliminary indicator of protective biological potential.

In antimicrobial investigations, plant extracts frequently exhibit inhibitory effects against both Gram-positive and Gram-negative bacteria, reflected by measurable zones of inhibition and reduced microbial growth. Flavonoids may contribute to this effect through disruption of microbial membranes, alteration of enzyme activity, interference with nucleic acid synthesis, and impairment of metabolic pathways. In cytotoxic evaluation, assays such as the MTT assay often demonstrate dose-dependent reduction in cell viability, suggesting selective ant proliferative potential. The cytotoxic action of flavonoids has been associated with induction of apoptosis, modulation of mitochondrial pathways, cell-cycle arrest, and regulation of oxidative stress–related signaling. Taken together, these findings indicate that antioxidant, antimicrobial, and cytotoxic activities are biologically interconnected and may arise through complementary mechanisms of action. Future research should focus on isolation, purification, and structural characterization of individual flavonoids responsible for the observed activities, followed by mechanistic studies at molecular and cellular levels. Standardization of extraction procedures, solvent systems, dosage ranges, and bioassay conditions will be essential for reproducibility and accurate comparison among studies. Further work should include in vivo pharmacological evaluation, toxicity profiling, bioavailability studies, and investigation of synergistic interactions among bioactive compounds. Advanced goals for future research include development of flavonoid-based lead molecules, Nano formulations, targeted delivery systems, and validation through preclinical and clinical studies. Such integrated research may support the development of safe, effective, and evidence-based therapeutic agents derived from medicinal plants.

## CONCLUSION

The present study indicates that the observed antioxidant, antimicrobial, and cytotoxic activities are closely related to the Bioactive compounds of the plant extract, particularly its flavonoid-rich fraction. The antioxidant activity observed in the DPPH assay demonstrates effective free-radical scavenging capacity, which may be attributed to the phenolic hydroxyl groups of flavonoids that donate electrons or hydrogen atoms and reduce oxidative stress. Since oxidative stress is associated with cellular injury, inflammation, and several chronic disorders, this finding supports the biological importance of the extract.

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