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In vitro Evaluation Of Biological Activities of Three Different Medicinal Plant Extracts

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This research evaluates the biological activities of three medicinal plants: Butea monosperma, Delonix regia, and Spathodea campanulata. These plants, belonging to the Fabaceae and Bignoniaceae families, were investigated for their antibacterial, antifungal, antioxidant, and cytotoxic properties. Phytochemicals were extracted from the flowers and leaves using methanol, ethanol, and acetone. The antibacterial and antifungal activities were assessed using a disc diffusion assay against pathogens S. epidermidis, B. cereus, E. coli, K. aerogenes, P. vulgaris, S. aureus, and C. albicans. The antioxidant potential was evaluated using the DPPH assay, and the cytotoxic activity was measured using the MTT assay on human lung cancer (A549) cells. Results indicated that ethanol extracts demonstrated the highest antimicrobial efficacy against both Gram-negative and Gram-positive bacteria, as well as the fungal strain Candida albicans. The extracts also demonstrated antioxidant activity, with the Butea monosperma ethanol extract showing the highest activity at an IC50 value of 61.55±1.22 μg/mL. Methanol extracts showed significant cytotoxic effects on the A549 cell line, with Butea monosperma having an IC50 value of 36.12±1.01 μg/mL. These findings suggest that the extracts from these plants have potential therapeutic applications against bacterial and fungal infections, oxidative stress, and cancer cells.

Keywords: Anticancer activity; Antimicrobial activity; Antioxidants; Butea monosperma; Delonix regia; Spathadea companulata.

For centuries, traditional medicinal plants have played a vital role in human health care, leveraging indigenous knowledge and practices to treat a variety of ailments[1]. These plants are abundant in phytochemical constituents such as alkaloids, flavonoids, terpenoids, and phenolic acids, which have shown potential in treating both acute and chronic health conditions. As the world seeks alternative solutions to combat the rise of antibiotic-resistant bacteria, fungal infections, oxidative stress, and cancer, research into plant-based therapies has gained momentum. This research is particularly crucial in the context of natural products, which are seen as a promising

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source for developing new drugs with minimal side effects[2].

Among the myriad of plants with purported medicinal properties, those belonging to the *Fabaceae* and *Bignoniaceae* families have shown significant potential. *Butea monosperma*, *Delonix regia* and *Spathadea companulata* are traditional medicinal plants that occur widely throughout the world. *Butea monosperma* and *Delonix regia* plants belong to the Fabaceae family[3], while *Spathodea campanulata* belongs to Bignoniaceae. These plants are native to tropical and subtropical areas but are widely distributed around the world, particularly in Asia, Africa, and Central and South America[4]. They are celebrated not only for their aesthetic value but also for their historical use in treating conditions such as infections, inflammation, fever, and even certain cancers. Despite their extensive ethnobotanical use, scientific validation of their biological properties remains insufficient, and comprehensive studies exploring their full potential are still lacking[5].

Therefore, this study intends to bridge this gap by investigating the biological activities of *Butea monosperma*, *Delonix regia*, and *Spathodea campanulata*, focusing specifically on their antimicrobial, antioxidant, and cytotoxic properties. The study involves extracting various phytochemicals from the leaves and flowers of these plants using the solvents methanol, ethanol, and acetone. These solvents were chosen to ensure the extraction of diverse biochemical constituents, as various solvents have varying abilities to dissolve both polar and non-polar compounds. By comparing the biological activities of the extracts obtained with different solvents, the study aims to identify the most effective extract for each activity. This work was initiated with the following objectives:

• To extract phytochemicals from *Butea monosperma* Leaves and Flowers of *Spathodea campanulata*, *Delonix regia* in various solvents (methanol, ethanol, and acetone).

• To screen antibacterial and antifungal potential of the extracts against various pathogens.

• To screen antioxidant potential of the plant extracts using DPPH *in vitro* free radical scavenging assay

• To evaluate the growth inhibitory activity of the extracts on A549 by MTT assay.

MATERIALS AND METHODS

Plant material

For this study, the leaves of *Butea monosperma* and flowers of *Spathodea campanulata* and *Delonix regia* were carefully collected from the forest area of Choutuppal, located in Nalgonda District, Telangana, India (17°152 033 N 78°532 503 E). The collection process was carried out during the appropriate season to ensure the optimal presence of bioactive compounds. Special care was taken to gather healthy, mature plant parts from different locations within the forest to ensure a representative sample. The collected specimens were then cleaned, air-dried, and stored in appropriate conditions before being processed for further analysis.

Solvent extraction

The Soxhlet extraction technique was employed to prepare the plant extracts. The leaves and flowers were washed with distilled water, air-dried in the shade for 4–5 days, ground into fine powder, and stored in airtight containers. The extraction process utilized three different solvents-methanol, ethanol, and acetone at a 1:10 ratio. The extracts obtained were labelled as follows: *B. Monosperma* Methanol (BM*Met*), *B. Monosperma* Ethanol (BM*Eth*), *B. Monosperma* Acetone (BM*Ace*), *S. Campanulata* Methanol (SC*Met*), *S. Campanulata* Ethanol (SC*Eth*), *S. Campanulata* Acetone (SC*Ace*), *D. Regia* Methanol (DR*Met*), *D. Regia* Ethanol (DR*Eth)* and *D. Regia* Acetone (DR*Ace)*.

Antimicrobial activity by disc diffusion assay

The antimicrobial activity of crude extracts (BMMet, BMEth, BMAce, SCMet, SCEth, SCAce, DRMet, DREth, and DRAce) was evaluated using the disc diffusion method against strains from the ATCC. Test organisms included three Gram-positive bacteria (*Staphylococcus epidermidis, Bacillus cereus, Staphylococcus aureus*), three Gram-negative bacteria (*Escherichia coli, Klebsiella aerogenes, Proteus vulgaris*), and one fungus (*Candida albicans*). Plates with Nutrient Agar or Potato Dextrose Agar were inoculated and incubated at 37°C—bacterial strains for 24 hours and the fungal strain for 48 hours[6]. A bacterial suspension (10x CFU/mL) was swabbed onto agar plates, and sterilized discs (Whatman

No. 2) impregnated with 500 µg/mL of extract or standard antibiotic were placed on the surface. Inhibition zones were measured after incubation, with tetracycline as the positive control.

Antioxidant activity by DPPH assay

The antioxidant activity of the plant extracts was evaluated using the DPPH free radical scavenging assay[7]. A 0.1 mM DPPH solution was prepared and kept away from light for 30 minutes. Plant extracts at varying concentrations (10–160 µg/mL) were mixed with 3.9 mL of DPPH solution, shaken, and incubated in the dark at room temperature for 45 minutes. Absorbance was measured at 517 nm using a Venchal Scientific Elisa Reader (Model: 512101).

$$\% \text{ DPPH scavenged} = \{(Ac - At)/Ac\} \times 100$$

The IC_{50} values were determined using linear regression analysis with ascorbic acid (10–160 µg/mL) as the reference standard.

Anticancer activity

Human cancer cell culture

The human lung cancer cell line (A549) was obtained from the National Centre for Cell Sciences (NCCS), Pune, India, and cultured in RPMI-1640 medium supplemented with 10% FBS, 100 IU/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were maintained at 37ºC, 100% humidity, and 5% CO_2 in a CO_2 incubator.

MTT assay

The cytotoxic effects of the extracts on A549 cells were assessed using the MTT assay[8]. Cells were seeded at 2×10t cells/well in 12-well plates and treated with varying extract concentrations upon reaching 80% confluence. Following 24 hours of incubation in a CO, incubator and PBS washing, 500 µL of MTT solution was added, followed by a 4-hour incubation. The obtained formazan crystals were then dissolved in 500 µL of DMSO, and the absorbance was measured at 540 nm. Paclitaxel was the standard drug, and 10% DMSO served as the negative control. IC_{50} values were calculated using linear regression analysis, and data were presented as a percentage of cell viability. The general formula used in estimating the percentage of viable cells:

$$\% \text{ Cell Viability} = \text{O.D of (treated cells)} / \text{(untreated cells)} \times 100$$

RESULTS

Anti-bacterial activity

The antimicrobial activity of the extracts from *Butea monosperma, Delonix regia, and Spathodea campanulata* showed variability depending on the solvent and microorganism tested. Among the three plants, *Butea monosperma* extracts demonstrated the most significant inhibition. The BM*Ace* extract showed a zone of inhibition up to 21±0.24 mm against *S. aureus*, indicating a strong antibacterial effect. Additionally, BM*Eth* and BM*Met* extracts exhibited substantial activity, particularly against *K. aerogenes* and *B. cereus* [Figure 1].

In comparison, *Delonix regia* showed relatively moderate antimicrobial activity, with the DR*Ace* extract having the highest zone of inhibition at 21.1±0.66 mm against *S. epidermidis*, but lower inhibition against *S. aureus* and *K. aerogenes* [Figure 2]. *Spathodea campanulata* exhibited a slightly lower range of inhibition zones. The SC*Ace* extract showed up to 18±0.40 mm against *P. vulgaris*. However, the SC*Eth* extract was particularly effective against *S. aureus* (17.4±0.26 mm), making it comparable to the other plants in specific cases [Figure 3]. Overall, while all three plants showed promising antimicrobial activity, *Butea monosperma* appeared to be the most effective across a wider range of microorganisms, particularly with its acetone and ethanol extracts, followed closely by *Spathodea campanulata* in ethanol extract and *Delonix regia* in acetone extract.

Anti-fungal activity

The methanol extract of *Spathodea campanulata* exhibited the strongest antifungal activity, with a zone of inhibition measuring 26.2±0.49 mm against *C. albicans*. Similarly, the BM*Eth* extract and the DR*Ace* extract showed notable inhibition zones of 25.9±0.23 mm. However, the SC*Eth* extract displayed the least activity, with a zone of 5.7±0.56 mm. Overall, these findings suggest significant antifungal potential of the plant extracts against *Candida albicans*, making them promising candidates for antifungal therapeutic applications [Figure 4].

Antioxidant activity

The antioxidant activity of the extracts was assessed using the DPPH *in vitro* free radical scavenging assay. The IC_{50} value, which indicates the concentration needed to capture 50% of the DPPH free radical, was calculated. Extracts with lower IC_{50} values demonstrate higher antioxidant activity. In this study, ascorbic acid served as the standard with an IC_{50} value of 46.67±1.15ìg/mL. The antioxidant activities of methanol, ethanol, and acetone extracts of *Butea monosperma*, *Delonix regia*, and *Spathodea campanulate*, along with ascorbic acid, against the DPPH free radical are depicted in Figures 5, 6, and 7.

The IC_{50} values for *Butea monosperma* extracts were 83.76±1.19ìg/mL (BM*Met*), 61.55±1.22ìg/mL (BM*Eth*), and 93.10±1.22ìg/mL (BM*Ace*). For *Delonix regia* extracts, the

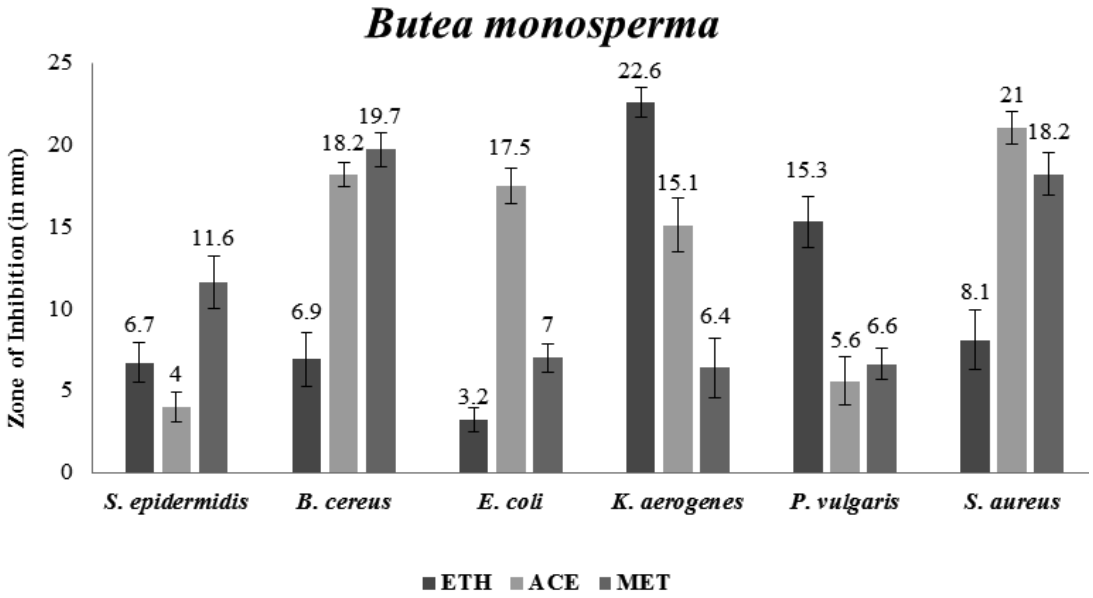

Fig. 1. Zone of inhibition of *Butea monosperma* plant extracts against various pathogens

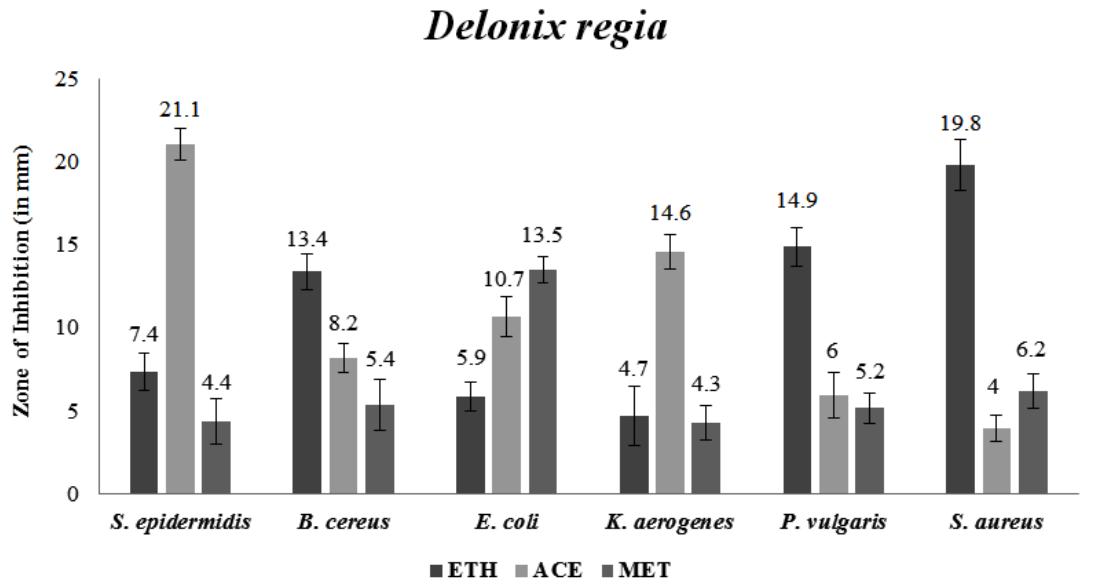

Fig. 2. Zone of inhibition of *Delonix regia* plant extracts against various pathogens

IC_{50} values were 80.22±1.29ìg/mL (DR*Met*), 69.29±1.09ìg/mL (DR*Eth*), and 89.41±1.17ìg/mL (DR*Ace*). *Spathodea campanulate* extracts had IC_{50} values of 78.29±1.18ìg/mL (SC*Met*), 68.66±1.05ìg/mL (SC*Eth*), and 83.89±1.11ìg/mL (SC*Ace*). The findings of this study reveal that the ethanol extract exhibited the highest activity, followed by the methanol extract, while the acetone extract displayed the least activity among the three.

Anticancer activity

The anticancer activity of the extracts was determined using the MTT assay. All the extracts

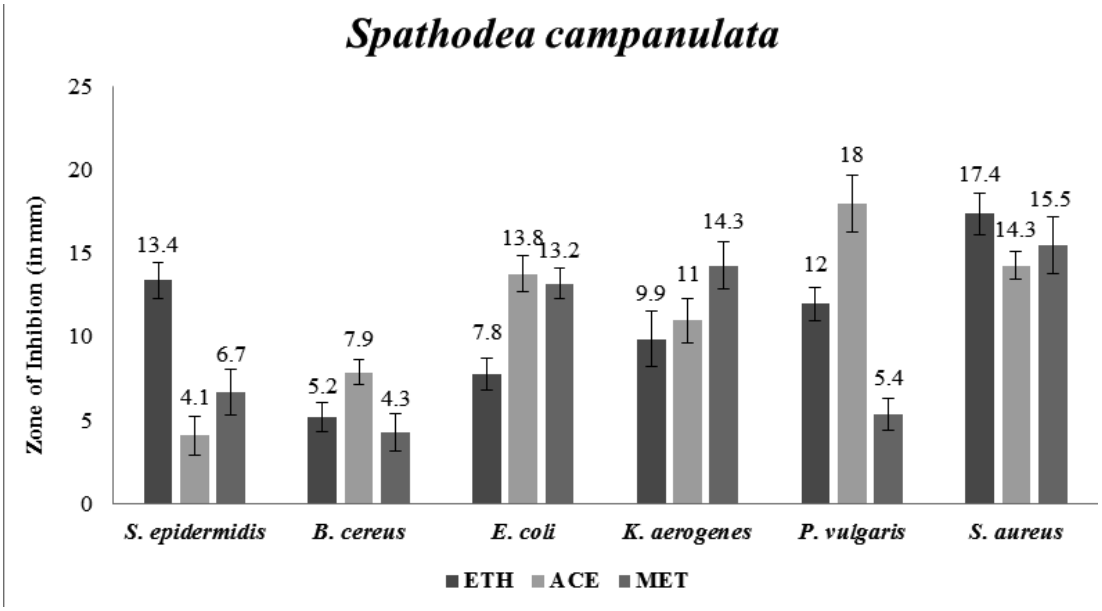

Fig. 3. Zone of inhibition of *Spathodea campanulata* plant extracts against various pathogens

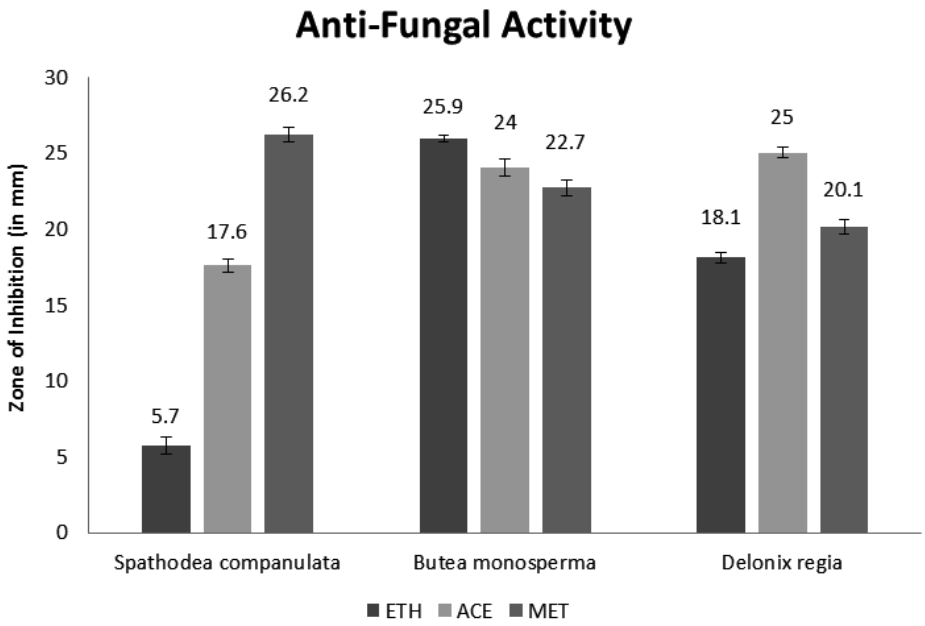

Fig. 4. Zone of inhibition of different solvent extracts of *Butea monosperma*, *Delonix regia* and *Spathodea campanulate* against *Candida albicans*

of the three plants showed a dose dependent cytotoxic activity against cell line A-549. In this study, Cisplatin served as the standard with an IC_{50} value of 32.76±1.25ìg/mL. The growth inhibitory ability of methanol, ethanol, and acetone extracts of *Butea monosperma*, *Delonix regia*, and *Spathodea campanulate*, along with Cisplatin, on A-549 cell line were depicted in Figures 8, 9, and 10.

For *Butea monosperma* extracts, the IC_{50} values were 36.12±1.01ìg/mL (BM*Met*), 51.07±1.12ìg/mL (BM*Eth*), and 71.03±1.29ìg/mL (BM*Ace*). *Delonix regia* extracts had IC_{50} values of 48.70±1.19ìg/mL (DR*Met*), 62.85±1.12ìg/mL (DR*Eth*), and 72.17±1.11ìg/mL (DR*Ace*). *Spathodea campanulate* extracts recorded IC_{50} values of 85.39±1.01ìg/mL (SC*Met*), 92.72±1.21ìg/mL (SC*Eth*), and 101.59±1.20ìg/mL (SC*Ace*). The findings of the current study reveal that, among the three extracts, the methanol extract demonstrated the highest cytotoxic activity, followed by the ethanol extract, with the acetone extract showing the least activity.

DISCUSSION

Medicinal plants serve as rich reservoirs of compounds essential for the development of pharmacopoeial, non-pharmacopoeial, and

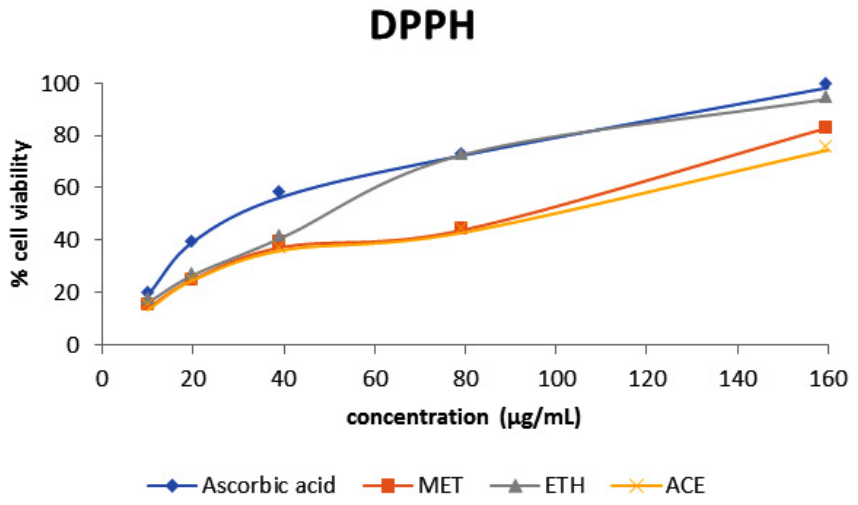

Fig. 5. DPPH scavenging activity of different solvent extracts of *Butea monosperma*

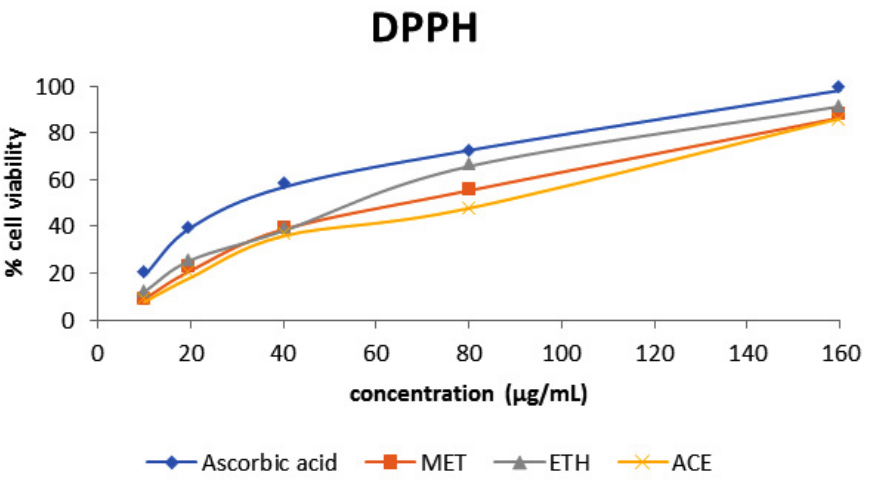

Fig. 6. DPPH scavenging activity of different solvent extracts of *Delonix regia*

synthetic pharmaceuticals. Amid the vast array of plants with medicinal properties, those from the Fabaceae and Bignoniaceae families have demonstrated remarkable potential[9]. This study builds on traditional knowledge by providing pharmacological evidence for the antimicrobial, antioxidant and anticancer activity of *Butea monosperma*, *Delonix regia*, and *Spathodea campanulata* plants.

Bacterial infection is a serious concerned global health issue in this 21st century. The upcoming issue of bacteria developing resistance to existing antibiotics is a crucial step to develop new antibiotics with novel mechanism of action to encounter a major number of health problems[10].

Previous investigations into the antimicrobial properties of *Butea monosperma* root extracts in petroleum ether against various bacterial strains have shown significant antimicrobial and antifungal effects, particularly against *A. hydrophilia*, *S. faecalis*, *S. typhae*, *S. cohni*, and *E. coli*[11]. Further research found that the MECL wood extract of *Delonix regia* exhibited significant antibacterial activity against *B. subtilis* (16.66 ± 0.57 mm), *S. lutea* (14.33 ± 1.15 mm), and *S. aureus* (20.66 ± 0.57 mm). The MECL bark

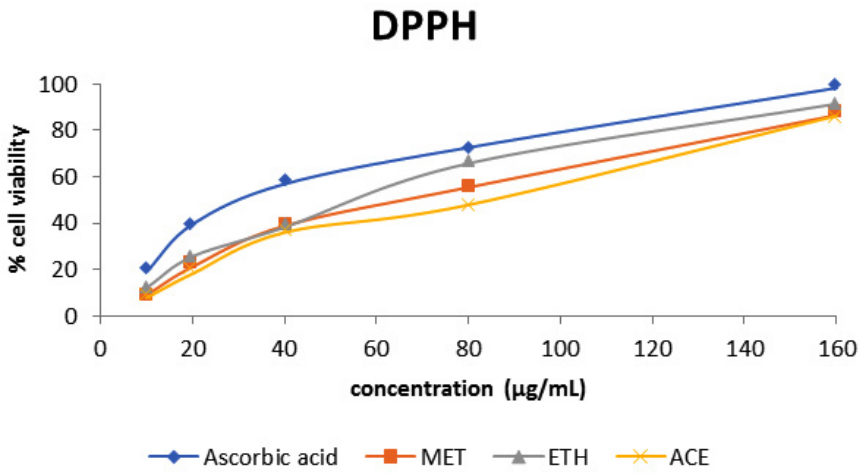

Fig. 7. DPPH scavenging activity of different solvent extracts of *Spathodea campanulata*

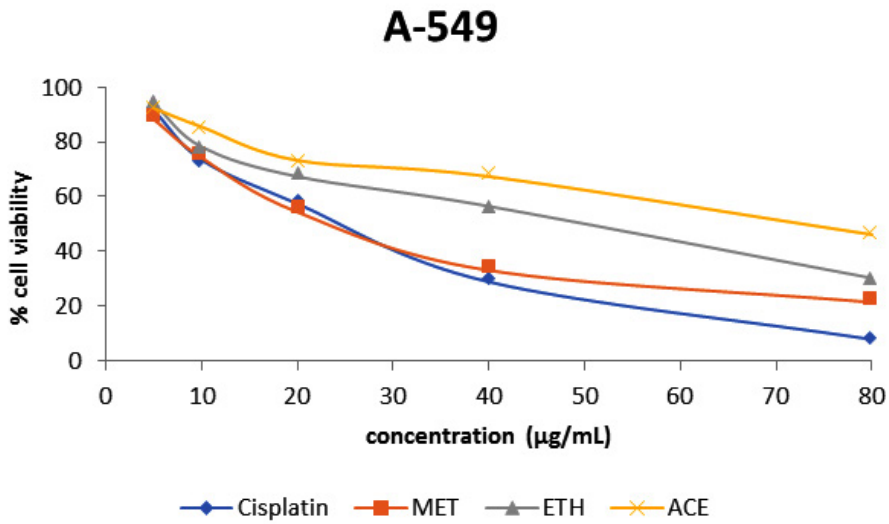

Fig. 8. Growth inhibitory activity of different solvent extracts of *Butea monosperma* against A549 cell line.

extract was most effective against *E. coli* and the essential oil exhibited significant activity against *P. carotovorum*. Additionally, the bark extract had the highest Suppression of mycelial growth against *P. variotii* and *P. selerotigeni*[12]. Similarly, a study on hexane, ethyl acetate, and ethanol extracts of *Spathodea campanulata* revealed significant antibacterial activity, with ethanol and ethyl acetate extracts performing comparably to Rifampicin[13]. These previous studies are consistent with the current study's results, showing that among the three extracts, the ethanol extract exhibited the highest activity, compared to methanol and acetone extract.

The *in vitro* antioxidant activity of the plant extracts was assessed using the DPPH assay, which revealed a high antioxidant scavenging ability. Previous research showed that the ethanolic

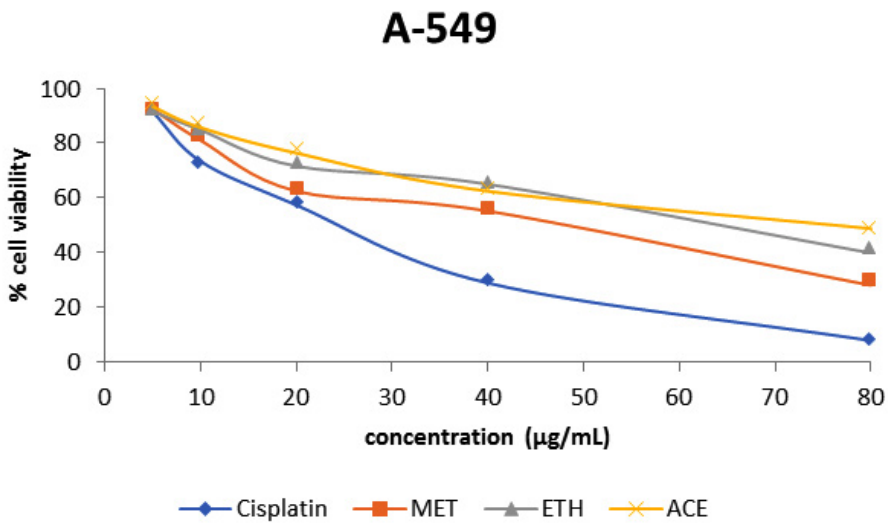

Fig. 9. Growth inhibitory activity of different extracts of *Delonix regia* against A549 cell line

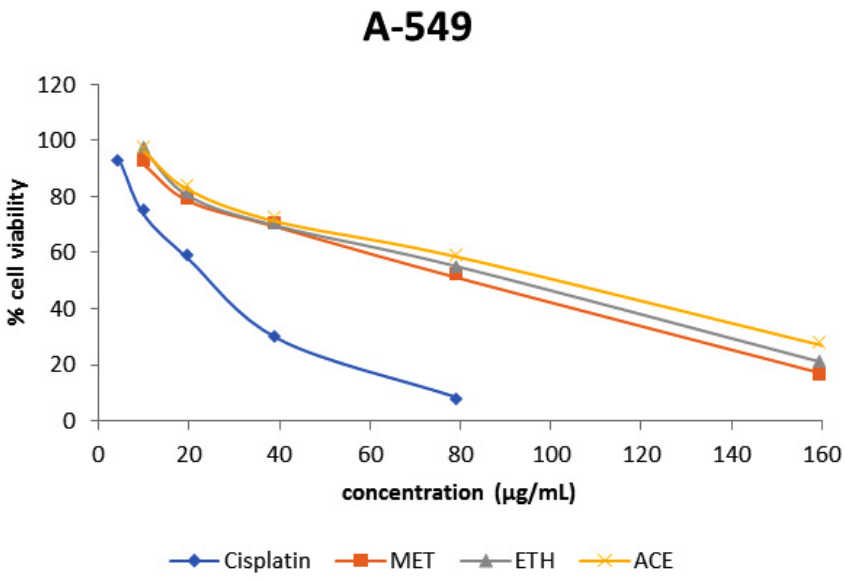

Fig. 10. Growth inhibitory activity of different extracts of *Spathodea campanulata* against A549 cell line

extract of *Spathodea campanulata* leaves exhibited high antioxidant activity against DPPH free radicals, outperforming chloroform and ethyl acetate extracts[14]. Another study assessed the DPPH scavenging activity of chloroform, ethyl acetate, and methanol extracts from *Butea monosperma* flowers, revealing the plant's significant antioxidant potential[15]. Similarly, an evaluation of the antioxidant potential of Delonix regia flowers found that the ethyl acetate extract exhibited significantly higher activity compared to other extracts[16]. These prior studies corroborate the current study's findings, as the ethanol extract exhibited the highest activity.

The antioxidants in the extracts are likely responsible for their anticancer effects, as they reduce the risk of cancer by scavenging free radicals[17]. The MTT assay evaluated the antiproliferative activity of the plant extracts, revealing significant inhibition of A549 cell growth in a concentration-dependent manner. The methanol extract exhibited the highest cytotoxic activity, followed by the ethanol extract, while the acetone extract showed the least activity. A study showed that the methanol extract of *Butea monosperma* had dose-dependent anticancer effects against the A-549 cell line[18]. Similar results were found for the cytotoxic effects of *Delonix regia* extracts against breast and liver cancer cell lines. Nanoparticles green-synthesized using *Delonix regia* plant extracts exhibited significant cytotoxicity against the A-549 cell line[19,20]. The lower cytotoxicity of *Spathodea campanulata* aligns with research findings, which noted that *Spathodea campanulata* extracts had weaker anticancer activity compared to other plants[21].

The cytotoxic effects of the extracts observed in this study are consistent with previous findings, which demonstrated strong growth inhibitory effects on U373, A549, MCF-7, EAC and SKMEL-28 cancer cells[14,15,21]. It supports the hypothesis that these solvents effectively extract bioactive compounds, such as flavonoids and alkaloids, known for their anticancer properties[22].

This research aims to enrich the existing scientific knowledge on the pharmacological properties of these plants and explore their potential in developing natural, plant-based therapies for various medical conditions. With increasing concerns over the adverse effects of synthetic drugs and the rise of antimicrobial resistance, the findings of this study could pave the way for the discovery of new, more sustainable therapeutic options derived from nature.

CONCLUSION

The study evaluates the antibacterial, antifungal, antioxidant, and anticancer activities of extracts from *Butea monosperma*, *Delonix regia*, and *Spathodea campanulata*. The antimicrobial activity of these extracts varied depending on the solvent and microorganism tested. *Butea monosperma* extracts demonstrated the most significant inhibition, particularly the BM*Ace* extract against *Staphylococcus aureus*. In comparison, *Delonix regia* and *Spathodea campanulata* showed moderate antimicrobial activity. The methanol extract of *Spathodea campanulata* displayed the strongest antifungal activity against *Candida albicans*, with *Butea monosperma* and *Delonix regia* extracts also showing notable inhibition zones.

The antioxidant activity, assessed using the DPPH assay, showed that ethanol extracts had the highest activity, followed by methanol and then acetone extracts. In anticancer activity assessments using the MTT assay, the methanol extract of *Butea monosperma* exhibited the highest cytotoxic activity against cell line A-549, followed by ethanol and acetone extracts. The findings indicate that *Butea monosperma* extracts are the most effective across various activities, underscoring their potential for developing new treatments targeting antimicrobial resistance and cancer. Further exploration of the therapeutic potential of these plant extracts could lead to significant benefits for human health and well-being.

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Conflict of interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

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

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Authors' Contribution

Kalyani Chepuri: conceptualized the study objectives, hypothesis and designed the experimental work; Chittepu Pranitha, Kathuroju Harikrishna, and Vadakavila Geethikalal: performed the experimental work, data collection, analysis, and writing; Chidepudi Devi Sri Lalitha Naga Tulasi : supervised the experimental studies, and Manikantha Dunna reviewed and edited the manuscript.

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