Current Botany 2025, 16: 292-301
doi: 10.25081/cb.2025.v16.9795
https://updatepublishing.com/journal/index.php/cb

Research Article

ISSN: 2220-4822

Pharmacological activities of *Alangium salviifolium* (L.f.) Wangerin extracts

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ABSTRACT

Tribal communities traditionally use *Alangium salviifolium* raw extracts to treat skin diseases. Inspired by tribal knowledge and the understanding that some skin conditions may be linked to liver health, the study explored the potential of A. *salviifolium* for treating liver pathogen infections. This study evaluates methanolic leaf, fruit, and seed extracts for their potential therapeutic effects against liver-related pathogens and conditions, aiming to explore their usefulness in treating liver diseases. The extracts were evaluated for antibacterial activity using the agar well diffusion method. Each bacterial strain was tested with a different control antibiotic: *Escherichia coli* (Chloramphenicol), *Streptococcus mutans* (Amikacin), *Salmonella typhi* (Ciprofloxacin), and *Klebsiella pneumonia* (Co-trimoxazole). The leaf extracts showed the strongest antibacterial inhibition at both concentrations, followed by moderate inhibition by the fruit and seed extracts. Antifungal activity was assessed using the Kirby–Bauer disk diffusion method against *C. albicans*, *C. tropicalis*, *C. parapsilosis*, and *C. glabrata*, with Fluconazole as the control. In the antifungal assays, each extract displayed a distinct performance against the tested fungal strains. The anti-inflammatory activity was analyzed using the bovine serum albumin denaturation assay, which showed IC_{50} values of 784 µg/mL for the leaf extract, 417 µg/mL for the fruit extract, and 354 µg/mL for the seed extract, with diclofenac as the control. Anticancer activity, tested on HepG2 cells using the MTT assay, showed IC_{50} values of 109.7 µg/mL for the fruit extract, 93.9 µg/mL for the leaf extract, and 49.5 µg/mL for the seed extract. The results indicate that the seed extract exhibits the most potent broad-spectrum bioactivities. Further *in vivo* and clinical studies are warranted to confirm therapeutic efficiency and safety for liver disease treatment.

Received: August 13, 2025
Revised: December 12, 2025
Accepted: December 15, 2025
Published: December 26, 2025

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KEYWORDS: Antibacterial, Antifungal, Anti-inflammatory, Anticancer, *Alangium salviifolium*

INTRODUCTION

Across the globe, liver disorders such as cirrhosis, hepatic steatosis, and hepatitis affect millions, leading to severe health complications and mortality. Recently, the medical terminology for non-alcoholic fatty liver disease (NAFLD) and its advanced stage, non-alcoholic steatohepatitis (NASH), has been updated by Addissouky (2025). With a focus on medicinal plants including milk thistle (*Silybum marianum*), turmeric (*Curcuma longa*), stonebreaker (*Phyllanthus niruri*), *Andrographis paniculata*, katuki (*Picrorhiza kurroa*), and licorice (*Glycyrrhiza glabra*) this highlights the role of oxidative stress in liver damage and antioxidant protection while also emphasizing issues with quality and drug interactions (Dash *et al.*, 2025). *Panax ginseng* (ginsenosides), *Lygodium flexuosum* (extract), *Silybum marianum* (silymarin), *Ganoderma lucidum* (polysaccharides, triterpenoids), and osthole (from *Cnidium monnieri*) are emphasized for their hepatoprotective and anti-fibrotic properties in chronic liver diseases. These effects are primarily achieved through antioxidant, anti-inflammatory, and anti-fibrotic mechanisms (Foghis *et al.*, 2023). Medicinal plants provide affordable antimicrobial compounds crucial for treating drug-resistant infections in low-income populations lacking access to synthetic drugs and modern healthcare in developing countries (Zahan *et al.*, 2012). Research into plant-based therapies for inflammation and related diseases is continuing because common NSAIDs like ibuprofen and diclofenac effectively treat inflammation but can also be expensive, cause long-term enteric pathologies, cardiocirculatory and renal issues. In contrast, medicinal herbs offer safer, less harmful, and more affordable anti-inflammatory alternatives (Afroz Shoily *et al.*, 2025). Liver cancer ranks sixth among malignant tumors and the fourth most lethal type of cancer among worldwide. The chemotherapy is still the standard treatment for liver tumors that cannot be cured, despite the development of other anticancer treatments. Using oat galactolipids as natural ASGPR ligands, GCLTVs exhibit better anticancer and liver-targeting properties than curcumin vesicles, which make them an attractive option for liver-targeted nano preparations (Ren *et al.*, 2025). In Siddha and Ayurveda, *Alangium salvifolium* bitter root bark is employed as an astringent, purgative, emetic, and febrifuge, highlighting

its therapeutic properties. Recent research has shown that it has *anti*-cancer properties and is an effective treatment for a variety of ailments, including leprosy, piles, rheumatism, diarrhea, back pain, vatha and kapha issues, and skin conditions. This herb is used for skin conditions in traditional formulations including Purayodum Virana Thailam and Naalpattapunnukupugai (Navinkumar *et al.*, 2019). A. *salviifolium*'s rich phytochemical profile is reflected in its leaves, which contain alangimarckine, deoxutubulosine, alangiside, and β-sitosterols; fruits, which contain iso-alangiside and demethylneoalangiside; seeds, which contain alkaloids like alangimarine, emetine, and cephaeline; and stem and root bark, which contain compounds like alangine A, B, ankoline, benzoquinolizidine, and stigmasterol (Venkateshwarlu *et al.*, 2011; Tanwer & Vijayvergia, 2014; Jha & Yogesh, 2015; Kumar, 2016; Kaur *et al.*, 2017; Shravya *et al.*, 2017). Its traditional use against skin cancer has been scientifically validated by the demonstration of dose-dependent antiproliferative and apoptotic activities of A. *salviifolium* methanolic bark extract on A431 and B16F10 skin cancer cells (Dhruve *et al.*, 2019). Previous study showed A. *salviifolium* methanol bark extract protects mice liver from CCl4 damage by enhancing detoxifying and antioxidant enzymes, reducing lipid peroxidation and liver damage markers (Dhruve *et al.*, 2022).

The aim of the present study is to evaluate the leaf, fruit, and seed extracts of A. *salviifolium* for anticancer activity against human liver cancer (HepG2) cells using the MTT assay, antimicrobial effects against microbial liver pathogens, and anti-inflammatory potential using a protein denaturation assay. There is no prior research has explored these combined activities of this A. *salviifolium* against liver-related ailments. The study focuses on discovering natural A. *salviifolium* extracts as safe and effective agents for liver cancer treatment and associated diseases.

MATERIALS AND METHODS

Plant Collection and Authentication

Alangium salviifolium (L.f.) Wangerin fresh leaves, fruits and seeds were collected in March 2023 insight Namakkal District Kadhapalli road, Tamil Nadu, India (Latitude 11.273918°, Longitude 78.138683°). The collected botanical sample was examined and validated by a taxonomist at the Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore, India and voucher specimen was deposited (BSI/SRC/5/23/2024/Tech/235).

Plant Extract Preparation

The leaves and fruits were handpicked, carefully and rinsed first with tap water followed by distilled water and dried under shade. The fruits were then separated into peels and flesh, while the seeds were washed, de-shelled, and their kernels dried separately. All plant parts were dried for 10-15 days, pulverized into coarse powder, and preserved in sealed containers. For the extraction process, 30g of each finely ground plant material was immersed in 300 mL of methanol, ensuring complete submersion. The containers were tightly sealed and kept at room temperature for a minimum7 days with periodic shaking. The solution was initially passed through standard filter paper and then further purified using Whatman No. 1 filter paper. The obtained crude samples were allowed to evaporate naturally at ambient temperature using glass Petri dishes, and the extract yield percentage was calculated on a dry weight basis using the following formula.

Percentage of yield= Dry weight of crude extract/Dry weight of initial sample x 100

Microbial Source and Identification

Clinical isolates were obtained and identified using the VITEK system at Saveetha University, Chennai. The microorganisms selected for this study were associated with hepatic diseases which includes *Streptococcus mutans* (MTCC 497), controlled with Amikacin; *Escherichia coli* (MTCC 443), controlled with Chloramphenicol; *Salmonella typhi* (MTCC 733), controlled with Ciprofloxacin; and *Klebsiella pneumoniae* (MTCC 109), controlled with Co-trimoxazole. The fungal strains tested were *Candida parapsilosis* (MTCC 2509), *Candida tropicalis* (MTCC 230), *Candida glabrata* (MTCC 3019), and *Candida albicans* (MTCC 227), all controlled with Fluconazole. Antimicrobial activity assays were carried out using extracts prepared from A. *salviifolium* parts, including leaves, fruits, and seeds. The extracts were tested against these microorganisms following standard protocols, with the results compared to those obtained from the respective standard antibiotics and also antifungal agents for each strain.

Well-diffusion Method

The antibacterial activity of plant extracts was examined against *S. mutans*, *E. coli*, *S. typhi*, and *K. pneumoniae* using the Agar well diffusion method. Tests were conducted on Mueller Hinton Agar (MHA; Himedia, India), prepared in distilled water at pH 7.0, through sterilized by autoclaving at 121 °C for 15 minutes. The sterilized medium was poured into Petri dishes kept allowed to solidify. The bacterial suspension containing about 10 µL CFU/mL of freshly grown cultures was evenly distributed over the plate surface with a sterile swab. Three circular wells (8 mm each) were created in the agar: two wells received 25 µL and 100 µL of each plant extract, while the third contained a reference antibiotic as a standard control. Plates were kept at room temperature for 4 hours for sample diffusion, then incubated at 37 °C for 24 hours. The antimicrobial action was assessed by measuring inhibition zones around the wells (Balouiri *et al.*, 2016).

Antifungal Assay

The antifungal efficacy of plant extracts was evaluated against four fungal strains such as *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, and *C. albicans* were using the Kirby-Bauer disk diffusion technique (Sharma *et al.*, 2016). Employing the lawn culture approach, fungal inocula were uniformly spread on

Mueller Hinton Agar (MHA) plates. Three circular depressions were made in the agar with a sterile borer, and each was filled with 25 µg/mL and 100 µg/mL concentrations of the respective samples. Fluconazole (25 µg) served as a reference antifungal control and was placed on the agar surface. The prepared plates were allowed to stand for 2 hours to facilitate the diffusion of the compounds into the medium, followed by incubation at 37 ºC for 24 hours. After incubation, the inhibition zones formed around each well were measured with the Hi Antibiotic Zone Scale to determine the antifungal potency of each sample (Hudzicki, 2009).

Protein Denaturation Assay

Bovine serum albumin (BSA) was procured from Hi Media, India, while all other reagents were obtained from Avra Chemicals, India. The anti-inflammatory potential of leaf, fruit, and seed extracts was assessed based on their ability to inhibit BSA denaturation, following the procedure outlined by Gunathilake *et al*. (2018). Various concentrations of the test samples were combined with 450 µL of a 0.5% bovine serum albumin (BSA) solution, and the total volume was brought to 1 mL using distilled water. The prepared mixtures were kept at 37 °C for 20 minutes, and then heated at 72 °C for 15 minutes to trigger protein denaturation. Once cooled, 2.5 mL of 0.2 M sodium phosphate buffer adjusted to pH 6.3 was incorporated into each mixture. The resulting solutions' turbidity was determined by recording absorbance at 660 nm with an Epoch 2 UV-Visible microplate reader (Bio Tek, USA). Control blanks containing the test substances without BSA were prepared to account for background interference, while a buffer-only sample served as the standard control (Akter *et al*., 2020). The proportion of protein denaturation suppression was determined using the subsequent equation.

$$\text{Antiinflammatory activity}(\%) = \left[\frac{\text{Absorbance}(\text{Control}) - \text{Absorbance}(\text{Sample})}{\text{Absorbance}(\text{Control})}\right] \text{x}100$$

Collection of Cell Line

The HepG2 cell line, representing human hepatic carcinoma, was obtained from the National Centre for Cell Science (NCCS) in Pune, India. The cells were cultivated in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution. The cultures were routinely passaged every three days within a CO_2 incubator sustained at 37 °C, under a controlled gaseous environment containing 5% CO_2 and 18-20% oxygen (Pfeifer *et al*., 2025).

Assessment of Cytotoxicity

A 200 µL cell suspension containing 20,000 cells per well was plated in a 96 well microplate and incubated for 24 hours to allow attachment. Different concentrations of leaf, fruit, and seed extracts (6.25-100 µg/mL) were then added, followed by another 24 hours of incubation at 37 °C in a humidified 5% CO_2 atmosphere. After treatment, the medium was removed and MTT reagent was added to reach a 0.5 mg/mL final concentration. The plates were covered to prevent light exposure and incubated for 3 hours. The reagent was then replaced with 100 µL of DMSO to dissolve formazan crystals, and gentle shaking ensured uniform dissolution. Absorbance was measured at 570 nm using a microplate spectrophotometer (Akhter *et al*., 2023). Cell viability percentage was calculated through the respective formula.

$$(\%)\text{Cell Viability} = \left[\frac{\text{Mean abs of treated cells}}{\text{Mean abs of Untreated cells}}\right] \text{x}100$$

Statistical Analysis

All *in vitro* experiments were performed in triplicate and duplicate. Data was analyzed by using SPSS version 25. Differences among the leaf, fruit, and seed extracts of *A. salviifolium* were evaluated by twoway ANOVA, with significance set at $P<0.05$. Results are reported as mean ± standard deviation (SD). Dunnett's posthoc test was used to compare treated samples with the control, with significance indicated by asterisks (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

RESULTS AND DISCUSSION

Antibacterial Activity

The antibacterial effects of methanol extracts from the leaf, fruit, and seed of *A. salviifolium* against *S. mutans*, *E. coli*, *S. typhi*, and *K. pneumoniae*. The inhibition zones indicate varying bacterial susceptibility among the tested species. Positive controls included Amikacin for *S. mutans*, Chloramphenicol for *E. coli*, Ciprofloxacin for *S. typhi*, and Co-trimoxazole for *K. pneumoniae*.

The results showed that the leaf extract demonstrated antibacterial activity against *S. mutans*, *E. coli*, *S. typhi*, and *K. pneumoniae*, strong activity was shown against *K. pneumoniae* and *S. typhi*, with moderate effects on *S. mutans* and *E. coli* (Table 1). The Results showed that the antibacterial activity of the fruit extract was concentration-dependent. Only *S. typhi* was suppressed at 25 µg/mL; these four bacteria were impacted at 100 µg/mL, with *K. pneumoniae* and *S. mutans* exhibiting the greatest suppression, indicating strong antibacterial potential (Table 2). This demonstrates that at a lower dosage of 25 µg/mL, the seed extract showed only modest inhibition against *S. typhi* and no antibacterial action against *S. mutans*, *E. coli*, or *K. pneumoniae*. All examined bacteria were suppressed by the seed extract at the greater concentration of 100 µg/mL, albeit it was less successful than conventional antibiotics (Table 3). So, the antibacterial activity of leaf, fruit, and seed extracts against all tested bacteria is shown, with efficiency varying based on extract type and concentration; these were compared with several conventional antibiotics.

Table 1: Antibacterial activity of *A. salviifolium* leaf extracts

		Zone of antibacterial inhibition (mm) in leaf extract		
S. No.	Name of the Pathogens	25 µg/mL	100 µg/mL	Positive Control
1	*S. mutans*	11.00±0.15	13.97±0.17	17.07±0.41 (Amikacin)
2	*E. coli*	10.90±0.29	14.01±0.19	19.02±0.26 (Chloramphenicol)
3	*S. typhi*	15.01±0.14	12.23±0.60	22.99±0.31 (Ciprofloxacin)
4	*K. pneumoniae*	17.00±0.20	11.83±0.90	5.12±0.10 (Co-trimoxazole)

Table 2: Antibacterial activity of *A. salviifolium* fruit extracts

		Zone of antibacterial inhibition (mm) in fruit extract		
S. No.	Name of the Pathogens	25 µg/mL	100 µg/mL	Positive Control
1	*S. mutans*	0.00±0.00	18.00±0.61	18.13±0.34 (Amikacin)
2	*E. coli*	0.00±0.00	16.00±0.72	23.88±0.42 (Chloramphenicol)
3	*S. typhi*	15.14±0.31	12.10±0.38	24.03±0.21 (Ciprofloxacin)
4	*K. pneumoniae*	0.00±0.00	19.49±0.18	13.99±0.30 (Co-trimoxazole)

Table 3: Antibacterial activity of *A. salviifolium* seed extracts

		Zone of antibacterial inhibition (mm) in seed extract		
S. No.	Name of the pathogens	25 µg/mL	100 µg/mL	Positive control
1	*S. mutans*	0.00±0.00	13.05±0.29	16.99±0.40 (Amikacin)
2	*E. coli*	0.00±0.00	15.03±0.56	24.02±0.25 (Chloramphenicol)
3	*S. typhi*	6.08±0.33	14.00±0.20	25.99±0.28 (Ciprofloxacin)
4	*K. pneumoniae*	0.00±0.00	16.11±0.18	14.17±0.39 (Co-trimoxazole)

Methanolic and ethanolic extracts derived from *Artemisia herba-alba* and its related species displayed notable antimicrobial potency against *S. aureus*, *E. coli*, and *K. pneumoniae*, producing inhibition zones between 18 and 26 mm. The antibacterial effect was mainly attributed to the presence of flavonoids and terpenoids, which disrupt bacterial membranes and hinder DNA gyrase function (Baraich *et al.*, 2025). *Psidium guajava* leaf extracts in methanol and chloroform showed inhibitory zones against *Pasteurella multocida* and *Mannheimia haemolytica* that ranged from 25 to 30 mm, above those of gentamicin and on par with oxytetracycline. The findings imply that fractions rich in saponins and phenolic chemicals have a greater ability to get through bacterial cell walls and block important enzymatic processes (Asfa *et al.*, 2025). Hydro methanolic extracts of nine medicinal plants showed antibacterial activity with inhibition zones of 6.0-23.0 mm and MIC values of 0.1-12.8 mg/mL. *Berberis vulgaris*, *Cistus monspeliensis*, and *Punica granatum* were most effective against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Enterobacter cloacae* (Bereksi *et al.*, 2018). Ethanol extracts of *Loranthus acaciae* and *Cymbopogon proximus* showed strong antibacterial activity with inhibition zones of 48.3-57.5 mm against multidrug-resistant *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* spp., and *Pseudomonas* spp (Jia *et al.*, 2025). Methanolic and acetonic extracts of *Cinnamomum verum* showed the highest antibacterial activity (22.3 mm) against *Staphylococcus aureus*, while ethanolic extracts of *C. verum* and *Trachyspermum ammi* were most effective against *S. typhi* (22.3 mm) (Irshad *et al.*, 2025).

Antifungal Activity

The antifungal effects of methanol extracts from the leaf, fruit, and seed of *A. salviifolium* against *C. albicans*, *C. tropicalis*, *C. parapsilosis*, and *C. glabrata*. The inhibition zones indicate varying antifungal potency among the tested plant parts, compared with fluconazole as the positive control.

The leaf extract inhibits all tested *Candida* species in a dose-dependent manner. While fluconazole is generally more effective, it fails against *C. glabrata*, unlike the leaf extract, which remains active and shows a broader range of antifungal activity (Table 4). The results illustrate the many ways in which the fruit extract inhibited *Candida* species, with *C. glabrata* exhibiting the greatest inhibition. Higher concentrations made the effect stronger. Fluconazole demonstrated little activity against *C. glabrata*, while it was more effective against *C. albicans*, *C. tropicalis*, and *C. parapsilosis*. This suggests that different species of *Candida* have different sensitivity levels (Table 5). The results demonstrated that the seed extract had potent antifungal activity against four species of *Candida*, which increased with concentration and was most successful against *C. albicans*. The extract slightly inhibited *C. glabrata* while Fluconazole was ineffective against it, suggesting a wider antifungal spectrum across clinically significant *Candida* strains (Table 6). Overall, these leaf, fruit and seed extracts significantly inhibit *Candida* species, including those resistant to Fluconazole, and exhibit dose-dependent antifungal action.

Quercus coccifera's methanolic extract showed efficacy similar to Fluconazole, rupturing fungal cell membranes and preventing biofilm formation by over 85%. Minimum Inhibitory Concentration (MIC) values for the extract were less than 25 µg/mL (Jaber *et al.*, 2025). Cupferron showed strong antifungal activity against *C. albicans* with MIC values between 50–100 µg/mL and inhibited fungal virulence at 50-12.5 µg/mL. It was non-toxic to human microglial cells at tested concentrations, indicating its potential as a promising antifungal agent (Palma *et al.*, 2024). Aqueous extract of *Mitragyna rubrostipulata* (ZOI: 18.00-38.33 mm; MIC: 3.13-20.83 µg/mL), methanol extract of *Khaya anthotheca* (ZOI: 10.11-15.11 mm; MIC: 1.04-12.50 µg/mL), and their combination with *Distimake dissectus* (ZOI: 7.89-19.67 mm; MIC: 0.78-50.00 µg/mL) showed broad-spectrum antifungal and fungistatic activity against multiple *Candida* species, outperforming standard drugs (Akwongo *et al.*, 2024).

Table 4: Antifungal activity of *A. salviifolium* leaf extracts

Zone of antifungal inhibition (mm) in leaf extract				
S. No.	Name of the pathogens	25 μg/mL	100 μg/mL	Fluconazole (Positive control)
1	*C. albicans*	14.13±0.61	22.00±0.15	32.00±0.28
2	*C. tropicalis*	9.90±0.31	15.97±0.17	27.14±0.46
3	*C. parapsilosis*	12.07±0.30	18.95±0.21	32.94±0.38
4	*C. glabrata*	13.13±0.38	17.06±0.21	0.00±0.00

Table 5: Antifungal activity of *A. salviifolium* fruit extracts

Zone of antifungal inhibition (mm) in fruit extract				
S. No.	Name of the pathogens	25 μg/mL	100 μg/mL	Fluconazole (Positive control)
1	*C. albicans*	9.99±0.14	15.00±0.48	32.00±0.26
2	*C. tropicalis*	10.05±0.15	16.00±0.60	27.01±0.12
3	*C. parapsilosis*	9.88±0.36	13.98±0.23	33.09±0.39
4	*C. glabrata*	11.12±0.33	24.03±0.61	0.00±0.00

Table 6: Antifungal activity of *A. salviifolium* seed extracts

Zone of antifungal inhibition (mm) in seed extract				
S. No.	Name of the pathogens	25 μg/mL	100 μg/mL	Fluconazole (Positive control)
1	*C. albicans*	10.00±0.54	17.05±0.28	36.00±0.60
2	*C. tropicalis*	9.93±0.23	14.89±0.32	25.98±0.28
3	*C. parapsilosis*	10.03±0.46	16.00±0.75	34.16±0.40
4	*C. glabrata*	6.79±0.10	11.14±0.29	0.00±0.00

Anti-inflammatory Activity

The anti-inflammatory activity was assessed by inhibition of protein denaturation using BSA as substrate, showing a concentration-dependent increase in inhibition across all samples. The leaf, fruit, and seed extract inhibited protein denaturation in a dose-dependent manner, demonstrating anti-inflammatory potential. The seed extract showed significant inhibition at low concentrations (8.92% at 100 μg/mL, IC_{50} - 354 μg/mL), indicating potent bioactive compounds. The fruit extract had the highest inhibition, reaching 84.40% at 750 μg/mL with an IC_{50} of 417 μg/mL, closely matching diclofenac's 86.18% at 250 μg/mL (IC_{50} - 145 μg/mL). The leaf extract showed moderate inhibition, increasing from 11.21% at 250 μg/mL to 75.38% at 1250 μg/mL, with an IC_{50} of 784 μg/mL. Diclofenac exhibited the strongest inhibition at the lowest doses. These results indicate fruit and seed extracts have promising anti-inflammatory activity comparable to diclofenac (Figure 1).

Commelina latifolia exhibited strong anti-inflammatory activity, attributed to the presence of flavonoids and tannins that inhibit inflammatory enzymes and stabilize cellular proteins (Tadege *et al.*, 2023). The role of phytochemicals in mitigating inflammation was further supported by the significant reduction in inflammatory mediators observed in the Indian medicinal herbs *Terminalia bellarica* and *Terminalia chebula* (Shaikh *et al.*, 2015). Similar to fruit extracts, studies on *Murraya koenigii* and *Neolamarckia cadamba* demonstrated a dose-dependent inhibition of protein denaturation (Shrivastava *et al.*, 2023). Ethyl acetate fractions of *Heliotropium indicum* exhibited varying degrees of anti-inflammatory activity, as supported by molecular docking and phytochemical analyses, revealing differences in the bioactivity among the plant fractions (Afroz Shoily *et al.*, 2025). Methanol extract of *Ajuga integrifolia* showed the highest anti-inflammatory activity with an anti-denaturation value of 3.75±0.28%, indicating strong potential to inhibit protein denaturation-related inflammation (Singh *et al.*, 2024). *Psychotria densinervia* leaf extract showed a strong anti-inflammatory effect with antiprotein denaturation IC_{50} of 257.0±7.51 μg/mL, and antiproteinase activity of 74.37±1.10 μg/mL, indicating higher efficacy than the bark extract (Mba *et al.*, 2022).

Anticancer Activity

The viability of HepG2 cells decreases progressively with increasing concentrations of leaf, fruit, and seed extracts, demonstrating a clear dose-dependent cytotoxic effect. In each case, untreated cells maintain full viability, while higher doses of the extracts significantly reduce cell survival, confirming enhanced cell death compared to the control. This combined observation indicates that all three extracts such as leaf, fruit, and seed-exert strong cytotoxic effects on HepG2 cells in a concentration-dependent manner (Figure 2).

In HepG2 and U87MG cell lines, *Argemone mexicana* exhibited greater cytotoxic activity, as indicated by lower IC_{50} values (91.3 μg/mL and 78.43 μg/mL, respectively), compared with *Prosopis cineraria* (261.43 μg/mL and 108.03 μg/mL), indicating its stronger anticancer potential (Vashishth & Kataria, 2025). *Cissus woodrowii* ethanolic and aqueous extracts capacity to prevent cancer in HepG2 cells. The ethanolic stem extract exhibited the highest inhibition and the lowest IC_{50} value. Ethanol-aqueous stem and leaf extracts exhibited notable cytotoxic and brine shrimp lethality activity (Patil *et al.*, 2025).

The methanolic extract of *Vernonia leopoldi* exhibited selective cytotoxicity against HepG2 cells, with an IC_{50} value of 9.2±0.88 μg/mL. The extract induced G2/M phase arrest, apoptosis, autophagy, and inhibited cell migration. LC-MS/MS analysis associated these biological activities with the presence of diverse metabolites (Mokbel *et al.*, 2025). The ethanolic extract of *Bacopa monnieri* showed higher phenolic and flavonoid levels, strong antioxidant activity, and potent cytotoxicity against HepG2 cells, inducing 58.65% apoptosis. Docking studies further indicated strong interactions between its active compounds and cancer-related proteins (Altaf *et al.*, 2025). Methanolic extracts from 11 Saudi Arabian plants were tested against HepG2 cells, with *A. monosperma*, *O. baccatus*, and *P. glutinosa* showing the strongest cytotoxic effects, reducing cell viability to 24-29%. GC-MS analysis identified 41 phytochemicals, including four key compounds with significant anticancer potential (Khan *et al.*, 2022).

The milk thistle (MT) extract exhibited notable anti-cancer activity by inducing apoptosis, causing G1 phase cell cycle arrest, and suppressing HepG2 cell proliferation, with an

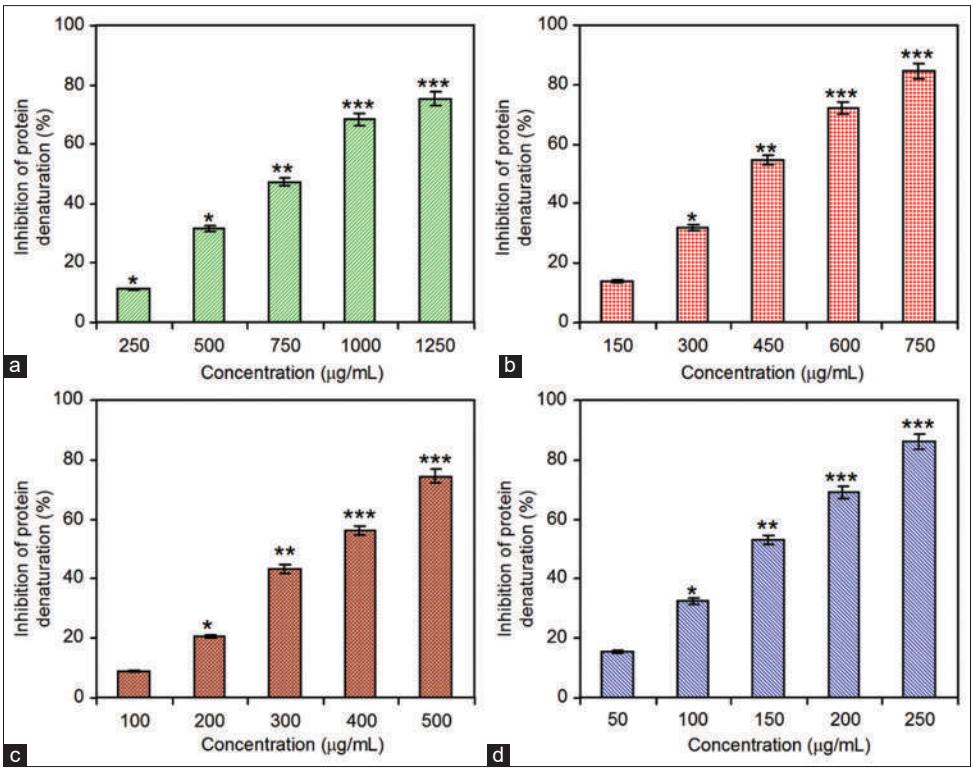

Figure 1: Anti-inflammatory activity of *A. salviifolium* extracts of a) leaf, b) fruit, c) seed and d) diclofenac. Data shown as Mean±SD for three replicate experiments. All the values are significant difference *P<0.005, **p<0.001; ***p<0.0001versus control

IC_{50} value of 21.73±0.89 µg/mL (Khateeb *et al.*, 2025).The anticancer activity of *Withania somnifera* leaf, stem, and root extracts was evaluated on HepG2 cell lines. The leaf extract showed the strongest cytotoxic effect with an IC_{50} of 43.06 µg/mL, subsequent to the stem (45.60 µg/mL) and root (314.4 µg/mL) extracts. When tested on normal L929 cells, all samples displayed markedly lower toxicity (Lingfa *et al.*, 2023). Flavonoid compounds from five plants were analyzed for anti-breast cancer potential using computational and experimental methods. Apigenin and luteolin showed strong binding with major cancer receptors and stable molecular dynamics. The plant extracts demonstrated natural cytotoxic effects on breast and liver cancer cells (Yasin *et al.*, 2025).

The ethanolic extract obtained from the leaves of *Mimosa rubicaulis* (Lam.) demonstrated significant cell-killing potential against HepG2 liver cancer cells, showing an IC_{50} value of 93.69 µg/mL. The treatment triggered programmed cell death, marked by condensed chromatin structure and the appearance of apoptotic vesicles (Tamboli *et al.*, 2024). Phytochemical composition and anticancer potential of *A. helferiana* were analyzed. LC-MS revealed seventeen active compounds, among which Quercetin and Schafroside exhibited strong affinity toward the PPAR-δ receptor. The extract demonstrated moderate cytotoxic effects against HepG2 cells, with an IC_{50} value of 236.93 µg/mL (Pandey *et al.*, 2024). Methanolic leaf extract of *Moringa oleifera* showed strong, concentration-dependent cytotoxic effects on HepG2 liver cancer cells, with an IC_{50} of 12.89 µg/mL. At higher doses, its impact matched that of quercetin and doxorubicin, indicating significant anticancer potential (Nejad *et al.*, 2020).

Pomegranate peel extracts significantly reduced HepG2 cell viability, with an IC_{50} value of approximately 83.9 µg/mL. The treatment induced DNA fragmentation, caused cell cycle arrest, downregulated oncogene expression, and upregulated pro-apoptotic genes, thereby exhibiting pronounced antitumor activity (Basal *et al.*, 2024). Passion fruit seed extract (PFSE) significantly attenuated oleic acid-induced hepatic steatosis in HepG2 cells. The extract exhibited anti-inflammatory and antioxidant activities by regulating lipid metabolism–related genes and pathways (Moolsup *et al.*, 2025). Both aqueous and ethanolic seed extracts of *Ammi majus* inhibited HepG2 cell proliferation, showing maximum growth suppression rates of 68.86% and 79.31% at 200 µg/mL, respectively (Ismael *et al.*, 2025). The root hexane extract of *Aquilaria agallocha* exhibited pronounced cytotoxic activity against HepG2 cells at 1000 µg/mL, inducing both apoptosis and necrosis while modulating the expression of p53, Bax, and Bcl-2 genes (Dalkılıç *et al.*, 2025). *Lantana camara* root extract, containing lupeol and other bioactive compounds, showed strong antioxidant and anticancer activity. It induced apoptosis and inhibited cell growth in MCF-7, HepG2, and A549 cancer cells, with minimal toxicity to normal cells (Shehzadi *et al.*, 2025).

Data demonstrate a dose-dependent decline onthe anticancer activity against HepG2 cells, measured by the MTT assay, varies among the extracts based on their IC_{50} values. The seed extract, with an IC_{50} of 49.5 µg/mL, shows the highest potency (lowest IC_{50} means stronger effect). The leaf extract, having an IC_{50} of 93.9 µg/mL, exhibits moderate anticancer activity. The fruit extract, with the highest IC_{50} of 109.7 µg/mL, demonstrates the lowest anticancer potency among the three (Figure 3).

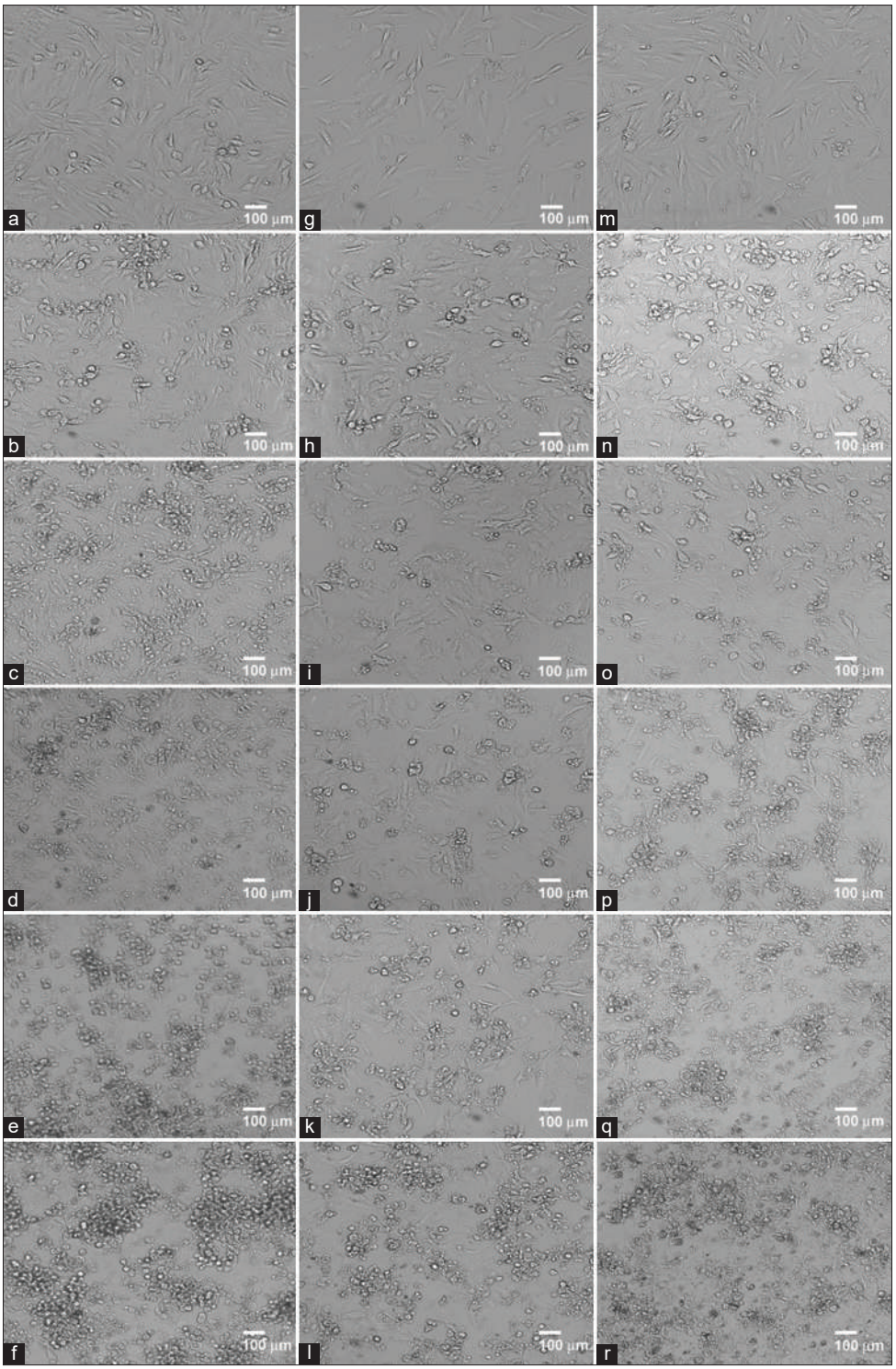

Figure 2: HepG2 cell line in MTT assay of *A. salviifolium* leaf extract. a) untreated control, b) 6.25 µg/mL concentration, c) 12.5 µg/mL concentration, d) 25 µg/mL concentration, e) 50 µg/mL concentration and f) 100 µg/mL concentration. *A. salviifolium* fruit extract g) untreated control h) 6.25 µg/mL concentration, i) 12.5 µg/mL concentration, j) 25 µg/mL concentration k) 50 µg/mL concentration and (l) 100 µg/mL concentration. *A. salviifolium* seed extracts m) untreated control n) 6.25 µg/mL concentration, o) 12.5 µg/mL concentration, p) 25 µg/mL concentration, q) 50 µg/mL concentration and r) 100 µg/mL concentration

The methanolic extract of green tea (GTME) exhibited selective cytotoxicity against HepG2 cells, with an IC_{50} value of 27.3 µL. The extract induced apoptosis, caused DNA damage and G1 phase cell cycle arrest, and reduced intracellular ROS levels (Hanna *et al.*, 2024). The petroleum ether fraction of guava leaves significantly inhibited HepG2 cell proliferation, exhibiting cytotoxic activity at concentrations of 250-500 µL with an IC_{50} value of 656 µg/mL (Kadhim & Kareem sultan,

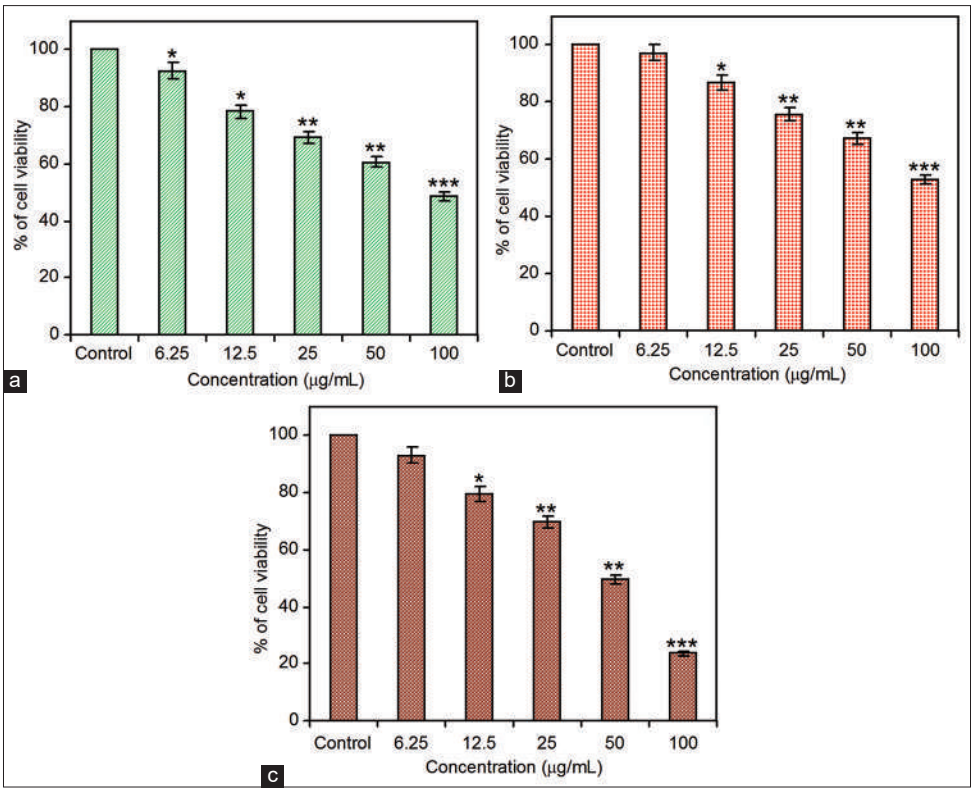

Figure 3: Anticancer activity of *A. salviifolium* a) leaf, b) fruit and c) seed extracts. Values shown as mean±S.D for three replicate experiments. All the values are significant difference *P<0.005, **P<0.001, ***P<0.0001 versus control.

2025). The leaf extracts of *Rivea hypocrateriformis*, namely the hydroethanolic (HEC) and hydroethanolic ethanol (HEE) fractions, exhibited notable cytotoxic activity against HepG2 cells, with 50% inhibition values of 61.49 µg/mL and 87.08 µg/mL (Bai & Rethnaswamy, 2025). The leaf extract of *Fagonia arabica* exhibited potent anti-cancer activity *in vitro* by markedly inhibiting HepG2 cell proliferation, reducing angiogenesis, inducing apoptosis, and promoting cell death (Fatima *et al.*, 2025). The dichloromethane fraction of *Calotropis gigantea* showed selective cytotoxicity toward HepG2 cells by inducing apoptosis through oxidative stress. It was less toxic to normal cells and moderately to strongly inhibited CYP3A4 and CYP2C9 enzymes, with IC_{50} values between 12.11 and 296.9 µg/mL, indicating strong anticancer potential (Suknoppakit *et al.*, 2023). *Celtis tournefortii* bark extract showed anticancer potential by inducing oxidative stress, increasing LDH levels, and reducing AKT gene expression in HepG2 liver cancer cells (Nadiger *et al.*, 2024).

CONCLUSION

This study demonstrates that A. *salviifolium* leaf, fruit, and seed extracts of the plant possess significant antibacterial, antifungal, anti-inflammatory, and anticancer activities relevant to liver-related pathogens. Among them, the seed extract showed the highest potency across all assays, while the leaf and fruit extracts also exhibited notable therapeutic effects, particularly in anti-inflammatory and antibacterial activities. Each extract displayed unique properties against hepatic pathogens. The findings provide scientific support for the traditional use of these plant parts by tribal communities in managing skin and liver disorders and highlight their potential as natural sources for developing treatments against infections and inflammation. These results further suggest that the leaf, fruit, and seed extracts could serve as alternative or complementary agents for liver disease treatment. Future *in vivo* studies and clinical trials are needed to confirm their efficacy, safety, and mechanisms of action for clinical application.

ACKNOWLEDGEMENTS

The authors recognize and thankful the Directorate of Collegiate Education, Chennai (Ref.R.C.No.00160/L/2022 dated 11.12.2023) (Under the Higher Education Department, Government of Tamil Nadu, India).

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