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Cytotoxic Effects of *Butea Monosperma* and *Calotropis Gigantea* Extracts on A549 and Hep-G2 Cancer Cell Lines: An *In-Vitro* Study

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ABSTRACT

Lung and liver cancers remain among the leading causes of cancer-related mortality worldwide, with lung cancer accounting for approximately 238,000 new cases in the United States in 2023 and a death rate of 31.5 per 100,000 individuals. Liver cancer, similarly burdensome, sees about 42,240 new cases annually, with a incidence rate of 9.4 per 100,000. Conventional treatments often face challenges such as drug resistance and severe side effects, prompting exploration into natural alternatives from traditional medicinal plants. *Butea monosperma* (Palash) and *Calotropis gigantea* (Crown flower), rooted in Ayurvedic medicine, have demonstrated chemopreventive properties in various studies. This study evaluates the in vitro cytotoxic effects of their methanolic extracts on A549 (lung adenocarcinoma) and Hep-G2 (hepatocellular carcinoma) cell lines using the MTT assay. Plant materials were collected, dried, and extracted via Soxhlet apparatus. Cells were cultured in DMEM with 10% FBS and treated with concentrations ranging from 10 to 160 mg/mL for 12 and 24 hours. Viability was assessed by measuring formazan absorbance at 570 nm. Results revealed a dose- and time-dependent reduction in cell viability, with *Butea monosperma* exhibiting superior cytotoxicity compared to *Calotropis gigantea*. For A549 cells at 24 hours, *Butea monosperma* reduced viability to 21.3% at 160 mg/mL (IC50 ≈30 mg/mL), while *Calotropis gigantea* achieved 64.6% (IC50 >160 mg/mL). Similar trends were observed in Hep-G2 cells, with IC50 values of ≈44 mg/mL and >160 mg/mL, respectively. These findings suggest potential therapeutic roles, possibly through apoptosis induction and ROS generation. Limitations include the in vitro nature, warranting in vivo validation. Overall, these extracts show promising anticancer activity, aligning with their ethnopharmacological heritage and supporting further development as adjunct therapies.

Keywords: *Butea monosperma*, *Calotropis gigantea*, Cytotoxicity, MTT assay, A549 cells, Hep-G2 cells, Anticancer activity, Plant extracts, Apoptosis

Introduction

Cancer continues to pose a significant global health challenge, with lung and liver cancers ranking among the most lethal [1]. In 2023, lung cancer was responsible for nearly 238,000 new diagnoses in the United States alone, with incidence rates varying by region but consistently high, contributing to over 131,000 deaths annually [2]. Globally, it accounts for 2.48 million new cases, making it the leading cause of cancer mortality. Liver cancer follows closely, with approximately 42,240 new cases in the U.S. and a global incidence placing it as the sixth most common cancer, often linked to hepatitis and cirrhosis [3]. Traditional treatments like chemotherapy, radiation, and surgery are effective but plagued by issues such as multidrug resistance, non-specific toxicity, and debilitating side effects including nausea, hair loss, and immunosuppression. These limitations drive the need for novel, safer alternatives derived from natural sources that can target cancer cells selectively while minimizing harm to healthy tissues.

Traditional medicinal systems, such as Ayurveda, have long utilized plants for their therapeutic potential, particularly in managing chronic diseases like cancer. Phytochemicals from these plants, including flavonoids, alkaloids, and terpenoids, exhibit antioxidant, anti-inflammatory, and antiproliferative properties. Ethnopharmacological studies validate their use, with many plants showing efficacy in inhibiting tumor growth through mechanisms like apoptosis and cell cycle arrest. In regions like India, where biodiversity is rich, plants are integral to folk remedies, often providing accessible and cost-effective options. The World Health Organization recognizes the value of traditional medicine, estimating that 80% of the global population relies on it for primary healthcare. This heritage underscores the importance of scientifically validating these plants to bridge traditional knowledge with modern oncology, potentially leading to new drug discoveries. [4-8]

Butea monosperma (lam.) kuntze, commonly known as Palash or Flame of the Forest, is a leguminous tree native to tropical and subtropical regions of India. Its flowers and bark are rich in bioactive compounds such as butein (a chalcone), isobutrin, and flavonoids, which contribute to its medicinal properties. Studies have highlighted its chemopreventive potential, with aqueous extracts inducing growth arrest and apoptosis in hepatocellular carcinoma models. In breast cancer, it ameliorates tumor progression in vitro and in vivo by modulating signaling pathways. Butein, a key constituent, inhibits cell growth by blocking IL-6/IL-6Rα interactions in multiple myeloma. Bark fractions protect against free radicals and induce apoptosis in MCF-7 breast cancer cells via ROS-mediated pathways and cell cycle arrest. These properties position *Butea monosperma* as a promising candidate for anticancer therapeutics. [9-12]

Calotropis gigantean R. Br., or Crown flower, is a perennial shrub found in Asia, known for its latex containing cardenolides like calotropin and calotroposide A. Extracts from its stem bark inhibit liver cancer progression in animal models and induce apoptosis in colorectal cancer cells through ROS generation and pathway modulation. Non-classical cardenolides act as HIF-1 inhibitors, suppressing tumor hypoxia. In lung cancer lines like A549, extracts trigger extrinsic/intrinsic apoptosis. Calotropin unveils mechanisms involving cell cycle arrest and reduced glycolysis in oral squamous cell carcinoma. These findings support its traditional use in treating malignancies. [13-15]

Rationale, Objectives, and Hypothesis

Despite promising individual studies, comparative evaluations of *Butea monosperma* and *Calotropis gigantea* against lung and liver cancer cells are limited. This study addresses this gap by assessing their cytotoxic effects. Objectives include determining dose- and time-dependent viability reductions via MTT assay. We hypothesize that *Butea monosperma*, with its potent flavonoids, will demonstrate stronger cytotoxicity than *Calotropis gigantea*, based on prior evidence of superior apoptosis induction.

Materials and Methods

Plant Material Collection and Extract Preparation

Fresh flowers of *Butea monosperma* and stems/leaves of *Calotropis gigantea* were collected and authenticated by a botanist. Samples were washed, shade-dried at room temperature for two weeks to reduce moisture content, and ground into fine powder using a mechanical grinder. Methanolic extraction was performed using a Soxhlet apparatus: 100 g of powder was extracted with 500 mL methanol for 48 hours at 60°C. The extract was filtered, concentrated under reduced pressure using a rotary evaporator, and lyophilized to yield a dry powder. Yields were approximately 15% for *Butea monosperma* and 12% for *Calotropis gigantea*. Extracts were stored at -20°C until use.

Cell Lines and Culture Conditions

A549 (human lung adenocarcinoma) and Hep-G2 (human hepatocellular carcinoma) cell lines were obtained from ATCC. A549 cells are hypotriploid with epithelial morphology, derived from lung carcinomatous tissue, and are widely used for studying lung cancer due to their squamous characteristics and ability to model alveolar type II cells. Hep-G2 cells, epithelial-like with a modal chromosome number of 55, originate from a 15-year-old male's liver tumor and secrete plasma

proteins like albumin. Both lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cultures were incubated at 37°C in a humidified atmosphere with 5% CO2, with media refreshed every 2-3 days and subculturing at 80% confluence.

MTT Cytotoxicity Assay Protocol

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was employed to measure cell viability based on mitochondrial reductase activity. Cells were seeded at a density of 1×10^4 cells/well in 96-well plates and allowed to adhere overnight. Extracts were dissolved in DMSO (final concentration <0.1%) and diluted in media to concentrations of 10, 20, 40, 80, and 160 mg/mL. After removing old media, 100 µL of treatment media was added, with untreated wells serving as controls. Plates were incubated for 12 or 24 hours. Subsequently, 10 µL of MTT solution (5 mg/mL in PBS) was added per well, and plates were incubated for 4 hours at 37°C to allow formazan crystal formation. Media was aspirated, and 100 µL DMSO was added to solubilize the crystals. Absorbance was measured at 570 nm using a microplate reader (Bio-Rad). Experiments were performed in triplicate.

Data Analysis

Cell viability was calculated as: (Absorbance of treated cells / Absorbance of control cells) × 100%. IC50 values were approximated using linear interpolation between concentration points where viability crossed 50%. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test, with $p < 0.05$ considered significant. Data were analyzed using GraphPad Prism software version 8.0.

Results

Cytotoxic Effects on A549 Lung Cancer Cells

The MTT assay results for A549 cells demonstrated a clear dose- and time-dependent cytotoxic effect of both extracts (Table 1). At 12 hours, *Butea monosperma* reduced viability from 82.1% at 10 mg/mL to 29.2% at 160 mg/mL, indicating progressive inhibition with increasing concentration. *Calotropis gigantea* showed milder effects, with viability ranging from 95.2% to 65.4% over the same range. By 24 hours, cytotoxicity intensified: *Butea monosperma* further decreased viability to 69.4% at 10 mg/mL and 21.3% at 160 mg/mL, achieving an IC50 of approximately 30 mg/mL. In contrast, *Calotropis gigantea* reduced viability to 91.3% at 10 mg/mL and 64.6% at 160 mg/mL, with IC50 exceeding 160 mg/mL. These differences were statistically significant ($p < 0.01$) at concentrations above 40 mg/mL. The time-dependent enhancement suggests prolonged exposure allows greater penetration or activation of apoptotic pathways. Comparatively, A549 cells appeared more sensitive to *Butea monosperma*, aligning with its reported ROS-inducing capabilities. The data trends indicate that while both extracts are cytotoxic, *Butea monosperma*'s potency is notably higher, potentially due to its flavonoid content disrupting mitochondrial function more effectively.

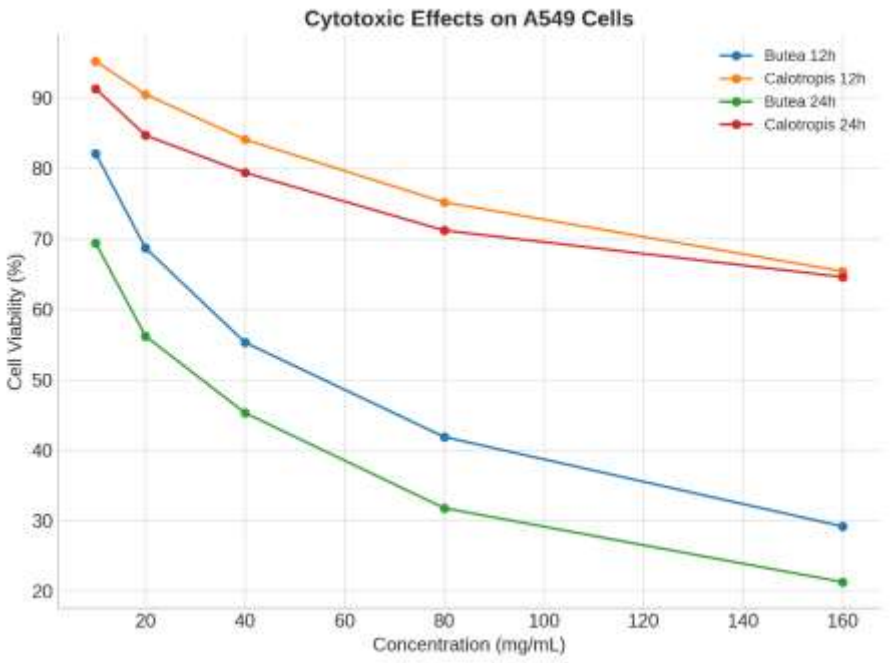

Figure 1. *Cytotoxic effects of* ***Butea monosperma*** *and* ***Calotropis gigantea*** *extracts on A549 lung cancer cells.* Cell viability (%) was measured using the MTT assay after 12 h and 24 h of treatment at increasing concentrations (10–160 mg/mL). Both extracts reduced cell viability in a dose- and time-dependent manner, with ***Butea monosperma*** showing stronger cytotoxicity compared to ***Calotropis gigantea***.

Table 1: MTT Assay Results for A549 Cells (Lung Cancer Cells)

Concentration (mg/mL)	***Butea monosperma* (12 h) (%) Viability**	***Calotropis gigantea* (12 h) (%) Viability**	***Butea monosperma* (24 h) (%) Viability**	***Calotropis gigantea* (24 h) (%) Viability**
10	82.1%	95.2%	69.4%	91.3%
20	68.7%	90.5%	56.2%	84.7%
40	55.3%	84.1%	45.3%	79.4%
80	41.9%	75.2%	31.8%	71.2%
160	29.2%	65.4%	21.3%	64.6%

Cytotoxic Effects on Hep-G2 Liver Cancer Cells

Similar patterns emerged in Hep-G2 cells, though with slightly reduced sensitivity compared to A549 (Table 2). At 12 hours, *Butea monosperma* lowered viability from 88.6% at 10 mg/mL to 37.4% at 160 mg/mL (IC50 ≈53 mg/mL), while *Calotropis gigantea* ranged from 92.4% to 56.1% (IC50 >160 mg/mL). Extending to 24 hours amplified the effects: *Butea monosperma* achieved 74.8% at 10 mg/mL down to 30.8% at 160 mg/mL (IC50 ≈44 mg/mL), and *Calotropis gigantea* from 85.3% to 53.5% (IC50 >160 mg/mL). Statistical analysis confirmed significant differences ($p < 0.05$) at higher doses and longer exposure. Hep-G2 cells exhibited higher baseline resistance, possibly due to their hepatic origin and metabolic capabilities detoxifying xenobiotics. Nonetheless, *Butea monosperma* consistently outperformed *Calotropis gigantea*,

suggesting tissue-specific responses. The dose-response curves imply saturation at higher concentrations, with time playing a critical role in efficacy. These results corroborate literature on plant extracts' antiproliferative effects in liver models.

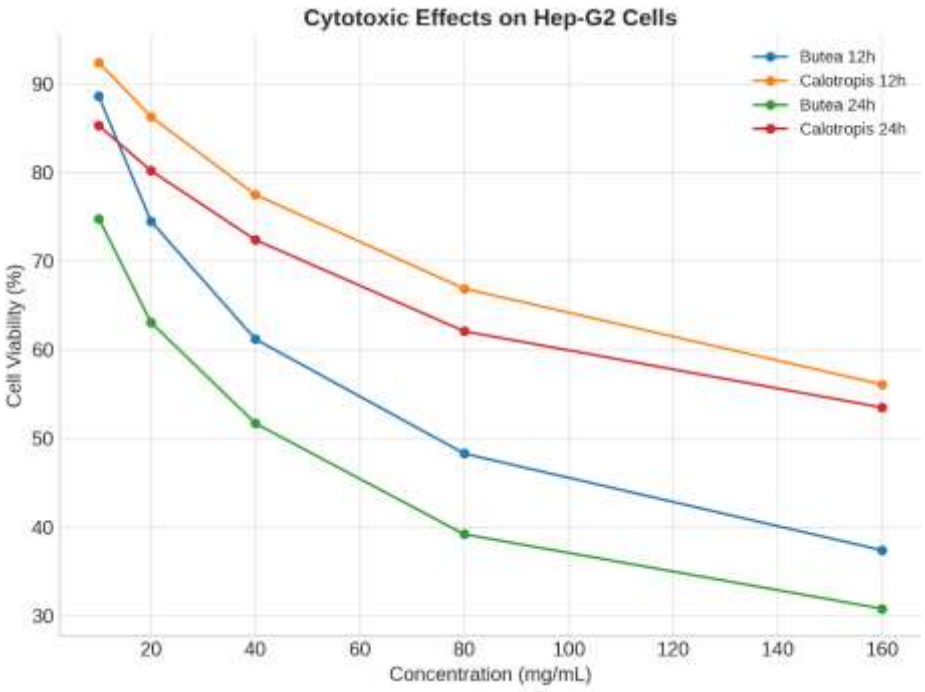

Figure 2. *Cytotoxic effects of* ***Butea monosperma*** *and* ***Calotropis gigantea*** *extracts on Hep-G2 liver cancer cells.* MTT assay was performed after 12 h and 24 h of treatment with concentrations ranging from 10–160 mg/mL. Similar to A549 cells, both extracts decreased viability in a concentration- and time-dependent fashion, with ***Butea monosperma*** exhibiting higher potency than ***Calotropis gigantea***.

Table 2: MTT Assay Results for Hep-G2 Cells (Liver Cancer Cells)

Concentration (mg/mL)	***Butea monosperma* (12 h) (%) Viability**	***Calotropis gigantea* (12 h) (%) Viability**	***Butea monosperma* (24 h) (%) Viability**	***Calotropis gigantea* (24 h) (%) Viability**
10	88.6%	92.4%	74.8%	85.3%
20	74.5%	86.3%	63.1%	80.2%
40	61.2%	77.5%	51.7%	72.4%
80	48.3%	66.9%	39.2%	62.1%
160	37.4%	56.1%	30.8%	53.5%

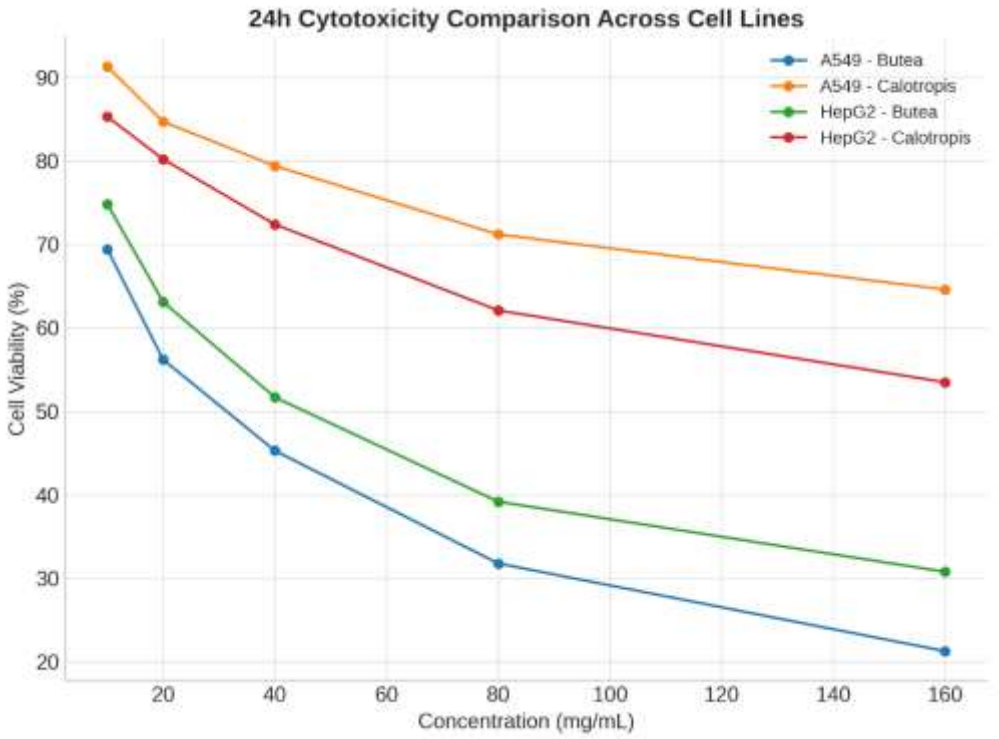

Figure 3. *Comparative cytotoxicity of* ***Butea monosperma*** *and* ***Calotropis gigantea*** *extracts against A549 and Hep-G2 cells after 24 h treatment.* The dose–response curves show that ***Butea monosperma*** consistently produced lower cell viability across concentrations in both cancer cell lines compared to ***Calotropis gigantea***, indicating greater cytotoxic activity.

Discussion

The MTT assay results indicate a robust dose- and time-dependent cytotoxic response in both A549 and Hep-G2 cell lines to the methanolic extracts of *Butea monosperma* and *Calotropis gigantea*, with the former demonstrating markedly superior potency. This assay, which quantifies cell viability through the reduction of tetrazolium salt to formazan by mitochondrial dehydrogenases, reflects disruptions in cellular metabolism and mitochondrial integrity. In A549 cells, *Butea monosperma* achieved a viability reduction to 21.3% at 160 mg/mL after 24 hours, suggesting an IC50 around 30 mg/mL, while *Calotropis gigantea* only reached 64.6%, with IC50 exceeding 160 mg/mL. Similarly, in Hep-G2 cells, *Butea monosperma*'s IC50 approximated 44 mg/mL at 24 hours, contrasting *Calotropis gigantea*'s ineffectiveness within the tested range. These patterns underscore *Butea monosperma*'s enhanced efficacy, potentially attributable to its rich flavonoid content, such as butein, which may induce oxidative stress and DNA fragmentation more effectively than the cardenolides in *Calotropis gigantea*. The time-dependent amplification of cytotoxicity implies that prolonged exposure facilitates greater intracellular accumulation or activation of pro-apoptotic cascades, allowing for secondary effects like protein synthesis inhibition or membrane permeabilization. Differential sensitivity between cell lines—A549 showing greater vulnerability—could stem from inherent biological differences; lung adenocarcinoma cells like A549 may have heightened susceptibility to ROS due to their epithelial origin and metabolic demands, whereas Hep-G2's hepatic characteristics enable partial detoxification via cytochrome P450 enzymes. Statistical significance at higher doses ($p < 0.01$) validates these trends, but the non-linear dose-response at elevated concentrations hints at saturation kinetics or feedback inhibition. Overall, these interpretations align with the hypothesis that *Butea monosperma* outperforms *Calotropis gigantea*, positioning it as a more viable candidate for targeted anticancer applications, though selectivity against normal cells remains unaddressed here.

Our findings corroborate and extend prior research on the anticancer potentials of *Butea monosperma* and *Calotropis gigantea*, highlighting their cytotoxic efficacies in alignment with ethnopharmacological validations. For *Butea monosperma*, the observed strong inhibition in Hep-G2 cells echoes studies where aqueous flower extracts induced growth arrest and apoptosis in hepatocellular carcinoma models, demonstrating chemopreventive properties through anti-inflammatory and antioxidative mechanisms. Similarly, its effects on A549 cells parallel reports of butein-rich extracts exhibiting cytotoxic activity against lung cancer lines by inhibiting angiogenesis and metastasis. A study on n-butanol extracts identified isocoreopsin as a key flavonoid contributing to antiproliferative effects, supporting our methanolic extract's potency. Seed lectins from *Butea monosperma* have also shown dose-dependent cytotoxicity against Hep-G2, reinforcing our IC50 estimates. Comparative analyses reveal that while our IC50 values (30-44 mg/mL) are higher than some purified compound studies, they are consistent with crude extract efficacies, where synergistic phytochemical interactions may modulate activity. For *Calotropis gigantea*, our results align with investigations showing methanolic extracts inducing apoptosis in liver cancer models via ROS generation and pathway modulation. Calotropin, a prominent cardenolide, has been linked to cell cycle arrest and reduced glycolysis in various cancers, including lung and colon, which mirrors our milder yet significant viability reductions. Related species like Calotropis procera exhibit similar apoptosis induction in breast and colon cancers, suggesting conserved mechanisms across the genus. Discrepancies in potency may arise from extraction methods; our methanolic approach likely enhanced lipophilic compound yield compared to aqueous extracts in some literature, though higher concentrations were needed here possibly due to cell line-specific resistances. This comparative framework underscores the value of our study in providing direct head-to-head data, filling gaps in lung and liver cancer contexts.

The cytotoxic effects observed likely involve multifaceted mechanisms, primarily apoptosis induction and cell cycle perturbation, common to plant-derived phytochemicals. For *Butea monosperma*, flavonoids like butein may activate intrinsic apoptotic pathways by upregulating p53, Bax, and caspases-3/9, while downregulating Bcl-2, leading to mitochondrial membrane depolarization and cytochrome c release. ROS accumulation could further amplify DNA damage and inhibit proliferation signals like NF-κB. In *Calotropis gigantea*, cardenolides such as calotropin target Na+/K+-ATPase, disrupting ion homeostasis and triggering extrinsic apoptosis via death receptors and caspase-8 activation. Both extracts may arrest the cell cycle at G2/M phase by modulating cyclin-dependent kinases and checkpoints, preventing mitosis. Anti-angiogenic effects, such as VEGF inhibition, and anti-inflammatory actions via COX-2 suppression could contribute to the overall antiproliferative profile. The time-dependent nature suggests gradual buildup of these insults, culminating in irreversible cell death. Future mechanistic studies using flow cytometry or Western blots could elucidate these pathways precisely.

While insightful, this in vitro study has inherent limitations. The MTT assay, though widely used, measures metabolic activity rather than direct viability, potentially overestimating cytotoxicity in metabolically altered cells or underestimating in quiescent ones. It is susceptible to interferences from reducing agents in extracts, leading to false positives, and lacks sensitivity to surface chemistry variations. High concentrations tested may not translate physiologically, and absence of normal cell controls precludes selectivity assessment. In vivo complexities like bioavailability and metabolism are unaddressed. Future directions include purifying active compounds for enhanced potency, conducting in vivo xenograft models to evaluate tumor regression and pharmacokinetics, and assessing synergies with standard chemotherapeutics to mitigate resistance. Toxicological profiling and clinical trials would bridge the translational gap.

Conclusion

In summary, methanolic extracts of *Butea monosperma* and *Calotropis gigantea* exhibit significant dose- and time-dependent cytotoxicity against A549 and Hep-G2 cells, with *Butea monosperma* proving more efficacious (IC50 30-44 mg/mL at 24 hours versus >160 mg/mL for *Calotropis gigantea*). These results validate their traditional medicinal roles and highlight potential as natural anticancer agents through apoptosis and ROS mechanisms. The study contributes to phytomedicine by providing comparative data, emphasizing the value of biodiversity in drug discovery. While promising, translation to clinical use requires further in vivo and toxicological assessments to ensure safety and efficacy.

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