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The Protective Effects of *Baeckea frutescens* Leaves Ethanolic Extract Against Cisplatin-induced Cell Death in Liver Cells

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ABSTRACT

Cisplatin is a cytotoxic drug that is commonly used to treat bladder, ovarian and testicular cancer. While treatment using cisplatin is effective, its potential hepatotoxic effects require careful monitoring and management. Adjuvants may help mitigate these effects by protecting liver cells through the antioxidant property, thereby improving the overall treatment outcome. This study investigated the potential of *Baeckea frutescens* leaves ethanolic extract, to mitigate cisplatin-induced hepatotoxicity. Using the human liver cancer cell line HEP-G2 as an *in vitro* model, the cytoprotective effects of *B. frutescens* extract was evaluated against cisplatin-induced cell death. The extract was co-administered with cisplatin, and cell viability was measured using the MTT assay. The results demonstrate that *B. frutescens* extract significantly protected HEP-G2 cells from cisplatin-induced cell death in a dose-dependent manner ranging from 6.25 to 100 µg/ml against 10 µM cisplatin. This protective effect is likely attributed to the antioxidant properties of the extract, which can scavenge ROS and reduce oxidative stress. These findings suggest the potential of *B. frutescens* to be developed as adjuvant therapy to ameliorate cisplatin-induced hepatotoxicity, thereby improving the therapeutic index of Cisplatin. Importantly, further research is warranted to elucidate the precise molecular mechanisms underlying this protective effect and to evaluate its efficacy *in vivo*.

INTRODUCTION

Cisplatin, a platinum-based alkylating agent, is a widely used chemotherapy drug (Aldossary, 2019; Domingo et al., 2022). It has proven efficacy against various cancers, including sarcomas, bone cancer, testicular cancer, and head and neck cancers (Romani, 2022). Cisplatin inhibits tumor cell division by

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forming mono-, inter-, or intrastrand DNA adducts through intercalation into actively dividing cells (Domingo et al., 2022; Katanić Stanković et al., 2023; Zhou et al., 2022). This process disrupts the cell cycle, inducing oxidative stress and initiating apoptosis which is a programmed cell death as a part of its cytotoxic effect (Aldossary, 2019; Gentilin et al., 2019). Despite its efficacy against various cancers, cisplatin can induce toxicities in normal cells, such as nephrotoxicity, ototoxicity, hepatotoxicity, gastrointestinal issues, and neurotoxicity, due to the disruption of cellular processes and elevated generation of reactive oxygen species (ROS) (Afsar et al., 2021; Aldossary, 2019; Domingo et al., 2022; Ghosh, 2019).Reactive oxygen species are a diverse group of highly reactive molecules, including both radicals and non-radicals, derived from oxygen through multiple exogenous and endogenous pathways (Lourenço et al., 2019; Pisoschi & Pop, 2015; Pizzino et al., 2017). Common ROS include superoxide radicals (O2•-) and hydroxyl radicals (OH-) while non-radicals such as hydrogen peroxide (H2O2) (Mar et al., 2023; Nabila et al., 2024). Most cellular ROS are generated during aerobic metabolism, particularly in the mitochondria during oxidative phosphorylation (Małecka et al., 2021; Sharifi-Rad et al., 2020). Additional sources include peroxisomal β-oxidation of fatty acids, microsomal cytochrome P450 activity, metabolism of xenobiotic compounds, phagocytosis induced by pathogens or lipopolysaccharides, metabolism of arginine and specific cellular enzymes in various tissues that promotes inflammation (Afsar et al., 2021).

Under physiological condition, ROS regulate pro-inflammatory and pro-fibrotic signaling, cell proliferation, apoptosis and various other biological processes without causing macromolecular damage (Brieger et al., 2012; Checa & Aran, 2020; Saleh et al., 2023). However, an imbalance between ROS and antioxidants leads to oxidative stress, which contributes to inflammation and tissue injury (Checa & Aran, 2020; Małecka et al., 2021). Excess ROS activate signaling pathways like MAPK (p38, JNK, ERK), STAT, and NF-κB, stimulating cytokine production and amplifying inflammation (Checa & Aran, 2020; Poljsak et al., 2013; Schieber & Chandel, 2014). To counter excessive ROS, the body relies on endogenous antioxidant systems dietary antioxidants (Checa & Aran, 2020; Sharifi-Rad et al., 2020). These systems scavenge ROS, prevent their formation, and repair oxidative damage through enzymes such as superoxide dismutase (Brieger et al., 2012; Małecka et al., 2021). Herbal adjuvant therapy, when used alongside conventional treatments such as surgery, chemotherapy, and radiotherapy, may enhance treatment efficacy, reduce side effects, and improve quality of life (Attia et al., 2020).

Natural compounds are well-documented for their ability to mitigate oxidative stress and its associated pathological consequences (Katanić Stanković et al., 2023). *Baeckea frutescens*, a member of the Myrtaceae family, has demonstrated notable antioxidant and anti-inflammatory properties (Navanesan et al., 2015; Shahruzaman et al., 2019). Commonly known as Cucur Atap, *B. frutescens* belongs to the Myrtoideae subfamily and is widely distributed across Southeast Asia, Australia, and the coastal regions of Malaysia (Navanesan et al., 2015; Nisa et al., 2017; Razmavar et al., 2014). Traditionally, it has been used in decoctions to treat various ailments, including fever, influenza, malaria, abdominal discomfort, and as a diuretic and emmenagogue (Huong et al., 2023; Razmavar et al., 2014). The plant's diverse pharmacological activities including antioxidant, anti-inflammatory, antibacterial, and skin-protective effects are attributed to its rich phytochemical profile, particularly flavonoids (Ayub et al., 2026; Kamarazaman et al., 2022; Navanesan et al., 2015; Nisa et al., 2017). While previous studies have focused primarily on the general biological activities of *B. frutescens*, its role in protecting against chemotherapy-induced organ toxicity has not been explored. In this study, we investigate the protective effect of *B. frutescens* ethanolic leaf extract against cisplatin-induced cytotoxicity in HEP-G2 liver cells using cell viability and morphological analyses. Although HEP-G2 is a hepatocellular carcinoma-derived cell line, it retains key liver-specific metabolic and oxidative stress response functions, making it a well-established in vitro model for evaluating hepatotoxicity and hepatoprotective agents. To our knowledge, this is the first report demonstrating the potential of *B. frutescens* to attenuate cisplatin-induced liver cell injury. Unlike other plant extracts previously studied for their nephroprotective or otoprotective effects, *B. frutescens* has not been evaluated in the context of liver protection. This work therefore provides novel insight into the

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potential use of *B. frutescens* as a complementary therapeutic agent to alleviate cisplatin-associated hepatotoxicity during chemotherapy.

EXPERIMENTAL

Baeckea frutescens leaves extract

The *Baeckea frutescens* plant was obtained from Setiu, Terengganu, Malaysia and verified by botanist, Ms. Tan Ai Lee at FRIM, Malaysia. The herbarium specimen (SBID: 044/20) was deposited at the Faculty of Pharmacy, Universiti Teknologi MARA Selangor. The plant material was cleaned and air-dried for a week before the leaves were separated from the branch. The leaves were then ground into a fine powder. The leaf powder of *B. frutescens* was soaked in 95% ethanolic solvent for 48 hours and filtered using Whatman filter paper. This maceration and filtration process was repeated twice, and the collected extracts were combined. The solvent was eliminated using a rotary evaporator. The resulting *B. frutescens* leaf extract was stored in a cool environment at −20 °C until ready for use (Kamarazaman et al., 2022).

Cell cultures

HEP-G2 cell lines were cultured in DMEM containing 10% fetal bovine serum (FBS) and 1% antibiotic (penicillin-streptomycin mixed solution). The cells were then maintained in the T25 flask in an incubator with 37◦C temperature and 5% CO_2 concentration. Then the cells were subculture twice a week (Kamarazaman et al., 2022; H. S. Kim et al., 2012).

Determination of cells viability

To determine cell viability, the MTT Assay was employed. Firstly, the cells were prepared and plated at a density of 10000 cells in 100 µL of media followed by a 24-hour incubation. After the incubation, the cells were treated with Cisplatin at the concentration of 1 – 100 µM to determine the IC_{25} of Cisplatin. Another set of cells were treated with *B. frutescens* leaves extract at the concentration of 6.25 - 100 µg/ml to assess cytotoxic effect of the extract. *B. frutescens* extract was dissolved in culture media, and all treatment and control wells received the same volume of media to ensure consistency and rule out any vehicle-related effects. Next, a set of cells were co-treated simultaneously with *B. frutescens* leaves extract (6.25–100 µg/mL) and cisplatin (10 µM, IC25) for 24 hours. Following this treatment phase, the culture mediums were removed and 100 µL of MTT solution (0.5 mg/ml) was added to each well. The plate was then incubated for 3 hours at 37◦C. Next, the medium was removed and substituted in each well with 100 µL of DMSO. Finally, the absorbance microplate reader was used to read each well's optical density at a wavelength of 570 nm (Kamarazaman et al., 2022; Shahruzaman et al., 2019; Silva et al., 2021).

The concentration range for *B. frutescens* extract (6.25 to 100 µg/mL) was selected based on previous studies demonstrating its biological activity without cytotoxic effects in similar cell lines (Kamarazaman et al., 2022). This range was also confirmed in our preliminary screening to be safe for HEP-G2 cells. For cisplatin, the IC25 value (10 µM) was used for subsequent co-treatment experiments to simulate sub-lethal stress. The use of IC25, rather than IC50, allows for detection of potential cytoprotective effects of the extract without overwhelming cell death that could obscure treatment benefits.

Statistical analysis

All data are expressed as mean ± standard deviation (SD) from three independent biological replicates (n = 3), each containing technical replicates. Statistical analyses were conducted using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). Data normality was assessed using the Shapiro-Wilk test. Comparisons between groups were performed using two-way ANOVA, followed by Tukey's post-hoc test. A p-value of less than 0.05 was considered statistically significant.

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RESULTS AND DISCUSSION

Cytotoxicity assessment of *B. frutescens* leaves extract

Various concentrations of *B. frutescens* were applied to HEP-G2 cells for 24 hours to evaluate its cytotoxic effects using the MTT assay. As shown in Fig. 1(A), there was a statistically significant difference ($p<0.05$) in the cell viability percentage of HEP-G2 cells when exposed to 50 and 100 µg/ml of *B. frutescens* leaves extract concentrations indicating the extracts were not cytotoxic. For 50 µg/ml concentration, the cells viability increased by approximately 20% and for concentration 100 µg/ml, the cells viability increased by around 40% when compared to the control. The control is untreated cells. The other concentrations of *B. frutescens* which were 6.25 µg/ml, 12 µg/ml and 25 µg/ml showed no statistically significant differences ($p>0.05$) when compared to the control group. Hence, the increased in cells viability in the presence of the extracts at all the concentration indicates the extracts were not cytotoxic to HEPG2 cells. Microscopic examination further revealed that treatment with *B. frutescens* extract alone did not cause any observable morphological alterations compared to untreated cells, indicating the absence of cytotoxicity at the tested concentrations, as shown in Fig. 1(B). Interestingly, phloroglucinols was previously reported in the *B. frutescens* leaves extract (Huong et al., 2023), the results obtained are similar to previous studies where there are no changes in cell viability of SH-SY5Y cells when treated with phloroglucinols (H. S. Kim et al., 2012). These results support that the concentration of extracts used in this study is safe to the HEP-G2 cells.

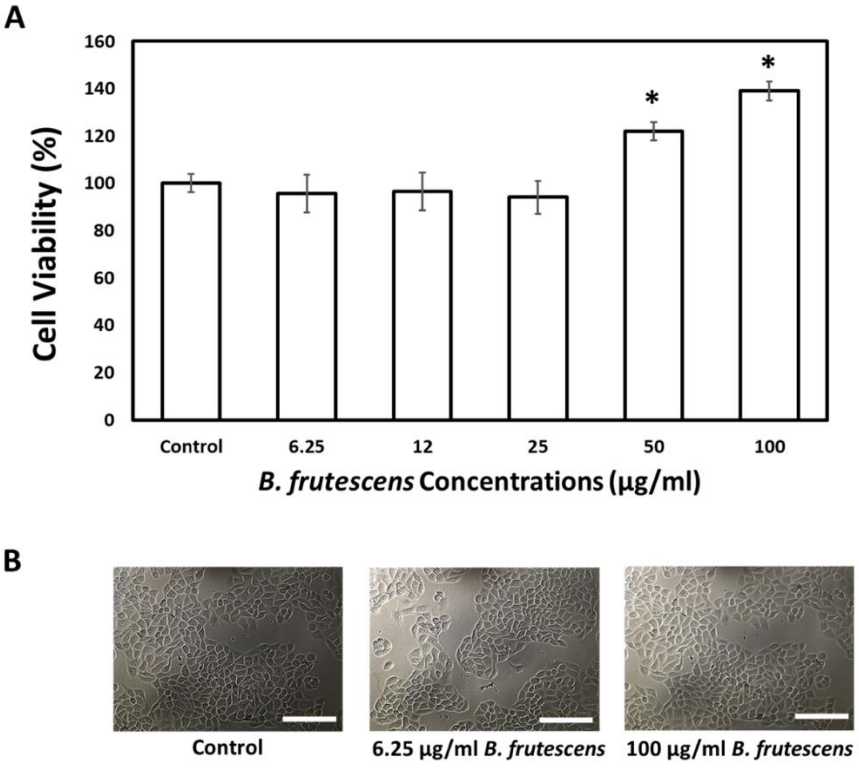

Fig. 1. (A) Cell viability of HEP-G2 cells treated with various concentrations of *B. frutescens* extract (6.25–100 µg/mL) for 24 hours, measured by MTT assay. (B) Representative phase-contrast images of HEP-G2 cells after treatment, showing cellular morphology. Scale bar = 500 µm. Data are presented as mean ± SD (n = 3). *$p < 0.05$ indicates significant difference compared to the untreated control.

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Cytotoxicity assessment of Cisplatin

HEP-G2 cells were treated with varying concentrations of Cisplatin which were 1, 5, 10, 25, 50 and 100 µM. Fig. 2(A) shows that there was significant reduction ($p<0.05$) in the cell viability for the concentrations 5 µM until 100 µM. It was observed that, at a concentration of 10 µM, 70% of the cells were viable but at a concentration of 100 µM, only about 20% of the cells remained viable. Conclusively, the percentage of cell viability decreased as the concentration increased. From these results, the 25% inhibitory concentration (IC_{25}) was determined to be 10 µM. The determination of the IC_{25} values was crucial, as the value was used for subsequent experiment. Cell morphology analysis revealed the presence of apoptotic bodies and shrunken cells in HEP-G2 cells when treated with Cisplatin, as depicted in Fig. 2(B). In contrast, the untreated cells (i.e., cells without cisplatin treatment) exhibited normal morphological structures.

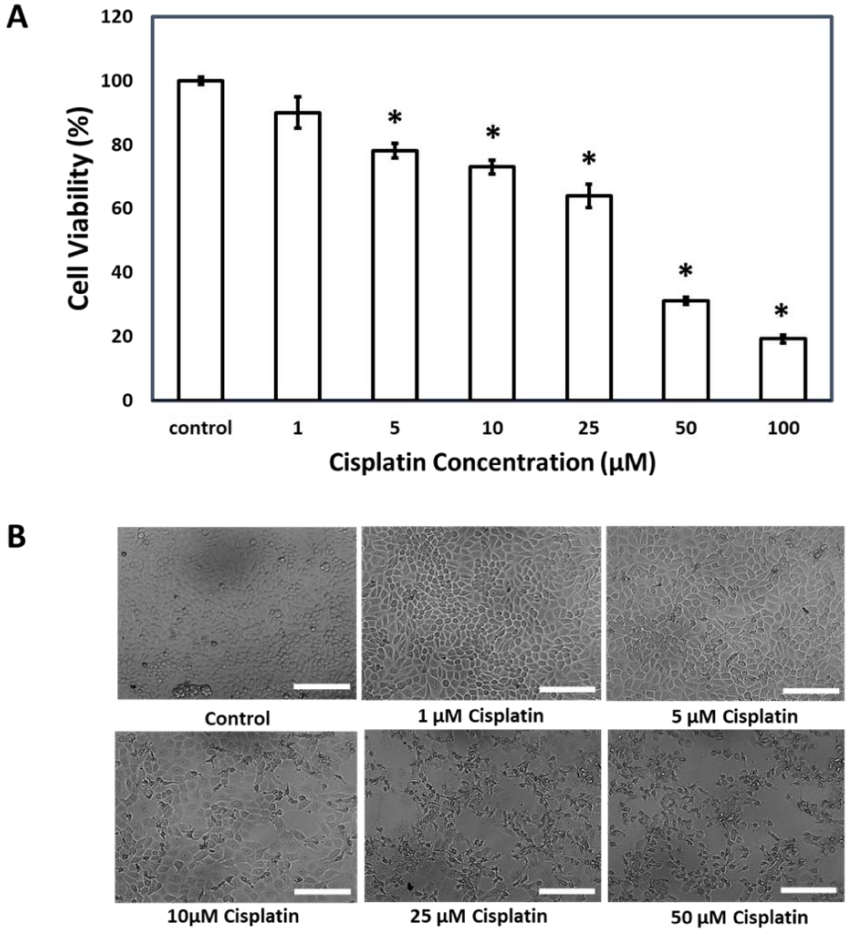

Fig. 2. (A) Cell viability of HEP-G2 cells treated with increasing concentrations of cisplatin (1–100 µM) for 24 hours, as determined by MTT assay. (B) Representative phase-contrast microscopy images showing morphological changes in HEP-G2 cells following cisplatin treatment. Cells exhibit signs of apoptosis, including shrinkage and formation of apoptotic bodies, in a concentration-dependent manner. Scale bar = 500 µm. Data are expressed as mean ± SD (n = 3). *$p < 0.05$ indicates significant difference compared to the untreated control group.

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Based on Fig. 2(B), the frequency of the dying cells increases as the concentration of Cisplatin increases as it works in a concentration and time-dependent process (Han et al., 2021; Katanić Stanković et al., 2023). The cells treated with 100 µM of Cisplatin shows shrinkage of cells as a sign of apoptosis. The mechanism for apoptotic cell death is typically characterized by several key features such as condensation of chromatin material, DNA fragmentation in the nucleus, cell shrinkage, dynamic cell blebbing and a loss of adhesion to cellular matrix. Cisplatin works as a cytotoxic drug, it triggers apoptosis using an intrinsic pathway or also known as a mitochondrial-mediated apoptotic pathway. In this pathway, anticancer drugs activate c-Jun N-terminal kinase (JNK) *via* mitogen-activated protein kinase kinase (MKK) 4/7 leading to dissociation of Bax and activation of Bak. Bax/Bak insertion into the mitochondrial membrane releases Cytochrome C (Cyt C) from the mitochondrial intermembrane into the cytosol. Cyt C combines with Apaf-1 and procaspase-9 to form apoptosome, a multi-protein complex with a seven-spoke ring-shaped. This complex activates caspase-9, which subsequently triggers caspase-3, initiating a caspase cascade that leads to apoptotic cell death (R. Kim et al., 2024; Yaghoubi et al., 2015).

Protective effect of *B. frutescens* leaves extract against Cisplatin

These results demonstrate the protective effect of *B. frutescens* against a sub-lethal dose of Cisplatin (10 µM) on HEP-G2 cells, as shown in Fig. 3(A) using the MTT assay. The cells were pre-treated with *B. frutescens* leaves extract with various concentration for 24 hours before treated with 10 µM of Cisplatin. Similar with Fig. 2(A), the cell viability reduced when treated with 10 µM of Cisplatin when compared to control. However, the cells treated in combination with Cisplatin and various concentrations of *B. frutescens* such as 6.25, 12, 25, 50, 100 µg/ml shows significant increase ($p<0.05$) in cell viability when compared with cell viability of Cisplatin (10 µM) only, indicating that the leaves extract have protective effects on the cells. The use of IC25 for cisplatin, rather than IC50, was selected to mimic sub-lethal cytotoxic stress, allowing a clearer assessment of potential protective effects by *B. frutescens*. Higher concentrations such as IC50 may result in irreversible cellular damage, thereby limiting the window to detect recovery or protection. This approach aligns with standard practice in cytoprotective screening models. The morphology of the cells is shown in Fig. 3(B), where the cells treated with 10 µM of Cisplatin display characteristics of apoptosis, consistent with previous data. However, at concentrations of 6.25, 12, 25, 50, and 100 µg/ml, the cells displayed protective effects against Cisplatin-induced cell death, although some characteristics of dying cells were still observed.

After co-treating HEP-G2 cells with Cisplatin and various concentrations of *B. frutescens* , the results show that the extract produces protective effects. Based on Fig. 3(A) treatment with 10 µM Cisplatin shows a reduction in cell viability as Cisplatin induces cell death compared to the control. Similarly, at concentrations of 6.25 µg/mLto100 µg/mL, it shows protective effects, as antioxidants from the leaves extract scavenge the ROS produced. This happens as *B. frutescens* scavenges the ROS production produced from Cisplatin activity and depletes it. Previous study shows that the potent antioxidant alpha-tocopherol reduces ROS production and prevents apoptosis in breast cancer cells caused by Cisplatin and suggests that antioxidants may hinder the effectiveness of anticancer drugs in patients (Karimian et al., 2022). The presences of antioxidants in *B. frutescens* leaves extract such as flavones for example Baeckeins A-E and 6-C-methylquercetin, with IC50 values ranging from 11.8 to 16.1 µM, demonstrated better DPPH radical scavenging activity compared to the standard compound quercetin which has IC50 of 18.2 µM (Huong et al., 2023). Moreover, quercetin, a family of flavones shows a great ability to remove excess ROS induced by Cisplatin. Based on previous studies, it can also inhibit the production of NF-κB, protecting against kidney damage induced by Cisplatin (Zhou et al., 2022).

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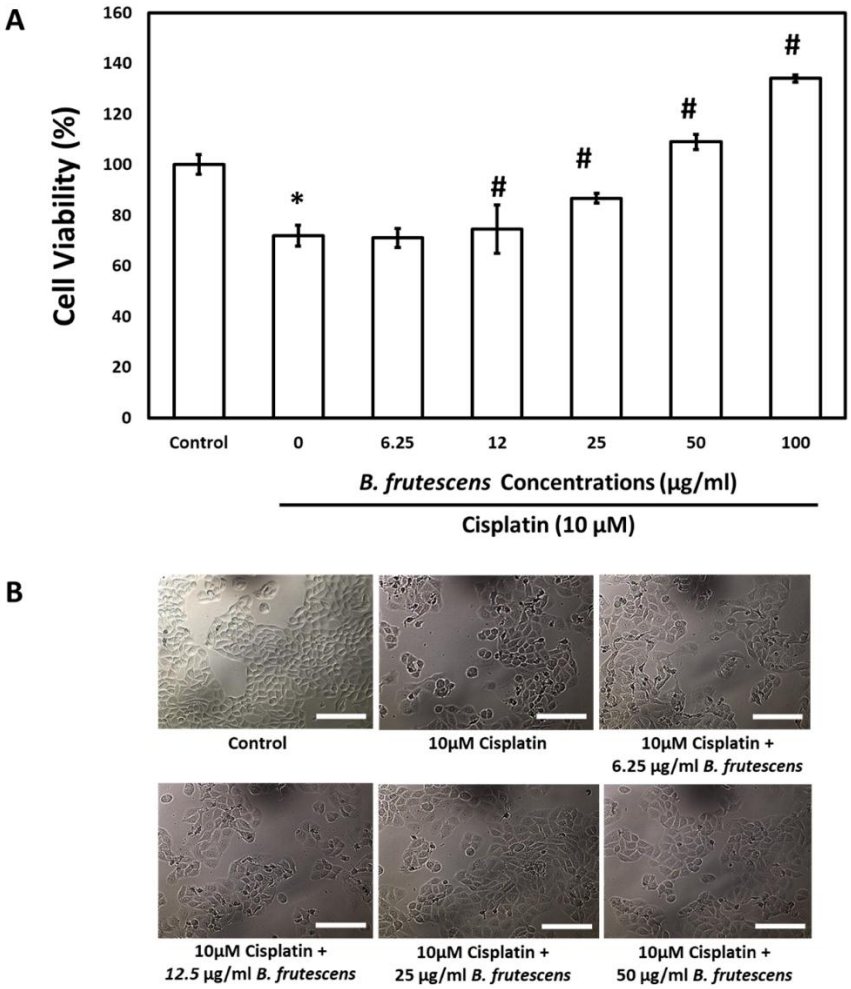

Fig. 3. (A) Cytoprotective effects of *Baeckea frutescens* extract (6.25–100 µg/mL) against cisplatin (10 µM, IC25)-induced cytotoxicity in HEP-G2 cells after 24-hour co-treatment, assessed by MTT assay. (B) Representative microscopic images showing cell morphology after co-treatment with *B. frutescens* extract and cisplatin. Cells treated with the extract displayed improved morphology and reduced apoptotic features in a dose-dependent manner. Scale bar = 500 µm. Data are presented as mean ± SD (n = 3). *p < 0.05 vs. untreated control group; #p < 0.05 vs. cisplatin (10 µM) alone group.

The phytochemicals of *B. frutescens*, exhibited anti-inflammatory effects by inhibiting the NF-κB signaling pathway through the suppression of p65 nuclear translocation. It reduced the expression of iNOS, decreased the production of NO and significantly inhibited the transcription of inflammatory cytokines, including IL-1β, IL-6, and TNF-α (Guo et al., 2025; Lin et al., 2021). As indicated by earlier studies, it shows that flavonoids can block the MAPK pathway which is an important signaling pathway in inflammatory macrophages by inhibiting phosphorylation of p38, JNK and ERK (Lin et al., 2021; Shi et al., 2024). Previous studies showed that *B. frutescens* leaves extracts are rich in phytochemicals with antioxidant properties such as phloroglucinols and flavonoids (Wang et al., 2026; Huong et al., 2023). Some studies have demonstrated the antioxidant properties of *B. frutescens* through DPPH radical scavenging and FRAP assay determinations. The IC_{50} value for DPPH radical scavenging was 22.87 µg/ml, which was not

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significantly different from the IC50 value of Green Tea at 16.69 µg/ml. Hence, the similar antioxidant properties of *B. frutescens* and green tea suggest that *B. frutescens* leaves extract is an excellent antioxidant that may offer similar health benefits as green tea (Kamarazaman et al., 2022). Flavonoids are good antioxidants because they are able to prevent the formation of ROS by chelating transition metal ions, primarily copper and iron, which catalyze the ROS-forming reactions such as hydroxyl radicals (OH-). Flavonoids also possess the ability to inactivate oxygen radicals, effectively by scavenging superoxide anion radicals (O2-), hydroxyl radicals (OH-), singlet oxygen and lipid radicals. Additionally, flavonoids have been shown to reduce the activity of xanthine oxidase, an enzyme that catalyze the formation of superoxide radical anions (Małecka et al., 2021).

Previous studies also show that the combination of Cisplatin together with quercetin significantly reduced or normalized the changes caused by Cisplatin (Sanchez-Gonzalez et al., 2011). Despite Cisplatin exerting cytotoxic effects that kill the cells, the *B. frutescens* leaves extract exerts protective effects on the cells at the same time. Supported by prior findings, numerous studies have confirmed that natural products can mitigate cisplatin-induced nephrotoxicity due to the presence of phytochemicals such as flavonoids, terpenoids and polyphenols. For example, natural products show protective effects against nephrotoxicity as they decrease the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), partly through the regulation of NADPH oxidase and inducible nitric oxide synthase (iNOS) and by boosting the antioxidant system, which includes both enzymatic and non-enzymatic substances where it helps to alleviate oxidative/nitrative stress induced by Cisplatin, thereby preventing kidney damage (Gao et al., 2021). Recent in vivo studies further support the therapeutic potential of plant-derived antioxidants in counteracting cisplatin-induced hepatotoxicity. For example, Alkhalaf *et al.* (2023) demonstrated that sodium salicylate-loaded nanoparticles significantly attenuated cisplatin-induced liver damage in rats by restoring markers of liver function and oxidative stress. More recently, Cesur *et al.* (2025) reported that obtusifolin effectively mitigates both hepatic and renal injury in mice via modulation of the Nrf2/HO-1 pathway, normalization of oxidative stress and inflammation biomarkers, and regulation of apoptotic protein expression. These findings underscore the translational relevance of in vitro cytoprotective observations involving *B. frutescens* and provide a rationale for advancing toward in vivo validation.

While our findings demonstrate the protective effects of *B. frutescens* extract against cisplatin-induced cytotoxicity in HEP-G2 cells, the mechanistic basis for this effect remains to be fully elucidated. The discussion of potential antioxidant mechanisms and involvement of signalling pathways such as NF-κB and MAPK is largely speculative and based on prior literature involving *B. frutescens* or related phytochemicals. A key limitation of this study is the absence of direct measurement of intracellular reactive oxygen species (ROS). Future studies should employ specific assays such as 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescence to quantify ROS levels and validate antioxidant activity. Additionally, Western blot analysis of key apoptotic (e.g., cleaved caspase-3, Bax, Bcl-2) and inflammatory (e.g., NF-κB p65, IκBα, p-p38, p-JNK, and p-ERK) markers is recommended to confirm the involvement of these pathways in mediating the protective effects of *B. frutescens*. Incorporating these molecular assays will not only validate the proposed mechanisms but also provide stronger evidence to support the development of *B. frutescens* as a hepatoprotective agent during chemotherapy.

The current study utilized HEP-G2 cells, a hepatocellular carcinoma cell line, to evaluate the protective effects of *B. frutescens* against cisplatin-induced toxicity. Although these cells are derived from a liver tumour, they remain one of the most commonly used in vitro models for hepatotoxicity studies due to their expression of liver-specific enzymes, oxidative stress-related pathways, and metabolic competence. These features make them suitable for preliminary cytoprotective screening of natural products. However, we acknowledge that the use of a cancer-derived cell line introduces limitations. Notably, cisplatin is a chemotherapeutic agent with well-documented cytotoxicity against cancer cells, including HEP-G2. Therefore, some of the observed reduction in cell viability may reflect cisplatin's anticancer effects rather than pure hepatotoxicity. This could potentially confound the interpretation of the extract's protective

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effects. To address this limitation, future studies should incorporate additional in vitro models such as non-tumorigenic hepatic cells (e.g., HepaRG, THLE-2, or primary human hepatocytes). These models would offer more physiologically relevant insight into hepatoprotection, especially in the context of drug-induced liver injury. Moreover, integrating 3D liver spheroids or organ-on-chip systems may provide a more accurate representation of in vivo liver responses. Another limitation of this study is the use of a relatively small number of biological replicates (n = 3), which, although commonly accepted in initial in vitro cytotoxicity screenings, may limit the statistical power and generalizability of the results. Future studies should include a larger number of independent experiments to enhance the robustness, reproducibility, and confidence in the observed protective effects of *B. frutescens*.

CONCLUSION

In conclusion, cisplatin induces concentration-dependent cytotoxicity in HEP-G2 cells, whereas non-toxic doses of *B. frutescens* leaves extract confer significant protective effects against cisplatin-induced cell death. This protection is likely due to the phytochemical richness of the extract particularly flavonoids with potent antioxidant and anti-inflammatory activities. The present findings provide the first *in vitro* evidence supporting the hepatoprotective potential of *B. frutescens* during chemotherapy. While the results are promising, further *in vivo* validation and mechanistic studies are required to confirm its protective role and assess its clinical relevance. These insights may lay the groundwork for developing *B. frutescens* as a complementary therapeutic strategy to mitigate cisplatin-associated hepatotoxicity.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in the present work.

AUTHORS' CONTRIBUTIONS

Nurhanani Ayub: Writing - Original draft, Methodology, Validation, Formal analysis, Investigation, Data Curation. Siti Nur Munirah Ismail: Writing - Original draft, Methodology, Investigation, Data Curation. Salfarina Ramli: Writing - Review and Editing, Conceptualization, Methodology, Validation. Richard Johari James: Writing - Review and Editing, Conceptualization, Methodology, Validation. Mohammad Zulfadhly Jan Jam: Writing - Review and Editing, Conceptualization, Methodology, Validation. Hasseri Halim: Project administration, Project acquisition, Formal analysis, Writing - Review and Editing, Conceptualization, Methodology, Validation, Resources, Data curation, Supervision.

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