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Research Article

NEUROPROTECTIVE POTENTIAL OF *DICTYOTA BARTAYRESIANA* CRUDE EXTRACTS: AN IN VITRO STUDY USING NEUROBLASTOMA CELL LINES (SH-SY5Y CELL LINE)

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ABSTRACT

Neurodegenerative diseases, characterized by progressive neuronal deterioration, pose a significant and growing global health challenge due to their increasing prevalence with an aging population and limitations of current symptomatic treatments. This study addresses the urgent need for novel therapeutic interventions by investigating the neuroprotective potential of crude extracts from the marine alga *Dictyota bartayresiana*. Using an *in vitro* model with SH-SY5Y neuroblastoma cells, the study employed MTT assays to evaluate both the extract's cytotoxicity and its capacity to alleviate hydrogen peroxide (H_2O_2)-induced oxidative stress. Key findings reveal that the *Dictyota bartayresiana* extract exhibits a concentration-dependent, biphasic effect. An intrinsic cytotoxic concentration (IC_{50}) of 63.53 µg/mL was determined. Importantly, the extract demonstrated significant neuroprotection against H_2O_2-induced oxidative stress within an optimal range of 25–50 µg/mL. However, protective effects waned at both lower and higher concentrations, highlighting a complex dose-response. These results underscore that *Dictyota bartayresiana* contains compounds with both neuroprotective and potentially cytotoxic properties, necessitating precise dose optimization for therapeutic application. The study highlights the promise of marine natural products as sources for new neuroprotective agents and emphasizes the need for further research to identify specific bioactive compounds and elucidate the molecular mechanisms underlying this intricate dose-dependent efficacy.

Keywords: *Dictyota bartayresiana,* Neuroprotection, Oxidative stress, SH-SY5Y cells, Biphasic dose-response.

INTRODUCTION

Neurodegenerative diseases constitute a diverse and devastating category of neurological disorders characterized by the gradual deterioration of neuronal structure and function, leading to significant cognitive and physical impairments (Prusiner, 2002). These conditions, encompassing Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis, constitute a substantial and escalating global healthcare challenge (Adamu *et al*., 2024; Dhariwal *et al*., 2025). Their frequency is anticipated to rise markedly with the aging global population, underscoring the urgent necessity for effective therapeutic interventions (Wu *et al*., 2021). Despite diverse pathogenic mechanisms related to distinct protein aggregation and genetic variations, chronic neuroinflammation is a prevalent characteristic among various neurodegenerative diseases (Zhang *et al*., 2023). Conventional management techniques for neurodegenerative illnesses have hurdles due to their multiple etiologies, progressive characteristics, and the difficulty of precisely targeting specific molecular pathways without causing systemic side effects (Bloomingdale *et al*., 2022). As a result, existing therapy strategies are primarily symptomatic and palliative, with minimal effectiveness in arresting or reversing disease development (Tondo and Marchi, 2022). The challenges associated with traditional treatment methods, along with the increasing global prevalence of these diseases, need the investigation of new therapeutic approaches. Natural sources, especially marine algae, offer a promising pathway for discovering new chemicals with potential neuroprotective and disease-modifying attributes, providing

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an optimistic route for future study and development. This research gap underscores the urgent necessity for thorough exploration of the neuroprotective attributes of natural compounds, especially those sourced from marine organisms such as *Dictyota bartayresiana*, which have already exhibited various pharmacological effects in different therapeutic domains (Mohideen *et al*., 2025). Various extracts of *Dictyota bartayresiana* have exhibited notable antibacterial, antioxidant, and anti-inflammatory effects, indicating a diverse phytochemical composition with extensive therapeutic potential.

MATERIALS AND METHODS

The study was approved by the Institutional Ethics Committee. All procedures were conducted in accordance with the ethical standards of the Declaration of Helsinki.

Plant identification and collection

Dictyota bartayresiana plants were gathered from the coastal area of Ramanathapuram district, meticulously rinsed with distilled water, and verified by a botanist to ensure taxonomic accuracy. Specimens were promptly conveyed to the laboratory for additional processing to maintain their phytochemical integrity. To enable the effective extraction of secondary metabolites, the collected plant material was systematically dried and ground in subsequent phases (Nahar *et al*., 2025). The raw ingredients were stored in screw-cap containers at ambient temperature, shielded from sunlight, until the extraction process commenced.

Preparation of plant extracts

Crude extracts are prepared using solvents such as ethanol solution via soxhlet extraction. 40g of powdered sample was dissolved in 200 mL of ethanol solvent and kept 72 hours on a rotary shaker at 150 rpm at 25 °C temperature. The entire mixture was filtrated and concentrated to dryness by evaporating on a vacuum evaporator as the slightly modified procedure of Pandey *et al.*, 2020. The extracted crude sample was kept in airtight glass vials and stored at 4 °C until further use (Figure 1). This approach ensures the preservation of the extract's bioactivity, crucial for accurate neuroprotective assessment. At the end of the procedure, 3.36 grams of crude extracts of *Dictyota bartayresiana* were obtained using ethanol as a solvent.

Neuroblastoma cell lines - SH-SY5Y

This study utilized a well-established neuroblastoma cell line (SH-SY5Y) as an in vitro model, closely resembling neuronal cells and extensively employed for neurotoxicity and neuroprotection investigations (Gallego *et al*., 2021; Silva *et al*., 2018). The SH-SY5Y cell line, a human neuroblastoma cell line, is particularly appropriate due to its neuronal properties and its capacity to differentiate into more mature neuronal phenotypes upon specific induction, rendering it a dependable model for assessing neuroprotective agents (Hassan *et al*., 2020) (Gallego *et al*., 2021). After being treated with plant extracts, the neuroblastoma cells were examined under a phase-contrast microscope to see if they had any cytoprotective effects (Mohideen *et al*., 2025). Subsequent quantitative evaluations of cell viability and metabolic activity were performed utilizing standard colorimetric assays, including the MTT assay, yielding a thorough assessment of the extract's influence on cell survival and proliferation (Bharathi *et al*., 2025).

Cytotoxicity of plant extract on SHSY5Y cell lines

The cytotoxic effects of *Dictyota bartayresiana* crude extracts on SH-SY5Y cells were evaluated using the MTT test to establish the concentration range for the subsequent neuroprotective investigations, with untreated cells acting as the control group. In this test, cells were inoculated at a density of 4 x 10^3 cells per well in 96-well plates and permitted to adhere overnight (Mohideen *et al*., 2025). Cell viability was then measured following a 24-hour application of the *Dictyota bartayresiana* extract at different concentrations. This initial cytotoxicity evaluation is essential for determining non-toxic quantities of the extract suitable for further neuroprotection studies, ensuring that observed benefits result from protective mechanisms rather than cellular damage (Bharathi *et al*., 2025).

Such studies are essential for determining the ideal therapeutic window, wherein the extract provides positive benefits without provoking detrimental cellular responses (Gallego *et al*., 2021). This initial screening identifies the half-maximal inhibitory concentration of the crude extracts, thereby informing the selection of suitable sub-cytotoxic doses for assessing neuroprotective activity (Silva *et al*., 2018) (Mohideen *et al*., 2025). The SH-SY5Y cells were subsequently exposed to different concentrations of the extract, from 6.25 µg/mL to 100 µg/mL, to determine a dose-response relationship, with control cells without the test plant extract for comparative analysis to establish relative potency (Mohideen *et al*., 2025). This systematic technique enables the accurate determination of an ideal concentration range in which the *Dictyota bartayresiana* extract may effectively reduce neurotoxicity without causing intrinsic cellular harm, so confirming its promise as a neuroprotective agent.

The cellular viability assay outlined herein was employed to assess cytotoxicity on the SHSY5Y cell line. As previously mentioned, 100 mL of crude extracts containing concentrations ranging from 6.25 to 100 µg/mL were added to incubated cells. The negative control lacked an extract. Cells were incubated in a 5% CO2 environment at 37°C for 24 hours. Subsequently, the medium was discarded, and 100 mL of 0.5 µg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in Dulbecco's phosphate-buffered saline (DPBS) was introduced, followed by a 4-hour incubation of the cells. Ultimately, the MTT solution was carefully eliminated, and 100 µL of dimethyl sulfoxide (DMSO) was used to solubilize the crystal formazan. Triton X-100 served as the positive

control, representing 100% cell mortality. The plate was agitated for 1 minute, and the absorbance of formazan in each well was quantified using a microplate reader at 570 nm. The cytotoxicity percentage relative to the negative control (untreated cells, regarded as 100% viability) was then computed.

To investigate the neuroprotective effects, oxidative stress was generated in SHSY5Y cells using 250 µM hydrogen peroxide (H2O2) for one hour, resulting in a usual reduction of cell viability by 70-80%. Prior to stress induction, cells were pretreated with *Dictyota bartayresiana* plant extracts at corresponding IC50 concentrations for six hours. Cell viability was subsequently assessed with the MTT test to evaluate the protective effects of the extracts on the cells. Untreated cells functioned as a 100% viability control, whereas H2O2-treated cells (lacking extracts) indicated 100% cell mortality.

RESULTS AND DISCUSSION

The research focused on evaluating the cytotoxicity and neuroprotective potential of a plant extract *Dictyota bartayresiana*, on SH-SY5Y cells, particularly in the presence of hydrogen peroxide (H_2O_2)-induced oxidative stress. The study utilized an MTT assay to assess cell viability. Two main experiments were conducted: Cytotoxicity of *Dictyota bartayresiana* alone: The percentage of cell viability for various concentrations of *Dictyota bartayresiana* plant extract (6.25, 12.5, 25, 50, and 100 µg/mL) was calculated relative to an untreated control, set at 100% viability. (Figure 2 and 3) Neuroprotective potential of *Dictyota bartayresiana*: SH-SY5Y cells were co-treated with varying concentrations of *Dictyota bartayresiana* extract (6.25, 12.5, 25, 50, and 100 µg/mL) and 100 µM H_2O_2 for 24 hours. This experiment aimed to determine if *Dictyota bartayresiana* extracts could protect cells from oxidative damage caused by H_2O_2. (Figure 4 and 5).

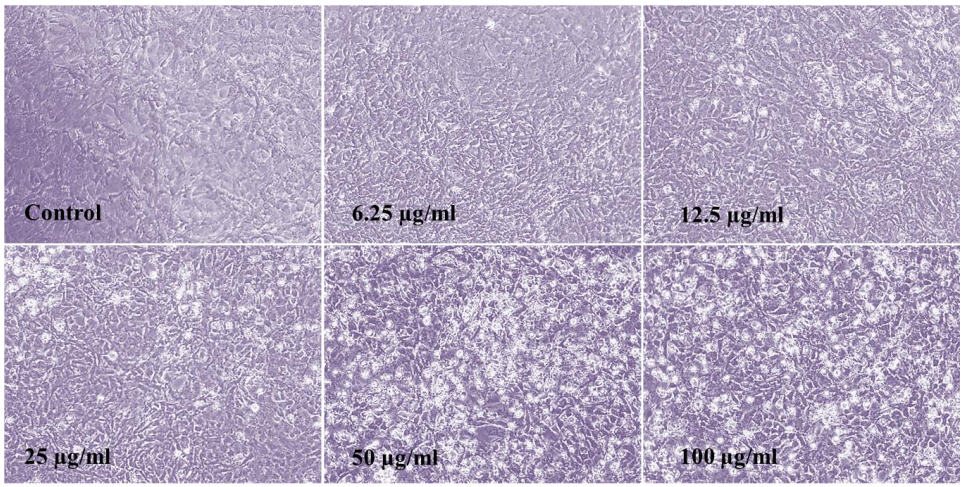

Figure 2. MTT assay showing the effect of *Dictyota bartayresiana* plant extract on SH-SY5Y cell viability at various concentrations (6.25, 12.5, 25, 50, and 100 µg/mL).

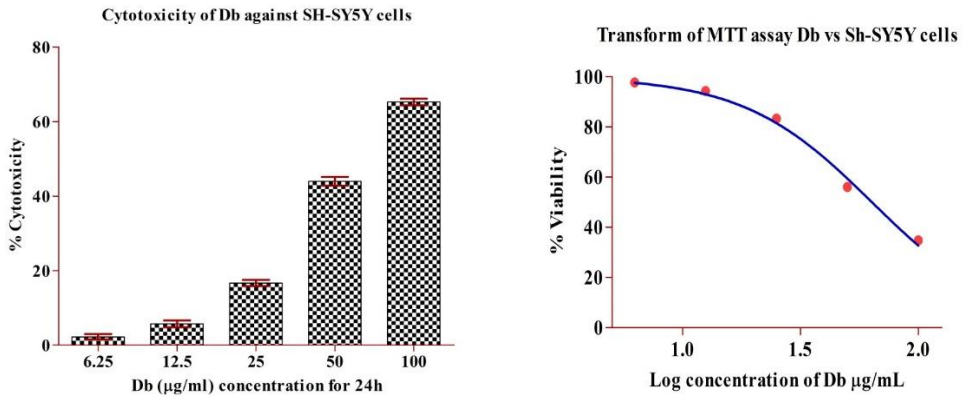

Figure 3. Dose–response curve for cytotoxicity of *Dictyota bartayresiana* extract on KB cells.

Non-linear regression analysis was performed using GraphPad Prism (version 5.0) with log-transformed concentrations of *Dictyota bartayresiana* extract. The curve represents % cell viability vs. log [extract concentration (µg/mL)], and the IC_{50} value was calculated from the fitted model, indicating the concentration required to reduce viability by 50%.

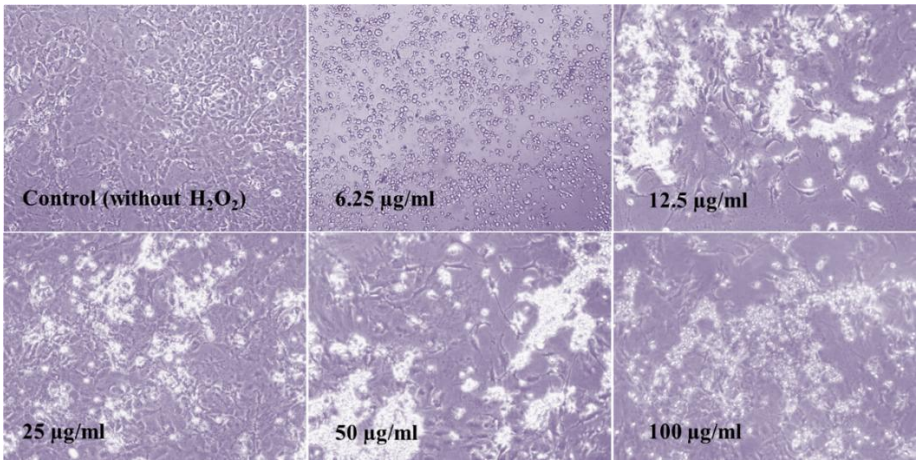

Figure 4. *Morphological changes in* SH-SY5Y cells *after treatment with Dictyota bartayresiana extract.* Representative microscopic images show untreated control cells with normal polygonal morphology and treated cells displaying dose-dependent morphological alterations such as rounding, shrinkage, and detachment, consistent with cytotoxic response.

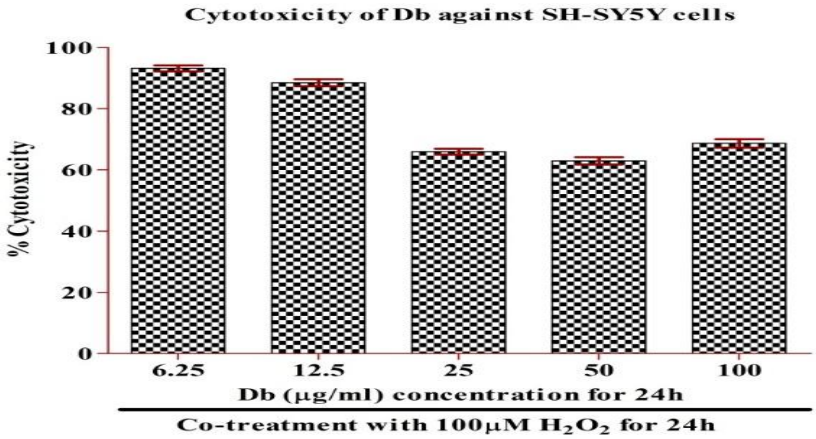

Figure 5. Effect of Db extract on H_2O_2-induced cytotoxicity in SH-SY5Y cells. Cells were co-treated with *Db* extract (6.25–100 µg/mL) and 100 µM H_2O_2 for 24 hours. The % cytotoxicity decreased significantly at 25–50 µg/mL, suggesting a protective effect of *Dictyota bartayresiana* against oxidative stress. Very low and high doses did not show protective effects.

Data analysis was performed using GraphPad Prism software, which fitted a non-linear regression curve to generate a sigmoidal dose-response curve. From this, the IC_{50} value (half-maximal inhibitory concentration) was calculated, representing the concentration required to reduce cell viability by 50%. The results were statistically validated, with error bars representing the mean ± standard deviation from three independent replicates. The study demonstrated a dose-dependent impact of the *Dictyota bartayresiana* extract on SH-SY5Y cells, specifically on its capacity to alleviate H_2O_2-induced cytotoxicity. The determined IC_{50} for the *Dictyota bartayresiana* extract is 63.53 µg/mL, with a 95% confidence interval of 60.33 to 66.90 µg/mL. Low Doses (6.25, 12.5 µg/mL): Co-treatment with *Dictyota bartayresiana* at these concentrations, in conjunction with H_2O_2, induced significant cytotoxicity, resulting in around 90–95% cell mortality. This indicates that *Dictyota bartayresiana* was not protective at these minimal concentrations. Moderate Doses (25–50 µg/mL): A notable reduction in cytotoxicity was seen at these doses, with cell mortality decreasing to around 60–65%. This suggests that *Dictyota bartayresiana*

provides partial neuroprotection against oxidative stress within this dosage range. Higher Dose (100 µg/mL): At the maximum tested concentration, cytotoxicity marginally rose to around 70%, indicating that the protective effect wanes beyond the optimum range. The results reveal that *Dictyota bartayresiana* has a dose-dependent protective window, with maximal neuroprotection observed at doses ranging from 25 to 50 µg/mL. At doses outside this range (either very low or extremely high), *Dictyota bartayresiana* failed to demonstrate protective properties, resulting in significant cellular damage. This concentration-dependent efficacy highlights the complex interplay between concentration-mediated toxicity, a feature shared by many chemicals produced from plants, and possible therapeutic advantages. The noted elevation in cytotoxicity at elevated concentrations corresponds with other studies on several plant extracts, which have demonstrated dose-dependent toxicity resulting in diminished cell viability with rising doses (Mohideen *et al*., 2025).

The present study seeks to investigate the impact of *Dictyota bartayresiana* extract on SH-SY5Y cells subjected to hydrogen peroxide-induced oxidative stress, highlighting a complicated, dose-dependent relationship that requires comprehension of its possible neuroprotective attributes. The principal discovery was the determination of an IC_{50} value of 63.53 for the *Dictyota bartayresiana* extract, signifying the concentration at which *Dictyota bartayresiana* alone diminishes cell viability by over 50%. This value sets a baseline for the extract's intrinsic cytotoxicity in SH-SY5Y cells without an additional stressor. The study unequivocally reveals a biphasic dose-response curve for the *Dictyota bartayresiana* extract in the context of neuroprotection against H_2O_2-induced oxidative stress. At minimal doses, *Dictyota bartayresiana* provided negligible protection, resulting in significantly diminished cell viability (90–95% cell death). In contrast, at moderate doses (ranging from 25 to 50 µg/mL), a notable neuroprotective effect was noted, decreasing cell death to 60–65%. Nevertheless, above this ideal range, at the highest evaluated doses, cytotoxicity marginally escalated to around 70%, indicating a reduced protective impact and maybe an enhancement in pro-oxidant activity or inherent cytotoxicity. This occurrence corresponds with prior studies on other plant extracts and natural chemicals, indicating that elevated quantities may result in diminished cell viability or pro-oxidant effects. The noted elevation in cytotoxicity at elevated concentrations, coupled with the absence of protective effects at diminished doses, indicates that *Dictyota bartayresiana* contains chemicals exhibiting both neuroprotective and potentially neurotoxic properties that are significantly concentration-dependent. This dual character is essential for future therapeutic considerations. The reduced protection at elevated concentrations may suggest a hermetic effect, wherein low amounts are stimulatory or protective, whereas higher dosages become inhibitory or harmful. This is consistent with research on *Dunaliella salina* extract, where concentrations over 20 µg/mL markedly impacted cell viability in HK-2 cells, as well as data about dieckol from Ishige okamurae, which reduced cell viability in HaCaT cells.

The difference in solvent polarity effects the extraction of beneficial chemicals such as flavonoids and may also affect overall biological activity. *Dictyota bartayresiana* exhibits neuroprotective properties within a precise dosage range (25-50 µg/mL) against oxidative stress; yet, its intrinsic cytotoxicity and the resultant escalation in cell mortality at elevated concentrations underscore the need for careful dose optimization. Additional research is necessary to identify and describe the particular chemicals responsible for the neuroprotective and cytotoxic effects at varying concentrations, hence aiding in the development of targeted therapy strategies. The mechanism behind this biphasic dosage response, in which moderate concentrations provide protection while both extremely low and high concentrations do not, requires further exploration to clarify the exact molecular processes involved. The intricate dose-response curve may be ascribed to diverse phytochemicals in the extract that exhibit both antioxidant and pro-oxidant characteristics contingent upon their concentration, as evidenced in research on other natural compounds (Durairaj and Andiyappan, 2020). Research on *Dictyota bartayresiana* demonstrated concentration-dependent cytotoxicity in HT29 colon cancer cells, with an IC50 value of 300 µg/mL, suggesting that elevated concentrations can result in enhanced cellular inhibition (Bharathi *et al*., 2025). A study on Ulva compressa extract shown neuroprotective potential but failed to mitigate H2O2-induced damage by 6-OHDA, indicating diverse modes of action among plant extracts (Silva *et al*., 2018). The neuroprotective properties of sulfated polysaccharides against cytotoxicity generated by oxidative stress further illustrate these diverse processes (Olasehinde *et al*., 2020). Moreover, prior research on other plant extracts has demonstrated analogous dose-dependent effects, wherein elevated concentrations of specific chemicals, such as γ-EC, may display cytotoxicity (Khaksar *et al*., 2024). The lack of neuroprotection at low concentrations may result from inadequate activation of intrinsic cellular defense mechanisms, whereas diminished protection at elevated concentrations could suggest a hermetic effect at supra-optimal levels (Jodynis-Liebert & Kujawska, 2020) (Khaksar *et al*., 2024). These biphasic responses highlight the essential requirement for precise dose adjustment in the development of plant-derived medicinal medicines to optimally utilize their neuroprotective advantages (Khaksar *et al*., 2024).

The presence of particular bioactive compounds in *Dictyota bartayresiana*, including long-chain fatty alcohols (n-pentadecanol and n-nonadecanol) and isoflavones, recognized for their antioxidant and cytotoxic properties, may influence this intricate dose-response relationship (Bharathi *et al*., 2025). The methanol extract of *Dictyota ciliolata* and *Dictyota bartayresiana* demonstrated significant phenol content, which coincides with their in-vitro antioxidant properties, underscoring the influence of solvent polarity in the extraction of these advantageous chemicals (Chellamanimegalai *et al*., 2025). The difference in solvent polarity markedly affects extraction yield and phytochemical composition, with research demonstrating that methanolic extracts frequently possess the largest

concentrations of advantageous chemicals, such as flavonoids (Bharathi *et al*., 2025) (Mohideen *et al*., 2025). The complex interaction of several chemicals, along with their concentration-dependent effects, requires additional isolation and characterisation of individual components to thoroughly clarify their specific roles in the noted neuroprotection and cytotoxicity (Mohideen *et al*., 2025). Subsequent research should concentrate on fractionating the *Dictyota bartayresiana* extract to isolate and identify the particular chemicals responsible for the neuroprotective and cytotoxic effects at different doses, potentially utilizing advanced analytical techniques. This would enable a more accurate comprehension of the mechanisms driving the observed biphasic dosage response and promote the creation of targeted treatment approaches.

CONCLUSION

The current study indicates that *Dictyota bartayresiana* extract demonstrates a multifaceted, dose-dependent influence on SH-SY5Y cells, exhibiting both cytotoxic and neuroprotective characteristics. An IC_{50} value of 63.53 signifies intrinsic cytotoxicity, although the extract exhibits considerable neuroprotection against H_2O_2-induced oxidative stress within an optimum range of 25–50 µg/mL. Outside this range, at both lower and higher concentrations, the protective effect wanes, underscoring a significant biphasic response. This highlights the necessity for precise dose adjustment to fully exploit the therapeutic potential of *Dictyota bartayresiana* and necessitates future research to clarify the individual chemicals and mechanisms underlying these concentration-dependent effects.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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