

Original Research Article

## Glycerol extract of Water hyacinth (*Eichhornia crassipes* (Mart.) Solms) attenuates hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-induced cell death in HT22 hippocampal neurons

Chi Fung Leong<sup>1</sup>, Amar Daud Iskandar Abdullah<sup>1</sup>, Hooi-Leng Ser<sup>1</sup>, Long Chiau Ming<sup>2,3</sup>, Mohamed Kheireddine Aroua<sup>4,5,6</sup>, Lai Ti Gew<sup>1\*</sup>

### Article History

Received: 07 April 2026;

Received in Revised Form:  
18 August 2026;

Accepted: 19 August 2026;

Available Online: 30  
September 2026

<sup>1</sup>Department of Biomedical Sciences, Jeffrey Cheah Sunway Medical School, Faculty of Medical and Life Sciences, Sunway University, Sunway City, Malaysia; 17021627@imail.sunway.edu.my (CFL); amara@sunway.edu.my (ADIA); hooilengs@sunway.edu.my (H-LS)

<sup>2</sup>School of Pharmacy, Sunway University, Sunway City, Malaysia; chiaumingl@sunway.edu.my (LCM)

<sup>3</sup>Department of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

<sup>4</sup>Department of Chemical Engineering, King Fahd University of Petroleum & Minerals, Dhahran, Saudi Arabia; mohamed.aroua@kfupm.edu.sa (MKA)

<sup>5</sup>Interdisciplinary Research Center for Hydrogen Technologies and Carbon Management, King Fahd University of Petroleum & Minerals, Dhahran, Saudi Arabia

<sup>6</sup>Saudi Aramco Chair in CO<sub>2</sub> Capture and Utilization, Dhahran, 31261, Saudi Arabia

\*Corresponding author: Lai Ti Gew; Department of Biomedical Sciences, Sir Jeffrey Cheah Sunway Medical School, Faculty of Medical and Life Sciences, Sunway University, No. 5, Jalan Universiti, 47500 Bandar Sunway, Selangor, Malaysia; janeg@sunway.edu.my (LTG)

**Abstract:** Neurological disorders continue to represent a significant global health burden, ranking among the leading causes of mortality and long-term disability. The persistent lack of effective therapeutic interventions reflects an incomplete understanding of the complex molecular mechanisms underlying neuronal cell death in the diseased brain. Among these, oxidative stress has been identified as a critical secondary injury mechanism that exacerbates neuronal damage. Recently, *Eichhornia crassipes* (Mart.), commonly known as water hyacinth, has emerged as a promising source of bioactive compounds with notable anti-inflammatory and antioxidant properties, making it a potential candidate for the development of neuroprotective therapies. This study aimed to assess the neuroprotective potential of *E. crassipes* extracts (ultrasound-assisted maceration), with glycerol extract (GE) emerging as the most effective extract, using a cell-based model of oxidative stress-induced by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in hippocampal HT22 cells. Cell viability of HT22 cells were measured through MTT assay, in order to determine cytotoxicity of extracts (0.5 mg/ml – 5 mg/ml) and protective effects of the extracts against H<sub>2</sub>O<sub>2</sub> (via co-treatment assay). Our study revealed

that H<sub>2</sub>O<sub>2</sub> was able to induce cell viability loss in HT22 cells in a dose-dependent manner, and the IC<sub>50</sub> value of H<sub>2</sub>O<sub>2</sub> was determined as approximately 655.3 µM. Following the co-treatment assay, GE at 0.8 mg/ml displayed a significant protective effect by alleviating H<sub>2</sub>O<sub>2</sub> (700 µM)-induced cell death as compared to the H<sub>2</sub>O<sub>2</sub> alone group (67.4 ± 4.4% viability vs. 52.4 ± 1.4%,  $p < 0.05$ ), while aqueous and mixed extracts did not display significant protective trend. These preliminary findings suggest that glycerol-based *E. crassipes* extract possesses underexplored neuroprotective potential against H<sub>2</sub>O<sub>2</sub>-induced oxidative stress in neuronal cells, though further mechanistic and *in vivo* investigation are required to validate these effects and establish translation relevance.

**Keywords:** Eichhornia crassipes; water hyacinth; hippocampal neurons; neuroprotective; oxidative stress; HT22 cells, antioxidants

---

## 1. Introduction

Oxidative stress arises from the accumulation of free radicals due to dysregulation of the redox homeostasis system, leading to persistent cellular damage [1, 2]. This prolonged condition is often overlooked as it may take years or even decades to display its observable impacts to human health [3]. The hallmark of prolonged oxidative insult is its contribution to neuropathogenicity, driving the development of acute and chronic brain diseases including stroke, Alzheimer's disease, Parkinson's disease, and Huntington's disease [4, 5]. Given that neurons are different from other cell types, they are particularly vulnerable to oxidative stress due to their high oxygen consumption, lipid-rich composition, and limited regenerative capabilities [6, 7]. These cascades collectively trigger downstream events including mitochondrial dysfunction, calcium dysregulation, autophagy dysregulation, neuroinflammation, and activation of programmed cell death pathways, ultimately leading to neuropathogenesis [8–15].

Despite the actual mechanisms of neurological diseases remain to be elucidated, it is proposed that they share similar neuropathological features with varying complexity and affecting different neuronal pathways and cell types [16, 17]. Furthermore, due to the scarcity of effective treatments, targeting the oxidative stress pathway offers a promising early intervention due to their central role in driving cellular-damaging downstream cascades in the mechanism, thereby impeding the disease progression and maintaining healthy brain functions [18, 19]. Thus, exogenous antioxidants come into play as they can effectively counteract the accumulated oxidant, especially when the endogenous antioxidant system is compromised [2, 20]. Plants, the cornucopia of beneficial bioactive compounds, have gained massive attention in searching novel intervention for neurological diseases due to the broad presence of antioxidants such as flavonoids, polyphenols, carotenoids, and alkaloids [21, 22].

*Eichhornia crassipes* (Mart.), generally known as water hyacinth, is a floating, flowering aquatic plant endemic to the Amazon Basin in South America [23, 24]. It has been introduced globally as an ornamental plant in the late 19<sup>th</sup> century, renowned for its alluring flower, as well as its robust adaptability and growth capacity [25, 26]. In the past decade,

improper management has turned this plant into an invasive species, leading to a range of ecological and socioeconomic challenges [27, 28]. Additionally, its pharmacological potential has been valued due to its diverse bioactive compounds such as alkaloids, phenolics, flavonoids, tannins, saponins, terpenoids, and sterols [29, 30]. Scientific studies have highlighted its activities in wound healing, hepatoprotection, antibacterial, antifungal, anti-cancer, antioxidant, and anti-inflammatory effects [31–33]. Focusing on its well-established antioxidant property, various extracts of water hyacinth from different plant parts have displayed ROS and free radicals scavenging, metal-ion chelating and lipid peroxidation-reducing activities *in vitro* while a reduction in oxidative stress marker *in vivo* [34–42]. On the other hand, studies have shown the anti-inflammatory effects of water hyacinth extracts *via* inhibiting albumin protein denaturation *in vitro* and reducing paw oedema *in vivo* [43, 44].

Despite its well-known antioxidant and anti-inflammatory activities which are key therapeutic approaches for neurological diseases, study on the neuroprotective potential of *E. crassipes* remains limited [45]. Two previous neuroprotective studies used ethanolic extracts of *E. crassipes* have shown anti-depressant, anti-epileptic and anti-anxiety effects in mice, as well as neuroprotection in cerebral ischemic mice model *via* reduction of infarct sizes, and oxidative stress markers [46, 47]. However, both studies did not clarify the direct protection of *E. crassipes* from oxidative injury at the cellular level, while the investigation on its neuroprotective potential using an *in vitro* oxidative stress neuronal model remains limited.

For *in vitro* cell-based study, hydrogen peroxide ( $H_2O_2$ ) is commonly employed to induce oxidative damage in cellular model including neuronal cell lines due to its ability to cross cellular membranes and mimic intracellular oxidative stress conditions at defined concentrations [48–51]. HT22, an immortalised mouse hippocampal cell line, was selected as the *in vitro* model mainly due to its well-characterized sensitivity to oxidative treatment [52, 53]. Additional reasons are due its widespread applications use in neuroprotection studies and relevance to hippocampal neurodegeneration associated with Alzheimer's disease [54, 55]. Given its known antioxidant activity and the recognized contribution of oxidative stress to neurodegeneration, we hypothesized that *E. crassipes* extracts could directly exert protective effects against oxidative injury in hippocampal neurons. Therefore, this study was aimed to assess the protective potential of water hyacinth against  $H_2O_2$ -induced cell death using HT22 hippocampal cell lines. Prior behavioural and neuroprotective outcomes following oral administration suggest the potential CNS bioavailability of *E. crassipes* bioactive compounds, however, direct evidence of blood-brain barrier penetration remains to be established [45, 46].

## 2. Materials and Methods

### 2.1. Chemicals

Solvent used for water hyacinth extraction was an ACS reagent graded plant-origin glycerol ( $\geq 99.5\%$  purity) from Supelco (Cat. No. 1.04057). Cell culture reagents include high glucose Dulbecco's Modified Eagle Medium (DMEM) from Sigma-Aldrich, (Cat. No. D6429), fetal bovine serum (FBS) from Capricorn (Cat. No. FBS-12A), penicillin-

streptomycin antibiotic solution (Pen-Strep) from Sigma Aldrich (Cat. No. TMS-AB2), phosphate buffer saline (PBS) from Sigma Aldrich (Cat. No. P7059), and accutase (Sigma Aldrich, Cat. No. A6964). Chemicals used for experimental assays were 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from Sigma Aldrich (Cat. No. M2128), AR graded ( $\geq 99.8\%$  purity) dimethyl sulfoxide (DMSO) from Fisher Scientific (Cat. No. FKCD/4121/PB17), and Puriss. p.a. graded 30%  $\text{H}_2\text{O}_2$  from Sigma Aldrich (Cat. No. 31642).  $\text{H}_2\text{O}_2$  stock solution was prepared freshly in 0.1 M concentration prior to every treatment.

## 2.2. Plant Collection and Extracts Preparation



**Figure 1.** Morphological characteristics of *Eichhornia crassipes* (Mart.) Solms collected from Kinta Nature Park, Batu Gajah, Perak, Malaysia. Flowering rosette of *E. crassipes* displaying leaf arrangement and inflorescence. Image was captured by author (Lai Ti Gew).

*Eichhornia crassipes* (Mart.) Solms whole plant (Figure 1) was collected from Kinta Nature Park located at Batu Gajah, Perak, Malaysia (approximate coordinates: 4.47° N, 101.03° E) by author (Lai Ti Gew) on 5<sup>th</sup> January 2023. Coordinates were determined with reference to a topographic map of the region (<https://en-bw.topographic-map.com/map-mxkz1h/Kinta/>. Accessed on 16 July 2025). The collected specimens were identified based on the following morphological features: leaves are green to dark green, round to bowl-shaped blades, with smooth margin and arcuate venation at the apex and base; the upper leaf surface bears a waxy cuticle, providing glossiness and water-repellency. Petioles are inflated and spongy, reflecting the aerenchymatous tissue characteristic of the species, which

functions in buoyancy and gaseous exchange. Roots are fibrous, dark purple to black in color, and hang freely in the water without anchorage to the substrate. Rosettes are interconnected by horizontal stolons, expediting the vegetative propagation process. The inflorescence bears lavender-to-violet flowers, the uppermost tepal marked with a yellow-centered spot on a blue-violet patch, resembling a peacock feather. The plants were observed forming dense floating mats along the shoreline and water surface (Figure 1), representing the species' known invasive growth pattern.

The fresh sample of *E. crassipes* whole plants were collected and air-dried at room temperature (25–30°C) for at least a week under shade, in a well-ventilated area. Once constant weight was achieved (no change >5%, over 24 h), the dried samples were kept air-tight bag and kept in non-humid environment for long term storage. For extraction process of water hyacinth, the ultrasound-assisted maceration method was applied. Generally, the dried whole plant was pulverized using machine grinder into fine powder. The powder was weighed at 500 mg and added to 25 ml of three types of solvents including, glycerol, deionized water, and water-glycerol with 1:1 ratio (v/v). The mixtures were vortexed at the highest speed (Sigma Aldrich, IKA® Vortex 3, Z654779) and sonicated at 40kHz, 150 W for 30 minutes under ultrasonic water bath (Cole Palmer, Model: 08895-11), followed by immersion at room temperature. These processes were repeated at every 24 hours intervals up to three cycles (total 72 hours). After 72 hours maceration, the mixture was centrifuged at 2000 rpm for 15 minutes at 4°C (Eppendorf, Model: Centrifuge 5810R), the supernatant was filtered using 0.45 µm followed by 0.22 µm nylon syringe filters (BioFlow, Cat. No. MALNY25045N and MALNY25022N) and stored at 4°C for subsequent experiments. The dry weight yields of extracts were not determined due to the hygroscopic nature of glycerol.

### 2.3. Cell Lines and Cell Cultures

The HT22 mouse hippocampal neuronal cell line was a precious gift by Assoc. Prof. Dr Audrey Lim Wei Ling from Sunway University which was originally purchased from Merck Millipore (SCC129). The cells were maintained in high glucose DMEM supplemented with 10% (v/v) FBS, and 1% (v/v) of 100× penicillin-streptomycin solution and incubated at 37°C with 95% humidified atmospheric air and 5% CO<sub>2</sub> levels. Once more than 80% of cell confluency was achieved in the T<sub>75</sub> culture flask, the cells were detached using Accutase for subsequent passaging, 1×10<sup>6</sup> cells were re-seeded into T<sub>75</sub> flask or transferred into cryovials for cryopreservation purpose. The passage number of HT22 cells was maintained below 10<sup>th</sup> passage throughout the study. Mycoplasma contamination was routinely checked for every post-thaw cell batch and thereafter on a monthly basis using mycoplasma PCR detection kit (Applied Biological Materials, Cat. No. G238).

### 2.4. Cell Viability Assays

The MTT reduction assay was applied to determine the HT22 cell viability after being exposed to various cytotoxicity screenings and co-treatment assays by following the manufacturer's manual [56, 57]. Prior to cell treatment, HT22 cells were seeded into 24-well plates with a density of 5×10<sup>4</sup> cells/well, and incubated for 24 hr. Instead of using 96-well

plate, 24-well plates provided greater growth surface area for HT22 cells, which are known to required higher seeding densities for optimal growth and to minimize the edge effects associated with 96-well plates in our laboratory setting.

For cytotoxicity and protective assays, the cells were then treated with a series of concentrations of  $\text{H}_2\text{O}_2$ , water hyacinth extracts, and their combinations for 24 hr. After treatment, each well was replenished with 200  $\mu\text{L}$  of fresh media containing 0.5 mg/ml MTT reagent and incubated for 3 hours to allow the formation of formazan crystal. The media was gently removed and 200  $\mu\text{L}$  of DMSO was added in to each well to dissolve the crystal. Additional wells with cell-free MTT solution were included to serve as the background readings of the assay; all measurements were subtracted from the averaged background reading on every experiment. To account for the potential interference of plant extracts with MTT measurement, cell-free wells containing extracts at the tested concentrations were also included, and their absorbance values were subtracted from the corresponding treatment wells. The absorbance signals were measured at 570 nm with additional reference wavelength at 630 nm using the Tecan Infinite M200 pro microplate reader. The difference between measurement and reference measurement of each well was calculated, the determined measurements of duplicated groups were then averaged, divided by that of the control and expressed as percentage cell viability relative to control. The results were presented in bar charts which were generated using GraphPad Prism 8.

#### 2.4.1. $\text{H}_2\text{O}_2$ cytotoxicity screenings on HT22 cells

This screening was aimed to evaluate the cytotoxic effect of  $\text{H}_2\text{O}_2$  and identifying its half-maximal inhibitory concentration ( $\text{IC}_{50}$ ) value on HT22 cells. A stock solution of 0.1M  $\text{H}_2\text{O}_2$  was freshly prepared by diluting 30% (v/v)  $\text{H}_2\text{O}_2$  using autoclaved deionised water for every experiment. During the 24 h cell treatment, a range of concentrations (50 – 1000  $\mu\text{M}$ ) were diluted from the stock and tested on HT22 cells. The testing concentrations were optimized after few rounds of screenings through applying higher range of concentrations and further reducing the concentrations until a dose-dependent cell viability reduction trend was observed. Non-linear regression model based on (log(inhibitor) vs. normalized response - variable slope) was plotted using GraphPad Prism 8 to estimate the  $\text{IC}_{50}$  value for the selection of a  $\text{H}_2\text{O}_2$  optimal concentration, which was required for co-treatment group design.

#### 2.4.2. Water hyacinth extracts cytotoxicity screenings on HT22 cells

This screening was performed to determine the cytotoxic thresholds of water hyacinth extracts. The initial stock concentrations of three extracts were 20 mg/ml and diluted into testing concentrations of (0.5 to 5.0 mg/ml) for 24 h cytotoxicity screenings on HT22 cells to determine the optimal concentrations of extracts that are not affecting HT22 viability. After statistical analyses, three optimal concentrations were applied based on the selected optimal concentration from each extract as co-treatment candidates in the subsequent neuroprotective assay. Glycerol-only vehicle control was not included in the experiment as glycerol was used as the extraction solvent and treated to cells at the diluted concentrations (< 1% in final volume of desired concentrations).

#### 2.4.3. Co-treatment of water hyacinth extracts on H<sub>2</sub>O<sub>2</sub>-treated HT22 cells

This assay was performed to determine the protective effect of water hyacinths extracts against H<sub>2</sub>O<sub>2</sub>-induced cell death in HT22 cells. During the treatment, the three replicates of each extract were co-treated with 700 µM H<sub>2</sub>O<sub>2</sub> (selected concentration) for 24 h. Additional experiment controls for this assay include the untreated control (control) as baseline cell condition, and H<sub>2</sub>O<sub>2</sub> alone group (700 µM H<sub>2</sub>O<sub>2</sub>) as negative control to induce oxidative damage, Trolox (100 µM) as an antioxidant positive control, while the Trolox alone group was also included to show its baseline cytotoxicity effect under the experimental condition, and 0.1% (v/v) DMSO as solvent control (DMSO) because Trolox (100 µM) was prepared using pure DMSO to rule out the cytotoxic effect of DMSO. While co-treatment was adopted as initial screening to assess direct protection of *E. crassipes* extracts against oxidative injury, pre-treatment settings are set as the part of the future mechanistic investigations to assess the prophylactic effects of *E. crassipes* (see Discussion).

#### 2.5. Statistical Analysis

All cytotoxicity screenings and neuroprotective assay were in performed three independent experiments with two technical duplicates each ( $n = 3$  biological, 2 technical = 6 data points per condition). Data were presented as the mean  $\pm$  SEM. Potential outliers were determined and removed using the ROUT method with the coefficient Q set to 1%, while the normality test were performed using the Shapiro-Wilk tests. Statistical analyses were conducted using GraphPad Prism 8, using One-way ANOVA to assess the statistical differences between the means among treatment groups. Post-hoc Bonferroni's multiple comparison test was applied to further compare the statistical differences between the means of each treatment group, with  $p < 0.05$  considered as statistically significant.

### 3. Results

#### 3.1. Cytotoxic effect of H<sub>2</sub>O<sub>2</sub> on HT22 viability

To identify the cytotoxic effects and its IC<sub>50</sub> of H<sub>2</sub>O<sub>2</sub> on HT22 cells, cell viability measurement was conducted. Figure 2A shows a dose-dependent cytotoxic effect of H<sub>2</sub>O<sub>2</sub> on HT22 cells with a range of concentration of 50 to 1000 µM. HT22 cells treated with 50 µM H<sub>2</sub>O<sub>2</sub> showed no toxic effects with cell viability measured at  $102.2 \pm 5.2\%$ . However, HT22 cells treated with 600 µM H<sub>2</sub>O<sub>2</sub> exhibited significant cell viability reduction effect on HT22 cell viability ( $p < 0.05$ ), while the effects shown in the subsequent increasing concentrations (700–1000 µM) were also significant ( $p < 0.001$ ). In the dose-dependent log curve (Figure 2B), the R<sup>2</sup> value for the non-linear regression curve was about 0.91, while the estimated IC<sub>50</sub> value of H<sub>2</sub>O<sub>2</sub> at approximately 655.3 µM [95% CI: 613.7–697.0 µM]. Based on the actual experiment, 700 µM H<sub>2</sub>O<sub>2</sub> elicited the closest effect to the IC<sub>50</sub>, reducing the percentage cell viability to  $47.3 \pm 11.9\%$ . Therefore, 700 µM H<sub>2</sub>O<sub>2</sub> was selected as the optimal concentration to induce oxidative cell death in HT22 for the subsequent assay. However, experimental variability across independent experiments was observed in the subsequent co-treatment

assays, 700  $\mu\text{M}$   $\text{H}_2\text{O}_2$  alone group consistently displayed approximately 52–55% viability (Figure 3).

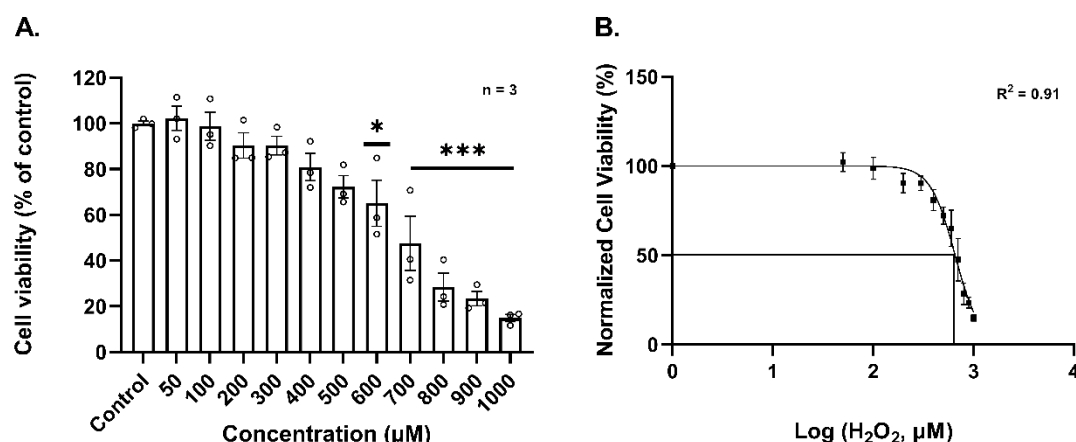


**Figure 2.** Cytotoxicity screening of  $\text{H}_2\text{O}_2$  on HT22 cells. (A) Percentage cell viability after 24 h treatments of  $\text{H}_2\text{O}_2$  (50–1000  $\mu\text{M}$ ) and its (B) dose-dependent non-linear regression log curve. \* $p < 0.05$  and \*\*\* $p < 0.001$  vs. control. Results are the mean  $\pm$  SEM of three independent experiments.

### 3.2. Cytotoxic Effect of Water Hyacinth Extracts on HT22 Viability

To evaluate the cytotoxic threshold and determine the non-toxic concentrations of water hyacinth extracts on HT22 cells, cytotoxic screenings of water hyacinth extracts were performed using a range of concentration from 0.5 to 5.0 mg/ml with an interval of 0.5 mg/ml for each treatment groups. In Figure 3A, GE demonstrated a significant reduction in cell viability in HT22 cells at concentration ranging from 1.5 mg/ml to 5.0 mg/ml as compared to control ( $p < 0.001$ ). The percentage cell viability decreased from  $85.1 \pm 1.9\%$  at 1.5 mg/ml to  $4.0 \pm 1.1\%$  at 5.0 mg/ml. Additionally, 1 mg/ml GE showed no toxic effects on HT22, with a recorded viability at  $104.8 \pm 2.9\%$ , as measured by the MTT assay. This slight increase in viability, though not statistically significant compared to the control, may be attributable to minor seeding inconsistency, MTT interference by phytochemicals, or a mild proliferative response. These possibilities were investigated further as the primary objective at this stage was to identify a safe concentration range for subsequent co-treatment assays. Therefore, three narrow concentrations including 0.8 mg/ml, 0.9 mg/ml, and 1.0 mg/ml were chosen as the optimal safe concentrations for further testing of their protective effects against  $\text{H}_2\text{O}_2$ -induced oxidative cell death.

In Figure 3B, WE significantly decreased HT22 viability starting from 2.5 mg/ml to 5 mg/ml as compared to control; the reduction in cell viability ranged from  $81.9 \pm 7.9\%$  to  $7.8 \pm 0.9\%$  ( $p < 0.001$ ). The HT22 cells viability was not affected by WE up to the concentration of 2 mg/ml, maintaining the cell viability at approximately  $97.11 \pm 3.0\%$ . Hence, three concentrations with shorter ranges which were 1.8 mg/ml, 1.9 mg/ml, and 2.0 mg/ml were selected for subsequent co-treatment group design. Cytotoxicity screening of WGE (Figure 3C) showed significant reduction in cell viability starting at the concentration of 1 mg/ml to 5 mg/ml. The reduction in percentage cell viability ranged from  $79.6 \pm 8.4\%$  to  $3.6 \pm 0.7\%$ . WGE at concentration of 0.5 mg/ml did not affect HT22 cell viability, while

the next tested concentration (1 mg/ml) displayed significant toxic effect on cell viability. Therefore, 50 mg/ml WGE was selected as the upper concentration limit for subsequent co-treatment assay, alongside 0.3 mg/ml and 0.4 mg/ml.

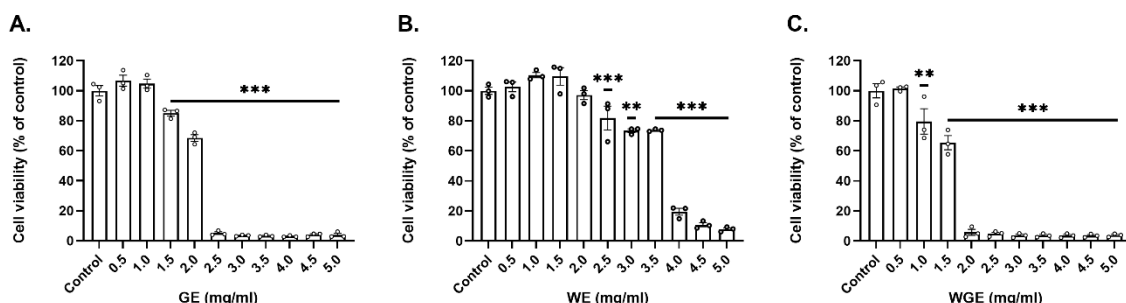


**Figure 3.** Cytotoxicity screening of water hyacinth extracts on HT22 cells. Percentage cell viability after 24 h treatments of (A) glycerol extract (GE), (B) water extract (WE), and (C) water-glycerol extract (WGE) with the testing concentration range of 0.01 mg/ml to 5.00 mg/ml. Estimated  $IC_{50}$  values based on non-linear regression model were 3.1 mg/ml (WE), 1.9 mg/ml (GE), and 1.5 mg/ml (WGE), respectively.  $**p < 0.01$  and  $***p < 0.001$  vs. control. Results are the mean  $\pm$  SEM of three independent experiments.

### 3.3. Protective Effect of Water Hyacinth Extracts Against $H_2O_2$ -Induced Oxidative Cell Death in HT22 Cells

To investigate whether different solvent type water hyacinth extracts confer neuroprotective effect against  $H_2O_2$ -induced oxidative cell death, the cell viability of HT22 were assessed after 24 h of co-treatment of three selected concentrations of water hyacinths extracts with 700  $\mu$ M  $H_2O_2$ . From the three independent co-treatment assays (Figure 4A–C), we demonstrated that Trolox alone and its solvent control (DMSO) groups did not significantly affect the HT22 cell viability as compared to the control, indicating that the concentrations of Trolox (100  $\mu$ M) and its solvent used (0.1% DMSO (v/v)) are fairly safe and independent to HT22 viability. The 700  $\mu$ M  $H_2O_2$  alone group from each assay significantly decreased HT22 cell viability as compared to control ( $p < 0.001$ ), keeping HT22 cell viability ( $52.4 \pm 1.4\%$ ,  $55.1 \pm 5.5\%$ , and  $52.7 \pm 4.4\%$ ). Additionally, the positive control group (Trolox with 700  $\mu$ M  $H_2O_2$ ) consistently displayed significant effect in all three co-treatment assays, by reducing the cell viability loss and keeping the percentage cell viability ( $78.7 \pm 3.0\%$ ,  $82.9 \pm 2.8\%$ ,  $84.0 \pm 4.0\%$ ).

In the co-treatment assay of GE (Figure 4A), the testing three concentrations displayed higher cell viability when co-treated 700  $\mu$ M  $H_2O_2$  than that of the 700  $\mu$ M  $H_2O_2$  alone group. Specifically, GE at 0.8 mg/ml significantly attenuated  $H_2O_2$ -treated viability loss demonstrated the highest viability as compared to the 700  $\mu$ M  $H_2O_2$  alone group ( $67.4 \pm 4.4\%$  vs.  $52.4 \pm 1.4\%$ ,  $p < 0.05$ ), representing a 28.6% relative increase over  $H_2O_2$  alone. On the other hand, WE co-treatment assay (Figure 4B) did not display significant protective effect against 700  $\mu$ M  $H_2O_2$ -treated cells, as the 1.8 mg/ml and 1.9 mg/ml of WE co-treatment groups exhibited slightly lower cell viability as compared to the 700  $\mu$ M  $H_2O_2$  alone group. However, WE at 2.0 mg/ml exhibited a non-significant trend toward increased viability as compared to the 700  $\mu$ M  $H_2O_2$  alone group ( $65.9 \pm 2.1\%$  vs.  $55.1 \pm 5.5\%$ ,  $p = 0.87$ ). Lastly, Figure 4C showed that WGE extracts reduced the cell viability loss when co-treated with 700

$\mu\text{M}$   $\text{H}_2\text{O}_2$  when compared to the  $\text{H}_2\text{O}_2$  alone group. However, significant difference was not identified during the statistical analysis, and the effects become more minimal in dose-dependent manner. WGE at 0.3 mg/ml displayed numerically higher in percentage of viability as compared  $\text{H}_2\text{O}_2$  alone group ( $71.2 \pm 4.9\%$  vs  $52.7 \pm 4.4\%$ ,  $p = 0.12$ ), which is statistically non-significant. Overall, the  $\text{H}_2\text{O}_2$  alone group showed consistency toxicity across three independent co-treatments (52.4–55.1% viability), supporting the reproducibility of the oxidative stress model. Last but not least, Table 1 was provided to present an overview of the key experimental findings.

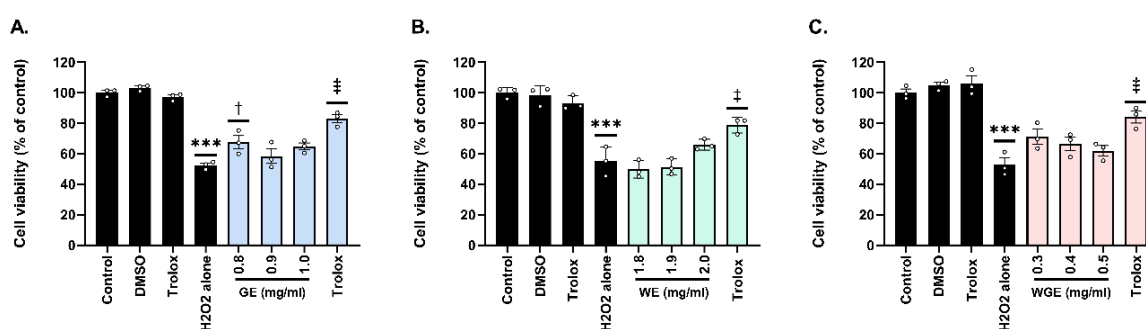


**Figure 4:** Effects of water hyacinth extracts against  $\text{H}_2\text{O}_2$ -induced oxidative stress in HT22 cells. Percentage cell viability after 24 h co-treatments of  $\text{H}_2\text{O}_2$  (700  $\mu\text{M}$ ) with (A) glycerol extract (GE) (0.80 – 1.00 mg/ml), (B) water extract (WE) (1.80 – 2.00 mg/ml), and (C) water-glycerol extract (WGE) (0.30 – 0.50 mg/ml), \*\*\* $p < 0.001$  vs. control. † $p < 0.05$ , ‡ $p < 0.01$  and □ $p < 0.001$  vs. 700  $\mu\text{M}$   $\text{H}_2\text{O}_2$  alone group. Results are the mean  $\pm$  SEM of three independent experiments.

**Table 1.** Summary of experimental results and statistical analysis of hydrogen peroxide and water hyacinth extract in HT22 cells.

| Assay        | Treatment                                       | IC <sub>50</sub> ( $\mu\text{M}$ ) | Selected Concentration ( $\mu\text{M}$ / mg/ml) | Selection rationale                                       | Key Outcome                                       | p-value                  |
|--------------|-------------------------------------------------|------------------------------------|-------------------------------------------------|-----------------------------------------------------------|---------------------------------------------------|--------------------------|
| Cytotoxicity | Hydrogen peroxide ( $\text{H}_2\text{O}_2$ )    | 655.3 $\mu\text{M}$                | 700 $\mu\text{M}$                               | Actual experiment displayed closest to IC <sub>50</sub> . | 600 $\mu\text{M}$ begins to reduce cell viability | $p < 0.05$ vs. control.  |
| Cytotoxicity | Water hyacinth glycerol extract (GE)            | -                                  | 0.8–1.0 mg/ml                                   | Safe dose (1.0 mg/ml)                                     | 1.5 mg/ml begins to reduce cell viability         | $p < 0.001$ vs. control. |
| Cytotoxicity | Water hyacinth water extract (WE)               | -                                  | 1.8–2.0 mg/ml                                   | Safe dose (2.0 mg/ml)                                     | 2.5 mg/ml begins to reduce cell viability         | $p < 0.001$ vs. control. |
| Cytotoxicity | Water hyacinth glycerol and water extract (WGE) | -                                  | 0.3–0.5 mg/ml                                   | Safe dose (0.5 mg/ml)                                     | 1.0 mg/ml begins to reduce cell viability         | $p < 0.01$ vs. control.  |

| Assay        | Treatment                    | Selected Concentration ( $\mu\text{M}$ / mg/ml)                 | Key Outcome                                                                                                                                                              | p-value                                            |
|--------------|------------------------------|-----------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|
| Co-treatment | GE + $\text{H}_2\text{O}_2$  | GE: 0.8–1.0 mg/ml + $\text{H}_2\text{O}_2$ : 700 $\mu\text{M}$  | 0.8 mg/ml GE + 700 $\mu\text{M}$ $\text{H}_2\text{O}_2$ significantly increased cell viability.<br><br>(67.4 $\pm$ 4.4% vs. 52.4 $\pm$ 1.4%,)                            | $p < 0.05$ vs. $\text{H}_2\text{O}_2$ alone group. |
| Co-treatment | WE + $\text{H}_2\text{O}_2$  | WE: 1.8–2.0 mg/ml + $\text{H}_2\text{O}_2$ : 700 $\mu\text{M}$  | 2.0 mg/ml WE + 700 $\mu\text{M}$ $\text{H}_2\text{O}_2$ displayed numerically higher in cell viability but not significant.<br><br>(65.9 $\pm$ 2.1% vs. 55.1 $\pm$ 5.5%) | $p = 0.87$ vs. $\text{H}_2\text{O}_2$ alone group. |
| Co-treatment | WGE + $\text{H}_2\text{O}_2$ | WGE: 0.3–0.5 mg/ml + $\text{H}_2\text{O}_2$ : 700 $\mu\text{M}$ | 0.3 mg/ml WE + 700 $\mu\text{M}$ $\text{H}_2\text{O}_2$ displayed numerically higher in cell viability but not significant.<br><br>(71.2 $\pm$ 4.9% vs 52.7 $\pm$ 4.4%)  | $p = 0.12$ vs. $\text{H}_2\text{O}_2$ alone group. |

#### 4. Discussion

To summarize the findings of the present study, we demonstrated that hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) significantly induced cell death in HT22 mouse hippocampal cells. The estimated  $\text{IC}_{50}$  values ranged from 500  $\mu\text{M}$  to 750  $\mu\text{M}$ , which also aligns with other findings using 6-well format and high seeding number ( $\text{IC}_{50}$ :  $\sim$  600 - 700  $\mu\text{M}$ )<sup>[58, 59]</sup>. Trolox, a water-soluble vitamin E analogue commonly used as a standard reference in oxidative stress models. In this study, Trolox was used at 100  $\mu\text{M}$  which is slightly higher than the 50  $\mu\text{M}$  in comparable studies<sup>[60, 61]</sup>. The use of 100  $\mu\text{M}$  was nonetheless justified, as Trolox vehicle control showed no significant effect on cell viability, while it significantly attenuated  $\text{H}_2\text{O}_2$ -induced cell viability loss across three independent co-treatments (78.7  $\pm$  3.0%, 82.9  $\pm$  2.8%, and 84.0  $\pm$  4.0%, respectively). We also revealed neuroprotective potential of water hyacinth extract in  $\text{H}_2\text{O}_2$ -treated HT22 cells. Specifically, the water hyacinth glycerol extract (GE) displayed significant protection in reducing cell viability loss by oxidative damage at a concentration of 0.8 mg/ml. While further study is warranted, these results suggest that the GE possesses unique bioactive compounds which may confer antioxidant-mediated neuroprotection, supporting the therapeutic relevance of *E. crassipes* as an underexplored medicinal plant.

Based on the co-treatment result, only GE at 0.8 mg/ml significantly alleviated cell viability of  $\text{H}_2\text{O}_2$ -treated HT22 cells (67.4  $\pm$  4.4% vs. 52.4  $\pm$  1.4%), while 0.9 mg/ml and 1.0 mg/ml did not show significant protection. This pattern is consistent with hormesis, a biphasic dose-response phenomenon commonly reported in plant extract studies, where low concentrations elicit positive effects while higher concentrations may instead induce cytotoxicity, especially in cells already subjected to oxidative stress. In this context, 0.9 mg/ml and 1.0 mg/ml may represent concentrations at which cytotoxic effects begin to offset the antioxidant benefit of GE. However, this interpretation requires confirmation through mechanism-based analyses.

The selective protective effect found in GE, but not in WE or WGE, is likely attributable to the uniqueness of their phytochemical compositions driven by different solvent polarity. Glycerol's moderate polarity facilitates phytochemical extraction profile, covering lipophilic compounds that are not readily extractable using water alone <sup>[62]</sup>. Phytochemical characterization of these extracts is currently underway and will be reported in detail separately. However, existing studies have reported that *E. crassipes* contain diverse classes of phytochemicals originating from different plant parts and solvent extraction, however, glycerol extraction was rarely observed <sup>[33]</sup>. Despite that, phenolic acids, flavonoids and terpenoids in *E. crassipes* are well-known free radical scavengers that display protective effect in H<sub>2</sub>O<sub>2</sub>-induced oxidative damage across neuronal cell models. It is therefore plausible that GE uniquely contains one or more of these compounds' classes, bolstering the observed protective effect.

The diversity of bioactive compounds reported in *E. crassipes* suggested that its neuroprotective mechanisms against oxidative damage in cells likely relies on antioxidant-mediated attenuation. Phenolic compounds and flavonoids are well known free radical scavengers which act as direct neutralizers of reactive oxygen species (ROS) and transition metal chelators <sup>[63]</sup>. On top of that, certain phenolic acids and flavonoids also exhibit indirect antioxidant effects *via* activation of the Nrf2/Keap1 signaling pathway, a central regulator of antioxidant defense <sup>[64]</sup>. Key enzymes such as heme oxygenase-1 (HO-1), superoxide dismutase (SOD), and glutathione peroxidase (GPx) are upregulated during the activation to enhance cellular oxidative stress metabolism <sup>[65]</sup>. This pathway has been widely studied as a key mechanism of neuroprotection in H<sub>2</sub>O<sub>2</sub>-treated HT22 cells in numerous plant extract studies <sup>[66–68]</sup>. Additionally, the lipophilic antioxidants reported in *E. crassipes* such as terpenoids which contribute mitochondrial membrane stabilization *via* inhibiting lipid peroxidation <sup>[69]</sup>. Beyond direct antioxidant activity, bioactive compounds such as phenolics and flavonoids have been shown to modulate autophagy-associated signaling pathways including AMPK, mTOR, and AKT signaling, which represent an alternative neuroprotective mechanism warranting investigation in context of GE <sup>[10, 70]</sup>. Hence, GE is likely to carry these proposed neuroprotective mechanisms above due to its capability in extracting broad range of antioxidants. The precise mechanistic contribution of each compound class within GE remains to be investigated.

Similar neuroprotective effects have been reported in various findings from other plant extracts using the same *in vitro* oxidative model. For instance, the ethanolic extract of *Chrysanthemum indicum* has elicited significant alleviation in H<sub>2</sub>O<sub>2</sub>-induced cell death in HT22 cells (200 µg/mL exhibit ~ 90% viability and ~120% LDH leakage vs. 500 µM H<sub>2</sub>O<sub>2</sub>) and suppressed ROS production *via* activation of Akt/Nrf-2 signaling pathway <sup>[66]</sup>. Additionally, *Euonymus alatus* ethanol extract demonstrated similar neuroprotective activities through reducing H<sub>2</sub>O<sub>2</sub>-induced cell death (100 µg/mL exhibit ~ 95% viability and ~ 2-fold apoptotic factors suppression) through activating the Nrf2/HO-1 pathway <sup>[59]</sup>. From the plant extracts mentioned, similar effects may be involved in GE *via* direct ROS scavenging and/or activation of endogenous antioxidant pathways such as Nrf2 in HT22 cells, though this remains to be confirmed. Nevertheless, direct comparisons across findings

are limited due to culture settings differences (differences in culture conditions, seeding density and concentration of H<sub>2</sub>O<sub>2</sub> used). Notably, the neuroprotective activities of glycerol-based extracts remain underexplored in neuronal oxidative models compared to common hydrophilic solvent extracts. This study thereby represents one of the early investigations into the protective potential of glycerol-based plant extract in HT22 cells, highlighting glycerol as a promising green solvent for bioactive compound extraction with translational relevance in both nutraceutical and pharmaceutical applications.

Several limitations of study should be acknowledged. First, the protective effects were solely evaluated in an *in vitro* cell-based model. Although HT22 cells are a well-established model for studying oxidative stress-induced neuronal death, findings based on a single cell type may not fully recapitulate the *in vivo* neuronal environments. Second, the yield of GE and WGE could not be determined on a dry weight basis due to the high viscosity and hygroscopic nature of glycerol whereby conventional drying methods (rotary evaporation and freeze drying) are not feasible to achieve. Hence, this limits direct comparison of extraction efficiency between the three extracts. Third, the lack of glycerol itself as vehicle control may leave the residual vehicle effects (cytoprotective or cytotoxic contributions) uncharacterized. Fourth, while Trolox itself is a notable antioxidant and being employed as the positive control in this study, inclusion of reference neuroprotective antioxidant compounds such as curcumin and quercetin would further contextualize GE's potency. Fifth, the use of 24-well plates rather than the conventional 96-well cell culture format was necessitated by practical considerations in our laboratory setup (higher seeding density for testing and labour-efficiency) without compromising data validity. Sixth, although MTT assay was commonly applied for cell viability measurement due to its convenient protocol, it is susceptible to interference from phenolic-rich or colored extracts [71]. Therefore, the absorbance readings may be artificially altered and potentially introduce false positive data. Seventh, the co-treatment setting employed in the present study allow the assessment on the extract's capability to counteract acute oxidative damage in cells. Despite that, the design does not reflect the full spectrum of clinical relevance whereby pre-treatment would be a better model to study a neuropreventive context, mimicking antioxidant supplementation prior to neuronal injury occurs. Lastly, the absence of well-established neuroprotective reference compounds such as curcumin or quercetin as positive comparators in the co-treatment assay may hinder the contextualization of GE's protective potency.

Future studies should focus on mechanistic validations including lactate dehydrogenase (LDH) release to validate cell survivability, intracellular ROS measurement, cell apoptosis determination, and Nrf2 nuclear translocation and activation of its downstream antioxidant enzymes. Compounds isolation of GE through bioassay-guided fractionation alongside comparative neuroprotective studies involving established antioxidant compounds (curcumin and quercetin) or isolated compounds of GE using the same model are warranted to further contextualize protective potency of GE. Moreover, incorporation of *in vivo* oxidative stress animal model testing should be assessed to study the pharmacokinetics, bioavailability, and CNS penetration of the bioactive compounds of GE. The phytochemical characterization of GE using GC-MS which will be reported in the companion study,

provides the foundation for compound identification and isolation which allows the application of molecular docking and *in silico* pharmacokinetic studies. Overall, the findings of the present study provide preliminary evidence that *E. crassipes* glycerol extract (GE) possesses the neuroprotective potential against oxidative stress-induced neuronal death, positioning this invasive aquatic plant as a worthy candidate for further investigation in the context of antioxidant-based neuropharmacology.

## 5. Conclusions

In conclusion, this study has shown the *E. crassipes* glycerol extract (GE) significantly attenuated H<sub>2</sub>O<sub>2</sub>-induced cell viability loss in HT22 mouse hippocampal cells at a concentration of 0.8 mg/ml, supporting its neuroprotective potential against oxidative stress conditions. Water and water-glycerol mixed extracts exhibited protective trends that did not reach statistical significance, and thus their neuroprotective relevance remains to be established. This study highlights GE as a promising candidate for further investigation, including identification of phytochemical profile, exploration of underlying antioxidant mechanisms, and evaluation of pre- and post-treatment models to better contextualize its neuroprotective potency. Optimization of the glycerol based extraction to enhance the bioactive constituents is also warranted in future studies. Collectively, these findings underline *E. crassipes* as an underexplored medicinal plant with potential application in antioxidant-based neuropharmacology. Not to mention, *E. crassipes* remains its notorious status as an invasive, therefore, its valorization as a source of neuroprotective bioactive compounds could simultaneously support ecological management effort and contribute to the development of botanical-based therapeutics.

**Author Contributions:** Conceptualization, CFL, ADIA, MKA, LTG; methodology, CFL, ADIA, MKA, LTG; validation, CFL, ADIA, and HLS; supervision, ADIA and LTG; resources, ADIA, MKA, LTG; writing—original draft preparation, CFL; writing—review and editing, CFL, ADIA, HLS, LCM, MKA, LTG.

**Funding:** This research was funded by Sunway University ASEAN Strategic Grant (Grant no.: GRTIN-ASGS-SET-01-2023) awarded by Sunway University, Malaysia.

**Acknowledgments:** This article is based upon work supported by King Fahd University of Petroleum & Minerals, and the author at KFUPM acknowledges the support.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Sies H. Oxidative stress: a concept in redox biology and medicine. *Redox Biology* 2015; 4: 180-3. doi: 10.1016/j.redox.2015.01.002.
2. Valko M, Leibfritz D, Moncol J, *et al.* Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology* 2007; 39(1): 44-84. doi: 10.1016/j.biocel.2006.07.001.
3. Liguori I, Russo G, Curcio F, *et al.* Oxidative stress, aging, and diseases. *Clinical Interventions in Aging* 2018; 13: 757-772. doi: 10.2147/CIA.S158513.
4. Barnham KJ, Masters CL, and Bush AI. Neurodegenerative diseases and oxidative stress. *Nature Reviews Drug Discovery* 2004; 3(3): 205-214. doi: 10.1038/nrd1330.

5. Uttara B, Singh AV, Zamboni P, *et al.* Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacology* 2009; 7(1): 65-74. doi: 10.2174/157015909787602823.
6. Sayre LM, Perry G, and Smith MA. Oxidative stress and neurotoxicity. *Chemical Research in Toxicology* 2008; 21(1): 172-88. doi: 10.1021/tx700210j.
7. Halliwell B. Oxidative stress and neurodegeneration: where are we now? *Journal of Neurochemistry* 2006; 97(6): 1634-58. doi: 10.1111/j.1471-4159.2006.03907.x.
8. Trushina E and McMurray CT. Oxidative stress and mitochondrial dysfunction in neurodegenerative diseases. *Neuroscience* 2007; 145(4): 1233-1248. doi: 10.1016/j.neuroscience.2006.10.056.
9. Lin MT and Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* 2006; 443(7113): 787-795. doi: 10.1038/nature05292.
10. Leong C, Ming LC, Aroua M, *et al.* Therapeutic and Pharmacological Role of Natural Products in Neurological Diseases: Targeting Autophagy Pathways. *Progress In Microbes & Molecular Biology* 2025; 8. doi: 10.36877/pmmb.a0000463.
11. Radi E, Formichi P, Battisti C, *et al.* Apoptosis and oxidative stress in neurodegenerative diseases. *Journal of Alzheimer's disease* 2014; 42 Suppl 3: S125-S152. doi: 10.3233/JAD-132738.
12. Teleanu DM, Niculescu AG, Lungu II, *et al.* An Overview of Oxidative Stress, Neuroinflammation, and Neurodegenerative Diseases. *International journal of molecular sciences* 2022; 23(11): 5938. doi: 10.3390/ijms23115938.
13. Talebi M, Mohammadi Vadoud SA, Haratian A, *et al.* The interplay between oxidative stress and autophagy: focus on the development of neurological diseases. *Behavioral and brain functions* 2022; 18(1): 3. doi: 10.1186/s12993-022-00187-3.
14. Buendia I, Michalska P, Navarro E, *et al.* Nrf2–ARE pathway: An emerging target against oxidative stress and neuroinflammation in neurodegenerative diseases. *Pharmacology & Therapeutics* 2016; 157: 84-104. doi: <https://doi.org/10.1016/j.pharmthera.2015.11.003>.
15. Paloczi J, Varga ZV, Hasko G, *et al.* Neuroprotection in Oxidative Stress-Related Neurodegenerative Diseases: Role of Endocannabinoid System Modulation. *Antioxidants & Redox Signaling* 2018; 29(1): 75-108. doi: 10.1089/ars.2017.7144.
16. Golde TE, Miller VM, Kokjohn TA, *et al.* Common features of neurodegenerative diseases. *Neurology* 2013; 81(2): 134-141. doi: 10.1212/WNL.0b013e3182988fcd.
17. Dugger BN and Dickson DW. Pathology of Neurodegenerative Diseases. *Cold Spring Harbor Perspectives in Biology* 2017; 9(7). doi: 10.1101/cshperspect.a028035.
18. Dias V, Junn E, and Mouradian MM. The role of oxidative stress in Parkinson's disease. *Journal of Parkinson's Disease* 2013; 3(4): 461-91. doi: 10.3233/JPD-130230.
19. Wang YJ, Wang LY, and Xu ZS. Targeting oxidative stress in neurodegenerative diseases: Current strategies and future directions. *Frontiers in Neuroscience* 2019; 13(428). doi: 10.3389/fnins.2019.00428.
20. Birben E, Sahiner UM, Sackesen C, *et al.* Oxidative stress and antioxidant defense. *World Allergy Organ J* 2012; 5(1): 9-19. doi: 10.1097/WOX.0b013e3182439613.
21. Spencer JP. Flavonoids: modulators of brain function? *British Journal of Nutrition* 2008; 99 E Suppl 1(E Suppl 1): ES60-77. doi: 10.1017/S0007114508965776.

22. Mandel S and Youdim MB. Catechin polyphenols: neurodegeneration and neuroprotection in neurodegenerative diseases. *Free Radical Biology and Medicine* 2004; 37(3): 304-17. doi: 10.1016/j.freeradbiomed.2004.04.012.
23. Ramezani M and Hajialyani M. The toxicology of *Eichhornia crassipes* and its potential as a resource for pharmaceuticals. *Environmental Toxicology and Pharmacology* 2017; 53: 143-149. doi: 10.1016/j.etap.2017.06.002.
24. Liao H and Chou C. Recent advances in the use of *Eichhornia crassipes* in phytoremediation and environmental management. *Environmental Science and Pollution Research* 2018; 25: 3479-3493. doi: 10.1007/s11356-018-1763-7.
25. Madsen JD and Boylen CW. The biology of water hyacinth (*Eichhornia crassipes*) and its impact on aquatic ecosystems. *Hydrobiologia* 1989; 269: 145-156. doi: 10.1007/BF00012558.
26. Zhang Y and Li J. Invasive species and their impact on biodiversity: A case study of water hyacinth. *Biological Invasions* 2020; 22: 1775-1789. doi: 10.1007/s10530-020-02331-9.
27. Bhagwat SA and Radhakrishna S. Invasive species and their role in ecological and economic damage: Focus on water hyacinth. *Ecological Applications* 2017; 27: 2081-2089. doi: 10.1002/eap.1485.
28. Yang G and Liu W. Water hyacinth as an invasive species in freshwater ecosystems and management strategies. *Aquatic Botany* 2019; 132: 11-22. doi: 10.1016/j.aquabot.2016.08.003.
29. Basak S and Chatterjee S. Therapeutic uses of bioactive compounds from *Eichhornia crassipes* (Mart.) in modern medicine. *Natural Product Research* 2020; 34: 2205-2212. doi: 10.1080/14786419.2019.1678832.
30. Ghosh S and Das S. Phytochemical analysis and pharmacological properties of water hyacinth: A potential therapeutic plant. *Journal of Ethnopharmacology* 2021; 260: 113096. doi: 10.1016/j.jep.2020.113096.
31. Rajeshkumar S and Naik P. Pharmacological and therapeutic potential of water hyacinth: A review. *Pharmacognosy Journal* 2017; 9: 115-121. doi: 10.5530/pj.2017.4.21.
32. Ahmed M and Alam S. A review on the pharmacological effects of water hyacinth and its phytochemicals. *Pharmacognosy Reviews* 2022; 16: 30-38. doi: 10.4103/PR.16.4.249.
33. Ben Bakrim W, Ezzariai A, Karouach F, *et al.* *Eichhornia crassipes* (Mart.) Solms: A Comprehensive Review of Its Chemical Composition, Traditional Use, and Value-Added Products. *Frontiers in Pharmacology* 2022; 13: 842511. doi: 10.3389/fphar.2022.842511.
34. Eden WT, Wahyuono S, Cahyono E, *et al.* Phytochemical, Antioxidant, and Cytotoxic Activity of Water Hyacinth (*Eichhornia crassipes*) Ethanol Extract. *Tropical Journal of Natural Product Research* 2023; 7(8): 3606-3612. doi: 10.26538/tjnpr/v7i8.5.
35. Kumar S, Kumar R, Dwivedi A, *et al.* In vitro antioxidant, antibacterial, and cytotoxic activity and in vivo effect of *Syngonium podophyllum* and *Eichhornia crassipes* leaf extracts on isoniazid induced oxidative stress and hepatic markers. *BioMed Research International* 2014; 2014. doi: 10.1155/2014/459452.
36. Andrade HM, Oliveira JA, Farnese FS, *et al.* Arsenic toxicity: cell signalling and the attenuating effect of nitric oxide in *Eichhornia crassipes*. *Biologia Plantarum* 2016; 60(1): 173-180. doi: 10.1007/s10535-015-0572-4.
37. Bodo R, Azzouz A, and Hausler R. Antioxidative Activity of Water Hyacinth Components. *Plant Science* 2004; 166(4): 893-899. doi: 10.1016/j.plantsci.2003.12.001.

38. Aboul-Enein AM, Shanab SM, Shalaby EA, *et al.* Cytotoxic and Antioxidant Properties of Active Principals Isolated from Water Hyacinth against Four Cancer Cells Lines. *BMC Complementary and Alternative Medicine* 2014; 14. doi: 10.1186/1472-6882-14-397.
39. Shanab SM, Shalaby EA, Lightfoot DA, *et al.* Allelopathic Effects of Water Hyacinth [*Eichhornia crassipes*]. *PLoS ONE* 2010; 5(10). doi: 10.1371/journal.pone.0013200.
40. Hamid HH, Ghaima KK, and Najem AM. Phytochemical, Antioxidant and Antibacterial Activities of Some Extracts of Water Hyacinth (*Eichhornia crassipes*) Leaves. *International Journal of Advanced Pharmaceutical Research* 2013; 4: 1847-1851.
41. Islam S. In Vitro Evaluation of Thrombolytic and Antioxidant Scavenging Activity of *Eichhornia crassipes*. *SEU Journal of Engineering Science* 2018; 12(2): 1.
42. Nugriani NO, Diah IWD, and Yusri S. *Antioxidant Stability Testing on Liquid and Powder Eichhornia crassipes Extract*. 2020.
43. Jayanthi P, Lalitha P, Sujitha R, *et al.* Anti-inflammatory Activity of the Various Solvent Extracts of *Eichhornia crassipes* (Mart.) Solms. *International Journal of PharmTech Research* 2013; 5(2): 641-645.
44. Sunitha P, Apparao S, Sandhya RM, *et al.* Evaluation of Antibacterial, Anti-inflammatory and Antioxidant Activities of Methanolic Extract of Whole Plant of *Eichhornia crassipes*. *International Journal of Pharmaceutical Sciences and Research* 2018; 48(1): 37-42.
45. Kumar A and Yadav P. Antioxidant and neuroprotective potential of water hyacinth: Implications for neurodegenerative diseases. *Neurochemistry International* 2020; 121: 131-142. doi: 10.1016/j.neuint.2018.12.004.
46. Farheen M, Arman S, Hussaini R, *et al.* Phytochemical Evaluation and Pharmacological Screening of Ethanolic Leaf Extracts of *Eichhornia crassipes* and *Nelumbo nucifera* for Neuropharmacological Activity in Psychoneurosis Induced Mice. *World Journal of Pharmacy and Pharmaceutical Sciences* 2015; 4(12): 874-904.
47. Bhavsar V, Vaghasiya J, Suhagia BN, *et al.* Protective Effect of *Eichhornia Crassipes* Against Cerebral Ischemia Reperfusion Injury in Normal and Diabetic rats. *Journal of Stroke and Cerebrovascular Diseases* 2020; 29(12). doi: 10.1016/j.jstrokecerebrovasdis.2020.105385.
48. Ransy C, Vaz C, Lombès A, *et al.* Use of H<sub>2</sub>O<sub>2</sub> to Cause Oxidative Stress, the Catalase Issue. *International journal of molecular sciences* 2020; 21(23): 9149. doi: 10.3390/ijms21239149.
49. Othman SB and Yabe T. USE OF HYDROGEN PEROXIDE AND PEROXYL RADICALS TO INDUCE OXIDATIVE STRESS IN NEURONAL CELLS. *Reviews in Agricultural Science* 2015; 3: 40-45. doi: 10.7831/ras.3.40.
50. Chen L, Xu B, Liu L, *et al.* Hydrogen peroxide inhibits mTOR signaling by activation of AMPK $\alpha$  leading to apoptosis of neuronal cells. *Laboratory Investigation* 2010; 90(5): 762-773. doi: 10.1038/labinvest.2010.36.
51. Ismail N, Ismail M, Azmi NH, *et al.* Modulation of Hydrogen Peroxide-Induced Oxidative Stress in Human Neuronal Cells by Thymoquinone-Rich Fraction and Thymoquinone via Transcriptomic Regulation of Antioxidant and Apoptotic Signaling Genes. *Oxidative Medicine and Cellular Longevity* 2016; 2016(1): 2528935. doi: <https://doi.org/10.1155/2016/2528935>.

52. Pfeiffer A, Jaeckel M, Lewerenz J, *et al.* Mitochondrial function and energy metabolism in neuronal HT22 cells resistant to oxidative stress. *British Journal of Pharmacology* 2014; 171(8): 2147-58. doi: 10.1111/bph.12549.
53. Song Q, Gou W-L, and Zhang R. FAM3A Protects HT22 Cells Against Hydrogen Peroxide-Induced Oxidative Stress Through Activation of PI3K/Akt but not MEK/ERK Pathway. *Cellular Physiology and Biochemistry* 2015; 37(4): 1431-1441. doi: 10.1159/000438512.
54. Mytych J, Zebrowski J, Lewinska A, *et al.* Prolonged Effects of Silver Nanoparticles on p53/p21 Pathway-Mediated Proliferation, DNA Damage Response, and Methylation Parameters in HT22 Hippocampal Neuronal Cells. *Molecular Neurobiology* 2017; 54(2): 1285-1300. doi: 10.1007/s12035-016-9688-6.
55. Lim J, Bang Y, Kim KM, *et al.* Differentiated HT22 cells as a novel model for in vitro screening of serotonin reuptake inhibitors. *Frontiers in Pharmacology* 2023; 13: 1062650. doi: 10.3389/fphar.2022.1062650.
56. Riss TL, Moravec RA, and Niles AL. *Cell Viability Assays*, in *Assay Guidance Manual*, S. Markossian, A. Grossman, and M. Arkin, Editors. 2013, Eli Lilly & Company and the National Center for Advancing Translational Sciences: Bethesda (MD).
57. Ser HL, Tan LT, Palanisamy UD, *et al.* *Streptomyces antioxidans* sp. nov., a Novel Mangrove Soil Actinobacterium with Antioxidative and Neuroprotective Potentials. *Frontiers in Microbiology* 2016; 7: 899. doi: 10.3389/fmicb.2016.00899.
58. Wu X, Luo P, Rao W, *et al.* Homer1a Attenuates Hydrogen Peroxide-Induced Oxidative Damage in HT-22 Cells through AMPK-Dependent Autophagy. *Frontiers in Neuroscience* 2018; 12: 51. doi: 10.3389/fnins.2018.00051.
59. You YL, Byun HJ, Lee JS, *et al.* Euonymus alatus and its compounds suppress hydrogen peroxide-induced oxidative stress in HT22 cells. *Food Science and Biotechnology* 2024; 33(3): 3567-3577. doi: 10.1007/s10068-024-01601-4.
60. Zou Y, Fan F, Fang Y, *et al.* Neuroprotective Effect of Alkylresorcinols from Wheat Bran in HT22 Cells: Correlation with in vitro Antioxidant Activity. *eFood* 2021; 2(1): 13-20. doi: 10.2991/efood.k.210125.001.
61. Zhang W, Guo Y, Cheng Y, *et al.* Ultrasonic-assisted enzymatic extraction of Sparassis crispa polysaccharides possessing protective ability against H<sub>2</sub>O<sub>2</sub>-induced oxidative damage in mouse hippocampal HT22 cells. *RSC Advances* 2020; 10(37): 22164-22175. doi: 10.1039/d0ra01581d.
62. de Nazaré de Oliveira A, Melchiorre M, Farias da Costa AA, *et al.* Glycerol: A green solvent for synthetic chemistry. *Sustainable Chemistry and Pharmacy* 2024; 41: 101656. doi: <https://doi.org/10.1016/j.scp.2024.101656>.
63. Platzer M, Kiese S, Tybussek T, *et al.* Radical Scavenging Mechanisms of Phenolic Compounds: A Quantitative Structure-Property Relationship (QSPR) Study. *Frontiers in Nutrition* 2022; Volume 9 - 2022. doi: 10.3389/fnut.2022.882458.
64. T LS, Rupasinghe HPV, Dellaire G, *et al.* Regulation of Nrf2/ARE Pathway by Dietary Flavonoids: A Friend or Foe for Cancer Management? *Antioxidants (Basel)* 2020; 9(10). doi: 10.3390/antiox9100973.

65. Vischer HF, Siderius M, Leurs R, *et al.* Herpesvirus-encoded GPCRs: neglected players in inflammatory and proliferative diseases? *Nature Reviews Drug Discovery* 2014; 13(2): 123-139. doi: 10.1038/nrd4189.
66. Jeong YH, Kim TI, Oh YC, *et al.* Chrysanthemum indicum Prevents Hydrogen Peroxide-Induced Neurotoxicity by Activating the TrkB/Akt Signaling Pathway in Hippocampal Neuronal Cells. *Nutrients* 2021; 13(11). doi: 10.3390/nu13113690.
67. Jia X, Liu Y, Li X, *et al.* Norcepharadione B attenuates H<sub>2</sub>O<sub>2</sub>-induced neuronal injury by upregulating cellular antioxidants and inhibiting volume-sensitive Cl<sup>-</sup> channel. *Experimental Biology and Medicine (Maywood)* 2019; 244(16): 1463-1474. doi: 10.1177/1535370219881358.
68. Byun E-B, Park W-Y, Kim WS, *et al.* Neuroprotective effect of Capsicum annuum var. abbreviatum against hydrogen peroxide-induced oxidative stress in HT22 hippocampus cells. *Bioscience, Biotechnology, and Biochemistry* 2018; 82(12): 2149-2157. doi: 10.1080/09168451.2018.1514248.
69. Baccouri B and Imen R. *Potential Antioxidant Activity of Terpenes*, in *Terpenes and Terpenoids - Recent Advances*, S. Perveen and A.M. Al-Taweel, Editors. 2021, IntechOpen: London. doi: 10.5772/intechopen.96638.
70. Fauzi A, Leong C, Ming LC, *et al.* Effects of Bioactive Compounds on Autophagy: A Systematic Review. *Progress in Microbes and Molecular Biology* 2025; 8.
71. Ghasemi M, Turnbull T, Sebastian S, *et al.* The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. *International journal of molecular sciences* 2021; 22(23). doi: 10.3390/ijms222312827.



Author(s) shall retain the copyright of their work and grant the Journal/Publisher right for the first publication with the work simultaneously licensed under:

Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0). This license allows for the copying, distribution and transmission of the work, provided the correct attribution of the original creator is stated. Adaptation and remixing are also permitted.