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NEUROPROTECTIVE EFFECTS OF DUNALIELLA SALINA EXTRACT IN SH-SY5Y CELL MODEL ARE EXAMINED.

Alok Kumar Yadav
Research Scholar, Sunrise University, Alwar, Rajasthan

Dr. K Saravanan
Research Supervisor, Sunrise University, Alwar, Rajasthan

ABSTRACT

Plant extracts are now being sought as a means of preventing neurodegenerative diseases around the globe. Alpinia galanga leaves, Alpinia galanga rhizomes, Vinifera seeds, Moringa oleifera leaves, Panax ginseng leaves, and Panax ginseng rhizomes ethanolic extracts were tested on human neuroblastoma (SHSY5Y) cells to determine their neuroprotective effects. VSE and MLE had DPPH radical scavenging rates of 81 percent and 58 percent, respectively, according to the results of this study. Ferric-reducing antioxidant powers were greater in ALE and MLE (33.57% and 26.76%, dry weight, respectively) than in any of the other extracts tested. Ferric-reducing antioxidant powers LC-QTOF/MS was used to identify MLE active chemicals in the liquid chromatography (LC). Researchers found that MLE and VSE exhibited substantial levels of oxygen scavenging in intracellular studies using the nitro-blue tetrazolium (NBT) test (0.83 0.09 mg/mL vs. According to the 2,7-dichlorodihydrofluorescein diacetate (DCFHDA) test, MLE scavenged the most ROS, followed by PRE (0.71 0.08 vs. 0.83 0.08 mg/mL, respectively). On SHSY5Y, the MTT cytotoxicity and neuroprotection assays indicated that PRE had a superior neuroprotective impact than MLE (viable cells 51% vs. 44%, IC50 1.92 0.04 mg/mL, respectively), whereas MLE was more hazardous (IC50 1.92 0.04 mg/mL). Among the plants investigated, MLE seems to have the most promising potential as a neuroprotective agent.

Keywords: Moringa oleifera; ROS; oxidative stress; superoxide anion; SHSY5Y neuroblastoma cell line

Introduction

The control of transcription, the distribution of proteins and metabolites, the metabolism of energy, the activation of cell death, and the folding and misfolding of proteins are all dependent on lipids. In many situations, we lack fundamental information of the cell lipid composition and how it changes as a result of the subcellular compartment or cell state. This insufficiency is in part due to the wide variety of lipid species that are found in the cell. The phospholipid class alone has hundreds of distinct lipid species due to the varying lengths and saturation levels of headgroups and fatty acid chains (FA). To make matters worse, reliable quantitative information is also desired.

For the neuroblastoma cell line SH-SY5Y, there is a lipidomic knowledge gap. For the first time, a human-derived cell line has been shown to be capable of differentiating into a neuron, and it is catecholaminergic. SH-SY5Y is the cell model of choice for research into neurodegenerative illnesses including Alzheimer's, amyotrophic lateral

sclerosis, and Parkinson's disease, in particular. Because of these characteristics (PD). The link between Parkinson's disease and lipids is especially robust. Presynaptic terminals in the brain are assumed to be controlled by the protein -synuclein, a crucial player in the disease, since it contacts the lipid membrane in a precise and reversible manner. Another factor that impacts its oligomerization is its misfolding, which is controlled by certain lipids and membrane properties. Disconcertingly, the SH-SY5Y cell type, which is one of the most important cell models in the study of Parkinson's disease (PD), has not yet been characterized in depth with regard to its lipid content.

LITERATURE REVIEW

Ana Luiza Sereia (2019) Alzheimer's disease (AD) is the most prevalent kind of dementia and there is currently no treatment for it. Antioxidative stress reduction, amyloid-beta regulation, and the prevention of tau protein hyperphosphorylation are needed to prevent the formation and progression of Alzheimer's disease (AD). The study's goal was to screen crude extracts (CEs) and ethyl-acetate fractions (EAFs) of Guazuma ulmifolia, Limonium brasiliense, Paullinia cupana, Poincianella pluviosa, Stryphnodendron adstringens and Trichilia catigua using preliminary in vitro bioassays (acetylcholinesterase inhibition, antioxidant activity and The EAF of S. adstringens also had an impact on mitochondrial membrane potential, lipid peroxidation, superoxide generation, and the mRNA expression of 10 genes linked to Alzheimer's disease (AD). according to the results of this study. To totally prevent mitochondrial depolarization (69 percent), superoxide generation (49 percent), and A25-35-induced lipid peroxidation, the EAF of S. adstringens at 15.62 g/mL proved effective (35 percent). For mRNA expression, the EAF of S. adstringens likewise blocked A25–35-induced MAPT overexpression (expression ratio 2.387x), which may be connected to tau protein phosphorylation. In this study, the neuroprotective benefits of these fractions were proven for the first time, and the electropherogram fingerprints for the EAFs of G. ulmifolia, L. brasiliense, P. cupana, P. pluviosa, and S. adstringens were also developed for the first time. This research adds to our understanding of the protective properties of the tested fractions in vitro as well as their quality control.

Koji Fujihara (2017) Alzheimer's disease (AD) is a devastating brain illness that mostly affects the hippocampus. The buildup of amyloid beta (A) peptides in the brain, which causes neuronal cell death, is a significant cause of Alzheimer's disease. Here, we isolated four saponins (1-4) and used 1D and 2D NMR as well as HRFABMS spectrum data to determine the structures of these compounds. 3 was confirmed to be a novel saponin with aglycon oleanolic acid, while the structures of 1 and 2 were determined to be aglycon cochalic acid. Chikusetsusaponin V (=ginsenoside R0) was identified as compound 4. These saponins (1-4) and six previously described saponins (5-10) were examined to see whether they inhibit A aggregation and protect cells from the harmful effects of A on the SH-SY5Y strain. A aggregation was inhibited by compounds 3 and 4, and SH-SY5Y cells were protected against the toxicity of A by compounds 5-8 as a consequence.

Priscila do Carmo Marchioro Raupp Torma (2017) Increases in bioactive substances and positive benefits on health have been the consequence of fruit breeding efforts. In this work, the hydroethanolic extracts of six açа (Euterpe oleracea) genotypes were evaluated for antioxidant activity and neuroprotective effects by ABTS, deoxyribose, and glutathione oxidation tests, as well as SH-SY5Y cells insulted

with H2O2. This study found that L22P13 genotype had the greatest total anthocyanin content, whereas L06P13 genotype had a high total carotenoids level. However, ABTS and deoxyribose tests indicated no variation in the antioxidant activity of the genotypes tested. Protective effects of açai hydroethanolic extracts on SH-SY5Y cells exposed to H2O2 at a concentration of 50 g/mL were observed by DCFH-DA assay. In the SRB experiment, no genotypes were shown to be cytotoxic except for L04P16. Reactive species produced in SH-SY5Y cells were reduced by açai genotypes, indicating a neuroprotective effect from hydroethanolic extracts of these fruits.

José DavidSánchez-Martínez (2022) In order to maximize the recovery of orange (Citrus sinensis) by-product terpenoids, pressurized liquid extraction (PLE) conditions were improved. A battery of in vitro assays (antioxidant (ABTS), reactive oxygen/nitrogen species (ROS/RNS) as well as enzymatic tests (acetylcholinesterase, butyrylcholinesterase, and lipoxygenase) were used to evaluate the neuroprotective potential of the PLE extracts. The PLE extracts with the best neuroprotective properties had a greater concentration of mono- and sesquiterpenoids, according to GC-q-TOF-MS analyses. In-silico molecular docking research revealed the unique interactions of terpenoids with enzymes active sites. Antioxidant, anti-cholinesterase, anti-inflammatory, and low-cytotoxicity qualities of the chosen extract at 100°C and 30 minutes were shown by the findings of the study. In cell models, the extract was found to be protective against L-glutamic acid and IL-6.

Liana M de Medeiros (2019) Memory, learning, and attention are all dependent on cholinergic transmission. Cognitive loss in Alzheimer's disease (AD) is linked to a particular degeneration of cholinergic neurons in the brain. There is no recognized medication that can effectively prevent or reverse the symptoms In order to test the neurotoxicity of OA or soluble amyloid- oligomers (AOs) in order to imitate tau and amyloid- disease, RA + BDNF-differentiated cells were used. Remarkable changes in neuronal morphology were seen as a consequence of RA + BDNF-induced differentiation. Compared to RA-differentiation, cholinergic markers were found to be more prominently expressed and active. Reduced neurite densities, an in vitro marker of synaptopathy, were seen after the administration of sublethal dosages of AOs and OA together. Sublethal dosages of OA and AO combined with RA+BDNF-differentiated SH-SY5Y cells produces an in vitro model that mimics the early-stage pathophysiology of cholinergic neurons afflicted by Alzheimer's disease.

MATERIALS AND METHODS

Plant Materials

Local gardens in Khon Kaen, Thailand, donated A. galanga, P. ginseng, and M. oleifera leaves, while the seeds of V. vinifera were given by Visootha (Nikki) Lohinavy of GranMonte Asoke Valley Winery from a winemaking facility in Pak Chong, Nakorn Rachasima, Thailand in October 2017, respectively. A hot air oven (Model FD240, Binder, Frankfurt, Germany) set at 65°C dried and pulverized the plant materials (Model 600W, Eindhoven, The Netherlands). At room temperature and away from the sun, the ground materials were stored in screwcap containers.

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Preparation Of Plant Extracts

Using a magnetic stirrer, all plant samples were extracted with ethanol at a 1:4 ratio and filtered using Whatman No. 1 filter paper for 8 hours (Camlab, Cambridge, UK). A. galanga leaves and rhizomes were extracted using 95 percent ethanol, whereas 70 percent ethanol was used to extract V. vinifera seeds, M. oleifera leaves and P. ginseng leaves and rhizomes. Using a rotary evaporator, we condensed the samples individually (Model Heidolgh VV2000, Heidolph Instruments Gmbh, Schwabach, Germany). An oily pale yellow top layer (PYL) and a brown bottom layer made up the A. galanga rhizomes extract (ARE) (BL). Due to the presence of essential oil in ARE (BL and PYL), the powdered extracts of A. galanga leaves, P. ginseng leaves and rhizomes, V. vinifera seeds, and M. oleifera leaves were all obtained. Separate extracts were stored at 4°C in screwcap vials.

Determination Of Antioxidant Capacity

- **Scavenging of the 1,1-Diphenyl-1-Picrylhydrazyl Radical (DPPH)**

describes the method used to estimate the free DPPH radical system. A final concentration of 200 ng/mL of each plant extract was added to 2.94 mL of 0.1 mM DPPH in methanol, along with 60 L of each plant extract. Methanol was used as a negative control and gallic acid as a positive control (with 95 percent DPPH scavenging). Afterward, the mixture was forcefully shaken for 20 seconds in a dark room for 30 minutes. A UV Probe-1800 spectrophotometer was used to record the reduction in absorbance at 516 nm (Shimadzu, Kyoto, Japan). The following equation was used to estimate the amount of DPPH radical scavenging:

$$\% \text{ DPPH scavenging} = [(Ab_0 - Ab_1/Ab_0)] \times 100$$

For example, Ab0 is the absorbance of the control, while Ab1 is the absorbance of the extract.

Ferric-Reducing Antioxidant Power (FRAP)

In accordance with, various adjustments were made to the FRAP method. In order to make the FRAP solution, 25 mL of pH 3 acetate buffer was mixed with 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in a 40 mM HCl solution and 2.5 mL of 20 mM Iron(III) chloride hexahydrate (FeCl3. 6H2O). For the negative control, distilled water was utilized; for the positive control, Trolox (0.03–0.1 mg/mL) was employed. It took 30 minutes of dark time for the reaction to begin after 100 L of crude extract was added to 2 mL of the FRAP working solution. At a wavelength of 593 nm, the ferrous tripyridyltriazine complex was shown to absorb light. Ferrous sulfate concentrations ranging from 5 to 100 M/mL were used to create the standard curve. Fe (II) concentrations were represented as a percentage of dry weight.

Neuroblastoma Cell Cultures (SHSY5Y)

The American Type Culture Collection , CRL2266TM, Manassas, VA, USA, got the SHSY5Y cell line (human neuroblastoma) from the Chulalongkorn University Institute of Biotechnology and Genetic Engineering in Bangkok, Thailand. At a 1:1 ratio, the SHSY5Y culture was cultivated in DMEM/F-12, a medium made up of Dulbecco's

modified Eagle medium (DMEM) and Ham's F-12 Nutrient Mixture. There were no significant differences in the cell viability between the two groups of cells, which were cultured in the presence of 10% heat-activated fetal bovine serum (FBS) and incubated at 37 °C in an incubator with an 85 % humidified environment containing 5% CO2 in a humidified atmosphere.

Culturing and Harvesting of the SHSY5Y Cells

Tweezers were used to remove cells from a flask of SHSY5Y cells, which were then resuspended in the culture media and placed in the wells of 96 well culture plates. They utilized the cells after 24 hours.

Cytotoxicity of Plant Extracts on the SHSY5Y Cell Line

cell viability assays were utilized to examine the SHSY5Y cell line for cytotoxicity. One hundred microliters (mL) of crude extracts were added to the cell cultures incubated in the preceding stage. The concentration ranged from 0.25 to 4 mg/mL. There was no extract in the negative control. For 24 hours, cells were incubated at 37°C in a 5 percent CO2 environment. After that, the medium was withdrawn, and cells were cultured for an additional 4 hours with 100 L of 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in Dulbecco's phosphate buffer saline (DPBS). DMSO was used to dissolve the crystal formazan after the MTT solution had been gently withdrawn. Positive control Triton X-100 was utilized (considered as 100 percent cell death). The absorbance of formazan in each well was measured using a microplate reader at 570 nm after the plate had been agitated for one minute. This equation was used to determine the cytotoxicity percentage in comparison to the negative control (untreated cells, which are assumed to be 100% viable):

$$\text{Cytotoxicity \%} = (\text{Ab. of control cells} - \text{Ab. of treated cells})/(\text{Ab. of control cells}) \times 100$$

Using the plot of the percentage cytotoxicity vs. sample concentration, the concentration of extract that killed 50% of the cells was determined (IC50).

Neuroprotection of Plant Extracts on the SHSY5Y Cell Line

In this experiment, oxidative stress was induced by H2O2. For this study, SHSY5Y cells were cultured for 24 hours with H2O2 concentrations ranging from 100–500 M and 100 L of 250 M was chosen to test the neuroprotective effects of plant extracts. Cells were pretreated for 6 hours with each plant extract tested (IC50 was chosen based on MTT assay) and then subjected to 250 M H2O2 for 1 hour to examine the dose-dependent neuroprotective effects of the plant extracts against cells exposed to oxidative stress. To determine the cytotoxic impact, cells were treated with 250 M H2O2 for 1 hour (the positive control was determined to be 100 percent cell death) and their viability was compared to that of cells that had not been treated (the negative control). the MTT test was used to evaluate cell vitality after each experiment.

Determination Of Intracellular O2 − And Ros Inside The Shsy5y Cell Line
Determination of O2 − by Nitro-Blue Tetrazolium (NBT) Reduction Test

The approach in was slightly modified to employ NBT reduction to insoluble blue formazan as a probe for O2 production within live cells. Supernatants from the cells in

were removed and replaced with a medium containing varying concentrations of tested plant extracts, and then 15 L of a 1 percent NBT solution was added, as reported in. Untreated cells and cells treated for one hour with 200 M H2O2 (the negative control) were compared for absorbance (positive control: considered as massive absorbance). Supernatants were removed after 3 hours of incubation, and the formazan result was dissolved in 100 L of DMSO. The absorbance of a colored formazan solution was measured at 620 nm using a Varioskan LUX multimode microplate reader after the wells had been mixed (Thermo Fisher Scientific, Waltham, MA, USA).

Determination of Intracellular ROS by DCFHDA Assay

2,7-Dichlorodihydrofluorescein diacetate (DCFHDA) was employed to study ROS production in SHSY5Y cells with and without plant ex-tracts [24]. The antioxidant system was induced in cells utilizing bioactive chemicals by incubating SHSY5Y cells with different amounts of plant extract (0.25, 0.5, 1, 2, and 4 mg/mL). It took 3 hours to incubate the cells, then 60 minutes in 10 M DCFHDA (Molecular Probes, Invitrogen, Basel, Switzerland) in complete medium at 37 degrees Celsius and 5 percent CO2. Intracellular oxidation was induced after 2 hours of treatment of the cells with 200 M H2O2 after a wash with DPBS. In the experiment, cells treated with 200 M H2O2 for one hour were used as a positive control and untreated cells as a negative control (both deemed to have a moderate absorbance) (considered as massive absorbance). Varioskan LUX multimode microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) was used to detect fluo-rescence intensity at an excitation wavelength of 485 nm and an emission wavelength of 528 nm, respectively. To test for cell survival, SHSY5Y cells were cultured in separate dishes with varying amounts of DMSO (a solvent used to solubilize plant extracts).

Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometer (LC–QTOF/MS) Analysis

Following ultrasonication for 20 minutes and centrifugation for 10 minutes at 4 C, an MLE sample with a concentration of 101.23 mg/mL in 50% methanol was obtained. It was necessary to filter the supernatant using nylon membranes of 0.2 m in length before injecting the filtrate into liquid chromatography in conjunction with a quadrupole time-of-flight mass spectrometry

II LC-6545 Quadrupole-TOF, Agilent Technologies, Santa Carla, CA, United States. II) Binary pump and online vacuum degasser were used in the liquid chromatographic system, which was linked to a Dual AJS ESI source mass spectrometer (Agilent Technologies, Santa Carla, CA, USA). Between m/z 100 and 1700, the full-scan mode was used. To do the analysis, the Zorbax Eclipse Plus column (Agilent Technologies in Santa Carla CA, USA) (C18 1.150mm, 1.78m) was employed. Mobile phase-gradient elution was performed as follows: Formic acid 0.1% in distilled water (solvent A) and 100 percent acetonitrile (solvent B) were utilized. 90% A, 0–2 min; 90% A, 2–25 min; 85% A, 25–40 min; 80% A, 40–48 min; 75% A, 48–68 min; 70% A, 68–80 min; 50% A, 80–85 min; 0% A, 85–90 min; 98% A, 90–100 min. Peaks were found at 254 and 280 nm in the spectrum. They were taken in both positive and negative ion auto MS/MS mode. The METLIN database's spectrum database for organic compounds was used to find the mass fragmentations (a cost-free reachable web-based data source,

has been developed to facilitate in a wide range of metabolite research and to assist natural products identification through mass analysis).

Statistical Analysis

A total of three experiments were performed in this research (n = 3). The data were presented as the mean standard deviation. With a p-value of 0.05, SPSS Statistics Base version 19 for Windows was used to analyze the differences among the treatments using Duncan's multiple range tests (DMRTs) (IBM Corp, Chicago, IL, USA).

RESULTS AND DISCUSSION

The antioxidant and neuroprotective properties of four Asian plants—M. oleifera, A. galanga, V. vinifera, and P. ginseng—were examined in the current research, and the results were promising. ligated. These plants' various components were extracted using ethanol (a polar solvent) at concentrations ranging between 70 and 95 percent. Because of this, the plant extracts included chemicals with a high polarity. FRAP and DPPH techniques were used to determine the extracts' antioxidant capability. Both hydrogen atom transfer (HAT) and single electron transfer (SET) methods are used to carry out the DPPH assay. Quenching DPPH radicals, VSE and MLE had the maximum activity with 81% and 58% activity, respectively (Table 1). According to, catechin and epicatechin are the most abundant molecules among VSE's phenolic components, and their capacity to scavenge DPPH radicals was supported by our findings using VSE and MLE extracted with a 70% ethanolic solvent.

According to one study, the antioxidant ability of MLE may be enhanced by luteolin. On the other hand, according to Table 1, ALE and MLE both had strong FRAP activity (33.57 and 26.76 mol Fe II/g dry weight, respectively). The ferric reducing activity of VSE was modest, with 19.45 mol Fe (II)/g dry weight. A lack of FRAP activity indicated that these three proteins had the lowest electron donating capacity. The galanga flavonoid, which was found in galanga plant ethanolic extract, may explain the FRAP action of ALE. Galanga flavonoids have previously been shown to be beneficial in scavenging free radicals and chelating metals. Meanwhile, polyphenolic components including phenolic acids and flavonoids, which are both classified as very efficient antioxidant molecules, are abundant in dried MLE. In both (DPPH and FRAP) tests, the extracts' ranking abilities altered, although MLE remained the second most active performance.

Table 1. Cellular viability and antioxidant capacity and activity of selected plant extracts estimated by various tests (1,1-diphenyl-1-picryl hydroxyl (DPPH), ferric-reducing antioxidant power (FRAP), nitro-blue tetrazolium (NBT), intracellular reactive oxygen species (ROS) level by 2,7-dichlorodihydrofluorescein diacetate (DCFHDA), and neuroprotection activity).

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Plant Extract	DPPH (Inhibition %)	FRAP (μmol/g)	Cytotoxicity by MTT Assay (IC_{50} mg/mL)	NBT Reduction Test (IC_{50} mg/mL)	Intracellular ROS Levels by DCFHDA (IC_{50} mg/mL)	Neuroprotection (% Cell Viability)
MLE	58	26.76 ± 0.3^{e}	2.7 ± 0.2^{a}	0.83 ± 0.09^{c}	0.71 ± 0.08^{c}	44
VSE	81	9.45 ± 0.08^{d}	2.43 ± 0.1^{b}	0.98 ± 0.08^{c}	1.24 ± 0.06^{a}	38
PLE	0.05	7.46 ± 0.2^{a}	1.96 ± 0.02^{c}	1.65 ± 0.04^{a}	1.34 ± 0.06^{a}	34
PRE	2	10.05 ± 0.08^{c}	1.92 ± 0.04^{c}	1.22 ± 0.06^{b}	0.83 ± 0.08^{c}	51
ALE	41	33.57 ± 0.2^{g}	2.53 ± 0.1^{b}	1.24 ± 0.05^{b}	2.48 ± 0.03^{d}	32
ARE (PYL)	0.01	5.62 ± 0.02^{b}	0.34 ± 0.02^{d}	1.62 ± 0.1^{a}	0.96 ± 0.1^{b}	40
ARE (BL)	31	24.19 ± 0.1^{f}	ND	ND	ND	ND

There was no significant difference in the mean cell viability (mol/g: mol of Fe (II) per grain of dry weight plant extract) or neuroprotection results among the three individuals who participated in the study. This extract could not be evaluated in a viable system due to its high cytotoxicity (the SHSY5Y cell line). Differential effects were found in terms of P-values (0.05). The following plants were used: M. oleifera, V. vinifera, P. ginseng, and A. galanga rhizomes, as well as extracts of the aforementioned plants' leaves, stems, and rhizomes (brown layer).

MLE was selected for LC–QTOF/MS investigation because of its excellent antioxidant potential to scavenge DPPH radicals and FRAP activity. Arabinoflavin, isorhamnetin, quercetin derivatives and caffeoylquinic acid and naringenin were discovered in MLE, according to Table 2 of the results. It's possible that these compounds, which enhance hydrogen-atom-transfer (HAT) and electron donation processes, may eliminate DPPH and reduce power (FRAP).

It is common knowledge in Asian society that the plants studied are well-known. The enhanced biological features of MLE were established via testing. above and above all else. It exhibited its unique antioxidant properties both in and outside of living systems. To find the most effective plant in the Asian diet, this research employed MLE to analyze its composition. It is possible to prevent or cure many diseases, such as Alzheimer's and Parkinson's disease, with the help of antioxidant compounds present in plants.

Aside from its anti-inflammatory and enzyme-inhibiting activities, polyphenolic substances also have gene expression and signal transduction characteristics. The neuroprotective properties of human neuroblastoma cell lines were investigated in this study. Considerations such as the following were taken into account while selecting this neuronal cell line: Free radical production and scavenging in the human brain may be examined utilizing cells as they share many biochemical and functional properties with neurons (a). This cell line was produced from a human origin. Due to these cells' oncogenicity, which enables them to proliferate and multiply to a large number, the experiment can be repeated with confidence since the cell population is rather homogenous.

Before looking for neuroprotective characteristics, plant extracts were tested for cytotoxicity on SHSY5Y cells using the MTT assay. Results of the extracts' 50% inhibitory concentration are summarized in Table 1 of this report (IC50). PYL (ARE) was the most lethal agent in this investigation, with an IC50 of 0.34 0.02 mg/mL for SHSY5Y cells. The cytotoxicity of MLE, ALE, and VSE was the lowest. In the study, the neuroblastoma cell line was harmed even at low concentrations of ARE (BL). The cytotoxicity of the extracts and the lethal dose of H2O2 were assessed on SHSY5Y cell

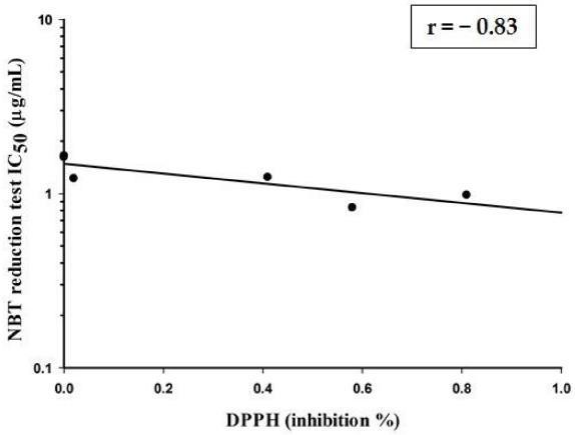
lines before the extracts' neuroprotective effect was investigated. More than half of the viable cells were protected by PRE, which was followed by MLE and ARE (PYL) with 44 percent and 40 percent, respectively.

Researchers have shown that the ginsenosides in PRE help to improve brain function, decrease inflammation, and minimize oxidative stress in the brain, as well as weaken or inhibit numerous neurodegenerative illnesses including Alzheimer's, HD, and traumatic brain injury. A combination of the antioxidant enzymes SOD and catalase as well as decreased lipid peroxide levels may help people with Alzheimer's disease improve their memory. Antioxidant effects may have a positive effect on cognitive performance. SOD and catalase, as well as other antioxidant substances including glutathione and other antioxidant molecules, ARE acts as a deterrent to brain lipid peroxidation by preserving the functioning of these enzymes. ROS scavenging by ARE in the form of aqueous and alcoholic extracts has been proven to promote neurodegenerative disorders.

The NBT test was used to determine if plant extracts could protect SHSY5Y cells against oxygen radicals, and the results are reported in Table 1. VSE had the lowest IC50 value, 0.98 0.08 mg/mL, which was closely followed by MLE in terms of NBT reduction (0.083 0.09). There was less reduction activity in ALE and PRE (1.24 0.05 mg/mL and 1.22 0.06 mg/mL, respectively) than in the other two compounds. ARE (PYL) and PLE had the lowest O2 radical scavenging capabilities, with concentrations of 1.62 0.1 mg/mL and 1.65 0.04 mg/mL, respectively. Figure 1 show SHSY5Y cells without MLE (darkly colored with high O2) and SHSY5Y cells treated with MLE (lightly colored with low O2) (light colored with low O2).

By using Pearson coefficient correlations, it was shown that extracts tested (MLE, VSE, PLE, ARE(PYL) and ALE) had similar scavenging effects on free radicals in the lab and in live cells, both in terms of scavenging O2 and intracellular ROS. As shown in there is a strong association between NBT reduction activity and quenching DPPH levels. Extracts with higher quenching DPPH levels were shown to be more effective at trapping O2 in the NBT test. According to the results, the remaining factors had no statistically significant correlations (p 0.05).

Figure 1. Nitro-blue tetrazolium (NBT) reduction test: (**A**) control cells (SHSY5Y cells without MLE); (**B**) SHSY5Y cells treated with MLE.



DCFHDA was used as a fluorogenic substrate to measure the ability of the plant extracts to scavenge ROS in SHSY5Y cells. By using a spectrophotometer, we were able to track the conversion of this substrate to extremely fluorescent DCFH. DCFHDA test results reveal the amounts of extracts that reduce ROS levels by 50%. The strongest ROS scavenging activity was found in MLE, PRE, and ARE (PYL), with IC50 values of 0.71 0.08, 0.83 0.08, and 0.96 0.1 mg/mL. VSE (1.24 0.06 mg/mL), PLE (1.34 0.06 mg/mL), and ALE (2.48 0.03 mg/mL) had the lowest antioxidant activity in SHSY5Y cells, whereas ALE had the highest. Furthermore, SHSY5Y cells that were treated with DMSO had no impact on the cells. In Table 2, quercetin derivatives, isorhamnetin glucoside, kaempferol, luteolin, ascorbic acid derivative, diosmetin, apigenin and its derivatives (Saponaria), hydroxy-tyrosyl 1-O-glucoside, HT, and esculetin are revealed to be present in MLE, which may explain why it has a unique antioxidant activity. The Bcl-2-associated X protein (BAX) pathway may help protect SHSY5Y against reactive oxygen species when ginsenoside, the primary active component of PRE, is administered. On 4T1 breast cancer cells as well as NIH-3T3 fibroblast cells, the volatile oil compounds coumarins, sesquiterpenes, and eugenol, as well as their derivatives in ARE, may prevent ROS.

It was clear from these findings that ethanolic MLE extract is pharmacologically active when it came in first or second place in all of the tests. This extract was shown to be more effective than the others we studied, perhaps as a result of the synergistic effects of many powerful components included in the complete extract. The low cytotoxicity and excellent antioxidant activity of MLE, which is cultivated in Thailand, make it a promising neuroprotective agent. This study's LC–QTOF/MS analysis (Figure 2 and), which was supported by MS spectra (see Supplementary Materials),

revealed that the extract was significantly richer in antioxidant ingredients (31 compounds) compared with constituents found in moringa leaves cultivated in China and India (11 compounds also present in our extract), whereas phytochemicals reported in a moringa extract from China were significantly lower than those in our extract As a result, moringa grown in Thailand is a top source of powerful antioxidant chemicals. The plants' adaptability to the climate and soil variables, such as soil type, pH, and soil nutrients, may explain the differences in phytochemical contents. Consequently, these elements are frequently connected with environmental and growth conditions. Table 2 shows that eriodyctiol and gallic acid are present in MLE and that they help to scavenge H2O2 and prevent oxidative stress-induced cell damage. Apigenin 7-rhamnosyl-(1!2)-galacturonide, Cartormin, Kaempferol 40-glucoside, Hesperidin, and Isorhamnetin 3-were all present, as were a number of other potent phenolic antioxidants (600-acetylglucoside). One of the most significant and distinctive biological roles of MLE is that it is an environmentally friendly and non-cytotoxic antioxidant agent, unlike the

other studied extracts.

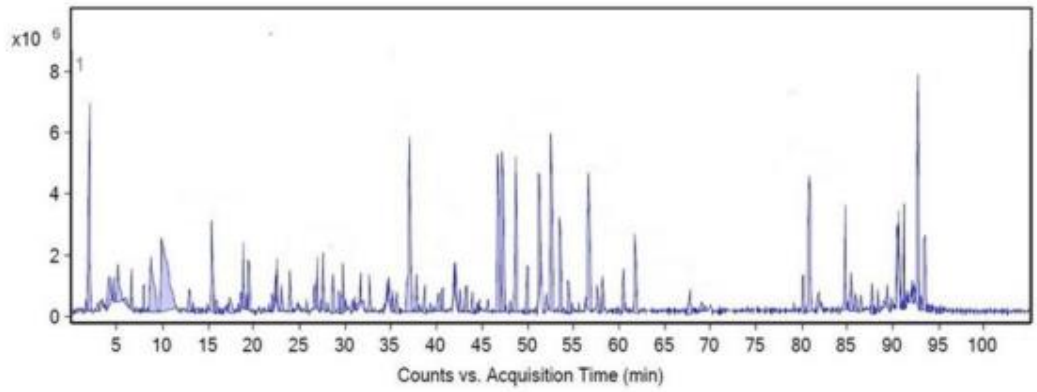


Figure 2. Liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC–QTOF/MS) chromatogram of MLE.

4. Conclusions

A variety of phytochemicals have been reported to prevent the risk of numerous diseases, including neurodegenerative diseases. In Asian countries, many researchers are seeking bioactive compounds from edible plants or herbs for the prevention or treatment of neurodegenerative diseases. A. galanga, V. vinifera, M. oleifera, and P. ginseng cultivated in Thailand were selected in the present study.

MLE showed high antioxidant activities (DPPH and FRAP assays) with low cytotoxic-it to SHSY5Y cell lines compared to the other studied plants. Its mechanism of action for neuroprotective effect on SHSY5Y cells is probably due to its high level of polyphenolic and other antioxidant compounds, and it possesses the ability to scavenge free radicals or activate the cellular antioxidant system. LC–QTOF/MS analysis confirmed that MLE con-sists of phenolic compounds, which are a good source of antioxidants. The determination of pure bioactive compounds involved in neurodegeneration contained in MLE should be the subject of further investigation.

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