Acetylcholinesterase Inhibition and Determination of Quality Parameters Polyherbal and Combination Extracts from *Moringa oleifera* Lam., *Phyllanthus niruri* L., and *Nigella sativa* L.

DOI : https://doi.org/10.22435/hsji.v14i2.6495

Sabrina K. Whardhani[1,2], Syamsudin Abdillah[2], Ratna Djamil[2]

[1]Faculty of Science and Technology, Universitas Pelita Harapan, Jakarta, Indonesia
[2]Faculty of Pharmacy, Universitas Pancasila, Depok, Indonesia

Corresponding author: Sabrina K. Whardhani
Email: sabrina.whardhani@uph.edu

Received: January 6, 2023; Revised: July17, 2023; Accepted: September 6, 2023

Abstract

Background: Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by degeneration and death of neurons. Cholinesterase inhibitor medicine, especially acetylcholinesterase inhibitors (AChE) are commonly used to manage AD. Huperzine A is a food supplement with AChE inhibition activity which has been approved for the treatment of AD in China. Therefore, further exploration on medicinal plants traditionally utilized in Indonesia is needed. This research aims to evaluate the potency and safety of several medicinal plants for future utilization to manage AD.

Methods: The acetylcholinesterase inhibitory activity was tested using Ellman method. The phytochemical screening of polyherbal, Moringa oleifera Lam., Phyllanthus niruri L., and Nigella sativa L extracts was carried out according to Materia Medika Indonesia. Determination of quality parameters used methods from Parameter Standar Umum Ekstrak Tanaman Obat.

Results: Active compounds have been successfully extracted. Each extract was subjected to different assays and shows that the polyherbal product has a low AChE inhibitory potential. Meanwhile, combination extracts formulated in this study have better AChE inhibitory potential.

Conclusion: The combined extract formulated in this study shows a potential to be applied as an AChE inhibitor. It may be utilized as a safe alternative treatment for people with AD.

Keywords: Polyherbal capsule, Moringa oleifera Lam., Phyllanthus niruri L., and Nigella sativa L extracts, Acetylcholinesterase inhibitor activity, Ellman method.

Abstrak

Latar Belakang: *Penyakit Alzheimer (AD) merupakan gangguan neurodegeneratif yang ditandai dengan degenerasi dan kematian neuron. Obat penghambat kolinesterase, terutama penghambat asetilkolinesterase (AChE) biasanya digunakan untuk mengelola AD. Huperzine A adalah suplemen makanan dengan kemampuan menghambat AChE dan telah disetujui untuk pengobatan AD di Cina.. Oleh karena itu eksplorasi lebih jauh terhadap tanaman obat tradisional di Indonesia perlu dilakukan. Penelitian ini bertujuan untuk mengevaluasi potensi dan keamanan beberapa tanaman obat agar dapat dimanfaatkan dalam pengobatan AD.*

Metode: *Uji aktivitas penghambatan asetilkolinesterase dilakukan menggunakan metode Ellman. Penapisan fitokimia ekstrak poliherbal, Moringa oleifera Lam., Phyllanthus niruri L., dan Nigella sativa L dilakukan berdasarkan metode dari Materia Medika Indonesia. Parameter mutu ditentukan berdasarkan Parameter Standar Umum Ekstrak Tanaman Obat.*

Hasil: *Senyawa aktif telah berhasil diekstraksi dari Moringa oleifera Lam., Phyllanthus niruri L., Nigella sativa L., dan poliherbal. Masing-masing ekstrak telah melalui beberapa pengujian dan hasil menunjukkan bahwa produk poliherbal memiliki potensi penghambatan AChE yang rendah. Sementara, kombinasi ekstrak yang diformulasikan dalam penelitian ini menunjukkan potensi penghambatan AChE yang lebih baik.*

Kesimpulan: *Kombinasi ekkstrak yang diformulasikan dalam penelitian ini menunjukkan potensi untuk diaplikasikan sebagai penghambat AChE. Ini dapat digunakan sebagai alternatif pengobatan yang aman untuk penderita AD.*

Kata kunci: *Kapsul poliherbal, ekstrak Moringa oleifera Lam., Phyllanthus niruri L., dan Nigella sativa L, aktivitas penghambatan asetilkolinesterase, metode Ellman*

INTRODUCTION

Alzheimer's Disease (AD) is a neurodegenerative disorder commonly occurred in older people and characterized by degeneration and death of neurons. It is predicted that the number of people with AD will reach 114 million in 2050.[1,2] AD in general threatens older people. Indonesia is currently entering population ageing,[3] thus the number of AD patients in Indonesia might increase as well.

Observation on AD patients resulted in three hypotheses of AD pathogenesis that generally appeared together, which are cholinergic hypothesis, amyloid deposition hypothesis, and abnormal tau accumulation hypothesis. The cholinergic hypothesis is the oldest AD pathogenesis theory. Cholinergic synapses are present in many parts of the human central nervous system, and cholinergic transmission is critical for memory, learning, attention, and other higher brain functions.[3–5]

Medicines that have been proven to manage AD are cholinesterase inhibitors, especially acetylcholinesterase inhibitors (AChEI). These medicines maintained the level of acetylcholine, and thus ultimately can improve cognition.[6] Tacrine, donepezil, rivastigmine, and galantamine are some AChEI-based medicines for the treatment of AD that have been approved by US Food and Drug Administration (FDA). Although these drugs have been approved by FDA, they still caused side effect, so there is a need for alternative treatments that are effective and save. Natural materials, especially medicinal plants, have a very significant contribution to the discovery and development of drugs that can fight or treat AD. Huperzine A, an alkaloid from *Huperzia serrata* with AchEI property, is used as a dietary supplement to improve memory function in the United States and has been approved for the treatment of AD in China.[7] In Indonesia, a polyherbal capsule which made from the extract of *Moringa oleifera* Lam., *Phyllanthus niruri* L., and *Nigella sativa* L. is commercially available. These plants have shown acetylcholinesterase (AChEI) inhibition, antioxidant, as well as neuroprotective and neurodegenerative properties. Therefore, the polyherbal product may has a potential to be utilized as treatment of AD.[8]

This research aims to evaluate the acetylcholinesterase inhibition property of the polyherbal capsule, and several combinations of *Moringa oleifera* Lam., *Phyllanthus niruri* L., and *Nigella sativa* L. extract. Determination of quality parameters was also carried out to ensure their safety and efficacy.

METHODS

Material

The sample used in this study were polyherbal capsules, *Moringa oleifera* Lam. powder from Balai Penelitian Tanaman Rempah dan Obat, *Phyllanthus niruri* L., *Nigella sativa* L., powder from Borobudur Extraction Center. The materials used in this study were quercetin, gallic acid, 96% ethanol, ddH_2O, Acetylcholinesterase Assay Kit (Colorimetric) (ab138871).

Tools

The tools used in this research were Heidolph rotary evaporator, ELISA Reader (iMark™ Microplate Absorbance Reader), Shaker, Barnstead International vortex mixer, Memmert water bath, glasswares, and micro pipette.

The Extraction of *Moringa oleifera* Lam., *Phyllanthus niruri* L., and *Nigella sativa* L

Each sample was extracted using 96% ethanol at a ratio of 1:20 (v/v), using the kinetic maceration method. The sample was soaked in 96% ethanol and stirred for 3 hours and then filtered. This process was repeated using the same amount of new solvent for several times until the sample has been extracted thoroughly.[9]

Measurement of Extract Yield

The macerate obtained was concentrated using a rotary evaporator to obtain a thick extract, then the yield was calculated using the following formula[9]: Calculation of extract yield is as follows:

$$\text{Extract yield} = \frac{\text{Extract weight (gr)}}{\text{Sample weight (gr)}} \times 100\%$$

Acetylcholinesterase Inhibition Assay

The acetylcholinesterase inhibition of the polyherbal capsule and the different combinations of *Moringa oleifera* Lam., *Phyllanthus niruri* L., and *Nigella sativa* L. extract were measured using an AChE assay kit (ab138871, Abcam, Cambridge, UK). The protocols were conducted according to the manufacturers' instructions.

50 µL buffer (as a positive control) and 50 µL F1-F5 was inserted into each well of the plate. Then 50 µL of acetylthiocholine reaction mixture was added to each well and allowed to stand for 10 – 30 minutes

in a dark room and at room temperature. After 10-30 minutes of incubation, the absorption was measured using a microplate reader at a wavelength of 410 ± 5 nm. The activity of AChE inhibition was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{A control} - \text{A test}}{\text{A control}} X\ 100\%$$

Where:
A control : absorption$_{\text{enzyme + substrate}}$
A test : absorption$_{\text{test+enzyme + substrate- test blank absorption}}$

IC_{50} is calculated by entering the percentage resistance of 50% in linear regression.[10]

Sample Preparation

The samples used were polyherbal capsules and combinations of *Moringa oleifera* Lam., *Phyllanthus niruri* L.*,* and *Nigella sativa* L.extracts at different ratios. All samples were prepared at a concentration of 100 ppm and the compositions are shown in Table 1:

Table 1. The Composition of *Moringa oleifera* Lam., *Phyllanthus niruri* L., and *Nigella sativa* L. Extract in Each Formula

Formula code	*Moringa oleifera* Lam. (ml)	*Phyllanthus niruri* L. (ml)	*Nigella sativa* L. (ml)
Formula 1 (F1)	4	0	0
Formula 2 (F2)	1.3	1.3	1.3
Formula 3 (F3)	2	1	1
Formula 4 (F4)	1	2	1
Formula 5 (F5)	0	4	0

Phytochemical Screening

The phytochemical screening of polyherbal and the combination of *Moringa oleifera* Lam., *Phyllanthus niruri* L.*,* and *Nigella sativa* L. extract was carried out using methods from Materia Medika Indonesia.[11]

Test for Alkaloids

Approximately 500 mg of sample powder was stirred with a few drops (?) of 2N HCl and 9 mL of ddH2O, then was heated in the water bath for 2 minutes. The mixtures were then cooled and filtered. The filtrate was used to perform saponins, tannin, phenolics, flavonoids, glycosides, and steroids assays.

1. **Saponins Assay:** Approximately 3 ml of plant extracts were added to 3 ml of ddH2O and shaken vigorously. The formation of a stable, persistent froth was taken as a positive test for saponins.Tannin Assay: About 0.5 g each portion was stirred with about 10 ml of distilled water and then filtered. A few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. The appearance of blue-black, green or blue-green precipitate indicates the presence of tannins.
2. **Phenolics assay:** A small amount of the ethanolic extract was mixed with 1 mL ddH2O in a test tube and 1 to 2 drops of Iron III chloride (FeCl3) was added. A positive result is indicated by color changes to blue, green, red, or purple.
3. **Flavonoids assay:** Approximately 3 mL of plant extract was treated with 1 mL of 10% NaOH solution. The formation of the intense yellow color indicated the presence of flavonoids.
4. **Glycosides assay:** Identification of glycoside compounds was carried out by addition of glacial acetic acid followed by addition of iron (III) chloride and concentrated sulfuric acid consecutively. The mixture was mixed by shaking. The appearance of a purple ring indicated the presence of glycosides.
5. **Steroids assay:** Approximately 2 mL of chloroform and concentrated H2SO4 were added to 5mL of the prepared plant extracts. A layer of red color indicated the presence of steroids in the lower chloroform phase.

Determination of Total Phenolics Content

0.2 ml of the extract was added with 0.2 ml of Folin Ciocalteu reagent (50%) in a test tube and then vortexed for 3 minutes. After a 3-minute interval, 0.2 ml of 7.5% Na2CO3 solution was added. Then the mixture was stored in a dark room for 30 minutes. The absorbance of the extract was read using a spectrophotometer at a wavelength of 765 nm. The results are expressed as gallic acid equivalents GAE/g of extract. The standard curve was prepared in the same way using gallic acid.

Determination of Total Flavonoids Content

1 ml of 50% (w/v) extract solution was added to 0.2 ml of 10% aluminum chloride which had been dissolved in ethanol, then vortexed and the absorbance was read

at a wavelength of 440 nm using a spectrophotometer. The total content of flavonoids was expressed as quercetin equivalents QE/g of extract. The standard curve was prepared in the same way as quercetin.

Determination of Quality Parameters

Determination of quality parameters of polyherbal and *Moringa oleifera* Lam., *Phyllanthus niruri* L.*,* and *Nigella sativa* L. extract was carried out using methods from *Parameter Standar Umum Ekstrak Tanaman Obat*. The measured parameters include water soluble compound, ethanol soluble compound, determination of water, ash, heavy metal, and yeast mold test.[12]

RESULTS

Extract Yield

Ethanolic extract has been successfully obtained from *Moringa oleifera* Lam., *Phyllanthus niruri* L.*,* and *Nigella sativa* L. by kinetic maceration method. The yield from the extraction process and the Farmakope Herbal Indonesia (FHI) requirements are shown in Table 2.

Table 2. The yield of *Moringa oleifera* Lam., *Phyllanthus niruri* L.*,* and *Nigella sativa* L. Extraction

No	Sample	Extract (%)	FHI Requirement
1	*Moringa oleifera* Lam.	17,49	≥ 9,2
2	*Phyllanthus niruri* L.	31,61	≥ 19
3	*Nigella sativa* L.	11,43	

Acetylcholinesterase Inhibition

Acetylcholinesterase inhibition activity of polyherbal and the combination of *Moringa oleifera* Lam., *Phyllanthus niruri* L.*, Nigella sativa* L. at different ratios have been measured. Each sample showed acetylcholinesterase inhibition as presented in Table 3. This result suggests that active compounds that are potential to be utilized as AChEI are contained in each plant.

Phytochemical Screening

The ethanolic extract of *Moringa oleifera* Lam., *Phyllanthus niruri* L.*, Nigella sativa* L. has been tested for its phytochemical content through phytochemical screening method. The screening result showed that each plant contained various active compounds as shown in Table 4.

Total Phenolic and Flavonoid Content of Extract

The measurement of the total phenolic content of each extract showed that *Nigella sativa* L. extract has the highest total phenolic content, at 161.14 mg GAE/g, among all samples followed by *Moringa oleifera* Lam. (59.22 mg GAE/g), polyherbal (46.55 mg GAE/g), and *Phyllanthus niruri* L. (8.37 mg GAE/g) (Table 5).

The measurement of the total flavonoid content of each extract showed that *Moringa oleifera* Lam. has the highest total flavonoid content, at 26.52 mgQE/g, among all samples followed by *Nigella sativa* L. (14.09 mg QE/g) polyherbal (10.52 mg QE/g), and *Phyllanthus niruri* L. (1.10 mg QE/g) (Table 6).

Determination of Quality Parameters

The ethanolic extract of each sample was tested for water-soluble compound, ethanol soluble compound, determination of water, ash content, heavy metal content, and yeast mold content. The result of these assays, as shown in Table 7, were compared against the standard established in Indonesian Herbal Pharmacopeia.

Table 3. AChE Inhibitory Activity of The Polyherbal and The New Extract Formula

No	Sample	Linear Regression	IC_{50} Value (µg/mL)
1	Polyherbal	y = 0.1616x - 7.5236	262.85
2	F1	y = 0.2524x - 1.758	191.13
3	F2	y = 0.1744x - 5.4348	255.53
4	F3	y = 0.216x - 2.5425	219.71
5	F4	y = 0.3147x - 3.8185	146.75
6	F5	y = 0.3133x - 6.1909	139.83

Table 4. Phytochemical Content of The Plant Extracts

No	Active Compounds	*Moringa oleifera* Lam. Extract	*Phyllanthus niruri* L. Extract	*Nigella sativa* L. Extract
1	Alkaloid	+	+	+
2	Saponin	+	+	+
3	Tanin	+	+	+
4	Phenolic	+	+	+
5	Flavonoid	+	+	+
6	Triterpenoid	+	+	+
7	Steroid	-	+	-
8	Glycosides	+	+	+

Note:
+ = detected
- = not detected

Table 5. Total Phenolic Extract Content

No	Sample	Linear Regression	R^2 Value	Phenolic Concentration	
				ppm	mg GAE/g
1	*Moringa oleifera* Lam.			32.22	59.22
2	*Phyllanthus niruri* L.	y = 0.0105x + 0.0084	0.9988	43.52	8.37
3	*Nigella sativa* L.			58.98	161.14
4	Polyherbal	Y = 0.0111x + 0.018	0.999	47.25	46.55

Table 6. Total Flavonoid Extract Content

No	Sample	Linear Regression	R^2 Value	Flavonoid Concentration	
				ppm	mg QE/g
1	*Moringa oleifera* Lam.			14.43	26.52
2	*Phyllanthus niruri* L.	y = 0.0366x - 0.0097	0.9994	5.73	1.10
3	*Nigella sativa* L.			5.16	14.09
4	Polyherbal	y = 0.0286x + 0.0089	0.9976	10.68	10.52

7. Determination of Quality Parameters

96% Ethanol extract / Content	Moringa	Requirement (%)*	Meniran	Requirement (%)*	Black cumin	Requirement (%)	Polyherbal	Requirement (%)*
Water soluble compound	29.4	≥ 4.9	26.6	≥ 20.3	73.2	≥ 22	35.01	-
Ethanol soluble compound	22.7	≥ 5	16.36	≥ 10.5	64.57	≥ 18	23.64	-
Lost of drying	13.37	≤ 10	17.68	≤ 10	5.48	≤ 10	5.21	≤ 10
Determination of water	3.22	≤10	3.92	≤17	3.09	≤10	6.35	≤10
Determination of ash	12.56	< 9	14.68	< 8.7	14.59	< 8	10.42	-
Determination of heavy metal:								
As	ND	≤ 10	ND	≤ 10	ND	≤ 10	ND	≤ 10
Cd	ND	≤ 0.3	ND	≤ 0.3	ND	≤ 0.3	ND	≤ 0.3
Hg	ND	≤ 0.5	ND	≤ 0.5	ND	≤ 0.5	ND	≤ 0.5
Pb	ND	≤ 5	ND	≤ 5	ND	≤ 5	ND	≤ 5
Determination of yeast mold	<10	$\leq 10^4$	<10	$\leq 10^4$	<10	$\leq 10^4$	<10	$\leq 10^4$

Note:
*= requirement based on Indonesian Herbal Pharmacopoeia
ND = Not Detected

DISCUSSIONS

Indonesia is located in the tropical region of earth which receives sufficient amount of sunlight all year round. This condition supports productive growth of various vegetations, and as the result Indonesian have traditionally utilized various plants for food and medicine. Plants such as *Moringa oleifera* Lam., *Phyllanthus niruri* L.*,* and *Nigella sativa* L. (also known as kelor, meniran hijau, and jintan hitam respectively in Bahasa Indonesia) have traditionally used within Indonesian household to prevent and even treat various diseases. However, these beliefs are lacking of scientific evidence and therefore further research is needed to support the belief scientifically. Several studies have shown that *Moringa oleifera* Lam., *Phyllanthus niruri* L.*,* and *Nigella sativa* L. have acetylcholinesterase inhibition activity which makes them potential to be utilized for the treatment of AD. Taking into account Indonesia's ageing population, who have greater risk to suffer from AD, further study on the potential of each plant as well as in combination may reveal greater benefit for AD treatment.

Ethanol 96% has been known as a universal solvent that is able to extract various compounds in plants. In this study, kinetic maceration method using ethanol 96% as the solvent has successfully extracted compounds contained in *Moringa oleifera* Lam., *Phyllanthus niruri* L.*,* and *Nigella sativa* L. as shown in Table 2. Each sample gave a good yield which meets the FHI requirements. Therefore, the extract can be used for further analysis to study the acetylcholinesterase inhibition activity and various assays to ensure their quality and safety.

Several previous studies stated that *Moringa oleifera* Lam. leaves contain various active compounds such as tannins, sterol terpenoids, flavonoids, saponins, anthraquinones, alkaloids, glucosinolates, isothio cyanates, oligosaccharides, and oxalates.[8] Study by Oyeniran OH *et al.* (2020), also showed that the dominant phenolic constituents in *Moringa oleifera* Lam. plants were gallic acid, quercetin, kaempferol, chlorogenic acid, ellagic acid, isoquercitrin, and quercitrin.[13] Study on purified extract of *Phyllanthus niruri* L. showed the presence of isocoralligin compounds that were successfully isolated, which are compounds capable of inhibiting cholinesterase.[14] Meanwhile, the main compound contained in *Nigella sativa* L. is thymoquinone, which is the most bioactive compound and shows wide therapeutic benefits.[15-17] In this study, phytochemical screening on the ethanolic extract of the samples was carried out to get a qualitative measure of each sample. The data presented in Table 4 show that various active compounds were present in the ethanolic extract of each sample, with only steroid was found to be absent in the extract of *Moringa oleifera* Lam. and *Nigella sativa* L. These results suggest that the compounds that have been successfully extracted were beneficial phytochemical compounds, in accordance with previous studies.

Next, quantitative assays were carried out to measure the amount of phenolic content of the extract. Phenolic compounds are the most prominent compounds in plants and often related to favorable health impact, mainly due to their antioxidant property. Table 5 shows the total phenolic content of each plant extract as well as the polyherbal. It was found that each extract has high phenolic content, which further indicates that the plants are promising sources of therapeutic agent to combat oxidative stress. Furthermore, as shown in Table 6, flavonoids were detected in the ethanolic extract of the samples. Flavonoids are a class of phenolic compounds and are often associated with health-promoting effects, including antioxidant property that is favorable for treating AD. Because flavonoids are a class of phenolic compounds, the total flavonoid content of each sample is lower compared to the total phenolic compounds. This result suggests that other types of phenolic compounds are present in the extract.

The main goal of this study is to evaluate the acetylcholinesterase inhibition activity of the extract for future utilization in AD treatment. As shown in Table 2, the polyherbal exhibit has the lowest inhibitory activity (262.85 µg/mL). This is probably due to the presence of fillers in the polyherbal capsules which can affect their IC_{50} value. Formula 5 which is a *Phyllanthus niruri* L.*,* extract exhibit the best acetylcholinesterase inhibition with an IC_{50} value of 139.83 µg/mL. These data also show that formulas containing more *Phyllanthus niruri* L.*,* extract have better inhibitory activity. This is supported by research conducted by Yee-Hui Koay *et al* (2014), which showed that the plant extract exhibited potential inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Drugs that have dual inhibition of AChE and BChE result in better clinical efficacy against Alzheimer's disease (AD) than drugs that selectively inhibit one enzyme.[13] From this study, it can also be seen that F5, F4 and F1 (IC_{50} = 139.83, 146.75, 191.13 µg/mL respectively), had moderate potential to inhibit AChE, while F3, F2

and polyherbal (IC_{50} = 219.71, 255.54, 262.85 µg/mL) had low AChE inhibition potential. This is based on the literature which states that for an extract to be considered as strong AChE inhibitor, IC_{50} value has to be < 20 µg/mL, while moderate inhibitor exhibit 20 < IC_{50} < 200 µg/mL, and weak inhibitor has 200 < IC_{50} < 1,000 µg/mL. The limits are set according to the average IC_{50} values described for galantamine.[18]

Plant extract must meet several quality parameters to be applied as medicine. The parameters were established in Indonesian Herbal Pharmacopeia by Indonesian Health Minister (Kementerian Kesehatan Indonesia) as the standard. Therefore, the ethanolic extract of each sample was tested for water-soluble compound, ethanol soluble compound, determination of water, ash content, heavy metal content, and yeast mold content. The results of these tests showed that the ethanolic extract of *Moringa oleifera* Lam., *Phyllanthus niruri* L.*, Nigella sativa* L. and the polyherbal have met most of the specified requirements based on *Parameter Standar Umum Ekstrak Tanaman Obat*[12] except for the ash content. The ash content of the ethanolic extract of all samples were higher than the specified requirements. Additionally, the results of the loss of drying test for the ethanolic extract of *Moringa oleifera* Lam., and *Phyllanthus niruri* L.*,* were higher than the specified requirements, while the ethanolic extract of *Nigella sativa* L. and polyherbal had met the specified requirement. The loss of drying might be improved by carrying out proper handling during the drying process by maintaining optimum temperature (between 35-45°C), sufficient air circulation, maintaining appropriate humidity, using proper drying methods and harvesting times so it could meet the specific requirement, while the ash content can be improved by harvesting herbs at the right time, using high quality soil, proper drying and storage of herbs, drying herbs at a suitable temperature with good ventilation, avoiding hazards, and supplementing the soil with minerals or by using mineral-rich fertilizers.

CONCLUSION

In this study, all extract combinations showed the ability to inhibit acetylcholinesterase. F5 has the best acetylcholinesterase inhibition value with an IC_{50} value of 139.83, and may be alternative for treating Alzheimer's disease and other neurodegenerative diseases. Determination of quality parameters were also carried out to ensure their safety and efficacy of each extract. The specified requirements established in Indonesian Herbal Pharmacopeia have been met by most of the sample. This research may also contribute to the evidence base of standard herbal medicinal products, which may provide a natural and safe alternative for treating Alzheimer's disease and other neurodegenerative diseases.

Acknowledgment

Sabrina Whardhani would like to thank Universitas Pelita Harapan and Universitas Pancasila for their support of her study, also to the Food Quality Control Laboratory – UPH and Mochtar Riady Institute for Nanotechnology (MRIN) for the technical assistance during the process of testing the multiplex real-time RT-PCR for this research.

REFERENCES

1. Lleó A, Greenberg SM, Growdon JH. Current pharmacotherapy for alzheimer's disease. Annu Rev Med. 2006;57:513–33.
2. Palmer AM. Pharmacotherapy for alzheimer's disease: progress and prospects. Trends Pharmacol Sci. 2002;23(9):426–33.
3. Hardy J. A hundred years of alzheimer's disease research. Neuron. 2006;52(1):3–13.
4. Galimberti D, Scarpini E. Progress in alzheimer's disease. J Neurol. 2012;259(2):201–11.
5. Hampel H, Mesulam M, Cuello AC, Farlow MR, Giacobini E, Grossberg GT, et al. The cholinergic system in the pathophysiology and treatment of alzheimer's disease. 2020;2020(2018).
6. Singh M, Kaur M, Kukreja H, Chugh R, Silakari O, Singh D. Acetylcholinesterase inhibitors as alzheimer therapy: from nerve toxins to neuroprotection. Eur J Med Chem [Internet]. 2013;70:165–88. Available from: http://dx.doi.org/10.1016/j.ejmech.2013.09.050
7. Rioux S. Acetylcholinesterase inhibitors. Rev Med Suisse Romande. 2003;123(7):471–4.
8. Thapa K, Poudel M, Adhikari P. Moringa oleifera: A review article on nutritional properties and its prospect in the context of Nepal. Acta Scientific Agriculture. 2019;3(11):47–54.
9. Djamil R, Pratami DK, Putri FA, Octavia TB. Determination of quality parameters and test antioxidant activities of 70% ethanol extract of seroja leaves (Nelumbo nucifera gaertn.). International Journal of Applied Pharmaceutics. 2021 Dec;13(Special Issue 2):30–3.
10. Abcam. Acetylcholinesterase Assay Kit (Colorimetric). 2017;(October).
11. Departemen Kesehatan Republik Indonesia. Materia Medika Indonesia. 1995.

12. Departemen Kesehatan RI. Parameter Standar Umum Ekstrak Tanaman Obat. Vol. 1. 2000. p. 10–1.

13. Oyeniran OH. Comparative study of the phenolic profile, antioxidant properties, and inhibitory effects of Moringa (Moringa oleifera Lam.) and Almond (Terminalia catappa Linn .) leaves on acetylcholinesterase and monoamine oxidase activities in the head region of of Fruitfly (Drosophila melanogaster Meigen) in vitro. 2020;(May):1–10.

14. Koay YH, Basiri A, Murugaiyah V, Chan KL. Isocorilagin, a cholinesterase inhibitor from Phyllanthus niruri. Nat Prod Commun. 2014;9(4):515–7.

15. Alhibshi AH, Odawara A, Suzuki I. Neuroprotective efficacy of thymoquinone against amyloid beta-induced neurotoxicity in human induced pluripotent stem cell-derived cholinergic neurons. Biochem Biophys Rep [Internet]. 2019;17(January):122–6. Available from: https://doi.org/10.1016/j.bbrep.2018.12.005

16. Sultana S, Asif HM, Akhtar N, Iqbal A, Nazar H, Rehman RU. Nigella sativa: monograph. J Pharmacogn Phytochem. 2015;4(4):103–6.

17. Yimer EM, Tuem KB, Karim A, Ur-Rehman N, Anwar F. Nigella sativa L. (black cumin): a promising natural remedy for wide range of illnesses. Evidence-based Complementary and Alternative Medicine. 2019;2019.

18. Hostettmann K, Borloz A, Urbain A, Marston A. Natural product inhibitors of acetylcholinesterase. Curr Org Chem. 2006;10(8):825–47.