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Inhibition of Acetylcolinesterase Enzyme from Pegagan Herb Extract (*Centella asiatica*), Kelor Leaf (*Moringa oleifera*) Extract and Combinations

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Abstract: Alzheimer's disease (AD) is a disease in which brain damage is characterized by decreased attention, memory, and personality. AD is one result of impaired acetylcholine function. In this case, acetylcholinesterase (AChE) is an enzyme that functions as a catalyst for the breakdown of acetylcholine (ACh) into inactive forms acetate and choline. The purpose of this study was to determine the treatment activity of AD through AChE inhibition by Centella asiatica extract, Moringa oleifera extract and both of combinations. The activity test was carried out based on the Ellman method wherein this method was based on the hydrolysis ATCh substrate reaction by AChE with DTNB which gave a yellow color and measured its absorption at a wavelength of 410 nm. The results of the AChE inhibition test showed that the combination of pegagan herbs extract and kelor leaf extract 1: 1 gave the best inhibition with an IC50 value of 217.588 ppm. Meanwhile, Eserin positive control with an IC50 value of 5.010 ppm. The combination of Centella asiatica extract and Moringa oleifera extract has a synergistic effect on the treatment of AD.
Keywords: Alzheimer, acetylcholinesterase, Centella asiatica, Moringa oleifera, Ellman's method.

1 Introduction

Alzheimer's disease is brain damage characterized by decreased attention, memory and personality. Cognitive function in people with Alzheimer's disease does not disappear at one time. The first decreased functions are attention and memory. Personality changes often occur when the sufferer becomes less spontaneous, more apathetic, and withdrawn. Alzheimer's is caused by damage or death of brain cells and has not been cured until now, is progressive and lasts a long time. Usually a person is diagnosed at the age of 60 years, but even young individuals can experience it. This can lead to a total vegetative state and subsequently death [1].
Alzheimer's is a result of acetylcholine dysfunction. In this case, acetylcholinesterase (AChE) is an enzyme that functions as a catalyst for the breakdown of acetylcholine (ACh) into inactive forms, namely acetate and choline.

Measurement of AChE enzyme activity can describe the accumulation of ACh in the body, which in this result shows that Alzheimer's patients have greater acetylcholinesterase enzyme activity. The compounds that can inhibit the activity of the AChE enzyme illustrate that there is a potential as a basic limit for the manufacture of Alzheimer's drugs [2].
The usefulness of doneprezil, rivastigmine, and galanthamine in the treatment of patients with Alzheimer's disease has been conducted in a meta-analysis study. In that study, AChE inhibitors, for example, doneprezil, galanthamine and rivastigmine, were able to stabilize or slow down the decline in cognitive function, behavior, and overall changes compared to placebo drug administration. There is no clear evidence of any difference in the efficacy of these three drugs [3].
Ilkay Erdogan Orhan conducted a study on the AChE inhibitory activity of Centella asiatica where the results of the AChE inhibition test using the Ellman method showed that 96% ethanol extract showed AChE inhibition[4]. Research on the AChE inhibitory activity of Moringa oleifera found that the AChE test using the Ellman method showed that the ethanol extract of Moringa leaves has AChE inhibition with IC50 measurement. The content of compounds found in gotu kola herb is asiaticoside, while in

moringa leaves there is quercetin where this compound is thought to have efficacy on the treatment of AD [5].

Based on the research data, it is necessary to do research on the activity of gotu kola herb extract, Moringa leaf extract and 1: 1 combination in vitro as AChE inhibition and its inhibition kinetics. This study aims to obtain AChE inhibitory activity and its inhibition kinetics in AD disease models.

2 Materials and Methods

2.1 Materials and Instrument

The test material was gotu kola herb (*Centella asiatica*) obtained from the Center for Research and Development of Traditional Medicinal and Medicinal Plants, Tawangmangu, Central Java and was determined at the Center for Traditional Medicinal and Medicinal Plants, Tawangmangu, Central Java, 96% ethanol (sigma aldrich), methanol pa (Merck), DTNB ((5,5'-ditiobis- (2-nitrobenzoic acid) (Sigma aldrich), AchI (acetylcholine iodide) (Sigma aldrich), Bovine Serum Albumin 0.1% (Sigma aldrich) , AchE (acetylcholinesterase) (Sigma aldrich); Sodium hydroxide (Sigma aldrich), eserin (Sigma aldrich). The tools used in this study were rotary evaporator (IKA), freeze dry (New Brunswick), ELISA reader (ELx 800), Shaker (Innova 40), glassware, micro pipette (Eppendorf).

2.2 Extraction of Gotu Kola Herbs

Gotu kola herbs obtained were cleaned and then made a powder with a size of 40 mesh. The powder of gotu kola herb was then extracted by maceration method in 96% ethanol for eight days. After that, the concentration of the extract is carried out with a Rotary Evaporator to obtain a thick extract. After that, Freeze Drying is carried out to remove moisture content and maintain the quality of an extract to make it more stable in storage.

2.3 Moringa leaf Extraction

The moringa leaves obtained were put into a container and extraction was carried out in which the moringa leaf powder was dissolved with 96% ethanol for eleven days. After that, the maceration extract results were concentrated using a rotary evaporator and freeze drying to make the extract stable in storage.

2.4 AChE inhibitory Activity Test

Testing of AChE inhibitory activity using the Ellman Method. The extract test used ATCh substrate, DTNB color indicator, and AChE enzyme. Donepezil HCl was used as a comparison. The absorbance of the color of the reaction was measured at 30 minutes with a wavelength of 400 nm [6].

2.5 AChE Inhibition Kinetics Testing.

The ethanol extract of gotu kola herb, the ethanol extract of Moringa leaves, and their combinations made different concentrations of 1: 1, 1: 2, 1: 3, and 2: 1. The test sample is incubated for 10 to 30 minutes at room temperature, protected from light. Absorption measurements are made at a wavelength of 410 nm. Equations are made with the x-axis as 1 / Velocity (1 / V) and the y-axis as 1 / Substrate Concentration (1 / [S]) of each concentration of the test solution. The intersection point of the equation determines the kinetics of AChE-I. The point of intersection of the equation with the x-axis is -1 / Km. The equation's intercept with the y-axis is 1 / Vmax [6, 7, 8].

3 Results and Discussion

3.1 Extract Yield

The maceration results of the simplicia Centella asiatica using 96% ethanol solvent obtained a yield of 33.68%, while in the simplicia Moringa oleifera with 96% ethanol solvent the yield was 46.71%. The selection of 96% ethanol in the maceration process of these two simplicia is to attract the compounds contained in each of these simplicia.

Table 1: The yield extract of *Centella asiatica* Linn.

No	Sample	Weight(g)	Yield extract (%)*
1	Ethanolic extract	333,4	33,68

Note: *calculated against 990g dry simplicial

Table 2: The yield extract of *Moringa oleifera* Lam.

No	Sample	Weight (g)	Yield extract (%)*
1	Ethanolic extract	467,12	46,71

Note: *calculated against 1000g dry simplicia

3.2 Phytochemical Screening

Phytochemical screening was carried out to determine the class of compounds contained in the extracts obtained from Centella asiatica and Moringa oleifera. The results of the phytochemical screening test are shown in table 3

Table 3: Phytochemical Screening Results.

No	Compound content	Pegagan's extract	Kelor's extract
1	Alkaloid	+	+
2	Saponin	+	+
3	Tanin	+	+

4	Fenolik	+	+
5	Flavonoid	+	+
6	Glikosida	+	+
7	Triterpenoid	+	-
8	Steroid	+	+

In the results of phytochemical screening, it was found that the extract of gotu kola herb (Centella asiatica Linn.) Contained many chemical compounds such as alkaloids, saponins, tannins, phenolics, flavonoids, glycosides, steroids, and triterpenoids. The main compounds found in gotu kola herbs are triterpenoid compounds known as asiatic acid, madecasic acid, asiaticoside, madekasosid, madasiatic acid, betulinic acid, tankunic acid, and isotankunic acid. In addition, there are also several derivatives of flavonoid compounds such as quercetin, kaempferol, patuletin, routine, apigenin, and mirisetin found in this gotu kola herb [9].

In the leaf extract of Moringa oleifera Lam. Several chemical constituents were found such as alkaloids, saponins, tannins, phenolics, flavonoids, glycosides, and steroids. The main compound found in Moringa leaves is a flavonoid compound known as quercetin. In addition, there are also ingredients such as cinnamic acid, phytosterols, vanillin, catechins, and epicatechins [10].

3.3 AChE Inhibition Activity Testing

The AChE inhibitory activity was tested using a microplate reader. The results of the AChE inhibitory activity test by Centella asiatica extract, Moringa oleifera, and a 1: 1 combination are shown in Table 4 below:

Table 4: Results of the AChE inhibitory activity test.

No	sample	Regretion	IC_{50} (ppm)
1	Eserin	y = 5.0196x + 24.851	5.010
2	Pegagan ethanolic extract	y = 0.0146x + 20.631	2011.575
3	Kelor's leaves ethanolic extract	y = 0.0443x + 13.685	819.7517
4	Combination 1:1	y = 0.0568x + 37.641	217.588
5	Combinations 1:2	y = 0.0288x + 5.9241	1530.4
6	Combinations 1:3	y = 0.0376x + 10.882	1040.372
7	Combinations 2:1	y = 0.0111x + 27.496	2027.387

In table 2, the standard unit used is IC50 to determine the inhibitory activity of extracts in compounds that can be used as Alzheimer's drugs. In this study, the extract chosen was a 1: 1 combination of gotu kola herb and moringa leaf because it had the smallest IC50 value, which was 217.588 ppm.

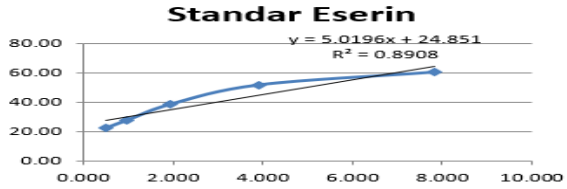

Fig. 1: Linear regression graph for the standard solution of Eserin.

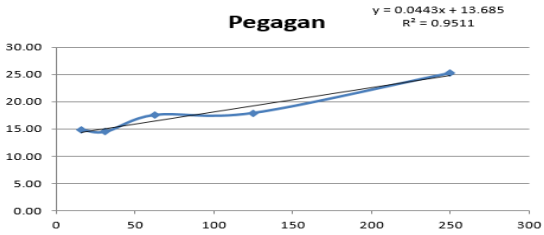

Fig.2: Graph of pegagan's Herb Extract Linear Regression.

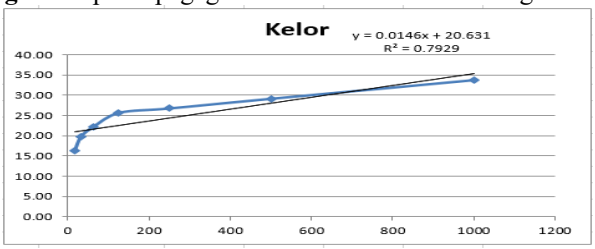

Fig. 3: Graph of Linear Regression of Moringa Leaf Extract.

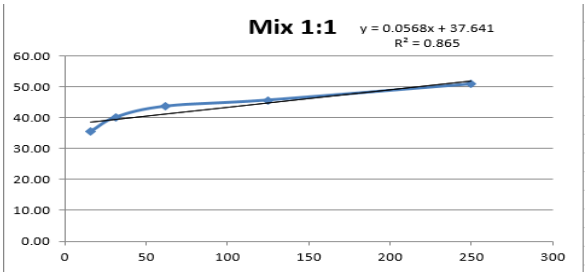

Fig. 4: Graph of 1: 1 Combination Linear Regression.

Fig. 5: Graph of 1: 2 Combination Linear Regression.

Fig. 6: Graph of 1: 3 Combination Linear Regression.

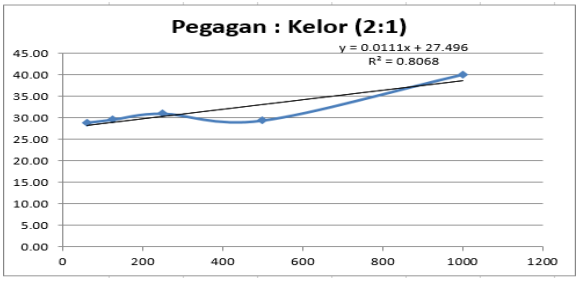

Fig. 7: Graph of 2: 1 Combination Linear Regression.

4 Conclusions

In the results of this study, it was found that a 1: 1 combination of gotu kola herb extract and Moringa leaf extract had an AChE inhibitory activity of IC50 217.588 ppm with a positive control of eserin at IC50 5.010 ppm.

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