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INTRODUCTION

Nowadays, the epidemiology study showed that over than 35 million people worldwide are affected by dementia[1] and prevalence is believed to be double every 20 years. Up to 70% of cases are Alzheimer's disease (AD). AD is a progressive neurodegenerative disease associated with oxidative stress[2]. That leads nerve cells to a degeneration and death which conduct to a deficiency of acetylcholine (ACh) in the brain, especially in the temporal lobe and hippocampus. As a result, patients are loss memory and function of daily living.

Corresponding to current therapy, donepezil, rivastigmine and galantamine are widely used to inhibit acetylcholinesterase (AChE) which is the hydrolyzed enzyme of ACh. However, these drugs still have some adverse effect such as excessive cholinergic stimulation with nausea, diarrhea, and vomiting[3].

Natural polyphenolic compounds can protect against various neurotoxic due to scavenging free radicals and increasing antioxidant capacities[2]. Several studies have shown that phenolic acids are efficient AChE inhibitors[4,5,6]. Gallic acid is one of potent antioxidative phenolic compound which widely found in plants. It would be interesting if gallic acid has a potential to inhibit AChE. Therefore, the aim of this study was to determine anti-AChE activity of gallic acid and its derivatives. Our result may provide some information for further development of Alzheimer's drugs.

MATERIALS AND METHODS

Chemicals: Gallic acid and iodomethane were purchased from Merck, German. Bromoethane was purchased from Fluka Chemika, Switzerland. *Electrophorus electricus* AChE, Acetylthiocholine iodide (ATCI) and 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co., USA. All other chemicals and reagents used in this study were of analytical grade and commercially available.

Synthesis of gallic acid derivatives: The alkylation and esterification of gallic acid were conducted. In alkylation reactions, 5 equiv of iodomethane and bromoethane were used to alkylated gallic acid in presence of 2.5 equiv $LiCO_3$ in DMF at room temperature for 3 days[7]. Additional, the esterification reactions were performed by using 1.5 and 3 equiv of dry methanol and dry ethanol, respectively, in presence of H_2SO_4 as a catalyst and refluxed at 75°C for 5 days. The products were checked by TLC and further purified by column chromatography. Structure elucidation was performed by using UV, IR, and NMR.

Structure identification: The spectral data form UV-Vis spectrophotometer (UV-2600, Bara Scientific), FT-IR (Nicolet 6700, Thermo Scientific) and NMR (Bruker) were used to determine the derivatives structures.

AChE inhibitory activity testing:

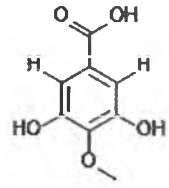
The AChE inhibition assay was measured using a microplate reader based on the modified Ellman method. Test samples and other chemicals were freshly prepared. Each sample was dissolved in DMSO to final concentration at 100 mcg/ml. Briefly, DTNB, ATCI and test solution in buffer were added into 96-well microplate and incubated for 5 min at 37°C. Then, the reaction was initiated by the addition of AChE. After 20 min incubation, absorbance was measured at wavelength 405 nm. The percentage of enzyme inhibition was calculated.

RESULTS

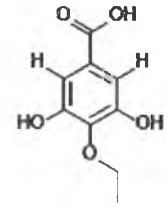
Synthesis of gallic acid derivatives:

The preparation of alkylated derivatives from gallic acid was performed by using alkyl halide and $LiCO_3$. Gallic acid reacted with 5 equiv of iodomethane, yield 4-*O*-methyl gallic acid (25.5% yield) and 3,5-di-*O*-methyl gallic acid (45.0% yield). Reaction of gallic acid with 5 equiv of bromoethane gave two ethylated compounds, 4-*O*-ethyl gallic acid and 3,5-di-*O*-ethyl gallic acid, with 45.7 and 11.5% yield, respectively. Esterification of gallic acid using MeOH and H_2SO_4 provided methyl gallate in good yield (55.3%). Similarly, EtOH and H_2SO_4 reacted with gallic acid, gave ethyl gallate in 58.7%yield. All gallic acid derivatives structures were shown in Figure 1.

Monosubstitutued alkylation

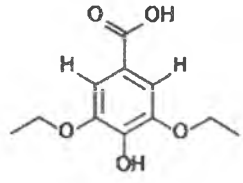


4-*O*-methyl gallic acid
(25.5%)

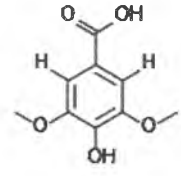


4-*O*-ethyl gallic acid
(45.7%)

Disubstituted alkylation

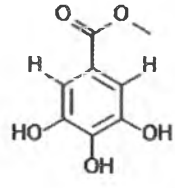


3,5-*O*-methyl gallic acid
(45.0%)

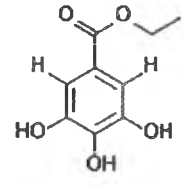


3,5-*O*-ethyl gallic acid
(11.5%)

Esterification



methyl gallate
(55.3%)



ethyl gallate
(58.7%)

Figure 1: Yield percentage (show in parentheses) and structure of gallic acid derivatives classified by substitution in gallic acid molecule

Acethylcholinesterase inhibitory activity testing:

The acetylcholinesterase inhibitory capacities of gallic acid and derivatives were depicted in Figure 2. Among all derivatives, ethyl gallate exhibited the most anti-AChE activity while 3,5-di-*O*-methyl gallic acid showed the least inhibitory activity. Interesting, ethyl derivatives of all groups tend to be more effective than methyl derivatives. However, starting material; gallic acid, and standard drug; galantamine, contained more inhibitory activity than all derivatives.

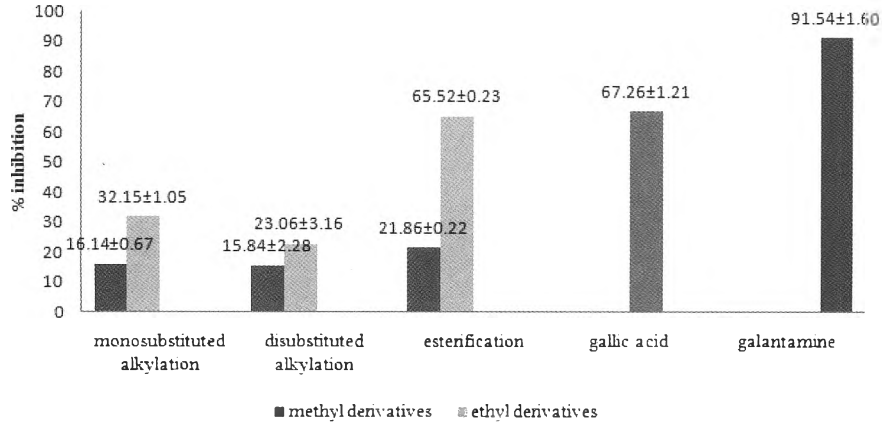


Figure 2: AChE inhibitory activity of galantamine, gallic acid and derivatives

DISCUSSION

According to the yield percentage as mentioned in the result, major product of methylation and ethylation reaction were 3,5-di-*O*-methyl gallic acid and 4-*O*-ethyl gallic acid, respectively.

Regarding the acyl pocket binding site of AChE, it may be possible that more hydrophobic property of derivatives would lead more binding capacity and inhibitory activity[8]. This is correspondence to what found in anti-AChE activity of ethyl derivatives which tend to be more effective than methyl derivatives. Further study about effect of molecular hydrophobicity on AChE inhibitory activity should be investigated to support and confirm result of this work.

CONCLUSION

Summarizing, six gallic acid derivatives, 4-*O*-methyl gallic acid, 3,5-di-*O*-methyl gallic acid, 4-*O*-ethyl gallic acid, 3,5-di-*O*-ethyl gallic acid, methyl gallate and ethyl gallate were synthesized and characterized their anti-AChE activities. Ethyl gallate is the most potent derivative which exhibiting 65.52% inhibition. The result from this study could be the basic knowledge for further development and discovery of Alzheimer's drugs.

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