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THE INHIBITORY EFFECTS ON ACETYLCHOLINESTERASE AND *α*-GLUCOSIDASE OF SOME COMPOUNDS FROM MEDICINAL PLANTS IN VIETNAM

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Summary

The Inhibitory Effects on Acetylcholinesterase and *α*-Glucosidase of some Compounds from Medicinal Plants in Vietnam

The study evaluates the inhibitory potential of several natural compounds on two enzymes, acetylcholinesterase and α-glucosidase, which are associated with Alzheimer's disease and diabetes. Six compounds from various medicinal plants in Vietnam, with a range of tested concentrations from 512 to 0.032 µg/mL, were used for bioassays, demonstrating varying degrees of enzyme inhibition. Specifically, for acetylcholinesterase, alpha- and beta-amyrin, 2*H*-chromen-2-one, and lupeol exhibited IC_{50} values of 348.70, 300.07, and 135.92 µg/mL, respectively. All six compounds showed IC_{25} values ranging from 65 to approximately 245 µg/mL. For *α*-glucosidase, GC1 and UGC9 showed IC_{50} values of approximately 69 and 295 µg/mL, respectively. The remaining compounds did not exhibit IC_{50} or IC_{25} values at the maximum tested concentration of 512 µg/mL. None of the six test samples caused hemolysis at the maximum tested concentration (2048 µg/mL), suggesting their safety. These results highlight the promising potential of these natural compounds for further research and therapeutic applications.

Keywords: *Acetylcholinesterase; α-Glucosidase; Inhibitory activity.*

1. Introduction

Acetylcholinesterase (AChE) and α-glucosidase are key enzymes involved in the regulation of neurotransmission and glucose metabolism, respectively. Inhibition of these enzymes has become a target for the treatment of diseases such as Alzheimer's disease and type 2 diabetes. Medicinal plants have been a rich source of bioactive compounds with potential therapeutic effects [1]. Vietnam, with its diverse flora, offers a wealth of medicinal plants that have long been used in traditional medicine. This article explores the inhibitory effects of various compounds from Vietnamese medicinal plants on AChE and α-glucosidase, shedding light on their potential as natural therapeutic agents. The compounds include GC1 (a mixture of alpha- and beta-amyrin) and GC16 (Kaempferitin) from *Gymnosporia chevalieri* [Unpublished], AL1 (2*H*-chromen-2-one) from *Aspidistra letrea* [2], UGC9 (Lupeol) from *Uvaria grandiflora* [3], UCE4I (Taraxerol) from *Uvaria cordata*, and LV1 (Asperuloside) from *Lasianthus verticillatus*. The findings aim to contribute to the growing interest in plant-based treatments for neurodegenerative and metabolic diseases.

2. Methods

2.1. Sample preparation

The purified samples were provided by the Faculty of Pharmacy, University of Medicine and Pharmacy, Hue University. The sample was extracted and isolated and its structure was determined using modern methods previously described [2],[3],[4]. The samples were prepared at a concentration of 20 mg/mL in dimethyl sulfoxide (DMSO) and then diluted in a suitable solvent for subsequent experiments.

2.2. AChE inhibitory activity

The AChE inhibitory activity of the samples was assessed using a modified Ellman's spectrophotometric method [5]. In a 96-well plate, 20 µL of different concentrations of test samples in phosphate buffer (pH 8.0), 20 µL of 19.2 mM 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB, TCI, Japan), 20 µL of 19.2 mM acetylthiocholine iodide (ATCI, Sigma-Aldrich, USA), 20 µL of 0.25 IU/mL acetylcholinesterase enzyme (AChE, Sigma-Aldrich, USA), and 120 µL of phosphate buffer (pH 8.0) were mixed. The mixture was incubated at room temperature for 30 minutes, and the absorbance was measured at 412 nm using a Varioskan Lux (Thermo Fisher). The AChE inhibition at concentrations ranging from 512 µg/mL to 0.032 µg/mL was calculated using the formula:

$$\textbf{Inhibition (\%)} = ((Ac - As) / Ac) \times 100$$

Where Ac refers to the absorbance of the control (ATCI, DTNB, and enzyme in buffer), and As refers to the absorbance of the test sample (or galantamine hydrobromide from TCI, Japan as a reference compound) with ATCI, DTNB, and enzyme in buffer.

2.3. α-Glucosidase assay

The α-glucosidase (CAS No 9001-42-7, Sigma-Aldrich, St. Louis, MO) inhibition assay was conducted as described previously [6]. The sample solution (dissolved in DMSO and distilled water to concentrations ranging from 512 to 0.032 µg/mL) and 0.2 U/mL α-glucosidase were mixed in 100 mM phosphate buffer (pH 6.8). Then, 2.5 mM p-nitrophenyl-α-D-glucopyranoside solution was added, and the mixture was incubated at 37°C for 30 minutes. The reaction was stopped with sodium carbonate, and the absorbance of released p-nitrophenol was measured at 412 nm using a Varioskan Lux (Thermo Fisher). Acarbose was used as a reference compound. The α-glucosidase inhibition was calculated as follows:

Inhibition (%) = ((Ac - As) / Ac) × 100

Where Ac refers to the absorbance of the control (p-nitrophenyl-α-D-glucopyranoside and enzyme in buffer), and As refers to the absorbance of the test sample or acarbose from Aksci (USA), p-nitrophenyl-α-D-glucopyranoside, and enzyme in buffer.

2.4. Hemolysis assay

Fresh rat blood was collected on heparinized centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min. The erythrocyte pellet was washed three times with an equal volume of NaCl 0.9%. The final erythrocyte pellet was suspended in NaCl 0.9% to make a 10% mouse red blood cell suspension. To each 1.5 mL Eppendorf tube, 100 µL of 10% mouse red blood cell suspension was mixed with 100 µL of each test sample and incubated at room temperature for 20 minutes. The mixture was then centrifuged at 6000 rpm for 2 minutes to separate the red blood cells, and the supernatant, containing hemoglobin from lysed cells, was collected. The supernatant was transferred to a 96-well plate, and absorbance was measured at 405 nm. Test samples were prepared at varying concentrations. Triton X100 (1%) from Sigma-Aldrich (USA) was used as a positive control, and 0.9% NaCl as a negative control [7]. The hemolysis percentage was calculated using the formula:

% Hemolysis = (A_sample / A_Triton) × 100

2.5. Data analysis

The experiment was conducted independently three times. Data were analyzed using GraphPad 4.0, and results are presented as mean ± SD. Inhibitory activity or hemolysis of each sample was expressed as the IC_{50} and IC_{25} or H_{50} and H_{25} values, representing the concentrations required to inhibit enzyme activity or hemolysis by 50% and 25%, respectively.

3. Results

3.1. Acetylcholinesterase (AchE) inhibition activity

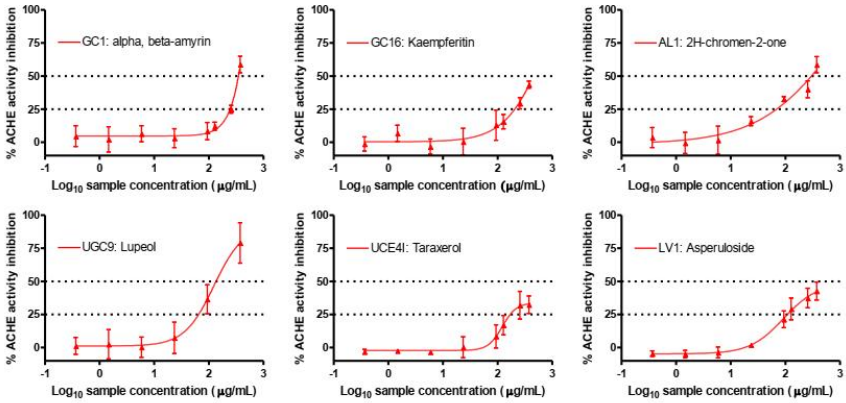

Fig. 1. AchE inhibition activity of the 6 compounds

Table 1. IC_{50} and IC_{25} of the 6 compounds on AchE

No	Sample	IC_{50} (µg/mL)	IC_{25} (µg/mL)
1	GC1: alpha-, beta-amyrin	348.70 (±2.35)	245.44 (±1.06)
2	GC16: Kaempferitin	> 512	206.09 (±0.57)
3	AL1: 2*H*-chromen-2-one	300.07 (±0.47)	69.09 (±0.31)
4	UGC9: Lupeol	135.92 (±0.47)	65.16 (±0.66)
5	UCE4I: Taraxerol	> 512	169.81 (±0.53)
6	LV1: Asperuloside	> 512	108.79 (±0.41)
7	Galantamine (Control)	0.77 (±0.10)	-

Based on the results in Fig. 1, we calculated the IC_{25} and IC_{50} values of the compounds (Table 1). The reference compound, galantamine, showed an IC_{50} value of about 0.77 µg/mL. Meanwhile, GC1, AL1, and UGC9 demonstrated IC_{50} values of 348.70, 300.07, and 135.92 µg/mL, respectively. IC_{25} is obtained from all six compounds, with concentrations ranging from 65 to approximately 245 µg/mL.

3.2. α-Glucosidase inhibition activity

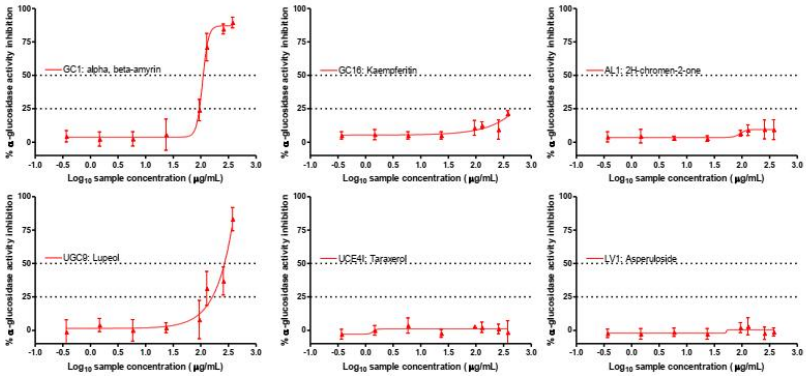

Fig. 2. α-glucosidase inhibition activity of the 6 compounds

Table 2. IC_{50} and IC_{25} of the 6 compounds on α-glucosidase

No	Sample	IC_{50} (µg/mL)	IC_{25} (µg/mL)
1	GC1: alpha-, beta-amyrin	68.83 (±1.05)	33.52 (±0.76)
2	GC16: Kaempferitin	> 512	>512
3	AL1: 2*H*-chromen-2-one	>512	>512
4	UGC9: Lupeol	294.89 (±1.23)	132.74 (±0.60)
5	UCE4I: Taraxerol	>512	>512
6	LV1: Asperuloside	>512	>512
7	Acarbose (control)	213.1 (±3.21)	-

Based on the results in Fig. 2, we calculated the IC_{25} and IC_{50} values of the compounds (Table 2). The reference compound, acarbose, showed an IC_{50} value of about 213 µg/mL. GC1 and UGC9 showed IC_{50} values of approximately 69 and 295 µg/mL, respectively, and IC_{25} values of approximately 33 and 133 µg/mL. The remaining compounds did not exhibit IC_{50} or IC_{25} values at the maximum tested concentration of 512 µg/mL.

3.3. Hemolysis

None of the six test samples caused hemolysis at the maximum tested concentration (2048 µg/mL) (**Fig. 3**)

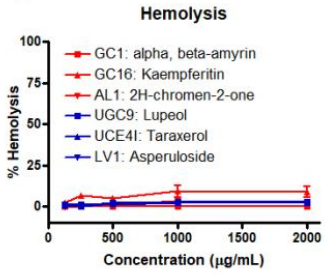

Fig. 3. Hemolytic activity of the 6 compounds

4. Discussion

This study evaluates the inhibitory potential of six compounds on two enzymes, acetylcholinesterase and α-glucosidase, which are associated with Alzheimer's disease and diabetes. The samples exhibited AChE inhibitory activity, with 3 out of 6 compounds showing IC_{50} values, specifically GC1, AL1, and UGC9, with IC_{50} values of 348.70, 300.07, and 135.92, respectively. All six compounds demonstrated IC_{25} values, with concentrations ranging from 65 to approximately 245 µg/mL. The inhibitory effect on α-glucosidase was more limited, with only GC1 and UGC9 showing IC_{50} values of approximately 69 and 295 µg/mL, respectively. The results indicate that GC1 and UGC9 have strong activity against both enzymes, consistent with several previous reports.

GC1 (α- and β-amyrin) have shown a range of pharmacological activities in both *in vitro* and *in vivo* studies. β-Amyrin (at 4 mg/kg) improved

scopolamine-induced memory impairment in mice and demonstrated acetylcholinesterase inhibitory activity [8]. The α- and β-amyrin isolated from the stem bark of *Vitex glabrata* R. Br. were also evaluated and exhibited strong inhibitory activity against the enzymes α-glucosidase and α-amylase [9].

UGC9 (Lupeol), a pentacyclic triterpenoid found in plants, fruits, and vegetables, displayed acetylcholinesterase inhibitory activity in LPS-induced neuroinflammation and amyloid beta model in the adult mouse hippocampus [10]. Lupeol isolated from *Vitex glabrata* R. Br. stem bark was also evaluated and showed strong inhibitory activity against α-glucosidase and α-amylase enzymes [9].

Although there are no published studies on the effects of AL1 (2*H*-chromen-2-one) from natural sources on α-glucosidase and acetylcholinesterase, several studies on chemically synthesized coumarin-chalcone derivatives containing the 2*H*-chromen-2-one group have shown strong inhibitory effects on α-glucosidase [11].

None of the six test samples caused hemolysis at the maximum tested concentration. This result suggests that these samples are safe at concentrations effective in inhibiting the enzymes. These findings highlight the potential for further research and application of these compounds in chronic diseases such as diabetes and Alzheimer's.

5. Conclusion

The study evaluated the inhibitory potential effects of six compounds on acetylcholinesterase and α-glucosidase enzymes, with varying levels of inhibition. Among them, GC1 and UGC9 showed good activity against both enzymes. For the samples with determined IC_{50} values, these were all lower than the concentrations that caused hemolysis, partly indicating their safety. These results show promising prospects for further research and applications.

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