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Anti-cholinesterase Activities of Hydrolysable Tannins and Polyhydroxytriterpenoid Derivatives from *Terminalia chebula* Retz. Fruit

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Abstract: In the present study, 48 hydrolysable tannins and 12 polyhydroxytriterpenoid derivatives were isolated from *Terminalia chebula* fruit and assessed for their inhibitory activities on cholinesterases *in vitro*. Among them, phyllanemblinin F (compound **35**), chebulanin (**36**), 23-Galloyl arjunolic acid (**55**), and Arjunetin (=24-deoxy sericoside) (**56**) showed strong inhibition against acetylcholinesterase (AChE) with IC_{50} values of 24.02, 21.36, 67.25, and 47.85 μM, respectively. Though corilagin (**15**) and several gallotannins (**8, 12, 14**) exhibited weak inhibitory activity against butyrylcholinesterase, the majority of compounds from *T. chebula* showed inhibition of AChE. Since cognitive dysfunction is closely related to diminution of cholinergic transmission, our results suggest that compounds from *T. chebula* could be used as potential treatment for dementia.

Keywords: *Terminalia chebula*; anti-cholinesterase; dementia.

1. Plant Source

Terminalia chebula fruits were obtained from Chong Kun Dang Pharmaceutical Corporation (Chong Kun Dang Co., Seoul, South Korea). A voucher specimen is deposited in the Herbarium in the Medicinal Plant Garden (Seoul National University, Seoul, South Korea) under number SUPH-2015-01. Sixty compounds were isolated from *T. chebula* fruit and investigated the inhibitory activities on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).

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2. Previous Studies

As a traditional medicinal plant, *Terminalia chebula* Retz. (Combretaceae) has been widely used to treat stomach ulcers and diabetes in Asia and India [1]. *T. chebula* extract has been reported to possess anti-oxidative, anti-inflammatory, and anti-cell death effects [2, 3]. In recent years, several studies have demonstrated *T. chebula*'s anti-cholinesterase (ChE) properties *in vitro* [4-8]. For example, methanolic extract of *T. chebula* at a concentration of 5 mg/mL reduced the activity of AChE and BChE by 89% and 95%, respectively [5]. A similar study using methanolic extract of *T. chebula* reported that its IC_{50} value was 180 ± 14.6 μg/mL [6]. On the other hand, the results from *in vitro* experiments done by Vinutha demonstrated that aqueous extract of *T. chebula* exhibited more inhibitory activity against AChE than the methanolic extract at a concentration of 100 μg/mL [7]. Another group showed that ethyl acetate fraction of *T. chebula* with a dose of 25 mg/mL reduced the AChE activity to 62.32% of the control [8]. However, these studies have mainly focused on anti-ChE properties of crude extract or fraction from *T. chebula*, while those of the active components in *T. chebula* have not yet been fully investigated. Since ChE can induce neurodegeneration, inhibition of ChE results in neuroprotective effects [9]. Thus, the present study aimed to isolate and investigate the active constituents from *T. chebula* responsible for anti-ChE activities.

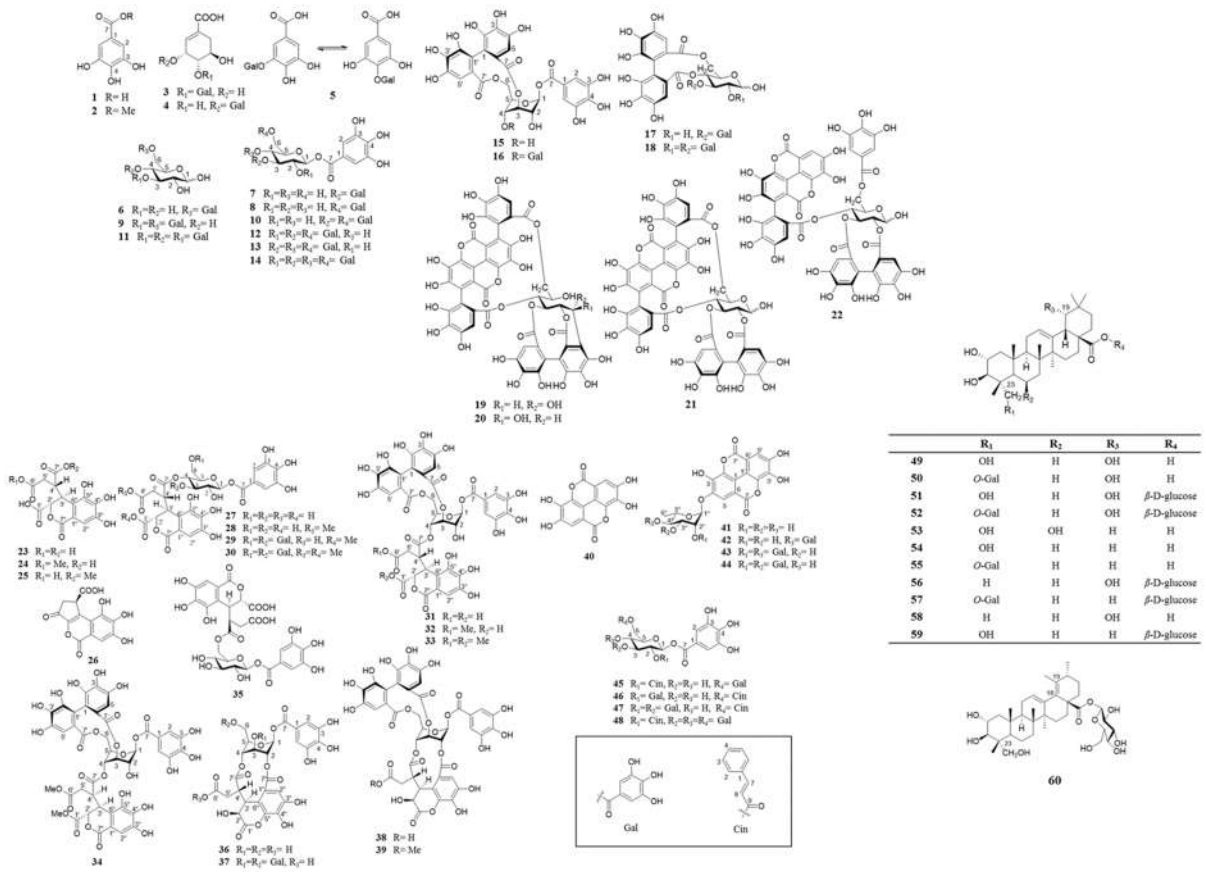

Figure 1. Structures of the compounds **1 – 60** from *T. chebula*

3. Present Study

Extraction: The dried fruits of *T. chebula* (1.8 kg) were powdered and macerated with MeOH at room temperature (8 L × 2 for 12 h × 2). The extracts were then filtered and evaporated *in vacuo*. Finally, dried total extracts (652.3 g) were obtained and stored in a freezer.

Table 1. Inhibitory activity of the compounds from *T. chebula* against AChE and BChE.

Compounds	% inhibition at 10 μM		IC_{50} values (μM)
	AChE	BChE	AChE
Gallic acid **(1)**	— [a]	6.7 ± 1.1	NT [b]
Methyl gallate **(2)**	—	7.6 ± 1.2	NT
4-*O*-Galloyl-(-)-shikimic acid **(3)**	—	6.7 ± 1.0	NT
5-*O*-Galloyl-(-)-shikimic acid **(4)**	—	5.7 ± 0.6	NT
Digallic acid **(5)**	—	6.5 ± 0.4	NT
6-*O*-Galloyl-D-glucose **(6)**	—	3.1 ± 0.3	NT
1,3-Di-*O*-galloyl-*β*-D-glucose **(7)**	—	1.6 ± 0.1	NT
1,6-Di-*O*-galloyl-*β*-D-glucose **(8)**	—	18.9 ± 0.9	NT
3,6-Di-*O*-galloyl-D-glucose **(9)**	—	15.2 ± 0.7	NT
1,3,6-Tri-*O*-galloyl-*β*-D-glucose **(10)**	—	5.9 ± 0.6	NT
3,4,6-Tri-*O*-galloyl-D-glucose **(11)**	—	7.7 ± 0.3	NT
1,2,3,6-Tetra-*O*-galloyl-*β*-D-glucose **(12)**	—	17.2 ± 0.1	NT
1,3,4,6-Tetra-*O*-galloyl-*β*-D-glucose **(13)**	—	15.5 ± 0.2	NT
1,2,3,4,6-Penta-*O*-galloyl-*β*-D-glucose **(14)**	17.9 ± 4.0	20.3 ± 0.3	NT
Corilagin **(15)**	—	20.3 ± 0.4	NT
Tercatain **(16)**	7.2 ± 0.7	10.5 ± 0.4	NT
Gemin D **(17)**	0.5 ± 1.2	—	NT
Telimagrandin I **(18)**	18.8 ± 3.7	—	NT
Punicacortein C **(19)**	17.0 ± 2.0	2.9 ± 0.3	NT
Punicacortein D **(20)**	13.8 ± 0.2	—	NT
Punicalagin **(21)**	7.1 ± 3.9	3.0 ± 0.1	NT
Terflavin A **(22)**	23.1 ± 6.9	4.2 ± 0.1	NT
Chebulic acid **(23)**	22.1 ± 6.6	1.5 ± 0.1	NT
11-Methyl chebulate **(24)**	17.3 ± 7.2	—	NT
13-Methyl chebulate **(25)**	22.6 ± 9.4	—	NT
Brevifolincarboxylic acid **(26)**	19.2 ± 2.7	—	NT
Phyllanemblinin E **(27)**	16.4 ± 6.4	—	NT
1′-*O*-Methyl neochebulanin **(28)**	19.9 ± 2.7	—	NT
1′-*O*-Methyl neochebulinate **(29)**	12.8 ± 3.6	—	NT
Dimethyl neochebulinate **(30)**	28.4 ± 2.2	—	NT
Neochebulagic acid **(31)**	25.4 ± 9.6	—	NT
6′-*O*-Methyl neochebulagate **(32)**	35.7 ± 10.4	—	>200
Dimethyl neochebulagate **(33)**	32.6 ± 6.6	—	>200
Dimethyl 4′-epi-neochebulagate **(34)**	37.9 ± 3.1	1.3 ± 0.5	>200
Phyllanemblinin F **(35)**	44.4 ± 5.6	—	24.02 ± 2.1
Chebulanin **(36)**	47.7 ± 5.8	—	21.36 ± 2.7

Chebulinic acid **(37)**	—	—	NT
Chebulagic acid **(38)**	—	—	NT
Methyl chebulagate **(39)**	9.0 ± 6.0	—	NT
Ellagic acid **(40)**	5.9 ± 10.3	—	NT
Eschweilenol C **(41)**	11.3 ± 2.0	5.7 ± 0.6	NT
4-*O*-(4"-*O*-Galloyl-*α*-L-rhamnopyranosyl)ellagic acid **(42)**	16.2 ± 6.1	2.4 ± 0.3	NT
4-*O*-(3",4"-Di-*O*-galloyl-*α*-L-rhamnopyranosyl)ellagic acid **(43)**	22.2 ± 6.0	3.7 ± 0.3	NT
4-*O*-(2",4"-Di-*O*-galloyl-*α*-L-rhamnopyranosyl)ellagic acid **(44)**	17.8 ± 2.5	1.6 ± 0.1	NT
2-*O*-Cinnamoyl-1,6-di-*O*-galloyl-*β*-D-glucose **(45)**	—	2.1 ± 0.3	NT
1,2-Di-*O*-galloyl-6-*O*-cinnamoyl-*β*-D-glucose **(46)**	—	1.8 ± 0.1	NT
1,2,3-Tri-*O*-galloyl-6-*O*-cinnamoyl-*β*-D-glucose **(47)**	8.2 ± 2.6	—	NT
1,3,4,6-Tetra-*O*-galloyl-2-*O*-cinnamoyl-*β*-D-glucose **(48)**	—	6.9 ± 0.3	NT
Arjungenin **(49)**	—	6.7 ± 0.1	NT
23-Galloyl arjunic acid **(50)**	—	6.7 ± 0.2	NT
Arjunglucoside I **(51)**	27.4 ± 1.1	5.7 ± 0.3	NT
Quercotriterpenoside I **(52)**	30.5 ± 6.8	6.2 ± 0.5	>200
Terminolic acid **(53)**	29.6 ± 0.2	4.7 ± 0.3	189.59 ± 5.6
Arjunolic acid **(54)**	39.2 ± 7.1	4.7 ± 0.4	126.63 ± 2.0
23-Galloyl arjunolic acid **(55)**	45.3 ± 4.3	2.0 ± 0.3	67.25 ± 1.2
Arjunetin (=24-deoxy sericoside) **(56)**	42.4 ± 3.2	8.0 ± 0.2	47.85 ± 0.7
23-Galloylarjunolic acid 28-*O*-*β*-D-glucopyranosyl ester **(57)**	30.3 ± 4.1	6.5 ± 0.1	117.17 ± 0.7
Arjunic acid **(58)**	31.6 ± 3.5	6.6 ± 0.1	>200
Arjunglucoside II **(59)**	9.9 ± 2.1	5.4 ± 0.2	NT
Pinfaenoic acid 28-*O*-*β*-D-glucopyranosyl ester **(60)**	15.1 ± 2.7	3.9 ± 0.2	NT
Donepezil [c]	97.2 ± 0.6	NT	0.099 ± 0.01
Galantamine [d]	NT	36.2 ± 0.5	NT

Data are presented as mean ± SD.
[a] —: not detected. [b] NT: Not tested. [c] Standard drug for AChE. [d] Standard drug for BChE.

Isolation: The extracts were suspended in distilled H_2O (4 L) and successively fractionated to afford *n*-hexane, $CHCl_3$, and *n*-BuOH extracts. The $CHCl_3$ fraction was subjected to silica gel column chromatography ($CHCl_3$—MeOH; 100:0 → 50:50 → 0:100) to obtain 11 sub-fractions. The *n*-BuOH fraction was subjected to HP-20 resin column chromatography (H_2O—MeOH; 75:25 → 50:50 → 25:75 → 0:100) to obtain four sub-fractions. Detailed information on the isolation from each sub-fraction was provided previously [10, 11]. From the extract of *T. chebula*, 48 tannins and 12 polyhydroxytriterpenoid derivatives were isolated and named **1–60** (Figure 1).

ChE inhibitory activity assay: To determine whether compounds from *T. chebula* have anti-ChE activity, we measured inhibitory effects of isolated compounds on AChE and BChE using an *in vitro* assay. The inhibitory activities against AChE and BChE were summarized in Table 1. We calculated IC_{50} values of compounds for ChE inhibition when the inhibitory activity is over 30 % at 10 μM.

The positive controls, donepezil and galantamine, showed significant inhibition of ChE. As seen in Figure S2, compounds exhibited considerable variations in ChE inhibition, owing to their structural differences. Most compounds showed inhibition of AChE but not inhibition of BChE. Although 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose (**14**) showed moderate AChE inhibitory activity (17.9% at 10 μM), most simple gallotannins and gallic acid (**1–13**) did not inhibit AChE. Conversely, di-, tetra-, and penta-galloyl glucose (**8, 9,** and **12–14**) showed moderate BChE inhibition (15.2–20.3% at 10 μM). Interestingly, compound **14,** which is the only compound from *T. chebula* reported in the literature to demonstrate ChE inhibition, showed dual AChE and BChE inhibition [5]. Non-chebulic ellagitannins (**15–22**) and chebulic acid derivatives (**23–25**) showed weak or moderate inhibition against AChE, except for coraligin, but no inhibition against BChE (**15**).

Amongst the hydrolysable tannins, phyllanemblinin F (**35**) and chebulanin (**36**) had strong inhibitory effects on AChE, with 44.4 and 47.7% inhibition at 10 μM, respectively. Their IC_{50} values were 24.02 and 21.36 μM, and their dose to inhibition graph was described in Figure 2. They have chebulic acid containing glucose in their structures, but their anti-AChE activity was about two times higher than that of chebulic acid alone (**23**), suggesting that chebulic acid combined with glucose may play a significant role in AChE inhibition. In addition, chebulinic acid (**37**), which has two more galloyl units than chebulanin and chebulagic acid (**38**) and contains hexahydroxyphenyl (HHDP), showed no inhibitory activity on AChE. These results indicate that additional galloyl units and HHDP do not have positive influences on AChE inhibitory activity. Rhamnosylated ellagic acid derivatives (**41–44**) and cinnamoyl-containing gallotannins (**45–48**) had low inhibitory activity against AChE and BChE.

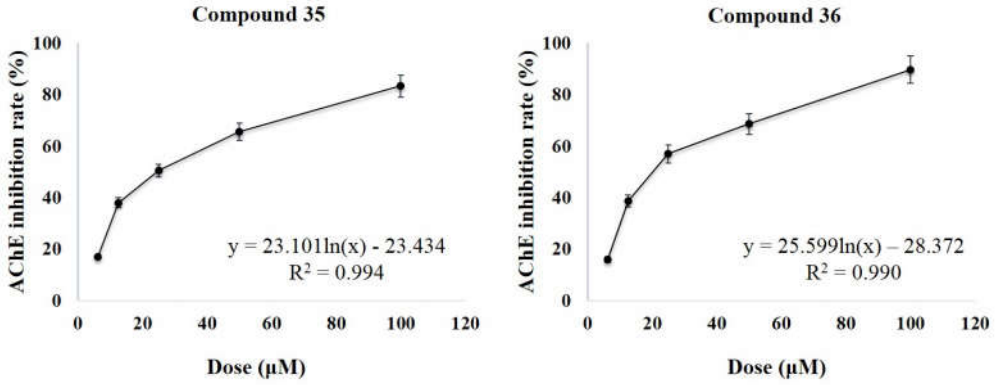

Figure 2. Effects of compounds **35** and **36** on AChE inhibitory activity at various concentrations

Polyhydroxytriterpenoid derivatives (**49–60**) isolated from *T. chebula* exhibited diverse degrees of ChE inhibition. Among them, 23-galloyl arjunolic acid (**55**) and arjunetin (**56**) were identified as the most active AChE inhibitors, with 45.3 and 42.4% inhibition at 10 μM, respectively (IC_{50}: 67.25 and 47.85 μM). The 23-galloyl arjunolic acid compound (**55**) possessing a galloyl unit at C23 displayed much greater inhibitory activity against AChE compared with that of arjunolic acid (**54**). By contrast, compounds **57** and **59**, with an additional glucose at C28, showed weaker AChE inhibitory activity than that of **54**. These results indicate that the presence of a galloyl unit at C23, as in **54**, enhanced AChE inhibition, while glucose at C28 seemed to reduce AChE inhibitory potency.

As a conclusion, we investigated 60 compounds from *T. chebula* for their inhibitory activities affecting ChEs. Compounds **35**, **36**, **55,** and **56** exhibited strong AChE inhibition, and compounds **8, 12** and **15** showed weak BChE inhibition. In addition, compound **14** displayed dual inhibitory activities against AChE and BChE. Meanwhile, the majority of compounds isolated from *T. chebula* showed potent inhibition of AChE compared to that of BChE. These results suggest that *T. chebula* could be a potential source of therapeutic compounds for the prevention and treatment of dementia.

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Supporting Information

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