



## Evaluating the acetylcholinesterase inhibitory activity of *Ferulago angulata* and *Ferulago subvelutina*

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### Abstract

Alzheimer is an age-dependent disease mostly with genetic origin. In this disorder, acetylcholine is decreased which results in deterioration of short-term memory. Many researches have been focused on finding new sources of medications with more potency and less side effects and investigating acetylcholinesterase inhibitors (AChEIs) has been the center of many researches. In the present study, the acetylcholinesterase inhibitory property of the total extracts and fractions of *Ferulago angulata* and *Ferulago subvelutina* have been evaluated according to Ellman method. The total extract and the 50% methanol fraction of *Ferulago angulata* demonstrated weak AChEI activity (15.8% and 9% in concentration of 300 µg/mL, respectively). The ability of the total extract and the *n*-hexane, dichloromethane, ethyl acetate and 50% methanol fractions of *Ferulago subvelutina* were found to be 19.7%, 15.4%, 32.2%, 14.5% and 11.7%, respectively. It was concluded that among the evaluated extracts and fractions, the dichloromethane fraction of *Ferulago subvelutina* demonstrated reasonable AChEI activity and it is suggested for further purification of its components.

**Keywords:** acetylcholinesterase, Alzheimer, Ellman assay, *Ferulago angulata*, *Ferulago subvelutina*

### Introduction

Acetylcholinesterase inhibitors (AChEIs) or anticholinesterases, have the ability to inhibit the hydrolysis of the neurotransmitter called acetylcholine and elevate its level in the synaptic cleft [1]. Currently, several AChEIs, such as donepezil, galantamine and rivastigmine are available for the symptomatic treatment of patients with mild to moderate Alzheimer disease

(AD) [2], which is the most common senile dementia affecting millions of people worldwide [3].

Plants secondary metabolites have been used as inhibitors of various classes of enzymes. Several thousand plant extracts have been screened against AChE from different parts of the world [4]. Within the structure diversity of the AChEIs,

plant alkaloids are the most studied already leading to the development of new drugs [5]. *Ferulago angulata* (Schlecht.) Boiss. and *F. subvelutina* Rech. f. (Apiaceae) are members of a genus with seven growing species in Iran [6]. The two mentioned species have been the center of some biological investigations before and in the present study, their ability to inhibit the acetylcholinesterase enzyme has been evaluated by the Ellman method.

## Experimental

### *Chemicals and Reagents*

Acetylcholinesterase (AChE) was purchased from Sigma (Germany). Acetylthiocholin iodide (ATCI) was prepared from Fluka (Germany). 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and other chemicals and solvents were provided from Merck (Germany).

### *Plant material*

The whole plant of *Ferulago angulata* and the aerial parts of *F. subvelutina* were collected from Kohgiluyeh va Boyer Ahmad and Khorasan-e-Razavi provinces of Iran, respectively. They were identified by the botanists of the Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences and their voucher specimens were deposited at TMRC Herbarium for future reference (No. 2800 TMRC and No. 3578 TMRC, for *Ferulago angulata* and *F. subvelutina*, respectively). The plant materials were dried in shade and ground.

### *Extraction*

100 g of the powdered *Ferulago angulata*/*F. subvelutina* was macerated with methanol 80% at room temperature for 4 days. The mixture was filtered every day and the solvent was refreshed. The filtrate was concentrated, dried and further used in Ellman assay.

### *Fractionation*

100 g of the dried powder of each plant was macerated with *n*-hexane at room temperature for

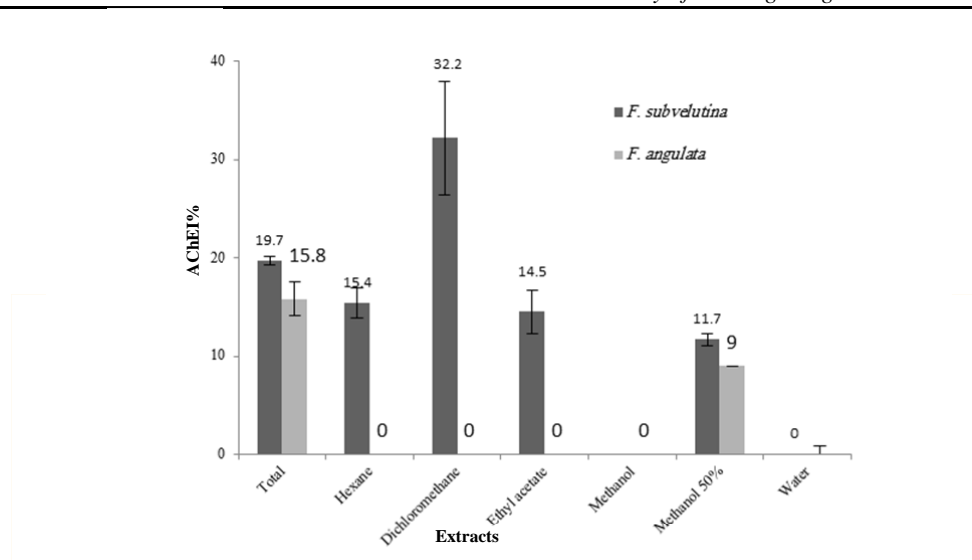
4 days. At the end of the fourth day, the residue of the plant was macerated with dichloromethane and the same procedure continued. The process was repeated with ethyl acetate, methanol, methanol 50% and water, successively and finally, the concentrated and dried fractions were used in acetylcholinesterase inhibitory assay.

### *Ellman assay*

In Ellman assay, the reaction of thiocholine (one of the products of enzymatic hydrolysis of ATCI) with DTNB (Ellman's reagent) forms a yellow product (5-mercapto-2-nitrobenzoic acid and its dissociated forms) at pH 8 which can be detected at 405 nm [7]. The method was established by Ellman *et al.*, to evaluate the cholinesterase activity *in vitro* [8]. In the present study, the above assay was conducted in 96-well plates [9-11]. The samples were dissolved in methanol (3000 µg/mL). 125 µL of 3 mM DTNB, 25 µL of 15 mM ATCI, 50 µL of phosphate buffer (pH 8), and 25 µL of the sample dissolved in methanol were added in wells of 96-well plates. The absorbance was recorded at 405 nm every 13 s for 65 s. 25 µL of 0.22 U/mL of AChE enzyme was then added and the absorbance was again measured every 13 s for 104 s using a TECAN ELISA reader at 405 nm. Absorbance vs time was plotted and the rate of the enzyme activity was compared to an assay using methanol without inhibitor. Any increase in the absorbance due to the spontaneous hydrolysis of substrate was corrected by subtracting the rate of the reaction before adding the enzyme from the rate after adding the enzyme. Inhibition percentage was obtained by comparing the rates of the sample to the blank (MeOH). Donepezil was used as the positive control.

## Results and Discussion

The results of the AChEI activity of *Ferulago angulata* and *F. subvelutina* extracts/fractions are demonstrated in figure 1.



**Figure 1.** AChEI activity of the extracts/fractions of *Ferulago angulata* and *F. subvelutina* in concentration of 300  $\mu\text{g/mL}$

*F. subvelutina* total extract demonstrated more ability to inhibit the acetylcholinesterase enzyme compared to *Ferulago angulata* and its dichloromethane fraction exhibited the most AChEI activity among all tested samples. The plants of *Ferulago* genus contain various secondary metabolites mostly coumarins which have different biological activities such as AChEI properties. The most investigations on AChEIs have been focused on alkaloids. More than 35 alkaloids have been purified from plants so far with AChEI activity [9] but there are a few reports about AChEI activity of other secondary metabolites. In a study, coumarins isolated from *Ferulago campestris* have demonstrated antioxidant, anti-bacterial, cytotoxic and anti-cholinesterase activities which is of interest because of their non-alkaloidal structure [12]. It has been established that three furanocoumarins of isoimperatorin, imperatorin and oxypeucedarin had anti-cholinesterase activity [13]. In addition, during an investigation on some coumarins with MAO A&B inhibitory activity, it was found that all examined coumarins had AChEI properties as well. It was suggested that AChE and MAO

inhibition may decrease  $\beta$ -amyloid peptide decomposition which is important in Alzheimer [14]. In another study, the anti-amnesic activity of decursin, a coumarin, has been evaluated *in vivo* using ICR mice with amnesia induced by scopolamine. It significantly ameliorated scopolamine-induced amnesia as measured in both the passive avoidance test and the Morris water maze test. Moreover, decursin significantly inhibited AChE activity by 34% in the hippocampus of the treated mice. The researchers had concluded that decursin may exert anti-amnesic activity *in vivo* through inhibition of AChE activity in the hippocampus [15]. AChEI property of coumarins has made them a good choice for derivatization in order to increase their effects. It has been proved that coumarin analogues with phenylpiperazine groups on position 3 and/or 4 have considerable anti-AChE activity [16].

These results have opened a new aspect for searching medicinal plants containing coumarins for treating Alzheimer. There is no previous report of investigating the AChEI activity of *F. angulata* or *F. subvelutina*. In the present study

the dichloromethane fraction of *F. subvelutina* demonstrated the most AChEI activity. Regarding the previous reports about the acetylcholinesterase inhibitory activity of coumarins, it could be proposed that the coumarins might be responsible for the AChEI activity observed in the present study, since coumarins are semi-polar compounds and they are abundant in the dichloromethane fraction which was the most active fraction of the examined samples. Further isolation of the active constituents of the dichloromethane fraction of *F. subvelutina* is necessary for examining this hypothesis.

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