

## A Preliminary in vitro Investigation of the Acetylcholinesterase and $\alpha$ -Amylase Inhibition Potential of Pomegranate Peel Extracts

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**Abstract :** The increasing prevalence of Alzheimer's disease (AD) and diabetes mellitus (DM) constitutes them major global health problems. Recently, the inhibition of key enzyme activity is considered a potential treatment of both diseases. Specifically, inhibition of acetylcholinesterase (AChE), the key enzyme involved in the breakdown of the neurotransmitter acetylcholine, is a promising approach for the treatment of AD, while inhibition of  $\alpha$ -amylase retards the hydrolysis of carbohydrates and, thus, reduces hyperglycemia. Unfortunately, commercially available AChE and  $\alpha$ -amylase inhibitors are reported to possess side effects. Consequently, there is a need to develop safe and effective treatments for both diseases. In the present study, pomegranate peel (PP) was extracted using various solvents of increasing polarity, while two extraction methods were employed, the conventional maceration and the ultrasound assisted extraction (UAE). The concentration of bioactive phytoconstituents, such as total phenolics (TPC) and total flavonoids (TFC) in the prepared extracts was evaluated by the Folin-Ciocalteu and the aluminum-flavonoid complex method, respectively. Furthermore, the anti-neurodegenerative and anti-hyperglycemic activity of all extracts was determined using AChE and  $\alpha$ -amylase inhibitory activity assays, respectively. The inhibitory activity of the extracts against AChE and  $\alpha$ -amylase was characterized by estimating their  $IC_{50}$  value using a dose-response curve, while galanthamine and acarbose were used as positive controls, respectively. Finally, the kinetics of AChE and  $\alpha$ -amylase in the presence of the most inhibitory potent extracts was determined by the Lineweaver-Burk plot. The methanolic extract prepared using the UAE contained the highest amount of phytoconstituents, followed by the respective ethanolic extract. All extracts inhibited acetylcholinesterase in a dose-dependent manner, while the increased anticholinesterase activity of the methanolic ( $IC_{50} = 32 \mu\text{g/mL}$ ) and ethanolic ( $IC_{50} = 42 \mu\text{g/mL}$ ) extract was positively correlated with their TPC content. Furthermore, the activity of the aforementioned extracts was comparable to galanthamine. Similar results were obtained in the case of  $\alpha$ -amylase, however, all extracts showed lower inhibitory effect on the carbohydrate hydrolyzing enzyme than on AChE, since the  $IC_{50}$  value ranged from 84 to 100  $\mu\text{g/mL}$ . Also, the  $\alpha$ -amylase inhibitory effect of the extracts was lower than acarbose. Finally, the methanolic and ethanolic extracts prepared by UAE inhibited both enzymes in a mixed (competitive/noncompetitive) manner since the  $K_m$  value of both enzymes increased in the presence of extracts, while the  $V_{max}$  value decreased. The results of the present study indicate that PP may be a useful source of active compounds for the management of AD and DM. Moreover, taking into consideration that PP is an agro-industrial waste product, its valorization could not only result in economic efficiency but also reduce the environmental pollution.

**Keywords :** acetylcholinesterase, Alzheimer's disease,  $\alpha$ -amylase, diabetes mellitus, pomegranate

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