

Isolation and characterization of acetylcholinesterase inhibitors from *Piper longum* Linn.

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The cholinergic deficit correlates with the severity of Alzheimer's disease. The cholinergic function can be improved by AChE inhibitors blocking this key enzyme in the breakdown of acetylcholine. Based on traditional medicine, during two last decades the use of herbal medicinal substances in dementia therapy has been studied [1]. The fruit extract of *Piper longum* Linn. (Piperaceae) has been used in several systems of traditional medicine with various therapeutic properties, among those for the enhancement of cognitive performance especially for memory improvement in several herbal drug mixtures with other plants. A previous study showed the AChE inhibitory activity of *P. longum* extract [2]. To find new natural compounds with AChE inhibitory effect, in this study the fruit of *P. longum* was investigated in details. First the fruits were obtained from herbal shop and identified scientifically. Then the fruits pulverized smoothly and extracted with dichloromethane by stirring after sonification. The extract was investigated by a respective enzymatic TLC bioautography assay [3] to identify the active zones. Then the active compounds were isolated using several chromatographic techniques such as vacuum liquid chromatography, column chromatography and size exclusion chromatography. The structures of the active components were characterized by different methods such as one and two-dimensional ¹H and ¹³C NMR spectroscopy, FTIR and mass spectrometry. These substances were identified as methylen-dioxide and piperine derivatives. The IC₅₀ values of them were determined using Ellman's chlorimetric assay [4]. Physostigmine was used as the positive control. The isolated compounds showed moderate AChE inhibition. As there are several compounds available in *P. longum* with AChE inhibition, the whole fruit can be considered as AChE inhibitor that proves the traditional use of that for memory enhancement.

Keywords: *Piper longum* Linn., AChE inhibitor, Alzheimer's disease, TLC bioautography, Ellman's assay

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