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Research Article

Evaluation of Acetylcholinesterase inhibition by *Alnus rugosa* L. stems methanol extract and phytochemical content

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Abstract

This study was carried out to evaluate acetylcholinesterase activity of methanol extract of *Alnus rugosa* L. stems and to determine the phytoconstituents in the plant extract. The acetylcholinesterase inhibition was detected using Ellman's method and the methanol extract was subjected for phytochemical analysis to identify different phytochemical constituents present in the extract. The methanolic extract of *A. rugosa* stems has shown (IC_{50} = 0.588 mg/mL), assuming that the extract has compounds with a similar activity to neostigmine (IC_{50} = 1.87 µg/mL) and galanthamine (IC_{50} = 0.37 x10^{-3} mg/mL) which are considered to be the most effective compounds in the treatment of Alzheimer's disease Phytochemical investigation of methanol extract of *Alnus rugosa* stems revealed the presence of triterpenes, flavonoids carbohydrates and tannins. These results prove that the methanol extract of *Alnus rugosa* stems seem of interest for further study as anti- Alzheimer agent.

Keywords: *Alnus rugosa*, stems, Anticholinesterase activity, Alzheimer's disease

1. Introduction

Alzheimer's disease (AD) is one of the most widespread neurodegenerative diseases. In a field of several theoretical options, the best approach has been the use of AChE inhibitors (AChEIs), which led to the introduction of tacrine as the first AChEI specifically approved for the treatment of AD. Now, several kinds of AChEIs, such as donepezil, galantamine and rivastigmine are available for the symptomatic treatment of patients with mild to moderate AD[1]. However, these compounds have been reported to have the problems associated with the gastrointestinal disturbances and bioavailability[2]. One of the best sources of new substances to treat AD are natural products and their derivatives. Traditionally, plants have been used to enhance memory and to alleviate other symptoms associated with AD[3]. The biologically active plant-derived substances that may be considered as a source of new anticholinesterase drugs come from different classes of compounds and are characterized by the diversity of their structures. The majority of bioactive substances are alkaloids, phenylpropanoids (furanocoumarins, xantones, and flavonoids) and terpenoids[3]. *Alnus rugosa* L. is a deciduous tree from Betulaceae family. It is in flower in May, and the seeds ripen in October. In traditional medicine, *A. rugosa* is used as alterative, anodyne, astringent; cathartic, emetic; febrifuge and tonic[4]. As far as we know, there are no reports about pharmacological activities or phytochemicals from *A. rugosa* plant. The aim of this study was to investigate possible acetylcholinesterase inhibition by methanol extract of *Alnus rugosa* stems and also determine the phytochemical content of the extract.

2. Material and Methods

2.1. Plant Material

Alnus rugosa stems were collected from Al-Zohiriya garden, Giza, Egypt in May 2011. The plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereez Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen was deposited in the herbarium of Al-Zohiriya garden, Giza, Egypt.

2.2. Plant extract preparation

The air dried stems of *Alnus rugosa* (450 g) were extracted with methanol: distilled water 80:20 (v/v) several times at room temperature by maceration. The extract was concentrated under reduced pressure to give 28 g of methanol 80% extract. The extract was phytochemically screened according to different chemical assays to identify the presence or absence of the phyochemical components according to Connolly[5] for sterols and/or triterpenes, Wolf[6] for carbohydrates and saponins, Harborne[7] for flavonoids and alkaloids**,** Farnsworth[8] for coumarins and Geissman[9] for tannins**.**

2.3. Acetylcholinesterase inhibition assay

The methanolic extract of *A. rugosa* was dissolved in methanol to prepare solution of 10 mg/mL. Then, 1.5 µL of the methanol extract of *A. rugosa* was spotted on the silica gel TLC plate and developed with chloroform: methanol 9:1 after which the enzyme inhibitory activity was detected using Ellman's method "in situ" on the plate[10,11]. The developed plate was sprayed with 1 mM DTNB and 1 mM ATCI in buffer A. It dried for 3-5 minutes, then an enzyme solution of AChE from an electric eel (type VI-s lyophilized, 261 U/mg solid, 386 U/mg protein) dissolved in buffer A (500 U/mL stock solution) was diluted with buffer A to obtain 5 U/mL enzyme and was then sprayed on the plate[11]. Yellow background with white spot for inhibiting extract was visible after about 5 minutes. These observation must be recorded within 15 minutes because they fade after 20-30 minutes. To observe whether the positive results of the extract in TLC or the microplate assay are due to enzyme inhibition or to the inhibition of the chemical reaction between DTNB and thiocholine, (the product of the enzyme reaction), 5 units/mL of AChE was premixed with 1 mM ATCI in buffer A and incubated for 15 minutes at 37^{o}C. This enzyme-substrate mixture was used as thiocholine spray[11]. The extract was spotted on the silica gel TLC plate developed as described above and sprayed with 1 mM solution DTNB followed by the thiocholine spray. White spot on a yellow background was observed for false positive extract.

The inhibitory effect quantitative of methanolic extract of *A. rugosa* on acetylcholinesterase activity is evaluated using and adaptation of the spectrophometric method of Ellman *et al*. (1961) modified by Rhee[11]. Five different concentrations were prepared in triplicate, starting from the methanolic extract of *Alnus rugosa* (1 mg/mL; 0.5 mg/ml; 0.25 mg/mL; 0.125 mg/mL and 0.0625 mg/mL). The reaction was monitored at 412 nm for 5 min in spectrophotometer.

In test tube is placed 100 µL of sample (concentration 0.1% solution in 50 mM Tris-HCl pH 8, and methanol 10%) was mixed with 100 µL of AChE 0.22 U / ml (22 U of enzyme diluted in 100 mL of 50 mM Tris-HCl pH 8, 0.1% BSA) and 200 µL of buffer (50 mM Tris-HCl, pH 8, BSA 0.1%). Incubating the mixture for 5 min at 30°C. Subsequently add, 500 µL of DTNB (concentration of the 3 mM in Tris-HCl pH 8, 0.1 M NaCl, 0.02 M $MgCl_2$) and 100 µL of ATCI (4 mM in water). 4. A blank should also be prepared by substituting AChE with 100 µL of buffer (50 mM Tris-HCl buffer pH 8, 0.1% BSA). The reaction is monitored for 5 min at 412 nm and initial velocity (V0) recorded. Anticholinesterase activity (%) was calculated:

$$I\ (\%) = (1 - \frac{V_o\ \text{sample}}{V_o\ \text{white}}) \times 100$$

Sample Vo and Vo represents the initial rates blank samples and white.

Inhibition concentration 50% (IC_{50}) values so obtained by plotting Log-Probit. Neostigmine (or other commercial acetylcholinesterase inhibitor) is used as positive control at the same concentration of the extract.

3. Results and Discussion

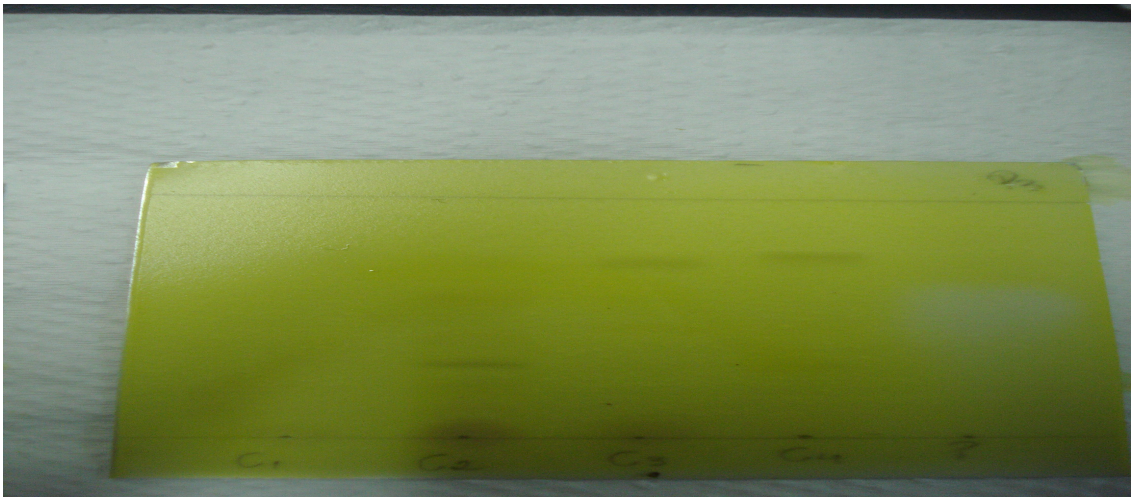
The qualitative results of inhibition of enzyme acetylcholinesterase in Thin Layer Chromatography (TLC) showed that the methanol extract the *A. rugosa* inhibited the enzyme by the appearance yellow backgrounds with white spots for inhibiting compounds were visible after about 5 minutes. This are the results of the first tests, yellow backgrounds with

white spots for inhibiting compounds were visible after about 5 minutes for methanolic extract of *A. rugosa* apparently tested positive enzyme inhibition in concentration of 10 mg/mL (**Figure** 1). The results of acetylcholinesterase inhibition quantitative for methanolic extract of *A. rugosa* that presented strong activity in both tests, the IC_{50} values were determined (IC_{50} = 0.588 mg/mL). The concentration of inhibition 50% (CI_{50}) was tested starting at five different concentrations (1 mg/mL; 0.5 mg/ml; 0.25 mg/mL; 0.125 mg/mL; 0.0625 mg/mL) tested in triplicate, shows the specie that showed higher inhibition activity (*A. rugosa* IC 50= 0.588mg/mL), in comparison to commonly used drugs neostigmine de (IC_{50} = 1,87 µg/mL) and galanthamine (IC_{50} = 0.37 x10^{-3} mg/mL). Galanthamine which is alkaloid considered to be the most effective compound in the treatment of Alzheimer's disease[12]. Phytochemical constituents of *Alnus rugosa* are shown in table 1.

Table 1. Phytochemical analysis of the different extracts from *Alnus rugosa* stems

Chemical Constituents	Methanol 80%
Carbohydrates and/or glycosides	+
Tannins a. Condensed tannins b. Hydrolysable tannins	 + +
Alkaloids and/or nitrogenous bases	-
Flavonoids	+
Sterols and/or triterpenes	+
Saponins	-
Coumarins	-
+ denotes the presence of the constituents - denotes the absence of the constituents	

Figure 1- Acetylcholinesterase inhibition in TLC showed that the methanolic extract the *A. rugosa* (C-2), (caffeine, is used as positive control for acetylcholinesterase inhibitor)



Alnus rugosa stems methanol extract seems of interest for further study. Plants that have shown favorable effects in relation to cognitive disorders, including anticholinesterase, anti-inflammatory and antioxidant activities or other relevant pharmacological activities are potentially of interest to clinical use for Alzheimer's disease[13]. Eighteen medicinal plants of Brazil were screened for inhibitory activity on AchE, the results show that various plants are very interesting for further isolation of acetylcholinesterase inhibitors, which are widely used in the treatment of Alzheimer's disease, galanthamine, an alkaloid from plants of the Amaryllidaceae family, is a selective reversible long-acting and competitive acetylcholinesterase inhibitor (AChEI). The extract is considered to be more effective in the treatment of Alzheimer's disease (AD) and to have fewer limitations than physostigmine and tacrine are relevant in terms of searching for novel formulations or compounds for

AD treatment[12]. This is the result of the first tests, yellow backgrounds with white spots for inhibiting extract was visible after about 5 minutes, and so *Alnus rugosa* stems methanol extract apparently tested positive enzyme inhibition in concentration of 10 mg/mL. The activity of the methanol extract of *A. rugosa* may be explained by the presence of carbohydrates, triterpenes, flavonoids and tannins. Many plants as *Sophora flavescens* showed a significant acetylcholinesterase inhibition and this activity is due prenylated flavonoid, 8-lavandulylkaempferol which exhibited significant inhibitory effects with IC_{50} values of 7.10 and 8.11μM for butyrylcholinesterase and acetylcholinesterase[14], also Inhibition of Acetyl Cholinesterase by *Indigofera* species extracts was due to the potential contribution of tannins and of flavonol present in the extracts.[15]

4. Conclusion

This research work deals with the Acetylcholinesterase inhibition by methanol extract of *Alnus rugosa* L. stems and also the phytoconstituents of the extract. The methanol extract of *A. rugosa* stems apparently tested positive enzyme inhibition, however notice an interference color in the extract in TLC. The methanolic extract has shown *A. rugosa* specie (IC_{50}= 0.588mg/mL), assuming there were compounds with a similar activity to neostigmine, which should contain about 1% of an active compound, or if present at lower levels even more active compounds than neostigmine (IC_{50} = 1,87 µg/mL) and galanthamine (IC_{50} = 0.37 x10 $^{-3}$ mg/mL), should be present. The results show that the extract is very interesting for further isolation of acetylcholinesterase inhibitors, which are widely used in the treatment of Alzheimer's disease and further work to isolate and characterize the constituents responsible for the biological activities of the plant is currently ongoing in our laboratory.

References

1. Racchi M, Mazzuchelli M, Porrello E, Lanni C, Govoni S. Acetylcholinesterase inhibitions: novel activities of old molecules. *Pharmacol Res* 2004; 50: 441-451.
2. Schulz V. Ginkgo extract or cholinesterase inhibitors in patients with dementia; what clinical trial and guidelines fail to consider. *Phytomed* 2003; 10 (Supplementary 4): 74-79.
3. Houghton PJ, Howes MJ. Natural products and derivatives affecting neurotransmission relevant to Alzheimer's and Parkinson's disease, *Neurosign* 2005; 14: 2–22.
4. Huxley. A. The New RHS Dictionary of Gardening, 1992. p. 28-46.
5. Connolly JD, Overton KH and Polonsky J. The chemistry and biochemistry of the linonoids and quassinoids. In: Reinhold l, Liwashitz Y (eds) Progress in phytochemistry. Wiley, London, 1970. p. 385-94.
6. Wolf HH, Swinyard EA, Goodman LS. Anticonvulsant properties of some N-substituted hydanoins. *J Phrama Sci* 1962; 51:74-76.
7. Harbone JB. Phytochemical methods. Chapman & Hall, London, 1973. p. 146-58.
8. Farnsworth NR. Biological and phytochemical screening of plants. *J Phrama Sci* 1966; 55: 225-276.
9. Geissman TA. The Chemistry of flavonoids compounds. Pergamon, London, 1962. p.126-38.
10. Ellman GL, Courtney DK, Andres VJR, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961; 7:88-95.
11. Rhee KI, Van Rijn MR, Verpoorte R. Qualitative Determination of false-positive effects in acetylcholinesterase assay using thin-layer chromatography. *Phytochem Anal* 2001; 14: 127-131.
12. Houghton PJ, Howes MJR. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. *Pharmacol Biochem Behav* 2003; 75: 513-527.
13. Feitosa CM**,** Freitas RM, Luz NNN, Bezerra MZB, Trevisan, Maria Teresa Salles . Acetylcholinesterase inhibition by somes promising *Brazilian medicinal plants. Braz J Biol* 2011; 71:783-789.
14. Jung Hyun Ah Takako Yokozawa, Byung-Woo Kim, Jee H. Jung and Jae Sue Choi. Selective Inhibition of Prenylated Flavonoids from Sophora flavescens against BACE1and Cholinesterases. *Amer J Chin Med* 2010; 38 (2): 415–429.
15. Bakasso S, Lamien-meda A, Lamien CE, Kiendrebeogo M, Coulibaly AY, M Compaoré, Male NR, *et al.*,. *In vitro* Inhibition of Acetyl Cholinesterase, Lipoxygenase, Xanthine Oxidase and Antibacterial Activities of Five *Indigofera* (Fabaceae) Aqueous Acetone Extracts from Burkina Faso. *Curr Res J Biol Sci* 2013; 5(3): 115-122.