

The Therapeutic Potential of *Berberis darwinii* Stem-Bark: Quantification of Berberine and *In Vitro* Evidence for Alzheimer's Disease Therapy

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Berberis darwinii is native to South America but has been widely distributed in Europe and other continents following its discovery by Charles Darwin. Herewith, the therapeutic potential of stem-bark of the plant for treating Alzheimer's disease was studied using an *in vitro* acetylcholinesterase inhibition assay. It was found that the methanolic extract of the stem-bark was a potent inhibitor of the enzyme with an IC₅₀ value of 1.23 ± 0.05 µg/mL. An HPLC-based berberine quantification study revealed an astonishing 38% yield of the dried extract.

Keywords: *Berberis darwinii*, Berberidaceae, berberine, acetylcholinesterase, Alzheimer's disease.

As a single chemical entity and a major constituent in plants extracts, berberine has been used as potent antimicrobial, anticancer, hepatoprotective, antidiabetic, cholesterol lowering effects, anti-inflammatory, immunomodulatory and many more activities [1a and references therein]. Recent studies further revealed that berberine has potent neuroprotective effects [1b]. Such neuroprotective effects combined with antioxidant activity are of particular significance to Alzheimer's disease (AD) where cholinergic neurons apoptosis is the major feature of the disease process. The other predominant feature of AD is the accumulation and aggregation of amyloid-β protein leading to the formation of intracellular neurofibrillary tangles and loss of normal neuronal functions [1c]. Interestingly, berberine has been shown to suppress the release of amyloid-β from the membrane-associated glycoprotein precursor amyloid protein [1d]. A further key therapeutic approach for AD is the inhibition of acetylcholinesterase (ACHE) enzyme thereby increasing the lifespan of the neurotransmitter, acetylcholine [1e]. Since berberine has also been demonstrated to potently inhibit ACHE [1f], berberine containing plants are likely to have therapeutic potential for AD.

Berberis darwinii Hook (Berberidaceae) is native to South America; southern Chile and Argentina. The plant is known to be discovered in 1835 by Charles Darwin during the voyage of the 'Beagle' and was named in his honor [2a]. Known by its common names such as Darwin's Barberry and (Argentinean-Chilean Spanish) Michay, the fruits are edible though they are acidic and rather favored

by birds [2a, personal observation]. *B. darwinii* is now one of the popular garden and hedge plant in Britain. Except for the earlier report by Cromwell in 1933 [2b] that documented the biosynthesis of berberine, no pharmacological or phytochemical studies have ever been reported on the plant. Owing to the above mentioned therapeutic potential of berberine and/or berberine-containing plants, the present study focused on establishing the therapeutic potential of *B. darwinii* for AD.

It was previously noted that the berberine content of *B. darwinii* was the highest in the root-bark of the plant with estimated average dry weight content of 11%, while the stem-bark was reported to contain 2.67-4.93% [2b]. In contrast to the bark of *B. darwinii*, the most common source of berberine plant, Goldenseal (*Hydrastis canadensis* L., *Ranunculaceae*), was shown to contain up to a maximum content of 4.62% in the dried Rhizome, while the other principal constituent, hydrastine, was 2.77% [2c]. The other well known berberine containing commercial plant is the dried root of *Coptis chinensis*, which is reported to contain up to a maximum level of 7.27% berberine together with small amount (less than 2%) of other principal constituents, palmatine and jatrorrhizine [2d]. Based on these literature information, there is no doubt that the bark of *B. darwinii* is just as good source (if not better) of berberine as Goldseal and Coptis.

An HPLC method with reverse phase C-18 column and ammonium acetate-acetonitrile gradient elution system was used for berberine analysis. The separations of the

Table 1: Anti-ACHE activity of Berberine, *B. darwinii* stem-bark extract and eserine.*

Drugs	IC ₅₀ , µg/ml	IC ₅₀ , µM
Berberine	0.44±0.02	1.18 ± 0.06
<i>B. darwinii</i>	1.23±0.05	-
Eserine	0.28 ±0.05	1.01 ± 0.16

*IC₅₀ values obtained from 4 independent experiments are shown.

stem-bark constituents were achieved during the first 10 minutes of the 25 min analysis time. The study revealed that berberine was the major constituent of the crude methanolic extract of *B. darwinii*. In order to quantify the content of berberine in the extract, a calibration curve for berberine as an external standard was plotted. Straight line equations with regression line, correlation coefficient (r^2) of 0.9998 to 0.9999, were obtained. Based on this analysis, the berberine content in the methanolic extract of the fresh stem-bark was calculated as $38.09 \pm 0.966\%$ (n=10). This means that the content of berberine in fresh stem-bark of *B. darwinii* was $4.57 \pm 0.12\%$ (n=10). Considering that a fresh plant material was used for the analysis, the observed high yield of berberine was rather astonishing.

With the level of berberine in the extract established, the therapeutic potential of the stem-bark of *B. darwinii* for AD was further assessed by analyzing its anti-ACHE effect using Ellman *et al.* [2e] method. In agreement with previous reports [1f] the present study demonstrated that berberine is a potent ACHE inhibitor (Table 1). The activity of berberine was comparable with the standard anti-ACHE drug, eserine (Table 1): the mean values were not significantly different from each other ($p > 0.05$; unpaired *t*-test). As evidenced by the IC₅₀ values (Table 1), the crude extract was about 3-fold less potent than berberine: which appear to be in good agreement with the observed percentage yield (38%) of berberine in the crude extract.

In conclusion, the stem-bark of *B. darwinii* may be used as an alternative source of berberine and has a potential to be

used for AD. Given that berberine has a wide range of other biological activities, further studies on the therapeutic potential of *B. darwinii* is well merited.

Experimental

Plant material and extraction: *B. darwinii* grown in our medicinal garden and believed to be over 20 years old has been used. The plant was authenticated and voucher specimens deposited at our laboratory collections. The fresh stem-bark (142 g) from the main stem of the plant flask. The plant material was then soaked in methanol (2.5 L) and left in the dark for two weeks. The resulting extract was chopped into pieces and placed in a large extraction was concentrated under reduced vacuum using rotary evaporator to yield 17 g of the crude extract.

Quantification of berberine by HPLC: Samples (20 µL) were injected onto a reverse phase column (Agilent – Eclipse XDB-C18, 5 µm, 4.6 x 150 mm). The mobile phase was a mixture of 10 mM ammonium acetate containing 0.08% formic acid (A) and acetonitrile (B). The composition of the mobile phase at a flow rate of 1 ml/min was rising from 30% to 70% B over a period of 20 minutes. The composition of B was further increased to 90% B over 5 minutes. The berberine concentration in the crude extracts was quantified from the standard curves constructed from two-fold standard berberine dilutions made from a stock solution of 1 mg/ml. All experiments were repeated at least ten times and the amount of berberine, based on the peak area at $\lambda = 280$ nm, was calculated using GraphPad InStat software (GraphPad, San Diego, USA).

Anti-ACHE assay: The Elman *et al.* [2e] colorimetric method of anti-ACHE assay was adopted for microtitre-based assay.

References

- [1] (a) Tang J, Feng Y, Tsao S, Wang N, Curtain R, Wang Y. (2009) Berberine and *Coptidis rhizoma* as novel antineoplastic agents: A review of traditional use and biomedical investigations. *Journal of Ethnopharmacology*, **126**, 5-17; (b) Zhou X-Q, Zeng X-N, Kong, H, Sun XL. (2008) Neuroprotective effects of berberine on stroke models *in vitro* and *in vivo*. *Neuroscience Letters*, **447**, 31-36; (c) Klein WL. (2002) Aβ toxicity in Alzheimer's disease: globular oligomers (ADDLs) as new vaccine and drug targets. *Neurochemistry International*, **41**, 345–352; (d) Asai M, Iwata N, Yoshikawa A, Aizaki Y, Ishiura S, Saido TC, Maruyama K. (2007) Berberine alters the processing of Alzheimer's amyloid precursor protein to decrease Aβ secretion. *Biochemical and Biophysical Research Communication*, **52**, 498-502; (e) Racchi M, Mazzucchelli M, Porrello E, Lanni C, Govoni S. (2004) Acetylcholinesterase inhibitors: novel activities of old molecules. *Pharmacological Research*, **50**, 441-445, (f) Huang L, Luo Z, He F, Lu J, Li X. (2010) Synthesis and biological evaluation of a new series of berberine derivatives as dual inhibitors of acetylcholinesterase and butyrylcholinesterase. *Bioorganic & Medicinal Chemistry Letters*, **18**, 4475-4484.
- [2] (a) Wikipedia: http://en.wikipedia.org/wiki/Berberis_darwinii#cite_note-0 Accessed – February 2011; (b) Cromwell BT. (1933) Experiments on the origin and function of berberine in *Berberis Darwinii*. *Biochemical Journal*, **27**, 860–872. Douglas JA, Follett JM, Parmenter GA, Sansom CE, Perry NB, Littler RA. (2010) Seasonal variation of biomass and bioactive alkaloid content of goldenseal, *Hydrastis canadensis*. *Fitoterapia*, **81**, 925-928; (c) Douglas JA, Follett JM, Parmenter GA, Sansom CE, Perry NB, Littler RA. (2010) Seasonal variation of biomass and bioactive alkaloid content of goldenseal, *Hydrastis canadensis*. *Fitoterapia*, **81**, 925-928; (d) Chen J, Wang F, Liu J, Lee FS-C, Wang X, Yang H. (2008) Analysis of alkaloids in *Coptis chinensis* Franch by accelerated solvent extraction combined with ultra performance liquid chromatographic analysis with photodiode array and tandem mass spectrometry detections. *Analytica Chimica Acta*, **613**, 184-195; (e) Ellman GL, Courtney KD, Andres V, Featherstone RM. (1961) A new and rapid colourimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, **7**, 88-95.