Flavonoids with xanthine oxidase inhibitory activity isolated by guidance of bioassay from *Artemisia asiatica* Nakai

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Artemisia asiatica herbs
http://131.230.176.4/users/pelserpb/8_8_11_1/8aug11a/artemisiaasiatica2.jpg

INTRODUCTION

Artemisia asiatica **Nakai has been used in the traditional oriental medicine for the treatment of cancer, gastritis, ulcers and other inflammatory disorders. Previous in vivo and human studies demonstrated the antioxidant and anti-inflammatory effects of its formulated EtOH extract DA-9601 on gastro-intestinal injuries. It was stated, that its therapeutic effect is mediated partly through the inhibition of gastric xanthine oxidase (XO) activity, which is a late enzyme of purine catabolism, well known as a major source of reactive oxygen species generation in the pathogenesis of various diseases.**

In our experiment the XO inhibitory activity of *A. asiatica* was investigated, followed by a bioactivity-guided fractionation aiming the isolation of the components responsible for the activity. Moreover, the free radical scavenging activity of the isolated compounds were also evaluated by DPPH test, in order to estimate their role in oxidative processes.

Artemisia asiatica herbs
http://131.230.176.4/users/pelserpb/8_10_11/10aug11/artemisiaasiatica.jpg 2012. 02.08.

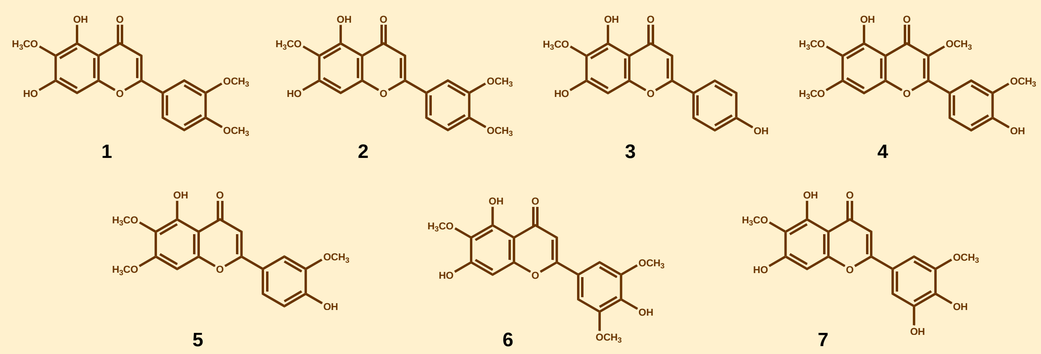
RESULTS

The MeOH extract of the aerial parts of *A. asiatica*, and its fractions obtained by CC on polyamide, significantly inhibited the XO induced uric acid production. The pure flavonoids were isolated using VLC, CPC and PLC (Fig 1) and identified as eupatilin (1), jaceosidin (2), hispidulin (3), chrysoplenetin (4), cirsilineol (5), 5,7,4'-trihydroxy-6,3',5'-trimethoxyflavon (6) and 5,7,4',5'-tetrahydroxy-6,3'-dimethoxy-flavon (7) by means of UV, NMR and MS. With except of chrysoplenetin (4) and cirsilineol (5), all compounds exerted remarkable XO inhibitory effect with IC_{50} values between 0.36-7.67 μM (Table 1, Fig 2). The highest activity was displayed by the main flavonoid eupatilin (1). The degree of XO inhibition of 1-3, 6, 7 suggest the importance of free OH group at C-7.

The isolated compounds 1-7 were also evaluated for their free radical scavenging activity by DPPH test (Fig 3), and found that substantial antioxidant activity is displayed by 5,7,4',5'-tetrahydroxy-6,3'-dimethoxy-flavon (7), having *ortho*-dihydroxy and 5-hydroxy groups in its structure. In summary, flavonoid-containig extract of *A. asiatica* have dual effect, acting by inhibition of XO (compounds 1-3, 6, 7), resulting the reduced generation of reactive oxygen species, and by scavenging free radicals (compound 7).

Samples	XO Inhibitory Activity (μM)	Antioxidant activity (μM)
eupatilin (1)	0.358	inactive
jaceosidin (2)	3.373	1284
hispidulin (3)	5.377	inactive
chrysoplenetin (4)	inactive	1233
cirsilineol (5)	inactive	inactive
5,7,4'-trihydroxy-6,3',5'-trimethoxyflavon (6)	7.669	319.7
5,7,4',5'-tetrahydroxy-6,3'-dimethoxy-flavon (7)	2.561	48.58
allopurinol	7.47	-
quercetin	-	31.11

Table 1. IC_{50} Values of the Xanthine Oxidase Inhibitory and Antioxidant Activities



Structures of Compounds Isolated from the *Artemisia asiatica* Herbs

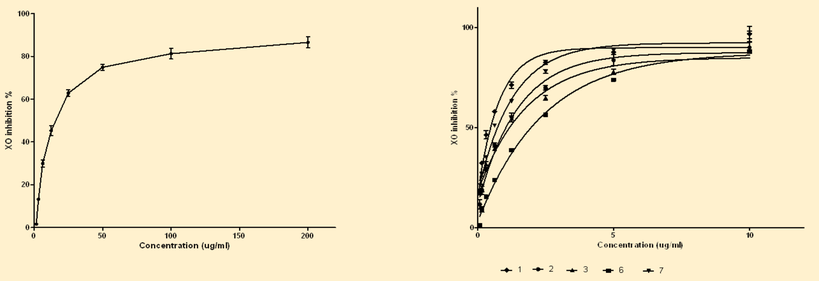


Figure 2. Dose-dependent XO Inhibition by 60% Methanolic Fraction of Polyamide CC (**B3**) and Pure Isolated Compounds

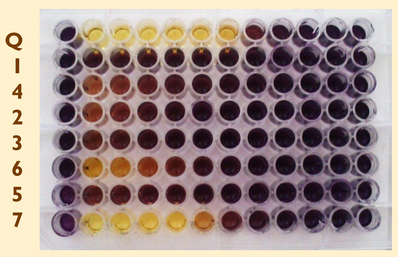


Figure 3. DPPH Test of Quercetin and Compounds **1-7**

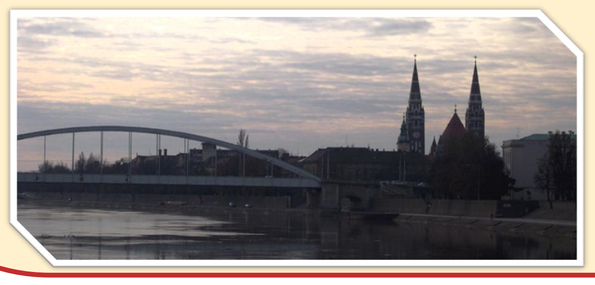
MATERIALS AND METHODS

Plant material: *A. asiatica* was gathered in September 2008 in the experimental field of Institute of Ecology and Botany of Hungarian Academy of Sciences, Vácrátót, Hungary.

Extraction and isolation: Dried and ground aerial parts were percolated with MeOH. The extract was concentrated and fractionated using solvent-solvent partition with n-hexane and chloroform. The $CHCl_3$ phase was subjected to CC on polyamide using MeOH-H_2O mixtures as eluents. Fraction eluted with 60% MeOH from polyamide CC afforded compounds (1-7) after VLC and crystallisation.

Structure determination: NMR spectra were recorded at 500 (^1H) and 125 MHz (^{13}C). Two-dimensional experiments were performed the standard Bruker protocol.

Xanthine oxidase assay: The method is based on a continuous spectrophotometric rate determination: the absorbance of XO enzyme induced uric acid production from xanthine was measured at 290 nm for 3 min. The enzyme-inhibitory effect was determined by the decreased production of uric acid. *Reagents*: 50 mM potassium buffer, pH 7.5 with 1M KOH, 0.15mM xanthine solution, pH 7.5, prepared by xanthine, XO enzyme solution 0.2 Units/ml prepared by XO. *Tests*: *A. asiatica* extracts, 12 g/ml and purified compounds 1-7, 600 μg/ml diluted in DMSO solution. The final reaction mixture in 300 μl well was: 100 μl xanthine, 150 μl buffer and 50 μl XO for enzyme-activity control. The reaction mixture for inhibition: 100 μl xanthine, 140 μl buffer, 10 μl Test and 50 μl XO. The IC_{50} values of the active compounds and extracts were calculated by analyzing the inhibitory percentage values of each concentration with GraphPad Prism 5.04.



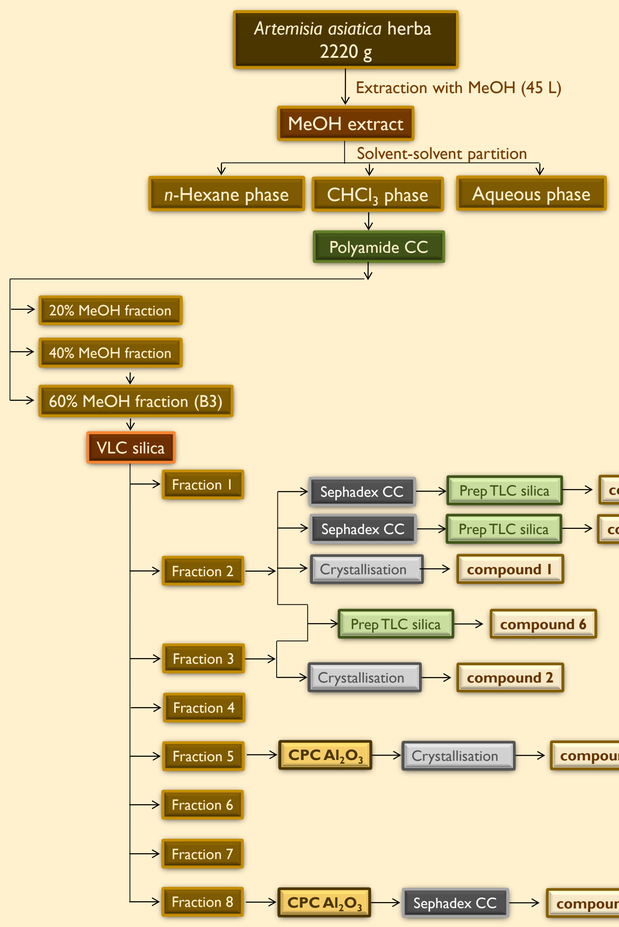


Figure 1. Isolation protocol of compounds **1-7**

ACKNOWLEDGEMENTS
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