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Xanthine Oxidase-Inhibitory Activity and Antioxidant Properties of the Methanol Extract and Flavonoids of *Artemisia Asiatica*

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Abstract: The MeOH extract of the aerial parts of *Artemisia asiatica* significantly inhibited the xanthine oxidase (XO) induced uric acid production. Flavonoids were isolated from the active extract and identified as eupatilin (**1**), hispidulin (**2**), jaceosidin (**3**), cirsilineol (**4**), 5,7,4',5'-tetrahydroxy-6,3'-dimethoxyflavone (**5**), 6-methoxytricin (**6**) and chrysosplenetin (**7**). With the exception of cirsilineol and chrysoplenetin, all these flavonoids exerted marked XO-inhibitory effects, with IC_{50} values in the range 1.33−6.13 µM. The degree of XO inhibition by **1**-**3**, **5** and **6** suggests the importance of the free OH group on C-7.
Evaluation of the isolated compounds **1**-**7** for their free radical scavenging activity in DPPH tests, revealed the substantial antioxidant activity of 5,7,4',5'-tetrahydroxy-6,3'-dimethoxy-flavone (**5**), which contains *ortho*-dihydroxy and 5-hydroxy groups. In summary, the flavonoid-containing MeOH extract of *A. asiatica* exhibits dual action, inhibiting XO (**1**-**3**, **5**, **6**) and resulting in a reduced generation of reactive oxygen species, and additionally scavenging free radicals (**5**).

Keywords: *Artemisia asiatica*; Asteraceae; Flavonoids; Xanthine oxidase inhibitory activity; Antioxidant activity.

1. Introduction

The genus *Artemisia* (Asteraceae), which includes some 500 species, occurs worldwide. Various Artemisia species are of importance as aromatic culinary herbs, e.g. *A. dracunculus* (tarragon), while others are widely used in medicine and display a wide range of biological activities, such as cytotoxic, antimalarial, antibacterial, antifungal, antihepatotoxic and antioxidant. The main classes of the constituents of this genus are terpenoids, flavonoids, coumarins, phenyl propanoids and acetylene derivatives. Moreover, very important drug leads have been discovered from this genus, e. g. the sesquiterpene artemisinin, a well-known antimalarial drug obtained from the Chinese herb *A. annua* [1].

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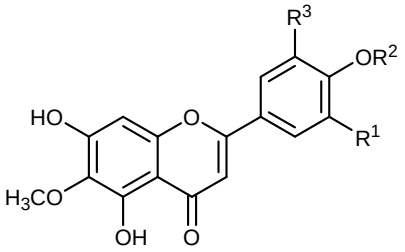
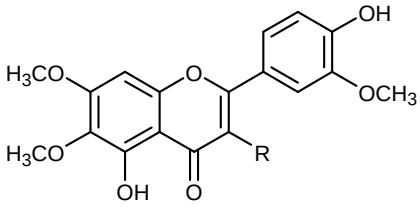
A. asiatica Nakai has been used in traditional oriental medicine for the treatment of cancer, gastritis, ulcers and other inflammatory disorders [1]. Previous studies have revealed that *A. asiatica* contains a high amount of eupatilin (**1**), a flavone which exhibits anti-tumour activity through the induction of a cell cycle arrest and the differentiation of gastric carcinoma and endometrial cancer cells [2]. Additionally, the formulated EtOH extract (DA-9601) of *A. asiatica* has pronounced antioxidative and anti-inflammatory activities and exerts cytoprotective effects against experimentally induced gastrointestinal, hepatic and pancreatic damage [3,4]. Previous *in vivo* and human studies revealed the antioxidant and anti-inflammatory effects of DA-9601 extract on gastrointestinal injuries [5,6]. Eupatilin (**1**) is regarded as the main pharmacologically active ingredient of the plant, since it was reported to have a variety of anti-inflammatory properties [7,8]. It was concluded that the therapeutic effect of the plant extract is mediated partly through the inhibition of the activity the of gastric xanthine oxidase (XO) [9], which is a late enzyme in the purine catabolism, well known as a major source of reactive oxygen species in the pathogenesis of various diseases. However, the compounds responsible for the XO-inhibitory activity have not been studied previously.

2. Results and Discussion

The crude MeOH and partitioned extracts (*n*-hexane, $CHCl_3$ and H_2O) of the aerial parts of *A. asiatica* were screened for XO-inhibitory activity. The crude MeOH extract displayed notable XO-inhibitory activity, with IC_{50} 2.93±0.80 µg/mL. Separation of the crude extract by solvent−solvent partition led to a higher activity of the $CHCl_3$ extract, which was therefore further purified to identify the constituents responsible for the activity. The flavonoid-containing fractions of the polyamide column, eluted with 60 and 80% MeOH, displayed the better XO-inhibitory activity, and were subjected to multistep chromatographic purification, including VLC, gel chromatography, CPC and preparative TLC, to afford compounds **1**-**7** in pure form.





	R^1	R^2	R^3
1	OCH_3	CH_3	H
2	H	H	H
3	OCH_3	H	H
5	OCH_3	H	OH
6	OCH_3	H	OCH_3

	R
4	H
7	OCH_3

Figure 1. Structures of the compounds isolated from *A. asiatica*: eupatilin (**1**), hispidulin (**2**), jaceosidin (**3**), cirsilineol (**4**), 5,7,4',5'-tetrahydroxy-6,3'-dimethoxyflavone (**5**), 6-methoxytricin (**6**) and chrysoplenetin (**7**)

The isolated compounds were identified by analysis of their ^{1}H-, ^{13}C-NMR, NOESY and ESIMS spectra as eupatilin (**1**), hispidulin (**2**), jaceosidin (**3**), cirsilineol (**4**), 5,7,4',5'-tetrahydroxy-6,3'-dimethoxy-flavone (**5**), 6-methoxytricin (**6**) and chrysoplenetin (7). Compounds **2**, **3**, and **5-7** were isolated for the first time from *A. asiatica*, while eupatilin (**1**) and jaceosidin (**3**) had been reported from this plant previously. The isolated compounds, with the exception of chrysoplenetin (**7**) and cirsilineol (**4**), were found to exert noteworthy XO-inhibitory effects with IC_{50} values ranging from 1.33 to 6.13 µM (Table 1).

Table 1. Xanthine oxidase-inhibitory and antioxidant activities (after 30 and 60 min of incubation) of *A. asiatica* extracts and compounds **1**-**7** (IC_{50}). Allopurinol and quercetin were used as positive controls in these experiments, respectively

	XO Inhibitory activity	**Antioxidant activity**
A. asiatica MeOH extract	2.93 ± 0.80 µg/mL	-
Eupatilin (**1**)	1.33 ± 0.21 µM	inactive
Hispidulin (**2**)	4.88 ± 1.21 µM	inactive
Jaceosidin (**3**)	3.27 ± 0.31 µM	inactive
Cirsilineol (**4**)	inactive	inactive
5,7,4',5'-Tetrahydroxy-6,3'-dimethoxyflavone (**5**)	2.59 ± 0.35 µM	58.74 ± 3.90 µM (30 min) 51.18 ± 5.23 µM (60 min)
6-Methoxytricin (**6)**	6.13 ± 0.34 µM	inactive
Chrysoplenetin (**7**)	inactive	inactive
Allopurinol	7.49 ± 0.29 µM	-
Quercetin	-	31.26 ± 2.11 µM (30 min) 26.31 ± 2.46 µM (60 min)

The highest activity was that of the main flavonoid, eupatilin (**1**), with IC_{50} 1.33 µM. Figure 2 illustrates the XO-inhibitory activities of eupatilin (**1**) and the positive control allopurinol (IC_{50} 7.49 µM, a well-known XO inhibitor used in the therapy of gout). Flavones **2**, **3**, **5** and **6** were also more potent inhibitors of XO than allopurinol. Analysis of the XO-inhibitory activities of compounds **1**-**7**, and their chemical structures indicates that the free OH group on C-7 plays a crucial role in the activity, since the compounds containing a 7-methoxy group (cirsilineol and chyrsoplenetin) were inactive in the XO assay. These data support the conclusion of Nguyen et al. that flavonoids with a hydroxy group on C-7 are more active than those without this hydroxy group [10]. The inhibition of XO by the flavonoid-containing extract of *A. asiatica* and flavonoids **1**-**7**, results in a reduced level of generation of reactive oxygen species, but their free radical-scavenging activity was also postulated. The free radical scavenging activities of the isolated compounds (**1**-**7**) were therefore investigated in DPPH tests, in order to estimate their roles in oxidative processes. The results are shown in Table 1. The highest antioxidant activity (51.18 µM) was found to be exerted by 5,7,4',5'-tetrahydroxy-6,3'-dimethoxyflavone (**5**), which contains *ortho*-dihydroxy and 5-hydroxy groups. Interestingly, all the other compounds, including eupatilin (**1**), were inactive in this assay.

In conclusion, the results of this study indicate that the MeOH extract and the pure compounds isolated from the aerial parts of *A. asiatica* exhibited significant XO-inhibitory activity. The highest effect was demonstrated by eupatilin (**1**), the main flavone constituent of the plant and its formulated ethanol extract (DA-9601). Other flavonoids, that were isolated for the first time from *A. asatica* (excluding jaceosidin (**3**), also proved to display XO-inhibitory activity with IC_{50} in the range 2.59-7.49 µM).

Evaluation of the free radical-scavenging activities of these compounds in DPPH decolouration tests revealed the scavenging ability of 5,7,4',5'-tetrahydroxy-6,3'-dimethoxyflavone (**5**).

Thus, the flavonoid-containing MeOH extract of *A. asiatica* has a dual effect, acting by inhibiting of XO, thereby resulting in the reduced generation of reactive oxygen species, and by scavenging free radicals.

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Conflict of interest

The authors have declared that there is no conflict of interest.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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