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Acetylcholinesterase inhibitors from galbanum, the oleo gum-resin of *Ferula gummosa* Boiss.

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ABSTRACT

Due to the correlation of Alzheimer's disease with a cholinergic deficit, acetylcholinesterase (AChE) inhibitors from herbal drugs or herbal medicinal preparations have gained a lot of scientific interest.

In a previous study, several substances with AChE inhibitory activity were detected in galbanum by TLC bioautography and two of them were identified as auraptene and farnesiferol A. The aim of present study was the isolation and characterization of the other active compounds in galbanum.

In an activity-guided approach, six components were isolated via column chromatography and solid phase extraction. The compounds were identified as deacetylkellerin, kellerin, umbelliferone, 5′-acetoxyauraptene, 5′-hydroxyauraptene and farnesiferol B and their concentrations in galbanum were determined by HPLC analysis with external standardization.

Kellerin showed the best AChE inhibition with an IC_{50} value of 10.6 μM which was about one third of the positive control physostigmine (IC_{50} 2.91 μM).

1. Introduction

Alzheimer's disease (AD) comprises neurodegeneration resulting in memory impairment, emotional disorder and personality changes (Bartolucci et al., 2001). It is well known that a cholinergic deficit correlates with the severity of Alzheimer's disease (Garcia-Alloza et al., 2006). Thus, one rational target to retard the progress of symptoms of Alzheimer's disease is the restoration of the cholinergic function. A prolongation of the availability of acetylcholine, which is the most important cholinergic neurotransmitter, can be achieved by using inhibitors of acetylcholinesterase (AChE) (Howes and Houghton, 2003).

Some herbal remedies have been used traditionally for improving cognitive functions for a long time (Ip et al., 2014). During the last two decades, such medicinal plants are under increasing investigation (Andrade et al., 2000), which also led to the discovery of promising drugs and drug leads. For instance, galantamine from *Galanthus nivalis* L. (Snowdrop) has been proved to be a potent AChE inhibitor and is currently one therapeutic option in the treatment of Alzheimer's disease (Hostettmann et al., 2006).

Ferula gummosa Boiss. (Apiaceae) is a perennial plant growing in Northern and Western parts of Iran. Galbanum, the oleo gum-resin of *F. gummosa*, is an Iranian traditional herbal drug that has been employed individually or in mixtures for several purposes, as a tonic, in epilepsy and chorea, or for memory enhancement (Zargari, 1997; Adhami et al., 2013b). It is also used in gastrointestinal disorders and for wound-healing in Iranian traditional medicine (ITM) (Amin, 2005).

Several biological activities, such as cardioprotective (Gholitabar and Roshan, 2013; Mahmoody et al., 2013), antibacterial (Abedi et al., 2008), antioxidant (Nabavi et al., 2010), anticonvulsant (Sayyah et al., 2002), spasmolytic (Sadraei et al., 2001) and antiepileptic effects (Sayyah et al., 2008), have been demonstrated for extracts from different parts of *F. gummosa*. Recent chemical research has mainly focused on volatile components of this plant (Jalali et al., 2011, 2012, 2013), whereas studies of non-volatile compounds remain scarce (Iranshahi et al., 2010; Saidkhodzhaev et al., 1991).

In a previous screening, the dichloromethane (DCM) extract of galbanum had shown AChE inhibitory activity (Adhami et al., 2011). Several substances in galbanum showing AChE inhibitory effects were detected by TLC bioautography and two of those were identified as auraptene and farnesiferol A (Adhami et al., 2013a). In

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continuation of our investigation, the present study reports the isolation and identification of further AChE inhibitors in galbanum.

2. Results and discussion

2.1. Structure elucidation

Two fractions from a dichloromethane extract of galbanum which have shown AChE inhibitory components were submitted to further separation steps by column chromatography on silica or Sephadex LH-20 as well as by solid phase extraction on RP-18 phases. The fractionation resulted in the isolation of compounds **1**–**8** (Figs. 1 and 2).

The identification of **2**, **3** and **5** as umbelliferone, auraptene and farnesiferol A, respectively, was achieved by TLC comparison with authentic compounds and LC–MS experiments. Umbelliferone (**2**) has been previously reported from *F. gummosa* (Saidkhodzhaev et al., 1991) and is known to act as a weak AChE inhibitor (Karimi et al., 2010). Auraptene (**3**) and farnesiferol A (**5**) were isolated and identified from *F. gummosa* in our previous study (Adhami et al., 2013a). **8** was identified as 7-({(2*E*)-5-[(1*R*,3*S*)-3-hydroxy-2,2-dimethyl-6-methylidenecyclohexyl]-3-methylpent-2-en-1-yl}oxy)-2*H*-chromen-2-one (farnesiferol B) by ESI-MS, as well as by 1D and 2D NMR-experiments. The resulting ^{1}H and ^{13}C chemical shifts match published data (Yadav et al., 2010), the stereochemistry was proven by 2D NOESY correlations. This compound was previously identified from *F. gummosa* as well (Saidkhodzhaev et al., 1991).

From the LC–MS analysis of **1**, a molecular weight of 400 Da was deduced. In the positive ion mode MS^2 spectrum a fragment ion at *m*/*z* 163.3 was characteristic for a hydroxycoumarin core structure, while a series of further fragment ions indicated an aliphatic side chain. The connectivity from the side chain to the hydroxyl group at C-7 of the coumarin was established by 2D HMBC long range signals from H-11′ to C-7. Based on the shifts of C-3′ and C-8′, the occurrence of two further hydroxyl groups in these positions was evident. From the ^{13}C and 2D NMR spectra, the structure of a 7-hydroxycoumarin bound to a bicyclic sesquiterpene was deduced. The stereochemistry was deduced from characteristic 2D NOESY cross peaks, e.g. from the angular CH_3-15′ to CH_3-14′ and the two axial protons H-2′ and H-6′, from the CH_2-11′ to H-5′, or from H-3′ to the CH_3-14′. The compound was unambiguously identified as 7-{[(1*R*,2*S*,4a*R*,6*R*,8a*S*)-2,6-dihydroxy-2,5,5,8a-tetramethyldecahydro-naphthalen-1-yl]methoxy}-2*H*-chromen-2-one (deacetylkellerin).

Compound **4** yielded a low abundant $[M+H]^+$ ion at *m*/*z* 443 and a dominant $[M\text{-}CH_3COOH+H]^+$ ion at *m*/*z* 383 in the positive ion mode, indicating acetylation of an aliphatic OH group. The further fragmentation pattern showed similarities to deacetylkellerin (**1**). ^{1}H and ^{13}C NMR data also showed high similarities with the NMR data of deacetylkellerin, additional ^{1}H (CH_3 δ = 1.73 ppm) and ^{13}C signals (CH_3 δ = 20.9 and C=O δ = 172.3 ppm) proved the acetyl group. The linkage of this acetyl group to the hydroxyl group at C-3′ was established by a HMBC cross peak from H-3′ to C-16′. Thus, the structure of **4** was elucidated as 7-{[(1*R*,2*S*,4a*R*,6*R*,8a*S*)-2-hydroxy-3-acetoxy-2,5,5,8a-tetramethyldecahydro-naphthalen-1-yl]methoxy}-2*H*-chromen-2-one (kellerin) in accordance with literature data

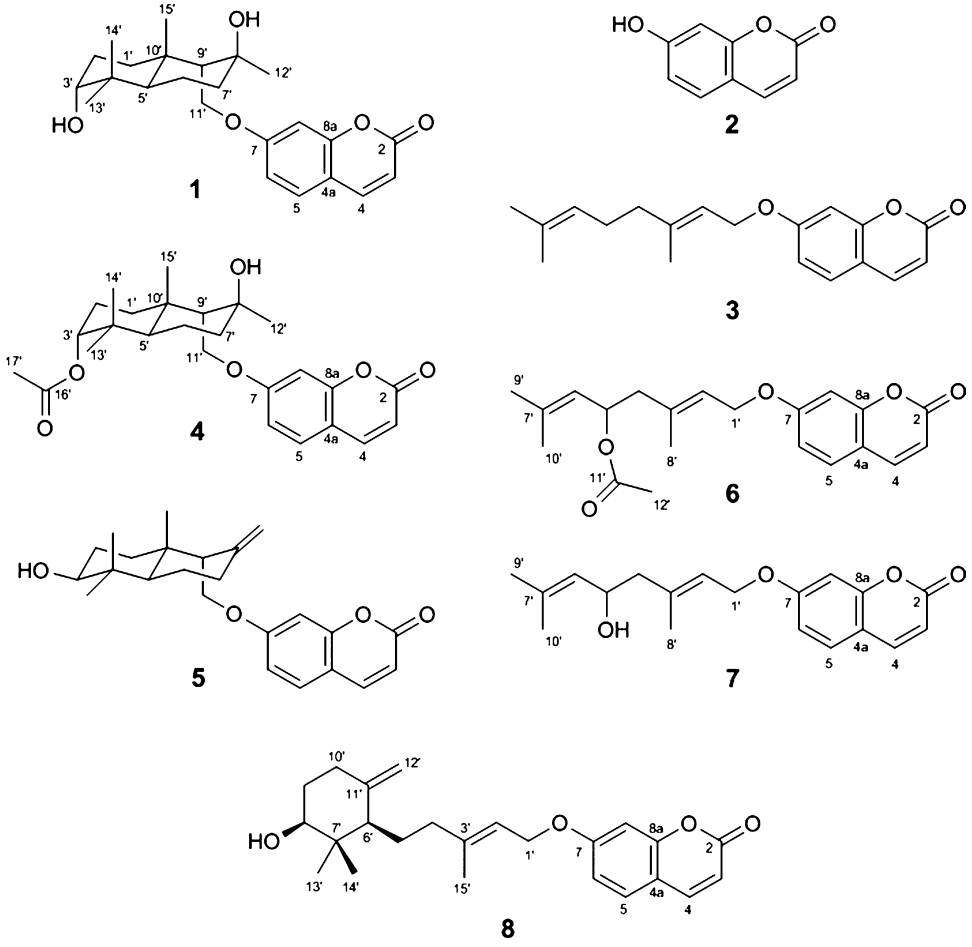

Fig. 1. Structures of the active compounds; **1**: deacetylkellerin, **2**: umbelliferone, **3**: auraptene, **4**: kellerin, **5**: farnesiferol A, **6**: 5′-acetoxyauraptene, **7**: 5′-hydroxyauraptene, **8**: farnesiferol B.

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