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SHORT COMMUNICATION

Study on constituents of *Scutellaria nepetifolia* as a potent source of phytochemicals with NO inhibitory effect

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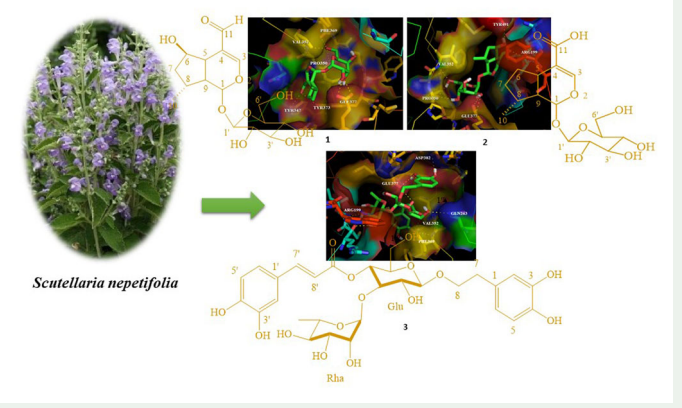
ABSTRACT

Based on the long history of *Scutellaria* plants in east traditional medicines, several species of *Scutellaria* showed promising antioxidant, anti-inflammation and neuroprotection effects in pharmacological researches. Using bioassay-guided fractionation of various extract of *Scutellaria nepetifolia*, an endemic species that grows widely in Iran, based on on nitric oxide (NO) inhibitory activity against H_2O_2 induced NO production in PC12 pheochromocytoma cells led to the isolation of two iridoid compounds namely, as 6β-hydroxy 8-epiboshnaloside (**1**) and 1,5,9-epideoxy loganic acid (**2**) and Verbascoside (**3**). Finally, the interaction of isolated compounds with inducible nitric oxide synthase (iNOS) protein was simulated by molecular docking study. It is the first report of these two iridoid glycosides from *Scutellaria* spp. All three isolated compounds showed strong interaction with iNOS enzyme in molecular docking simulations. So, they possibly contributed in the NO inhibitory effect of *S. nepetifolia*.



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1. Introduction

The genus *Scutellaria* (Lamiaceae) has about 360 species growing worldwide, usually in temperate and mountain regions of Europe, North America and Asia. In Iran there are 27 species of *Scutellaria* which 12 of them are endemic. This genus has a great place in traditional medicines, especially in China and Korea, and they have been used for memory-enhancing and antiepileptic purposes for many centuries (Shang et al. 2010; Jamzad 2012; Orhan et al. 2012). Nowadays, numerous researches revealed antioxidant, anti-inflammatory (Cui L et al. 2019; Jeong et al. 2019; Zengin et al. 2019), neuroprotective (Sashourpour et al. 2017; Jin et al. 2019), antidiabetic (Zhang et al. 2019) and antitumor (Wang M et al. 2019) properties of *Scutellaria* species and their constituents. Phytochemical studies showed that these plants are rich sources of phenolic (Wang G et al. 2011; Matsa et al. 2019), alkaloid (Han QT et al. 2018), diterpene and monoterpene compounds (Gousiadou et al. 2013; Matsa et al. 2019; Wang M et al. 2019).

Scutelaria nepetifolia is one of the endemic species of Iran which is commonly found in mountain region of the west and centre of Iran (Jamzad 2012). Following our previous work on *S. nepetifolia* which showed strong inhibitory effect against nitric oxide (NO) production during oxidative stress in PC12 cells (Parsa Khankandi et al. 2019), the present article studied the effect of various extracts of *S. nepetifolia* on NO production and the constituents of the methanol extract. In addition, the interaction of the isolated compounds with inducible nitric oxide synthase (iNOS) enzyme was simulated through docking method.

2. Results and discussion

The Griess assay results showed that all of the extracts except to the n-hexane extract halted the increase of NO production at 25 and 100 μg/ml concentrations and at 50 μg/ml concentration all of the extracts were effective. However, the methanol and ethyl acetate extracts were more effective than others at the lowest concentration (Figure S1). Subsequent phytochemical analysis of the methanol extract through vacuum liquid chromatography (VLC) and high-pressure liquid chromatography (HPLC) methods led to isolation of two terpenes (compound **1** and **2**) and a phenolic compound (compound **3**).

The structure of isolated compounds (Figure 1) was identified explicitly as 6β-hydroxy 8-epiboschnaloside (**1**), 1, 5, 9-epideoxy loganic acid (**2**) and Verbascoside (**3**) basis on their H and C-NMR (nuclear magnetic resonance) data (Table S2 and S3). The isolated compounds belong to iridoid and phenylethanoid classes which has well conformity with previous reports which showed that the iridoid and phenylethanoid compounds, especially in glycoside forms, are main constituents of many species of *Scutellaria* genus (Shang et al. 2010; Jamzad 2012). However, this is the first report of these two specific iridoid glycoside from *Scutellaria* genus. Before present study, 6β-hydroxy 8-epiboschnaloside (**1**) was isolated from *Cordylanthus kingii* (Justice et al. 1992) and 1, 5, 9-epideoxy loganic acid (**2**) was reported from three *Nepeta* species including *N. cadmea* (Takeda et al. 1998), *N. cataria* (Murai et al. 1984) and *N. grandiflora* (Nagy et al. 1998). On the other hand, Verbascoside is a well-known compound which was reported copiously from many plants, particularly which belongs to Lamiacea family (Saracoglu et al. 1995;

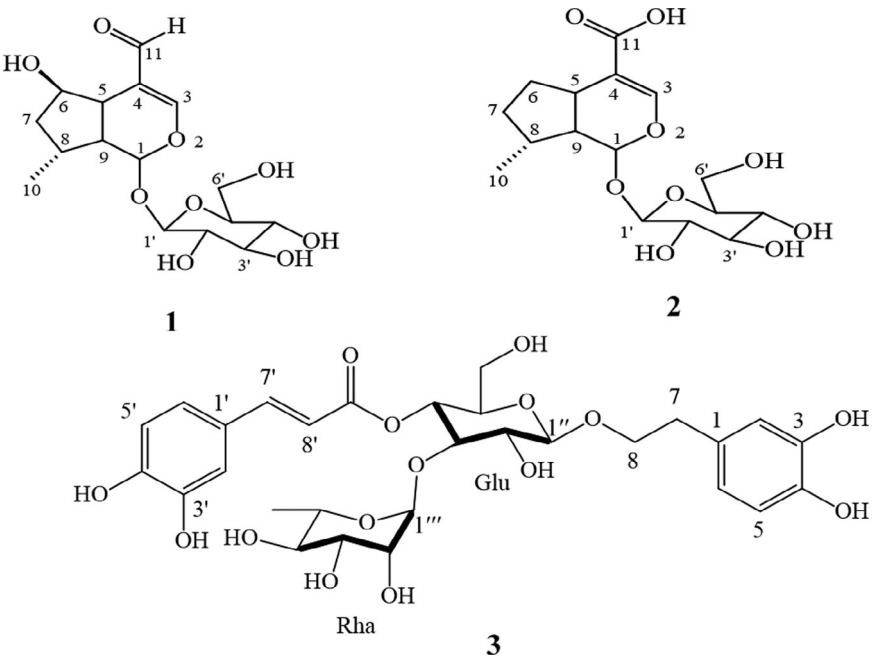

Figure 1. Structures of compounds **1**–**3**.

Zhou et al. 1997; Xie et al. 2002; Gousiadou et al. 2007; Rungsimakan and Rowan 2014). However, there are no bioassay reports available from the effect of two isolated iridoids either in vitro or in vivo, several studies reported inhibitory effect on production of inflammatory mediators such as NO for similar compounds (Cui Y et al. 2018; Tran et al. 2019). The biological effects of Verbascoside were studied extensively in last decades and several studies showed the inhibiting effect on NO production for this compound (Sheng et al. 2002; Han M-F et al. 2018).

The total extract of *S. nepetifolia* in our previous study (Parsa Khankandi et al. 2019) and the methanol extract of that in present study, proved strong effect in restraining of NO production during oxidative stress. Though, there is a good possibility of contribution of the isolated compounds in present study in the NO inhibitory effect of *S. nepetifolia*. In addition, inhibition of the iNOS enzyme is a presumable mechanism for active compounds of *S. nepetifolia* extract. The result of conducted docking study was corroborated this supposition. According to the results of molecular docking simulation (Table S4 and Figure S2), the isolated compounds had strong interaction with the iNOS protein. In addition, they could bond to key residues in active site of the iNOS enzyme, which are very important for inhibitory effect basis on previous iNOS studies (Garcin et al. 2008).

3. Conclusion

The methanol extract of *S. nepetifolia* showed strong NO inhibitory effect, and three compounds including two iridoid glycoside and a well-known phenylethanoid glycoside, Verbascoside were isolated from the effective extract. Furthermore, docking study

proved the strong interaction between the isolated compounds and the iNOS protein through targeting key residues in the active site. These results proposed the isolated compounds were possibly responsible to at least some of the NO production inhibitory effect of *S. nepetifolia* by harnessing the iNOS enzyme, and the isolated compounds together with the methanol extract of *S. nepetifolia* could be the potent subjects for more studies developing anti-inflammatory pharmacophores.

Disclosure statement

No potential conflict of interest was reported by the authors.

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