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SHORT COMMUNICATION

Glutaminase inhibitory activities of pentacyclic triterpenes isolated from *Thymus vulgaris* L.

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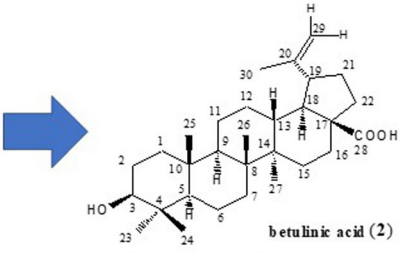
ABSTRACT

Glutaminase is an important target that is often over expressed in neurodegenerative and lifestyle related diseases but few effective inhibitors of this enzyme have yet reached clinical trials. Ursolic acid (**1**), betulinic acid (**2**) and oleanolic acid (**3**), three pentacyclic triterpene acids, have been isolated from the leaves of *Thymus vulgaris* L. Enzyme inhibition experiments demonstrated their inhibitory effects against glutaminase activity. Compound **2** significantly inhibited the glutaminase activity with IC_{50} of 0.31 mM, stronger than the positive control 6-diazo-5-oxo-L-norleucine (DON) with IC_{50} of 0.57 mM. Compound **2** may serve as a potential lead compound for the prevention and treatment of neurodegenerative diseases and lifestyle related diseases by targeting glutaminase. This is the first report on glutaminase inhibitory activities of **1**-**3** isolated from *T. vulgaris* L.

Thymus vulgaris L.



Glutaminase inhibition
IC_{50} = 0.31 mM

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1. Introduction

Glutaminase is the first key enzyme in glutamine metabolism that catalyzes the hydrolysis of glutamine into glutamate and is associated with central nervous system (CNS) disorders, cancer cells, type 2 diabetes (T2D) and obesity (Busque et al. 2014; Thomas et al. 2014; Griffin et al. 2018). L-Glutamate is the major excitatory neurotransmitter in the mammalian CNS and regulates all functions of the CNS including memory, cognition sensation and behavior. Microglial glutaminase activity levels increase under conditions of neuroinflammation, resulting in increased CNS glutamate production and therefore excess extracellular glutamate causes neuronal dysfunction and death, a process known as excitotoxicity. Chronic glutamate excitotoxicity is responsible for neurodegenerative diseases such as amyotrophic lateral sclerosis, Huntington's disease and Alzheimer's disease (Sugiyama et al. 2017). Glutaminase controls the first step in the glutaminolysis which serves as a significant role in many energy-generating and biosynthesis process for the accelerated growth and proliferation in many malignant tumors (Thangavelu et al. 2014; Xu et al. 2019). Some oncogenes including c-Myc, Raf, Ras and Rho GTPase could up-regulate glutaminase expression in many cancer cells and treating tumor cells with kidney-type glutaminase specific siRNA induces apoptosis under oxidative stress (Yang et al. 2019). T2D is a chronic and metabolic disease characterized by hyperglycemia due to insulin resistance and beta cell dysfunction. Excess glutamate in the liver is deaminated and fed into the citric acid cycle, and then glutamine-derived gluconeogenesis contributes to the high blood glucose levels observed in T2D. Additionally, glutamate has been found to increase insulin secretion by amplifying the incretin pathway in beta cells (Griffin et al. 2018). Glutaminase inhibition led to AMP-activated protein kinase phosphorylation, which facilitates energy expenditure, lipid oxidation and thermogenic capacity and exerts protective effects against obesity (Momcilovic et al. 2017; Kim et al. 2019).

Glutaminase has become a potential therapeutic target for the development of the prevention and treatment of neurodegenerative and lifestyle related diseases, but there are few known potent and selective glutaminase inhibitors available. Although 6-diazo-5-oxo-L-norleucine (DON), a glutaminase inhibitor isolated from *Streptomyces* bacteria, exhibited neuroprotective, anticancer, anti-T2D and antiobesity effects (Thangavelu et al. 2014; Koizumi et al. 2020a and Koizumi et al. 2020b), it could not be widely applied due to its non-specific selectivity, poor solubility and dose-limiting toxic gastrointestinal-related side effects (Thomas et al. 2014; Hollinger et al. 2020). On the other hand, the use of plant extracts and phytochemicals can contribute to reduce the intake of pharmaceutical drugs and side effects. Therefore, we need to seek new glutaminase inhibitors from the daily intake of food.

Thymus vulgaris L. (common thyme) belonging to the *Lamiaceae* family which is composed primarily of thyme, basil, oregano, rosemary, and sage, is an aromatic medicinal herb native to Mediterranean region, widely utilized in food seasoning, herbal tea, folk medicine and essential oils. Thyme shows antioxidant, anti-inflammatory, anticancer and immunomodulatory activities (Mirjalili et al. 2016). Additionally, rosmarinic acid from *T. vulgaris* L. remarkably inhibited growth of human breast cancer cell line (Darwish et al. 2020).

Here, we report the isolation, structural identification of three pentacyclic triterpenes, betulinic acid, oleanolic acid and ursolic acid from *T. vulgaris* L. and those glutaminase inhibitory activities.

2. Results and discussion

The hydroethanolic extracts from the leaves of *T. vulgaris* L. and the EtOAc-soluble extracts inhibited glutaminase activities by 33% and 41% at a concentration of 10 mg.mL^{-1}, respectively. A component in the EtOAc-soluble extracts was purified by column chromatography, HPLC and recrystallization to afford three compounds. Three compounds were identified on the basis of spectroscopic data, especially using 1 D and 2 D NMR as well as EI-MS and comparison of the data with literature values as ursolic acid (**1**) (Shimada and Inagaki 2014), betulinic acid (**2**) (Peng et al. 1998) and oleanolic acid (**3**) (Mshelia et al. 2019), respectively (Fig. 1). Compounds **1**-**3**, pentacyclic triterpene acids, are secondary plant metabolites widespread in herbs and fruits (Jäger et al. 2009), but there is no information regarding glutaminase inhibitory activities of **1**-**3**.

Compounds **1**-**3** and DON isolated from *Streptomyces* bacteria used as a positive control were examined for their effects on glutaminase from *Escherichia coli*. Compound **2** exhibited potent glutaminase inhibitory activity with an IC_{50} value of 0.31 mM and was stronger than that of DON. Compounds **1** and **3** showed weaker glutaminase inhibitory activities than that of DON (Table S1). Compounds **1**-**3** have two active positions in their structures, namely the secondary hydroxy group at C-3 position and the carboxyl group at C-28 position, because those functional groups might form hydrogen bonding interactions with the active-site residue Ser66 in *E. coli* glutaminase (Brown et al. 2008). Compound **2** has also the other active position, the isopropenyl side chain at C-19, in its structure, because the bulky side chain might form the hydrophobic interactions with the active-site residues Tyr29, Tyr192 and Tyr244 in *E. coli* glutaminase (Brown et al. 2008). Those results suggested that the hydroxy and carboxyl groups in the molecules of **1**-**3** and the isopropenyl group at C-19 in the molecule of **2** might play the important role in the glutaminase inhibitory activity.

Consequently, the daily intake of herbs and fruits such as thyme, rosemary, sage, apple and olive could be valuable for supplying a high concentration of **1**-**3** (Jäger et al. 2009) and might play an important role in the prevention of chronic neurodegenerative diseases, anticancer, anti-T2D and antiobesity therapy because biological

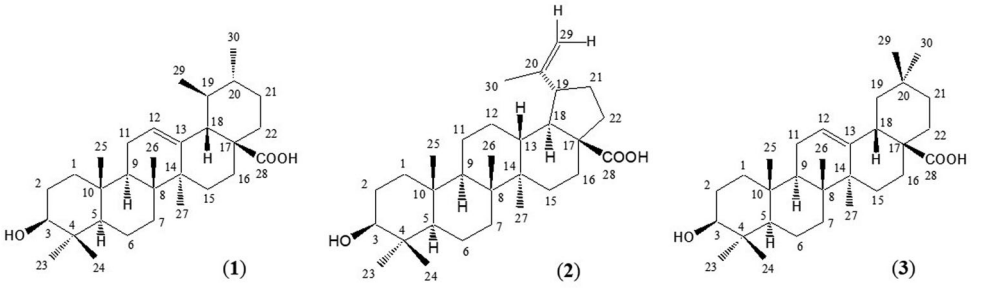

Figure 1. Chemical strucutres of ursolic acid (**1**), betulinic acid (**2**) and oleanolic acid (**3**).

activities of **1**-**3** had already been reported to exhibit pharmacological activities against neurodegenerative disorders (Ruszkowski and Bobkiewicz-Kozlowska 2014; Kaundal et al. 2018), cancer cells, type 2 diabetes and obesity (Silva et al. 2016; Bildziukevich et al. 2019) in addition to showing glutaminase inhibitory activity. Compounds **1**-**3**, especially betulinic acid, might be lead compounds for the prevention and treatment of neurodegenerative diseases and lifestyle related diseases which have not been known until now. This is the first report on glutaminase inhibitory activities of **1**-**3** isolated from *T. vulgaris* L.

3. Conclusions

The EtOAc-soluble extracts from the leaves of *T. vulgaris* L. were purified by column chromatography, HPLC and recrystallization to afford three compounds. Those structures were established on the basis of 1 D and 2 D NMR spectroscopic data as ursolic acid (**1**), betulinic acid (**2**) and oleanolic acid (**3**). Compound **2** exhibited potent glutaminase inhibitory activity with an IC_{50} value of 0.31 mM. This is the first report on glutaminase inhibitory activities of **1**-**3** isolated from *T. vulgaris* L.

Disclosure statement

No potential conflict of interest was reported by the authors.

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