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rvH1N1 Neuraminidase Inhibitory Activities of Phenolics from *Perilla frutescens* (L.) and Comparison of Their Contents Among Cultivars and Germplasms

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[Introduction]

Pharmacological strategies for dealing with influenza pandemic is now based on antiviral drugs, in which neuraminidase (NA) inhibitors are the most important. There is no doubt that the agricultural production is the most important of *Perilla frutescens*. In comparison with the production, the phytopharmacology is not fully investigated in biological activity, although it is widely used as traditional Chinese medicines for various diseases, such as cold due to wind-cold, headache and cough. To the best of our knowledge, the investigation regarding NA inhibition of this plant has still not been reported. This prompted us to examine major four phenolic compounds isolated from Perilla to gain new insights into their NA inhibitory action on a molecular basis. We also quantified the content of these compounds of Korean perilla germplasms and cultivars.

[Materials and Methods]

Recombinant influenza A virus H1N1 neuraminidase was purchased from R&D Systems. 2'-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid, acetic acid, $CaCl_2$, DMSO, Tris, and NaCl were obtained from Sigma Chemical Co. Throughout the experiment, 4-MUNANA was used as a substrate. In a fluorescence experiment, the cleaving activity of the NA was monitored at 37 ºC by Spectra MAX plus spectrophotometer. The quantification of four major phenolic compounds in the seeds of perilla germplasm and cultivars were carried out using Ultimate 3000 HPLC analysis. A 20 μ l sample of the 80% methanol extract was injected into an analytical Eclipse XDB-C18 column (Agilent Technologies, 150× 4.6 mm I.D., 5 μ m, Palo Alto, CA, USA).

[Results and Discussions]

Four major polyphenolic compounds including rosmarinic acid-3-*O*-glucoside (**1**), rosmarinic acid (**2**), luteolin (**3**), and apigenin (**4**) from *P. frutescens* were evaluated for their inhibitory effect on recombinant virus H1N1 neuraminidase. Among the test compounds, **2** and **3** inhibited the rvH1N1 NA with an IC_{50} of 46.7 and 8.4μ M, respectively. The inhibition kinetics analyzed by Dixon plots indicates **2** and **3** are noncompetitive inhibitor and inhibition constant, K_i, were established as 43.9 and 14.3 μ M, respectively. In addition, 578 genetically diverse accessions and 39 cultivars of *P. frutescens* were analyzed using HPLC to characterize the diversity of polyphenolic composition and concentration. The individual and total compositions exhibited significant difference ($p < 0.05$), especially **2** was detected as the predominant (59%) metabolite in all accessions and cultivars. Compound **2** and **3** contents ranges from 280.8 to 3,230.2 μ g/g and 0.3 to 889.8 μ g/g in germplasm respectively. Additionally, Yeupsil and Sangback cultivars exhibited the highest rosmarinic acid (3,393.5 mg/g) and luteolin (383.3 mg/g) content respectively.

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