

Abstract

Evaluation of Anti-Asthmatic Activity of Essential oil from *Vitex rotundifolia* Fruits in LPS-Stimulated NCI-H292 Cells [†]

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Abstract: Background: Medicinal plants continue to be an interesting source of natural products for treating various health conditions. It is estimated that more than 150,000 plant species have been studied, many of which contain valuable therapeutic agents, and the applications of novel compounds from plants for pharmaceutical purposes have been gradually increasing in recent years. Essential oils are volatile compounds with an oily fragrance obtained from the different plant parts, flowers, leaves, stems, bark, and roots. These volatile oils showed the different kinds of biological activities, including antibacterial, antioxidant, antiviral, insecticidal, etc. The importance of essential oils is increasing because they are mostly used in the beverage and food industries, cosmetics and fragrance industries to make valuable perfumes, and with many biological activities. Medicinal and aromatic plants are extensively used as natural organic compounds and as medicines. Also, essential oils of aromatic plants have been used to treat various sorts of infectious diseases. In recent years, numerous efforts are made to explore essential oils used to treat various diseases. *Vitex rotundifolia* L. is a deciduous shrub belonging to the Verbenaceae. Its fruits have been used in traditional medicine to treat colds, headaches, chronic otitis media, neuralgia, and various diseases. The extractives of *Vitex rotundifolia* L contain various chemical components, such as flavonoids, diterpenes, terpenoids, and lignans. In addition, it has various bioactivities such as antioxidant, anti-cancer, anti-inflammatory, anti-allergic, and soothing effects. **Objective:** The purpose of this study was to determine the potential as an inhibitor of mucus production through evaluating the anti-asthmatic effect of essential oil from *V. rotundifolia* fruit. **Methods:** The essential oil was extracted from *V. rotundifolia* fruit by hydrodistillation method. Chemical compositions of the essential oil were identified by GC-MS analysis. GC-MS was equipped with a VF-5MS column. The anti-asthmatic activity of essential oil was examined using the LPS-stimulated NCI-H292 cells. NCI-H292 cells were cultured in a 37°C, 5% CO₂ incubator, followed by treatment with various concentrations (10⁻³%~10⁻⁷%) of essential oil from *V. rotundifolia* fruit. After 24 h incubation, CCK-8 solution was added to each well, and the 96-well plate was incubated at 37°C for 1 h. The percentage of cells showing was determined relative to the control group. Relative gene expression changes of MUC4, MUC5AC, and MUC5B were investigated in the LPS-stimulated NCI-H292 cells treated with essential oil. **Results:** As a result of GC/MS analysis, α -pinene (38.07%), 1,8-cineol (12.73%), 5 α ,9 α ,10 β -kaur-15-ene (7.53%), β -pinene (6.20%), and α -terpinyl acetate (5.22%) were identified as major components of the essential oil. The essential oil exhibited toxicity in a dose-dependent manner. The cell viability was higher than 80% at oil concentrations of 10⁻⁷%~10⁻⁵%, and almost no cytotoxicity was observed at a concentration lower than 10⁻⁵% in NCI-H292. When LPS-stimulated NCI-H292 cells were treated with essential oil (10⁻⁷%), the relative expression levels of the mucus genes MUC4, MUC5AC, and MUC5B were significantly reduced compared to the LPS-treated group (NC). According to the results of the relative expression level of mucus genes, the expression level of the MUC4, membranous mucin, was decreased by about 42% compared to the negative control. The relative expression of MUC5AC, the most important secretory mucus gene among the mucus genes, was reduced by about 73.6% compared to the negative control. In MUC5B, another secretory mucinous, the essential oil inhibited the gene expression of MUC5B effectively. The MUC5B expression was decreased by about 69.3% in

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the LPS-stimulated NCI-H292 cells. Budesonide, the positive control, was reduced the relative expression of mucin genes MUC4, MUC5AC, and MUC5B by 7.4%, 18.6%, and 86.6%, respectively. The anti-asthmatic activity of essential oil was very good compared with budesonide. **Conclusions:** The anti-asthmatic effect of essential oil from *V. rotundifolia* fruit was confirmed. The anti-asthmatic activity of essential oil was superior to budesonide, an anti-asthmatic treatment. These results are suggested that that the essential oil of *V. rotundifolia* fruit can reduce asthma response by suppressing the expression of the mucus gene.