Identification of Biological Effective Constituents from the *Potentilla supina*

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RURAL DEVELOPMENT ADMINISTRATION

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Introduction


Potentilla supina (PS) are a member of the R osaceae family that is native to China, Japan, Korea, India, and Malaysia. They produces o ne or more erect stems from a branching cau dex and system of rhizomes. They grows 20 to 60 cm tall, and is slightly hairy to nearly hairless. The leaves are ternate, divided into three leaflets. The basal leaves are largest, borne on long petioles. Each has oval leaflets up to 3 cm long which are deeply cut into blunt teeth. Smaller leaves occur higher on the stem. The inflorescence is a cyme of one or more flowers. The flower has usually five yellow petals up to a cm long on a calyx of pointed sepals and narrower pointed bractlets.

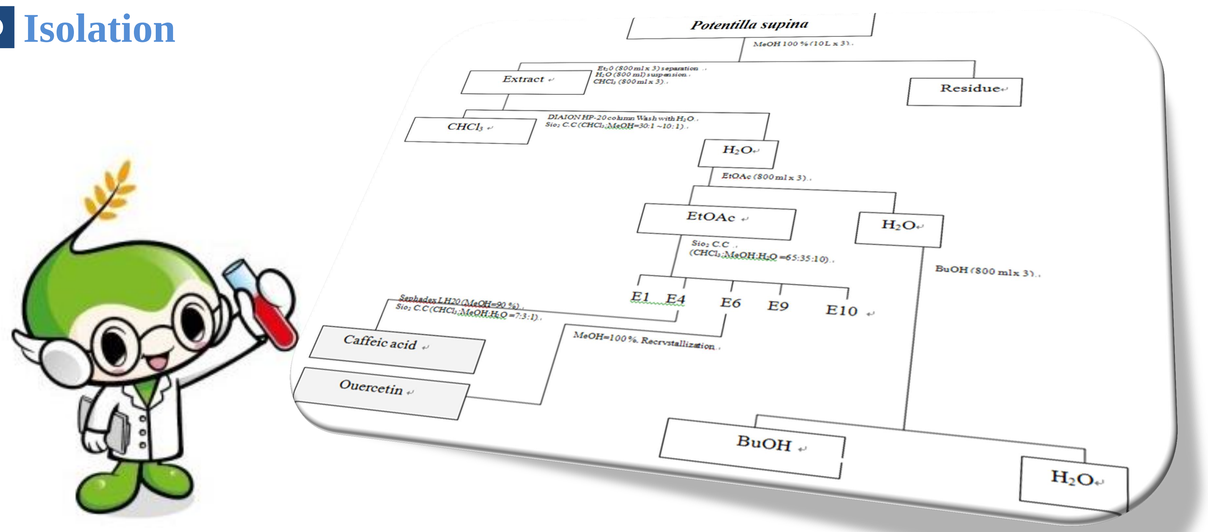
PS often used in Korean traditional systems of medicine as a remedy for hemostasis, dysentery cough, pertussis, sore throat, and external bleeding. Generally drugs that are used for arthritis have antinociceptive and anti-inflammatory properties.

The purpose of this study was to determine whether the total polyphenol contents, anti-oxidative and anti-inflammatory activities were examined on the extract from PS and their biologically active constituents were identified.

Materials & Methods

Plant Material - PS was collected on May 2011, near the paro lake, Yang-gu County, Gang-won Province, Korea and was identified by Prof. H. J. Park (Department of Pharmaceutical Engineering, College of Health Sciences, Sangji University, Won-ju, Korea). A voucher specimen (NIHA.P-04) is deposited in the herbarium of Highland Agriculture Research center, Pyeong-chang, Korea. Dried substance of PSwere cut and extracted three times with EtOH under reflux and evaporated to give a viscous mass. This material was successively suspended in 2L H_2O and then partitioned with each 2L of Hexane, $CHCl_3$, EtOAc. And BuOH Each layer was dried *in vacuous*.

Isolation



Measurement of total polyphenol contents - Determined by Folin-Denis method

Radical scavenging activity - Anti-oxidant effects was performed by using DPPH assay

Cell culture - RAW 264.7 murine macrophages were obtained from American Type Culture Collection

MTT assay for cell viability - Cytotoxicity of extraction and fractions on RAW 264.7 cells, was carried out by MTT assay.

Nitric oxide & ProstaglandinE_2 assay

- The Inhibitory effect on NO & PGE_2 production in RAW 264.7 cells was evaluated by measuring nitrite (Griess reaction) and Prostaglandin E_2 in the medium as an indicator of nitric oxide production.

Results

Table 1. Measurement of total polyphenol contents of PS extract and fractions.

Total polyphenol contents	
Sample	*μg/g*
PS Hexane fr.	0.8±5.7
PS EtOH ex.	53.1±2.8
PS EtOAc fr.	316..4±11.8
PS $CHCl_3$ fr.	49.2±7.3

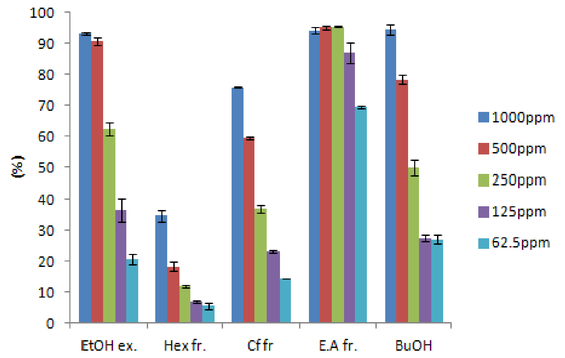


Fig. 1. Radical scavenging activity of PS extract and fractions.

Table 2. Cytotoxicity of PS extract and fractions.

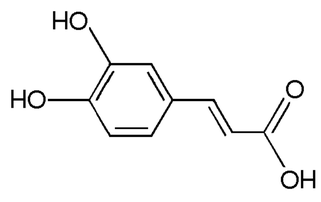
MTT assay	
Sample	IC_{80}(μg/ml)
PS Hexane fr.	>100
PS EtOH ex.	>100
PS EtOAc fr.	>100
PS $CHCl_3$ fr.	>100

Table 3. Effects of PS extract and fractions on LPS induced NO production.

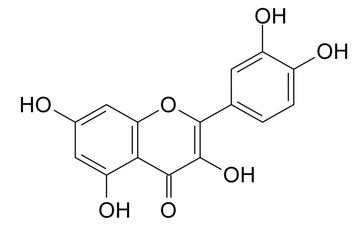
NO assay	
Sample	IC_{50} (μg/ml)
PS EtOH ex.	56.07
PS Hexane fr.	60.30
PS EtOAc fr.	29.34
PS $CHCl_3$ fr.	>10

Table 4. Effects of PS extract and fractions on LPS induced PGE_2 production.

PGE_2	
Sample	IC_{50} (μg/ml)
PS EtOH ex.	47.46
PS Hexane fr.	54.96
PS EtOAc fr.	50.75
PS $CHCl_3$ fr.	>10



Caffeic acid(1)



Quercetin(2)

Fig 2. The structure of two compounds isolated from PS

Conclusion

The total polyphenol contents appeared to be highest in EtOAc fraction(316.4±11.8mg/g) of PS. The DPPH radical scavenging activity was highest in an EtOAc fraction of PS of 95.4±0.3% at a concentration of 250μg/mL ($p<0.05$).The anti-inflammatory activities of EtOAc fraction of PS was evaluated for inhibitory activities against lipopolysacchride(LPS) induced nitric oxide(NO) and prostaglandinE_2(PGE_2) production in RAW264.7 celllines. The EtOAc fraction of PS was high inhibitory activity for both tests withIC_{50} values showed in the ranges of 29.34~50.75μg/ml.

The biological effective compounds in the whole parts of PS were isolated by a bioassay guided purification using the anti-oxidative and anti-inflammatory activities. The repeated column chromatography of ethyl acetate-soluble layer in this ethanol extract led to isolation two constituents such as caffeic acid(1) and quercitrin(2). The structures of these compounds were identified by spectroscopic methods and by comparing their data to those in the literature. As far as we know, two compounds were isolated for the first time from this plant. Both compounds were already reported ingredients but two compounds are considered to exhibit a high physiological activity. This result revealed that EtOAc fraction of PS is expected to be good candidate for development into source of prophylactic agent.

References

Uttara B, Singh AV, Zamboni P, Mahajan RT(2009) Oxidative stress and neurodednergeat ive iseases : a review of upstream and downstream antioxidant therapeutic options. Curr. Neuropharmacol. 7:65-74