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야관문 에탄올 추출물의 항산화 및 Metalloproteinase 저해 활성

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Antioxidant and Metalloproteinase Inhibitory Activities of Ethanol Extracts from *Lespedeza cuneata* G. Don

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Received: 23 October 2017/ Revised: 30 October 2017/ Accepted: 1 December 2017

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Abstract

BACKGROUND: *Lespedeza cuneata* G. Don is a well-known medicinal plant. In this study, the biological activities of *L. cuneata* extracts were investigated.

METHODS AND RESULTS: *L. cuneata* shoot was extracted with 30% ethanol and further fractionated with organic solvents. Total phenolic and flavonoid content, antioxidant activity, and matrix metalloproteinase inhibition effect of the extract and fractions were measured. Among the tested extract and fractions, the highest contents of total phenolic and flavonoids were found in ethyl acetate fraction (117.8 mg GAE/g and 35.9 mg QE/g, respectively). Ethyl acetate fraction showed the highest DPPH and ABTS radical scavenging activity, and the antioxidant activity of the other fractions followed the order n-hexane fraction>ethanol extract>methyl chloroform>n-butanol fraction. Inhibitory effect on the expression of matrix metalloproteinases (MMP1 and MMP3) was highest in the fraction of ethyl acetate, and n-butanol fraction also significantly inhibited the expression of MMP3. Antioxidant activities of *L. cuneata* extracts were significantly positively related to their phenolic and flavonoid content.

CONCLUSION: Ethyl acetate fraction of *L. cuneata* ethanol extract showed potent antioxidant and matrix metalloproteinases inhibitory activities. Those activities might be related to the high total phenolic and flavonoid content of the extract.

Key words: Antioxidant, Flavonoid, *Lespedeza cuneata*, Matrix metalloproteinase, Phenol

서론

(*Lespedeza cuneata* G. Don)

가

al, 2014).

가

1991).

(collagen)

(elastin)

. Collagen matrix metalloproteinase-1 (MMP1) elastin

(Jung et

가

(Stich,

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matrix metalloproteinase-3 (MMP3) 가 (Rhie *et al.*, 2001; Tsuji *et al.*, 2001). MMPs 가 collagen elastin 가 MMPs (Stich, 1991). flavonoid phenol quercetin kaempferol (Numata *et al.*, 1980). tyrosinase ethyl acetate 가 (Cha *et al.*, 2010; Lee *et al.*, 2011). elastase 가 (Kim and Kim, 2014). collagenase elastinase human dermal fibroblast (HDF)

gallic acid equivalent(GAE) . Kim (2011) 200 µL 10% aluminum nitrate 100 µL 1 M potassium acetate 100 µL 4.6 mL 80% 가 417 nm 3 quercetin quercetin equivalent(QE) **항산화 활성 측정** DPPH ABTS radical DPPH radical Kim (2011) 20 µL 0.15 mM 1,1-diphenyl-2-picrylhydrazyl(DPPH) 180 µL 가 96-well plate 30 multi-micro plate reader (Spectra Max i3X, CA, USA) 517 nm 3 DPPH radical , DPPH radical 50% DRC50

재료 및 방법

시료 및 추출물의 제조

500 g 30% (v/v) 1:15 24 n-hexane, methyl chloroform, ethyl acetate, n-butanol, aqueous 3

$$\text{Inhibition rate (\%)} = [1 - (A - B) / C] \times 100$$

A: absorbance of sample

B: absorbance of blank

C: absorbance of control

ABTS radical Ko (2017) 7.4 mM 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) 2.6 mM potassium persulphate ABTS

총 페놀 및 플라보노이드 함량 분석

Folin-Ciocalteu (Kim *et al.*, 2011). 30% 100 µL 500 µL Folin-Ciocalteu 50 µL 5 20% sodium carbonate 300 µL 20 725 nm 3

ABTS 가 734 nm 가 1.0 20 µL multi-microplate reader 734 nm 3 , ABTS radical 50% ARC50

MMP1과 MMP3 저해 활성 측정

MMPs Cho (2016) Human dermal

fibroblast (HDF) 1×10^5 cells/well
 6-well plate tumor necrosis factor alpha
 (TNF- α) 10 ng/mL MMPs
 48 phosphate buffered
 saline
 protein extraction solution 가 1.5
 mL tube 99°C 10 가
 -70°C bicinchoninic acid protein
 assay kit 10% SDS
 polyacrylamide gel nitrocellulose membrane
 membrane 5% skim milk/TBST
 (tris-buffered saline and 0.1% Tween 20) 1
 blocking 1 antibody membrane 가
 가 TBST 5 5 가
 1 antibody peroxidase 2 가
 antibody 1
 Membrane EZ-Western lumi pico reagent 가
 ChemiDoc image analyzer(Bio-Rad)
 ImageJ program 가
 (Kim *et al.*, 2011; Senguttuvan *et al.*, 2014),

통계 처리
 test ,
 가 .
 Duncan's multiple range
 Peason's correlation coefficient

결과 및 고찰
 페놀, 플라보노이드 함량
 Table 1
 가 , 30%
 가 ,
 가
 , 30%
 가

Table 1. Total phenol and flavonoid contents in 30% ethanol extract and soluble fractions of *Lespedeza cuneata* G. Don

Extract and Fractions	Total phenol (mg GAE/g) ^{a)}	Total flavonoid (mg QE/g) ^{b)}
30% Ethanol extract	36.21±0.24 e ^{c)}	5.13±0.54 e
<i>n</i> -Hexane fraction	79.74±0.78 b	6.36±1.20 d
Methyl chloroform fraction	39.06±1.10 d	7.93±0.43 c
Ethylacetate fraction	117.77±2.86 a	35.89±0.55 a
<i>n</i> -Butanol fraction	45.40±0.80 c	14.71±0.23 b
Aqueous fraction	13.31±0.57 f	Not detected

^{a)}GAE: gallic acid equivalent

^{b)}QE: quercetin equivalent

^{c)}Each value represents mean±SD (n=3), and same letters within a row indicate no significant difference at $p < 0.05$.

Table 2. Radical scavenging activity of 30% ethanol extract and soluble fractions of *Lespedeza cuneata* G. Don

Extract and Fractions	DRC ₅₀ (μ g/mL) ^{a)}	ARC ₅₀ (μ g/mL) ^{b)}
30% Ethanol extract	178.94±5.46 c ^{d)}	59.60±0.80 b
<i>n</i> -Hexane fraction	124.11±2.18 de	51.13±0.26 c
Methyl chloroform fraction	190.49±3.77 c	58.72±1.22 b
Ethylacetate fraction	91.63±1.96 e	26.82±0.61 d
<i>n</i> -Butanol fraction	157.38±4.17 cd	60.21±0.87 b
Aqueous fraction	833.24±63.1 a	161.36±8.14 a
BHT ^{c)}	241.35±8.48 b	< 6.25

^{a)}Concentration required to scavenge DPPH radical by 50%

^{b)}Concentration required to scavenge ABTS radical by 50%

^{c)}Positive control (Dibutylated hydroxytoluene),

^{d)}Each value represents mean±SD (n=3), and same letters within a row indicate no significant difference at $p < 0.05$.

가 . 가 . ABTS radical radical

항산화 활성

butylated (Zheng

hydroxy toluene (BHT) . DPPH radical and Wang, 2001; Wojdylo *et al.*, 2007).

ABTS radical 50% 30%

ARC₅₀ Table 2 DRC₅₀ 야관문 추출물의 MMPs 억제 활성

DPPH radical 가 , Matrix metalloproteinase (MMP) collagenase (MMP1, MMP8, MMP13), gelatinase (MMP2, MMP9), stromelysin (MMP3, MMP10) 3 , collagenase

DPPH radical collagen collagen

Stromelysin collagen

ABTS radical 가

DPPH radical 가 MMP1 MMP3

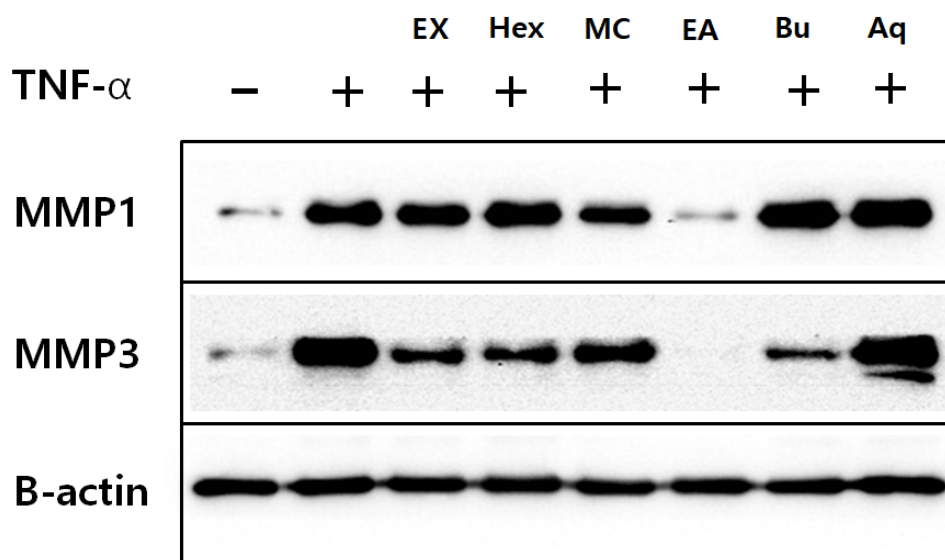


Fig. 1. Effect of ethanol extract and soluble fractions of *Lespedeza cuneata* G. Don on MMP1 and MMP3 protein expression. Cells were stimulated by TNF- α for 48 hour in the presence of ethanol extract and soluble fractions of *Lespedeza cuneata* G. Don. Ex: ethanol extract, Hex: *n*-hexane fraction, MC: methyl chloroform fraction, EA: ethyl acetate fraction, Bu: *n*-butanol fraction, Aq: aqueous fraction.

Table 3. Correlation between total phenol and flavonoid content and biological activities of the ethanol extract and soluble fractions of *Lespedeza cuneata* G. Don

	TPC ^{a)}	TFC ^{b)}	DRC ₅₀ ^{c)}	ARC ₅₀ ^{d)}	MMP1	MMP3
TPC	1	0.8479***	-0.6461**	-0.7427**	-0.7687**	-0.8505***
TFC		1	-0.5289*	-0.6435**	-0.8269***	-0.8946***
DRC ₅₀			1	0.9841***	0.5003*	0.7775***
ARC ₅₀				1	0.6359**	0.8411***
MMP1					1	0.6948**
MMP3						1

^{a)}Total phenolic content determined as gallic acid equivalent

^{b)}Total flavonoid content determined as quercetin equivalent

^{c)}Concentration required to scavenge DPPH radical by 50%

^{d)}Concentration required to scavenge ABTS radical by 50%

Fig. 1 . 30%
 MMP1 MMP3
 가 가 , TNF- α
 control cell (-TNF- α) MMP1
 MMP3가 ,
 , MMP3 , 30%
 ,
 . 30% MMP1
 MMP3 .
 MMP1 MMP3
 (Kang *et al.*, 2007; Jung *et al.*, 2014),

상관관계 분석

Table 3 .
 radical DRC50 ARC50
 , collagen elastin
 MMP1 MMP3

요 약

30% DPPH
 ABTS radical matrix metalloproteinase
 MMP1 MMP3
 가
 MMP1, MMP3
 가
 MMPs 가 가

Notes

The author declare no conflict of interest.

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