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Research Article

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Inhibitory effects of phenolic compounds from stems of *Acer ginnala* on nitric oxide production

Eun-Kyung Ko and Sun-Eun Choi*

Department of Cosmetology Science, Nambu University, GwangJu Metropolitan City, Republic of Korea

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ABSTRACT

*Activity guided isolation of 80% acetone extract from the barks of Acer ginnala Maxim. yielded five gallotannins[6-galloyl-1,5-anhydroglucitol (ginnalin B) (**1**), acertannin (3,6-digalloyl-1,5-anhydroglucitol) (**2**), methyl gallate (**3**), acertannin(2,6-digalloyl-1,5-anhydroglucitol) (**4**) and gallic acid (**5**)].In order to evaluate the anti-inflammatory effects of these compounds, their inhibitory activities agains tnitric oxide production in lipopolysaccharide(LPS)-stimulated RAW 264.7 cells were examined.*

Keywords: *Acer ginnala*, gallotannin, Anti-inflammatory activity, Nitric oxide

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INTRODUCTION

The inflammatory response is a defense mechanism against infection or injury and leads to the over-expression of several pro-inflammatory enzymes[1]. In particular, inducible nitric oxidesynthase (iNOS) produce nitric oxide (NO), respectively, which play a critical role in disease pathophysiology.

Nitric oxide plays a role in vasodilation, neurotransmission, blood coagulation and immune regulation, and is mainly divided into cNOS and iNOS. Of these, iNOS is involved in the massive production of NO when it is activated by various cytokines(IFN-γ, TNF-α and IL-1β) or bacterial Lipopolysaccaride (LPS). An excessive presence of NO aggravates the inflammatory response[2].

Massive amounts of NO produced by iNOS under pathological conditions (e.g., inflammatory diseases) are potentially harmful, especially when time-spatial regulation of iNOS expression becomes compromised. During inflammation associated with different pathogens, NO production increases significantly and may become cytotoxic. Moreover, the free radical nature of NO and its high reactivity with oxygen to produce peroxynitrite makes NO a potent pro-oxidant molecule able to induce oxidative damage, and to be potentially harmful towards cellular targets. Thus inhibition of NO production in response to inflammatory stimuli might be a useful therapeutic strategy in inflammatory diseases[4,5].

Acer ginnala(AG) is a plant species with woody stems native to northeastern Asia from easternmost Mongolia east to Korea and Japan, and north to southeastern Siberia in the Amur River valley. AG is a deciduous spreading shrub or small tree growing to 3-10 m tall, with a short trunk up to 20-40 cm diameter and slender branches. The bark is thin, dull gray-brown, and smooth at first but becoming shallowly fissured on old plants. The sap of AG has been used for treatment of stomachic and diarrhea. The leaves and barks of AG have been used for treatment of wound healing and eye disorder. Recently, phytochemical investigations on leaves of AG were reported the identification of various compounds such as gallotannins, phenolcarboxylic acids, flavonoids and saponins[9-14]. Also anti-oxidative[11,13,15]anti-bacterial[16] and cytotoxic activities[14] of leaves of AG have been reported. In the previous study, we isolated several anti-oxidative phenolic compounds from barks of AG. In this continued search for anti-inflammatory phenolic compounds from AG, I isolated the major components of the stems of AG and

evaluated NO production in this study.

EXPERIMENTAL SECTION

General experimental procedures

The stationary phases for the column chromatographic isolation were performed on Sephadex LH-20 (10-25 μm, GE Healthcare Bio-Science AB, Uppsala, Sweden), MCI-gel CHP 20P (75-150 μm, Mitsubishi Chemical, Tokyo, Japan) and ODS-B gel (40-60 μm, Daiso, Osaka, Japan). ODS-B gel was used as stationary phase on middle pressure liquid chromatography (MPLC) system. Sample injector was Waters 650E (Waters, Seoul, Korea), detector was 110UV/VIS detector (Gilson, Middleton, WI) and pump was TBP5002 (Tauto Biotech, Sanghai, China). Thin layer chromatography (TLC) was carried out using a pre-coated silica gel 60 F_{254} plate (Merck, Darmstadt, Germany) on chloroform, methanol and water (6:4:1, volume ratio). The spots were detected under UV radiation (254 nm) and by spraying with $FeCl_3$ and 10% H_2SO_4 or anisaldehyde-H_2SO_4 followed by heating.

The components from the AG were identified by several instrumental analyses. The 1 D NMR such as ^{1}H- (300 or 600MHz) and ^{13}C- (75 or 125MHz) nuclear magnetic resonance (NMR)experiments were recorded with Gemini 2000 and VNS (Varian, Palo Alto, CA, USA) at center for research facilities on Chung-Ang University. Low resolution fast atom bombardment mass spectrum (LRFAB-MS) were recorded with JMSAX505WA (JEOL, Tokyo, Japan) at National Center for Inter-University Research Facilities on Seoul National University.

Plant material

AG was botanized 5.5 kg from Korea National Arboretum in Pocheon-si, Gyeonggi-do (Korea), on August of 2009, certificated by Dr. Choi (Pharmacognosy Lab, College of Pharmacy, Chung-Ang University). And, a voucher specimen (AGM2009-08) was deposited at the herbarium of the College of Pharmacy, Chung-Ang University.

Extraction and isolation

The 5.5 kg of AG were extracted several times with 80 % acetone at room temperature. Concentration that is removing the acetone under vacuum afforded to 387.87 g of extraction. The only 142.87 g of extraction was dissolved in water and this aqueous solution was filtered through Filter paper No. 20 (Hyundai micro, Anseong, Korea). And, purification and isolation were performed by below liquid column chromatographic methods with TLC monitoring. The concentrated filtrate applied to the Sephadex LH-20 (2 kg, 10 × 80 cm) equilibrated with water. The column was eluted with water-methanol gradient system and then yielded eight fractions. And, 4.60g of fraction 3 on Disogel (300 g, 3 × 50 cm) with water-100% methanol and 10%-80% methanol gradient in MPLC system (10 ml/min, 280 nm)resulted in **ginnalin B** (**1**, 0.5g) and **gallic acid** (**5**, 0.85g), respectively. 13.86g of fraction 4 on Sephadex LH-20 (200g, 2.5 × 30 cm) water-100% methanol and Disogel (300 g, 3 × 50 cm) with 10%-80% methanol gradient in MPLC system (10 ml/min, 280 nm)resulted in **ginnalin B** (**1**, 10.22g). 6.07g of fraction 5 on Disogel (300 g, 3 × 50 cm) with 20%-100% methanol gradient in MPLC system (10 ml/min, 280 nm)resulted in **methyl gallate**(**3**, 2.5g) and **gallic acid** (**5**, 0.38g), respectively. Also, 5.54g of fraction 6 on Disogel (300 g, 3 × 50 cm) with 20%-100% methanol gradient in MPLC system (10 ml/min, 280 nm)resulted in **methyl gallate**(**3**, 0.99g) and **gallic acid** (**5**, 1.36g), respectively. 14g of fraction 7 on Disogel (300 g, 3 × 50 cm) with 20%-100% methanol gradient in MPLC system (10 ml/min, 280 nm) yielded three sub-fractions. Among these sub-fractions, fraction 7-2 on Disogel (300 g, 3 × 50 cm) with 30%-100% and 10%-80% methanol gradient in MPLC system (10 ml/min, 280 nm) and Sephadex LH-20 (200g, 2.5 × 30 cm) 50% methanol isocratic resulted in **acertannin(3,6-digalloyl-1,5-anhydroglucitol)**(**2**, 7.74g) and **methyl gallate**(**3**, 0.04g), respectively. Finally, 13.45g of fraction 8, crystallizationyielded**acertannin(2,6-digalloyl-1,5-anhydroglucitol)**(**4**, 8.37g) and 4.08g of water soluble layer on ODS-B gel (50 μm, 300 g, 3 × 40 cm) 50% methanol isocratic and Disogel (300 g, 3 × 50 cm) with 20%-100% methanol gradient in MPLC system (10 ml/min, 280 nm)resulted in **acertannin(3,6-digalloyl-1,5-anhydroglucitol)**(**2**, 0.15g) and **acertannin (2,6-digalloyl-1,5-anhydroglucitol)**(**4**, 2.87g), respectively(**Fig. 1**).

6-galloyl-1,5-anhydroglucitol (ginnalin B) (1)

white amorphous powder, $[\alpha]_D$: + 30.8° (*c*=0.005, acetone), Negative FAB MS: *m/z* 315 $[M-H]^-$, ^{1}H-NMR (600MHz, DMSO-d_6+D_2O) : δ 3.03 (1H, t, *J*=10.8Hz, H-1), 3.15 ~ 3.11 (2H, m, H-3,4), 3.31 ~ 3.25 (2H, m, H-5,2), 3.72 (1H, H-1), 4.14 (1H, dd, *J*=12.0Hz, 6.0Hz, H-6b), 4.40 (1H, dd, *J*=12.0Hz, 1.8Hz, H-6a), 6.93 (2H, s, galloyl-H), ^{13}C-NMR (125MHz, DMSO-d_6+D_2O) : δ 64.3 (C-6), 69.8 (C-1), 69.9 (C-2), 70.3 (C-4), 78.2 (C-3), 78.6 (C-5), 108.9 (C-2',6'), 119.8 (C-1'), 138.6 (C-4'), 145.7 (C-3',5'), 166.2 (C-7')

acertannin (3,6-digalloyl-1,5-anhydroglucitol) (2)

white amorphous powder, $[\alpha]_D$: + 45° (*c*=0.005, acetone), Negative FAB MS : *m/z* 467 $[M-H]^-$, ^{1}H-NMR (600MHz, DMSO-d_6+D_2O) : δ 3.21 (1H, t, *J*=10.8Hz, H-1), 3.40 (1H, t, H-4), 3.47 (1H, m, H-5), 3.56 (1H, m, H-2), 3.84 (1H,

dd, *J*=11.4Hz, 5.4Hz, H-1), 4.22 (1H, dd, *J*=12.0Hz, 5.4Hz, H-6b), 4.41 (1H, br d, *J*=12.0Hz, H-6a), 4.93 (1H, t, *J*=9.0Hz, H-3), 6.94, 6.95 (each 2H, s, galloyl-H),^{13}C-NMR (125MHz, DMSO-d_6+D_2O) : δ 63.9 (C-6), 68.2 (C-2), 68.4 (C-1), 69.9 (C-4), 78.6 (C-5), 79.5 (C-3), 109.0, 109.1(C-2',6',2",6"), 119.7, 120.5(C-1',1"), 138.3, 138.7 (C-4',4"), 145.6, 145.7 (C-3',5',3",5"), 166.2, 166.0 (C-7',7")

methylgallate (3)
gray amorphous powder, $[\alpha]_D$: –3.4° (*c*=0.005, acetone), DIP EI MS : *m/z* 183 [M], ^{1}H-NMR (600MHz, DMSO-d_6+D_2O) : δ 6.91 (2H, s, H-2,6), 3.71 (3H, s, -OCH_3), ^{13}C-NMR (125MHz, DMSO-d_6+D_2O) : δ 52.0 (-OCH_3), 108.8 (C-2,6), 119.7 (C-1), 138.7 (C-4), 145.8 (C-3,5), 166.8 (C-7)

acertannin (2,6-digalloyl-1,5-anhydroglucitol) (4)
white amorphous powder, $[\alpha]_D$: + 27.6° (*c*=0.005, acetone), Negative FAB MS : *m/z* 467 $[M-H]^-$, ^{1}H-NMR (600MHz, DMSO-d_6+D_2O) : δ 3.24 (1H, t, *J*=10.8Hz, H-1), 3.32 (1H, t, *J*=9Hz, H-3), 3.45 (2H, m, H-3,5), 3.91 (1H, dd, *J*=10.8Hz, 5.4Hz, H-1), 4.21 (1H, dd, *J*=12.0Hz, 5.4Hz, H-6b), 4.44 (1H, br d, *J*=12.0Hz, H-6a), 4.72 (1H, m, H-2), 6.95 (2H×2, s, galloyl-H), ^{13}C-NMR (125MHz, DMSO-d_6+D_2O) : δ 64.0 (C-6), 66.5 (C-1), 70.6 (C-4), 72.0 (C-2), 75.1 (C-3), 75.7 (C-5), 109.0, 109.2(C-2',6',2",6"), 119.6, 119.7(C-1',1"), 138.8 (C-4',4"), 145.8 (C-3',5',3",5"), 166.9, 166.2 (C-7',7")

gallic acid (5)
gray amorphous powder, $[\alpha]_D$: −3.2° (*c*=0.005, acetone), DIP EI MS :*m/z* 170 [M], ^{1}H-NMR (600MHz, DMSO-d_6+D_2O) : δ 6.96 (2H, s, galloyl-H)

Cell culture
The murine macrophage cell line, RAW 246.7 cells were purchased from the Korean Cell Line Bank. These cells were grown at 37℃ in a humidified atmosphere (5 % CO_2) in a DMEM medium (Sigma, St. Louis, MO, USA) containing 10 % fetal bovine serum, 100 IU/ml penicillin G and 100 mg/ml streptomycin (Gibco BRL, Grand Island, NY, USA)[17] and were used after cell counting with hemocytometer.

MTT assay for viability
Before the biological assay, the cytotoxicity was measured by the mitochondrial- dependent reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium -bromide] (Sigma, St. Louis, MO, USA) to formazan[17]. After each cells were cultured in a 96-well plate and incubated for 2 h, the cells were treated with the samples (12.5, 25. 50 and 100 ug/ml or uM). The cells were incubated for an additional 24 h, and the medium was replaced with fresh medium contained 0.5 mg/ml MTT and the incubation continued for a further 4 h at 37℃. The medium was then removed and the MTT-formazan produced was dissolved in 200 ul DMSO. The extent of the reduction of MTT to formazan within the cells was quantified by measuring the absorbance at 540 nm using an ELISA reader (TECAN, Salzburg, Austria)[17]. The cytotoxicity was calculated as cell viability (%) = sample *O.D.* / blank *O.D.*× 100

Measurement of NO production
NO production was assayed by measuring the nitrite in the supernatants of cultured RAW 264.7 cells. Briefly, the cells were seeded at a density of 5 x 10^5/ml in 96 well culture plates. After pre-incubation for 18 hours, the cells were pretreated for 30 min with Com. **1-5** (10, 50 and 100 μM) and then stimulated with LPS (200ng/ml) for 24 hours. The supernatant was then mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediaminedihydrochloride, and 2.5% phosphoric acid) and then incubated at room temperature for 5 min. The concentration of nitrite was then determined by measuring the absorbance at 570 nm and comparing the values to a standard curve generated using sodium nitrite ($NaNO_2$).

Statistical analysis
All data were expressed mean ± S.D. Values were analyzed by Dunnett's t-test using Statistical Package for the Social Sciences (SPSS) software pack and considered to be significantly different when the *p* value was less than 0.05. Values bearing different superscripts in the same column are significantly different.

RESULTS AND DISCUSSION

Identification of phenolic compounds

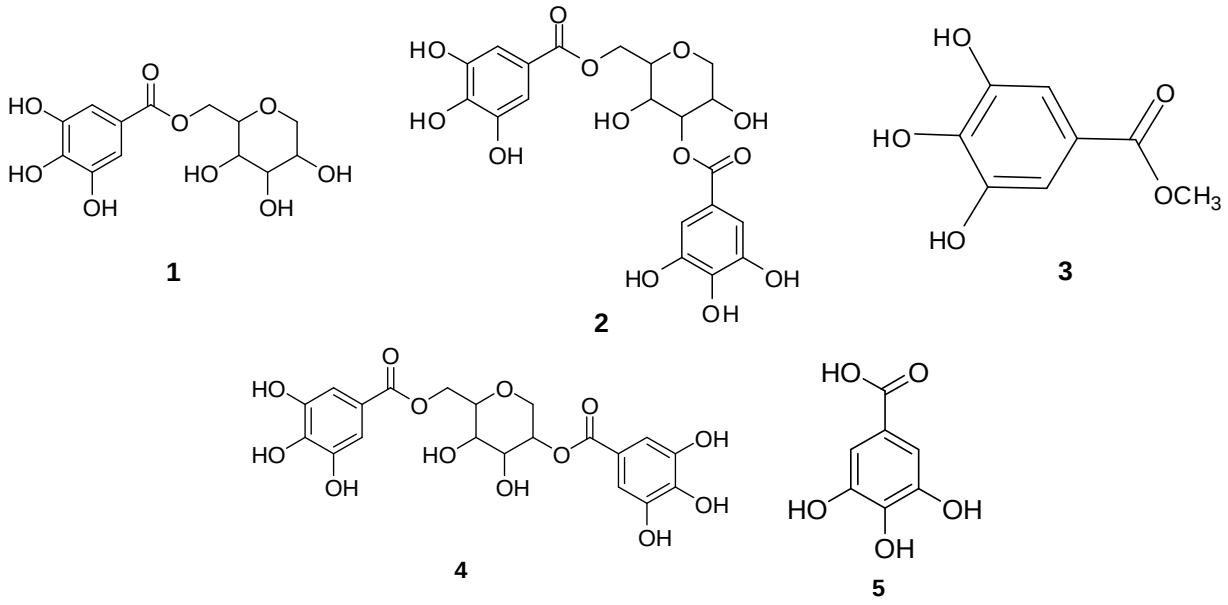

Fig 1. The structures of compounds 1-5 from AG

Compound 1

Compound **1** is a white powder, where *m/z* 315 $[M-H]^-$ion peak could be observed on the negative FAB-MS. On ^{1}H-NMR spectrum, there was a singlet signal corresponding to 2H at δ 6.92 ppm in the aromatic field. Based on this finding, galloyl group is presumed to exist within the molecule. On ^{13}C-NMR spectrum, there were signals (166.2, 145.7, 138.6, 119.8 and 108.9) originating from one galloyl group. Besides, six oxygen-bearing methine signals (78.6, 78.2, 70.3, 69.9, 69.8 and 64.3) were observed. This indicates the presence of hexose. In regard to hexose, based on the findings that 1 mol of oxygen (-16)was deficient as compared with normal types of hexose on MS and there was a absence of anomeric carbon signals at the 110 ~ 90ppm, the substance was presumed to be 1,5-anhydroglucitol. Accordingly, compound **1** is assumed to be monogallate of 1,5-anhydroglucitol. In regard to the binding site of galloyl group, there was a down-field shift of two proton signals (4.14 and 4.40) corresponding to H-6 of sugar moiety on ^{1}H-NMR spectrum. Based on the above results, compound **1** was identified as **6-galloyl-1,5-anhydroglucitol (ginnalin B)**. This was also in agreement with the spectra data described in literatures[19].

Compound 2

Compound **2** is a white powder, where *m/z* 467 $[M-H]^-$ion peak could be observed on the negative FAB-MS. On ^{1}H-NMR spectrum, there was a singlet signal corresponding to 2H at δ 6.94 and 6.95 ppm in the aromatic field. Based on this finding, galloyl group is presumed to exist within the molecule. On ^{13}C-NMR spectrum, there were signals (166.2, 166.0, 145.7, 145.6, 138.7, 138.3, 120.5, 119.7, 109.1 and 109.0) originating from two galloyl groups. Besides, six oxygen-bearing methine signals (79.5, 78.6, 69.9, 68.4, 68.2 and 63.9) were observed. This indicates the presence of hexose. In regard to hexose, based on the findings that 1 mol of oxygen (-16)was deficient as compared with normal types of hexose on MS and there was a absence of anomeric carbon signals at the 110 ~ 90ppm, the substance was presumed to be 1,5-anhydroglucitol. Accordingly, compound **2** is assumed to be digallate of 1,5-anhydroglucitol. In regard to the binding site of galloyl group, there was a down-field shift of three proton signals (4.22, 4.41, 4.93) corresponding to H-3 and 6 of sugar moiety on ^{1}H-NMR spectrum. Based on the above results, compound **4** was identified as **3,6-digalloyl-1,5-anhydroglucitol (acertannin)**. This was also in agreement with the spectra data described in literatures [10, 20].

Compound 3

Compound **3** is a white powder, where *m/z* 183 [M] ion peak could be observed on DIP EI-MS. On ^{1}H-NMR spectrum, there was a singlet signal corresponding to 2H at δ 6.91 ppm in the aromatic field. Thus, the proton signal originating from galloyl group. At δ 3.71 ppm, there was a proton signal corresponding to methoxy group. On ^{13}C-NMR spectrum, there was a typical presence of two strong carbon signals at δ 108.8 and 145.8. Besides, there were also acidic carbonyl signal at δ 166.8 and quaternary carbon signal at δ 119.7 and 138.7. Finally, at δ 52.0, there was a typical presence of carbon signal corresponding to methoxy group. Following a comparison with the data which has been described in literatures, the substance was identified as **methyl gallate**[21].

Compound 4

Compound **4** is a white powder, where *m/z* 467 $[M-H]^-$ion peak could be observed on the negative FAB-MS. On ^{1}H-NMR spectrum, there was a singlet signal corresponding to 2H at δ 6.95 ppm in the aromatic field. Based on this finding, galloyl group is presumed to exist within the molecule. On ^{13}C-NMR spectrum, there were signals (166.9, 166.2, 145.8, 138.8, 119.7, 119.6, 109.2, 109.0) originating from two galloyl groups. Besides, six oxygen-bearing methine signals (75.7, 75.1, 72.0, 70.6, 66.5, 64.0) were observed. This indicates the presence of hexose. In regard to hexose, based on the findings that 1 mol of oxygen (-16)was deficient as compared with normal types of hexose on MS and there was a absence of anomeric carbon signals at the 110 ~ 90ppm, the substance was presumed to be 1,5-anhydroglucitol. Accordingly, compound **4** is assumed to be digallate of 1,5-anhydroglucitol. In regard to the binding site of galloyl group, there was a down-field shift of three proton signals (4.21, 4.44, 4.72) corresponding to H-2 and 6 of sugar moiety on ^{1}H-NMR spectrum. Based on the above results, compound **4** was identified as **2,6-digalloyl-1,5-anhydroglucitol (acertannin)**. This was also in agreement with the spectra data described in literatures[10, 20].

Compound 5

Compound **5** was gray amorphous powder, where *m/z* 170 [M] ion peak could be observed on DIP EI-MS. The spot was detected under UV radiation at 254 nm, the black spot was detected by spraying with $FeCl_3$ and soft violet spot were detected by spraying 10 % H_2SO_4 followed by heating.

^{1}H-NMR spectrum showed the only one singlet (δ 6.88) of two protons because of its H-2 and 6 were magnetically equivalent. Thus, compound **5** was identified as **gallic acid** comparing the spectral data with the values reported in the literature[22].

Cell viability

Before the measurement of inhibitory activity on NO production in RAW 264.7 cells, MTT assays were performed to test cytotoxicity of each compound from AG. None of compounds from AG exhibited cytotoxicity at experimental doses compared with blank (**Fig. 2**). These results demonstrated that inhibitory activities on NO production of each compounds from AG were not related to its cytotoxicity.

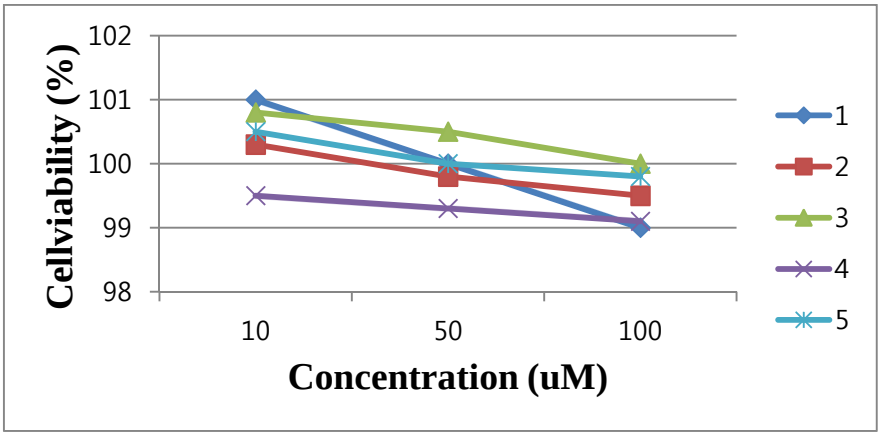

Fig 2.Effects of compounds from AG on the viability of mouse RAW 264.7 macrophage cells

The cell viability was measured by MTT assay. Results were expressed as % of control absorbance. Compound 1-5 meant6-galloyl-1,5-anhydroglucitol (ginnalin B) (1), acertannin(3,6-digalloyl-1,5-anhydroglucitol) (2), methyl gallate (3), acertannin(2,6-digalloyl-1,5-anhydroglucitol) (4) and gallic acid (5) respectively

Inhibitory activity on NO production

NO is known to be associated with many conditions, including inflammation. To examine the effect of Com. **1**-**5**on LPS-induced NO production in RAW 264.7 cells, cells were treated with or without Com. **1**-**5**for 30 min and then treated with LPS (200 ng/ml) for 24h. The cell culture medium was then harvested, after which the NO levels were determined using the Griess reaction. The LPS treated cells produced approximately 2.5-fold more NO than the control cells; however, treatment with Com. **1**-**5** inhibited this NO production in a concentration-dependant manner (**Fig.3**-**7**).

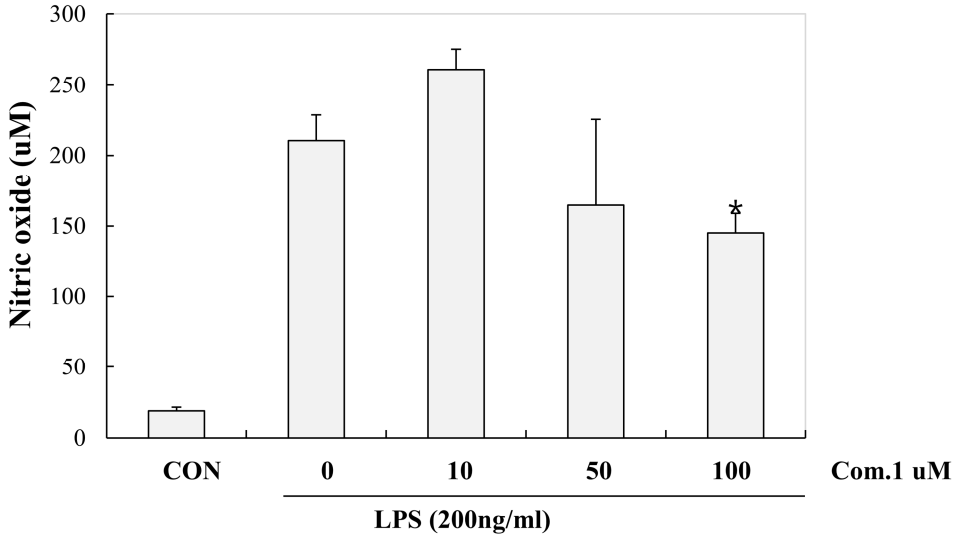

Fig 3.Effect of Compound 1 on the expression of NO in LPS-stimulated RAW 264.7 cells

*RAW 264.7 cells were pretreated with the indicated concentration (10,50 and 100 uM) of Com.1 for 30 min prior to being incubated with LPS (200 ng/ml) for 24 hours. The culture supernatant was then isolated and analyzed for nitrite production. Statistical significance: *P<0.05, as compared to the LPS treated group. Significant differences between treated groups were determined using the Dunnett's t-test. Values shown are the mean ± S.E. of duplicate determinations from three separate experiments.*

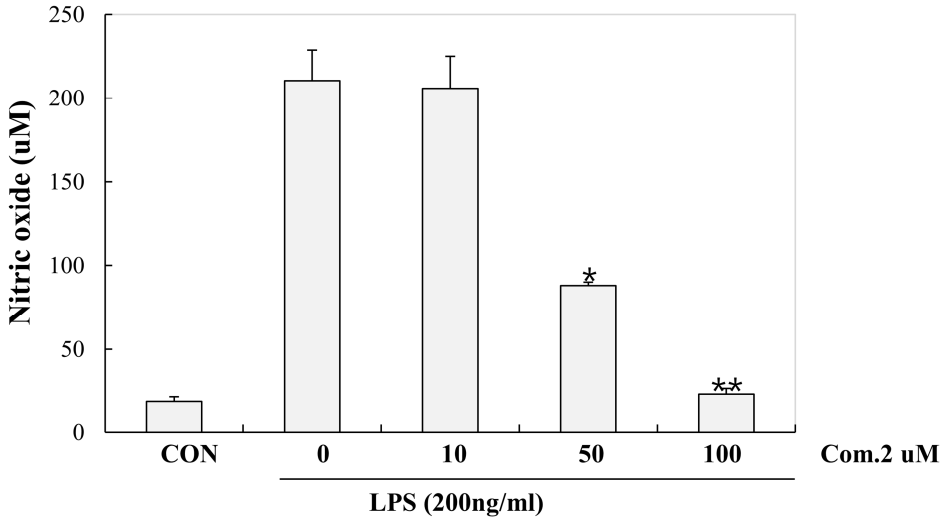

Fig 4.Effect of Compound 2 on the expression of NO in LPS-stimulated RAW 264.7 cells

*RAW 264.7 cells were pretreated with the indicated concentration (10,50 and 100 uM) of Com.2 for 30 min prior to being incubated with LPS (200 ng/ml) for 24 hours. The culture supernatant was then isolated and analyzed for nitrite production. Statistical significance: *P<0.05, as compared to the LPS treated group. Significant differences between treated groups were determined using the Dunnett's t-test. Values shown are the mean ± S.E. of duplicate determinations from three separate experiments*

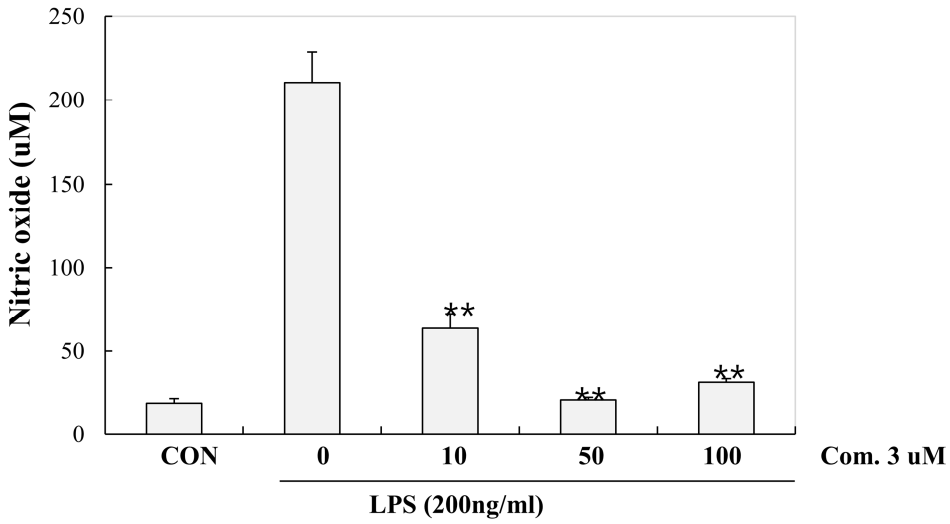

Fig 5.Effect of Compound 3 on the expression of NO in LPS-stimulated RAW 264.7 cells

*RAW 264.7 cells were pretreated with the indicated concentration (10,50 and 100 uM) of Com.3 for 30 min prior to being incubated with LPS (200 ng/ml) for 24 hours. The culture supernatant was then isolated and analyzed for nitrite production. Statistical significance: *P<0.05, as compared to the LPS treated group. Significant differences between treated groups were determined using the Dunnett's t-test. Values shown are the mean ± S.E. of duplicate determinations from three separate experiments*

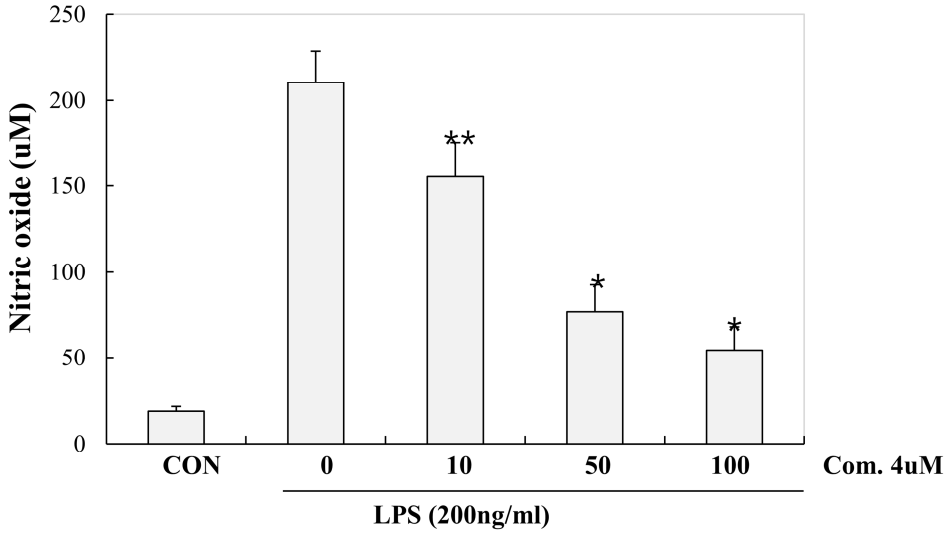

Fig 6.Effect of Compound 4 on the expression of NO in LPS-stimulated RAW 264.7 cells

*RAW 264.7 cells were pretreated with the indicated concentration (10,50 and 100 uM) of Com.4 for 30 min prior to being incubated with LPS (200 ng/ml) for 24 hours. The culture supernatant was then isolated and analyzed for nitrite production. Statistical significance: *P<0.05, as compared to the LPS treated group. Significant differences between treated groups were determined using the Dunnett's t-test. Values shown are the mean ± S.E. of duplicate determinations from three separate experiments*

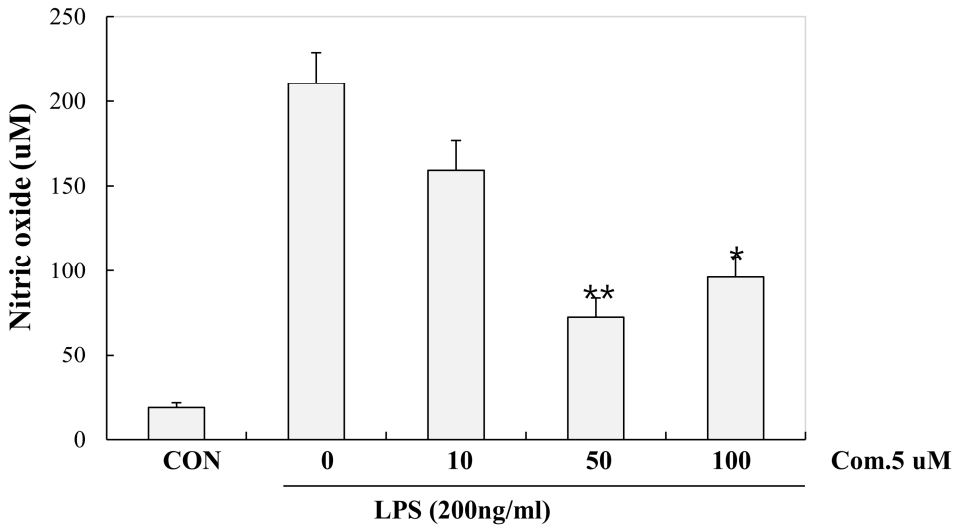

Fig 7.Effect of Compound 5 on the expression of NO in LPS-stimulated RAW 264.7 cells

*RAW 264.7 cells were pretreated with the indicated concentration (10,50 and 100 uM) of Com.5 for 30 min prior to being incubated with LPS (200 ng/ml) for 24 hours. The culture supernatant was then isolated and analyzed for nitrite production. Statistical significance: *P<0.05, as compared to the LPS treated group. Significant differences between treated groups were determined using the Dunnett's t-test. Values shown are the mean ± S.E. of duplicate determinations from three separate experiments*

CONCLUSION

In conclusion, phenolic compounds of AG were found to possess outstanding significant anti-inflammatory effects in cell models. Particularly, compounds **3** and **4** were potent shown to inhibit the inflammatory mediators, such as NO, in RAW 264.7 cells, suggesting that these compounds may be a potential source of anti-inflammatory agents, which is advantageous for pharmaceutical applications.

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