



***In vitro* evaluation of the pharmacological properties of crude methanol extract and its fractions of *Aconitum austrokoreense* aerial parts**

**Evaluación *in vitro* de las propiedades farmacológicas del extracto de metanol crudo y sus fracciones de partes aéreas *Aconitum austrokoreense***

H. J. Ju<sup>1,†</sup>, T.K. Yoo<sup>1,†</sup>, S. Jin<sup>1</sup>, H. Kim<sup>2</sup>, T. K. Hyun<sup>1\*</sup>

<sup>1</sup>Department of Industrial Plant Science and Technology, College of Agricultural, Life and Environmental Sciences, Chungbuk National University, Cheongju 28644, Republic of Korea.

<sup>2</sup>Gangwon Forest Science Institute, Chuncheon 24207, Republic of Korea.

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**Abstract**

Although the dried tuberous roots of *Aconitum austrokoreense* Koidz has been traditionally used to treat various diseases, its aerial part has been considered a useless by-product. In this study, we determined the antioxidant, antibacterial, anti-inflammatory and anti-cancer activities of the aerial part of *A. austrokoreense* to assess its potential benefit to human health. The antioxidant activity was analyzed using two different methods, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and reducing power assays and suggested that the crude methanol extract and butanol fraction possessed strong antioxidant activity. Additionally, the aqueous fraction exhibited not only remarkable antibacterial activity against Gram-negative bacteria but also significant inhibition of lipopolysaccharide-induced nitric oxide production in RAW 264.7 cells and cytotoxic activity against human cancer cell lines. Furthermore, correlation analysis between the polyphenolic content and biological activities of the aerial part of *A. austrokoreense* suggests that phenolic compounds might be major contributors to the DPPH free radical scavenging and anti-cancer activities. Taken together, these findings suggest that the nonmedicinal parts of *A. austrokoreense* can also be used as a potential natural resource to develop effective dietary health supplements.

**Keywords:** *Aconitum austrokoreense* Koidz, antioxidant, antibacterial, anti-inflammatory, anti-cancer.

**Resumen**

A pesar de que las raíces tuberosas secas de *Aconitum austrokoreense* Koidz tradicionalmente han sido utilizadas para tratar varias enfermedades, su parte aérea ha sido considerada como un sub-producto inútil. En este estudio se determinaron las actividades antioxidante, antibacteriana, antiinflamatoria y anticáncer de la parte aérea de *A. austrokoreense* para evaluar su potencial benéfico para la salud humana. La actividad antioxidante se analizó por el método del radical libre 2,2-difenil-1-picrilhidrazilo (DPPH) y también con ensayos del poder reductor. Ambos métodos sugieren que tanto el extracto metanólico crudo y la fracción butanólica una fuerte actividad antioxidante. Adicionalmente, la fracción acuosa exhibió no tan solo una notable actividad antibacteriana en contra de bacterias Gram-negativas, sino también inhibió significativamente la producción de óxido nítrico inducido por lipopolisacárido en células RAW 264.7 y la actividad citotóxica en contra de líneas celulares cancerosas humanas. Más aún, el análisis de correlación entre el contenido de polifenoles y las actividades biológicas de la parte aérea de *A. austrokoreense* sugiere que los compuestos fenólicos podrían contribuir de forma importante a las actividades del radical libre DPPH y anticáncer. Estos hallazgos, en conjunto, sugieren que las partes no-medicinales de *A. austrokoreense* también pueden ser utilizadas con un recurso natural con potencial para desarrollar complementos alimenticios efectivos.

**Palabras clave:** *Aconitum austrokoreense* Koidz, antioxidante, antibacteriano, antiinflamatorio, anticancerígeno.

**1 Introduction**

Because the average lifespan in modern society has lengthened and living standards have improved,

being healthy has also increased. Consequently, the demand for health supplements is increasing and developing natural medicines with fewer side effects than synthetic medicines has received more attention (Vicentini *et al.*, 2016). Approximately 40% of current mono-molecular medicines are derived directly or

\* Corresponding author. E-mail: taekyung7708@chungbuk.ac.kr

†These authors contributed equally to this work.

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indirectly from plant species and their preparations (Silano *et al.*, 2011). Additionally, FDA (Food and Drug Administration) data suggest that 40% of the approved molecules are natural compounds or their derivatives, of which 74% are used in anticancer therapy (Seca and Pinto, 2018). Thus, identifying new plant resources has attracted worldwide interest regarding functional applications in the cosmetic and pharmaceutical industries.

*Aconitum austrokoreense* Koidz is a perennial herb belonging to the genus *Aconitum* in the family Ranunculaceae (Luo *et al.*, 2005) and an endemic species with a very limited geographic distribution in the Korean peninsula (Yun *et al.*, 2015). The tuberous roots of genus *Aconitum* contains are a rich source of alkaloids including alkaloids benzoylmecasonine, mesaconitine, aconitine, hyaconitine, heteratisine, heterophyllisine, heterophylline, heterophyllidine, atidine, isotisine, hetidine, and hetsinone and benzoylheteratisine, which are known to be very toxic (Chan, 2009; Srivastava *et al.*, 2010). However, boiling, decoction or alkaline treatment through deacetylation, debenzoylation, or oxidation reaction hydrolyze aconite alkaloids into less toxic and non-toxic derivatives (Srivastava *et al.*, 2010) such as benzyaconine, and aconine that exhibit some pharmacological activities (Zeng *et al.*, 2016). Therefore, the dried tuberous roots of *A. austrokoreense*, along with those of other *Aconitum* species, have been used in traditional herbal medicine to treat various diseases, such as diarrhea, syncope, rheumatic fever, bronchial asthma, painful joints, gastroenteritis, edema, and various tumors (Singhuber *et al.*, 2009). Thus, the tuberous root extract of *A. austrokoreense* has potential uses as a crude drug and dietary health supplement, whereas its aerial part has been considered a useless by-product. However, phytochemical investigations of aerial parts of *Aconitum* species have revealed the presence of multiple active ingredients, flavonoid glycosides (Yin *et al.*, 2019). For example, quercetin derivatives, kaempferol derivative, cloven, robinin, liquiritigenin, and liquiritin were identified from the methanol extract of aerial parts of *A. anthora*, *A. burnatii*, and *A. variegatum* (Mariani *et al.*, 2008; Vitalini *et al.*, 2010), and these compounds were studied for their pharmacological activities (Mariani *et al.*, 2008; Vitalini *et al.*, 2010), suggesting the potential of the aerial part *A. austrokoreense* for drug and phytomedicine development, although no systematic study regarding the pharmaceutical benefit of the aerial part of *A. austrokoreense* is available.

To address this gap, in this study, we evaluated the pharmaceutical value of the aerial part of *A. austrokoreense* by analyzing its antioxidant activity, antibacterial activity, anti-inflammatory effect, and cytotoxic activity against cancer cell lines. These results are expected to motivate further interest in using the aerial part of *A. austrokoreense* as a regular source of food and as a dietary health supplement.

## 2 Materials and methods

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### 2.1 Materials

The methanol (MeOH) extract (GNEP-AA-001; AA01) of the aerial part of *A. austrokoreense* and its fractions [butanol fraction (GNEP-AA-004; AA04) and aqueous fraction (GNEP-AA-005; AA05)] were distributed from the Nature Environment Research Park of Gangwon Province, South Korea. All the test strains used to analyze the antibacterial activity were obtained from the Korean Agricultural Culture Collection (KACC) in South Korea. Bacterial lipopolysaccharide (LPS) from *Escherichia coli* 0127, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), sodium nitrite, and Griess reagent were obtained from Sigma-Aldrich. Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from GE Healthcare Life Sciences.

### 2.2 Analysis of the total phenolic and total flavonoid contents

The total phenolic content (TPC) and total flavonoid content (TFC) were determined according to the Folin-Ciocalteu method and colorimetric method, respectively, as described by Jin *et al.* (2019). The total phenolic content (TPC) and total flavonoid content (TFC) were determined according to the Folin-Ciocalteu method and colorimetric method, respectively, as described by Jin *et al.* (2019). 100  $\mu$ L of MeOH extract (100  $\mu$ g/mL) and its fractions (100  $\mu$ g/mL) was mixed with 50  $\mu$ L of 2 N Folin-Ciocalteu reagent and incubated for 5 min at room temperature. The resultant blue color was read at an absorbance of 725 nm. The calibration curve was prepared using gallic acid (0-1000  $\mu$ g/mL), and the TPC in the MeOH extract and its fractions was expressed in milligrams of gallic acid equivalents (mg GAE/g extract).

For TFC analysis, a total of 0.5 mL of MeOH extract (100 µg/mL) and its fractions (100 µg/mL) was mixed with 0.1 mL of 10% aluminum nitrate (w/v), 0.1 mL of 1 M potassium acetate, and 4.3 mL of 80% ethanol. After 40 min incubation at room temperature, the absorbance was determined at 415 nm. The TFC was calculated in milligrams of quercetin equivalents (QE) per gram of extract using the equation obtained from the standard quercetin graph prepared using various concentration of quercetin (0-1,000 µg/mL).

### 2.3 Determination of the antioxidant activity

The total free radical scavenging activity of the MeOH extract and its fractions was estimated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. A mixture of the sample and DPPH (0.4 mM in MeOH) was reacted for 10 min in the dark, and the absorbance was measured at 520 nm using an iMark<sup>TM</sup> microplate reader (Bio-Rad). The RC<sub>50</sub> (50% reduction of DPPH radicals) was calculated as described by Jin *et al.* (2019). Butylated hydroxytoluene (BHT) was used as a positive control.

To analyze the total reducing power of the MeOH extract and its fractions, 10 µL of MeOH added to 20 µL of each sample (10 mg/mL) was mixed with 200 µL of 0.2 M sodium phosphate buffer (pH 6.6) and 200 µL of 1% potassium ferricyanide, and then the mixture was incubated at 50 °C for 20 min. After stopping the reaction by adding 1 mL of 10% trichloroacetic acid, the mixture was centrifuged at 6,500 rpm for 10 min, the supernatant (500 µL) was mixed with the same volume of deionized water (500 µL) and 100 µL of 0.1% ferric chloride, and the absorbance was measured at 750 nm using an ultraviolet-visible spectrophotometer. Ascorbic acid was used as a positive control.

### 2.4 Determination of the antibacterial activity

The bacterial test strains used in this study comprised *Enterobacter cloacae* (E.c., KACC 11958), *Micrococcus luteus* (M.l., KACC 14819), *Listeria monocytogenes* (L.m., 19115), *Staphylococcus aureus* (S.a., KACC 1916), *Salmonella enteritidis* (S.e., KACC 12021), *Pseudomonas aeruginosa* (P.a., KACC 2004), and *Salmonella enterica* subsp. *enterica* (S.s., KACC 10769). The minimum concentration of each sample to inhibit bacterial growth (MIC) was determined according to the serial two-fold dilution

method as described by Kwon *et al.* (2017). Briefly, 96-well microtiter plates were prepared by dispensing 180 µL of the bacterial suspension (10<sup>6</sup> CFU/mL) into the first row of wells and 100 µL into the remaining wells. 20 µL of each sample (10 mg/mL) were added to the first row and 100 µL was serially diluted into the remaining wells. The final volume in each well was 100 µL. After 24 h incubation, the growth of the bacteria was evaluated based on the degree of turbidity of the culture using the naked eye.

### 2.5 Cell culture

The RAW 264.7 (murine macrophage cell line), A549 (human lung adenocarcinoma epithelial cell line), HCT15 (human adenocarcinoma colon cancer cell line), SK-MEL-2 (human melanoma cell line) and SKOV3 (human ovarian cancer cell line) cell lines were purchased from the Korean Cell Line Bank (KCLB; Seoul, Korea). The cells were cultured in DMEM or Roswell Park Memorial Institute medium supplemented with 10% fetal bovine serum and antibiotics in an atmosphere of 5% CO<sub>2</sub> at 37 °C.

### 2.6 Determination of the nitrite oxide level and cell viability

The nitric oxide (NO) concentration in the culture medium was analyzed by the Griess reaction test as described by Choi *et al.* (2017). RAW264.7 cells were treated with or without LPS (1 µg/mL) in the presence of each sample. After 24 h of incubation, 100 µL of Griess reagent [1% sulfanilamide (w/v) and 0.1% (w/v) N-(1-Naphthyl)ethylenediamine dihydrochloride in 2.5% phosphoric acid (v/v)] was mixed with an equal volume of cell supernatant, followed by incubation at room temperature for 10 min, and the cells were treated with MTT solution (5 mg/mL in phosphate-buffered saline) for 4 h. The absorbance at 540 nm was measured, and the NO concentration in each sample was calculated according to the standard curve generated from sodium nitrite. Dexamethasone (DEX) was used as a positive control. Additionally, the formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and the absorbance was measured at 520 nm to determine the cell viability.

### 2.7 Cytotoxicity assay of the human cancer cell lines

The cytotoxicity effects of the MeOH extract and its fractions were assessed by the MTT assay. The human

cancer cells were plated into 96-well plates ( $5 \times 10^3$  cells/well) and were incubated at 37 °C for 24 h. Next, the cells were treated with 200 µg/mL of each sample for 24 h before adding MTT solution into each well. DMSO was added to each well to dissolve the formazan crystals and produce a uniform dark purple color before measuring the absorbance at 520 nm using an iMARK<sup>TM</sup> microplate reader (Bio-RAD).

### 2.8 Statistical analysis

All the experiments were performed independently three times, and the data were expressed as the mean ± standard error. Statistical analysis was performed using SPSS (IBM, USA), and the significance level was expressed using Duncan's multiple range test ( $p < 0.05$ ).

## 3 Results and discussion

### 3.1 Total phenolic and flavonoid contents in the aerial part extract of *A. austrokoreense*

Polyphenolic compounds are plant secondary metabolites that exhibit antioxidant activity based on multiple phenolic functionalities (Scalbert *et al.*, 2005; Zhao *et al.*, 2014). Recently, polyphenolic compounds have gained increasing interest in the science and food industries because they can protect the human body from oxidative stress-related diseases, such as atherosclerosis, cancer, cardiovascular diseases, coronary heart disease, inflammation, and aging-related disorders (Kaur and Kapoor, 2001; Alfadda and Sallam, 2012). The TPC and TFC in the crude

MeOH extract of the aerial part of *A. austrokoreense* were  $6.48 \pm 1.04$  mg GAE/g extract and  $6.62 \pm 0.24$  mg QE/g extract, respectively (Table 1). Additionally, the highest level of TPC was analyzed from the BuOH fraction, which contained  $7.82 \pm 1.18$  mg GAE/g extract, whereas the MeOH extract contained the highest flavonoid content. Polyphenolic compounds are more soluble in polar organic solvents containing a hydroxyl group such as MeOH (Wang and Weller, 2006), and solvent polarity is likely important to enrich phenolic and flavonoid compounds (Hyun *et al.*, 2016). Thus, the presence of a high concentration of phenolic compounds in the BuOH fraction compared with other samples is due to the solvent polarity.

### 3.2 Antioxidant activity of *A. austrokoreense* aerial part

Antioxidant compounds play important roles as health-protective factors against reactive oxygen species (ROS). Because of the long-term safety of natural antioxidants compared with synthetic antioxidants, folk plants, including spices and herbs, have been used as primary sources of naturally occurring antioxidants (Atawodi, 2005). Therefore, to investigate the antioxidant properties of the aerial part extract of *A. austrokoreense*, we analyzed the antioxidant activities based on the DPPH free radical scavenging activity and reducing power. As shown in Fig. 1a, the highest DPPH free radical scavenging activity was observed in the BuOH fraction ( $RC_{50} = 593.2 \pm 23.5$  µg/mL extract), followed by the crude MeOH extract ( $RC_{50} = 852.4 \pm 6.8$  µg/mL). The aqueous fraction revealed the lowest DPPH-free radical scavenging activity (Fig. 1a) and reducing power (Fig. 1b) compared with the other samples.

Table 1. Total phenolic content and total flavonoid content of *A. austrokoreense* methanol extract and its fractions.

Extract and fractions	Total phenolic content (µg GAE/mg) <sup>1)</sup>	Total flavonoid content (µg QE/mg) <sup>2)</sup>
MeOH extract	$6.48 \pm 1.04^b$	$6.62 \pm 0.24^c$
BuOH fraction	$7.82 \pm 1.18^b$	$1.67 \pm 0.03^b$
Aqueous fraction	$2.64 \pm 0.57^a$	$0.14 \pm 0.09^a$

<sup>1)</sup>Total phenolic content measured by calibration curve of gallic acid equivalent (µg of gallic acid equivalent / mg of extract or fractions).

<sup>2)</sup>Total flavonoid content measured by calibration curve of quercetin equivalent (µg of quercetin equivalent / mg of extract or fractions).

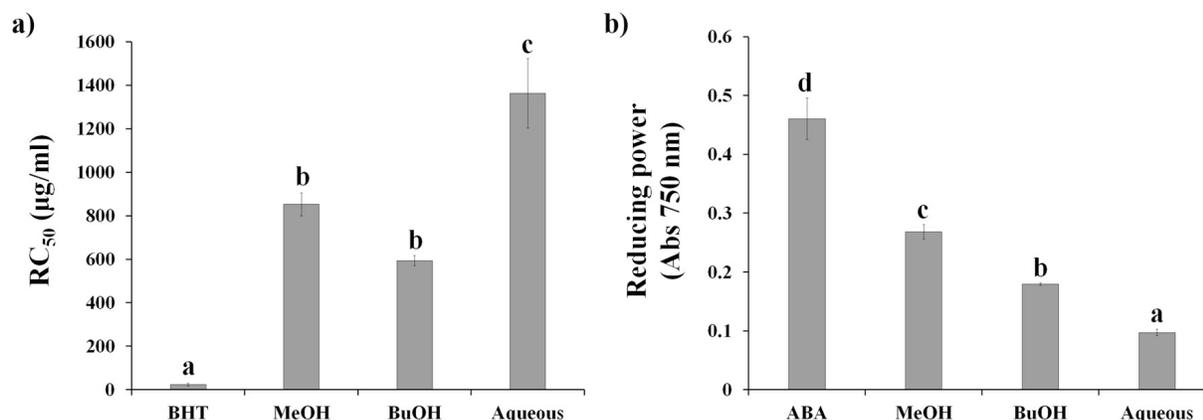


Fig. 1. Antioxidant activity of *A. austrokoreense* aerial part extract and its fractions. The antioxidant activities were measured by the DPPH free radical scavenging activity (a) and reducing power (b). RC<sub>50</sub> is concentration of the sample which is required to scavenge 50% of radicals. Each value is mean ± SE of triplicate determinations. Values in the same column with different superscript letters are significantly different ( $p < 0.05$ ). BHT, butylated hydroxytoluene; ABA, ascorbic acid.

Notably, the crude MeOH extract (OD<sub>750</sub> value = 0.27) exhibited better reducing power than the BuOH fraction (OD<sub>750</sub> value = 0.18) but lower DPPH-free radical scavenging activity than the BuOH fraction (Fig. 1). The reducing power assay is a well-known electron transfer (ET) reaction-based method, whereas the DPPH-free radical scavenging assay involves both ET and hydrogen atom transfer (HAT) reactions (Choi *et al.*, 2017). Thus, the aerial part extract of *A. austrokoreense* might prevent the initiation of free radical-mediated oxidative damage by stabilizing reactive oxygen species via ET or HAT.

### 3.3 Antibacterial activity of *A. austrokoreense* aerial part

Although antibacterial agents are important in clinical medicine, agriculture and veterinary medicine, the indiscriminate and inappropriate use of antibiotics has led to the emergence and dissemination of multidrug-resistant pathogenic bacteria that pose a significant public health threat. This situation has led to the search for plant-derived antibacterial compounds to discover new structures that show immense potential to combat multidrug-resistant pathogenic bacteria without any known side effects (Chandra *et al.*, 2017). To investigate the antibacterial activity of aerial part extract of *A. austrokoreense*, we determined the MIC of its extract and fractions using the serial two-fold dilution method and the results are expressed as MIC values (Table 2). Overall, the aqueous fraction exhibited good antibacterial activity against all the

tested bacteria, whereas the MeOH extract and BuOH fraction exerted no effect. Additionally, the aqueous fraction exhibited greater antibacterial activity against Gram-negative bacteria than Gram-positive bacteria (Table 2). Usually, Gram-negative bacteria are more resistant to antibodies and antibiotics than Gram-positive bacteria, mainly due to the low permeability of the cell membrane of Gram-negative bacteria (Górniak *et al.*, 2019). However, some phytochemicals are potent antibacterial agents against Gram-negative bacteria through inhibiting bacterial efflux pumps and nucleic acid synthesis (Górniak *et al.*, 2019). Although the antibacterial mechanism of the aqueous fraction remains unknown, this result indicates that the aerial part of *A. austrokoreense* can be further subjected to the isolation of antibacterial compounds and further pharmacological evaluation.

### 3.4 Anti-inflammatory effect of *A. austrokoreense* aerial part

Chronic inflammation plays a role in the development of various progressive diseases, including cancer, neurological disease, metabolic disorders, and cardiovascular disease (Choi *et al.*, 2017). Recently, various nonsteroidal anti-inflammatory drugs (NSAIDs) have been used to control chronic inflammation, although severe side effects of NSAIDs, such as gastrointestinal ulceration, perforation, obstruction and bleeding, have been reported

Table 2. Antibacterial activity of *A. austrokoreense* methanol extract and its fractions.

Samples	MIC ( $\mu\text{g/ml}$ ) <sup>1)</sup>						
	S.s <sup>2)</sup>	L.m <sup>2)</sup>	S.a <sup>2)</sup>	M.l <sup>2)</sup>	P.a <sup>2)</sup>	E.c <sup>2)</sup>	S.e <sup>2)</sup>
MeOH extract	1000	500	>1000	1000	1000	>1000	>1000
BuOH fraction	1000	250	>1000	>1000	>1000	1000	>1000
Aqueous fraction	250	250	500	500	500	250	250
Ampicillin	15.625	125	62.5	125	15.625	31.25	31.25

<sup>1)</sup> MIC (minimal inhibitory concentration) values against bacteria were determined by the serial two-fold dilution method.

<sup>2)</sup> *Salmonella enterica* sub sp. enterica KACC 10769 (S.s), *Listeria monocytogenes* KACC 19115 (L.m), *Staphylococcus aureus* KACC 1916 (S.a), *Micrococcus luteus* KACC 14819 (M.l), *Pseudomonas aeruginosa* KACC 2004 (P.a), *Enterobacter cloacae* KACC 11958 (E.c), *Salmonella enteritidis* KACC 12021 (S.e).

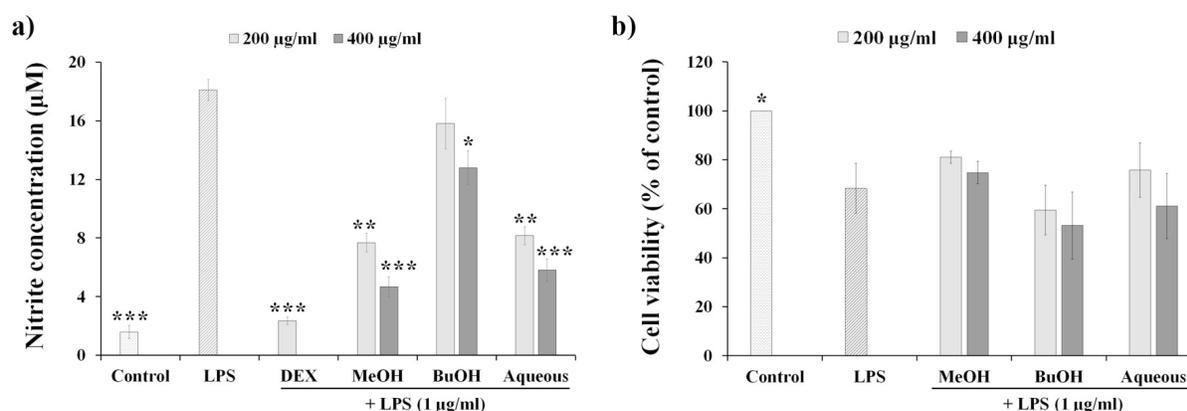


Fig. 2. Effects of *A. austrokoreense* aerial part extract and its fractions on NO production (a) and cell viability (b) in LPS-stimulated RAW 264.7 cells. Values are the mean  $\pm$  SE of triplicate experiments. \*  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$  represent the significant differences in comparison with the cells treated with LPS alone. DEX, dexamethasone.

(Vonkeman and van de Laar, 2010). Therefore, an increasing need exists to develop a natural, safe product with low or no side effects. To investigate the anti-inflammatory effect of the aerial part of *A. austrokoreense*, the ability of each sample to inhibit NO production was determined in LPS-stimulated RAW 264.7 cells. As shown in Fig. 2a, the MeOH extract of the aerial part of *A. austrokoreense* and its aqueous fraction markedly inhibited LPS-induced NO production dose-dependently. The MeOH extract and aqueous fraction at 200  $\mu\text{g/mL}$  inhibited NO production by greater than 60% compared with that generated by LPS. To elucidate whether this NO decrease resulted from the cytotoxic effects of the aerial part extract of *A. austrokoreense*, the cytotoxic effect of the MeOH extract and its fractions on LPS-stimulated RAW 264.7 cells was determined by the MTT assay. The MeOH extract (at 200 and 400

$\mu\text{g/mL}$ ) and aqueous fraction (at 200 and 400  $\mu\text{g/mL}$ ) did not affect cell viability with or without LPS for 24 hours (Fig. 2b), suggesting that the inhibitory effect of both samples on LPS-induced NO production is not attributed to cytotoxicity. The antioxidant activities of phytochemicals contribute to their anti-inflammatory actions by interrupting the ROS-inflammation cycle (Yahfoufi et al., 2018). In fact, a number of plant extracts including *Calophyllum brasiliense* leaf extract and *Abeliophyllum distichum* extract exhibited strong anti-inflammatory activity mediated by their antioxidant activities (Choi et al., 2017; Cisneros-Torres et al., 2020). However, the aqueous fraction exhibited the lowest antioxidant activity compared with the other samples, indicating that the aqueous fraction inhibited LPS-induced NO production by a

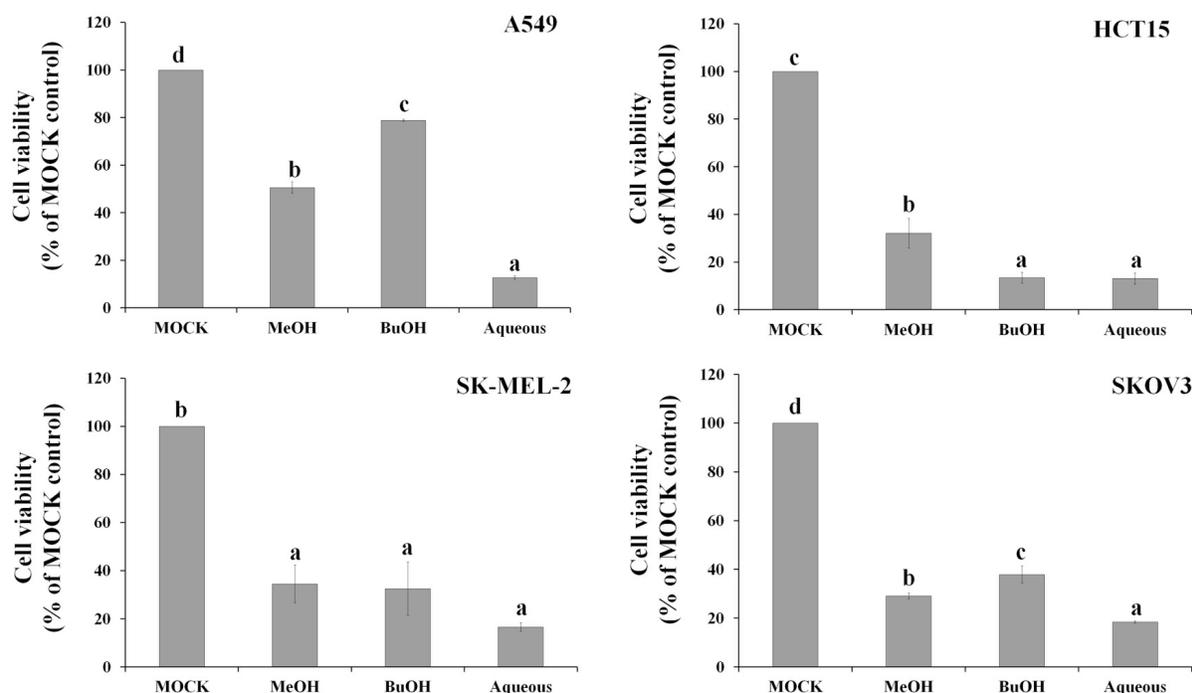


Fig. 3. Antiproliferative properties of *A. austrokoreense* aerial part extract and its fractions against A549, HCT15, SK-MEL-2 and SKOV3 cell lines. Each value represents the mean  $\pm$  SE, and the means were significantly different as calculated from a paired Duncan's test, with  $p < 0.05$ .

ROS-independent mechanism. Taken together, these findings suggest that the aerial part of *A. austrokoreense* may prevent inflammation-mediated diseases through inhibition of NO production in response to inflammatory stimuli.

### 3.5 Anti-cancer effect of *A. austrokoreense* aerial part

Since the initial search for novel anticancer agents from natural sources in the early 1950s, plant-based drugs such as vinca alkaloids (vinblastine, vinorelbine, vincristine, and vindesine) have been approved for use in cancer treatment combined with chemotherapy (Martino *et al.*, 2018). Additionally, due to our interest in identifying new plant-derived cancer agents that overcome multiple side effects of chemotherapy, extensive research on plant-based natural products has been focused on discovering a new generation of anticancer agents. In this study, the effect of the MeOH extract of the aerial part of *A. austrokoreense* and its fractions on the growth of tumor cell lines was analyzed to investigate their anti-cancer potential. As shown in Fig. 3, treatment with 200  $\mu$ g/mL of MeOH extract and its fractions significantly inhibited the proliferation of all the tested cancer cell lines.

Notably, the aqueous fraction exhibited the lowest viability of all the cell lines, ranging from 12.7% to 18.4%. Thus, the aqueous fraction is a potent source of anti-cancer compounds, although further study is necessary to analyze its effect on non-tumorous cells.

### 3.6 Correlation between the phytochemical content and biological activity of the aerial part of *A. austrokoreense*

Plant-derived polyphenolic compounds have attracted much attention because of their pharmaceutical properties (Es-Safi *et al.*, 2007; Wahle *et al.*, 2010; Gülçin, 2012), which are related to the TPC and TFC (Zou *et al.*, 2012; Hyun *et al.*, 2013). Regarding the aerial part of *A. austrokoreense*, the TPC exhibited good correlation with DPPH free radical scavenging activity and anticancer activity against A549 cells, and the correlation of TFC with reducing power was significant (Table 3). Although lycotonine-type norditerpenoid alkaloid (swatinine), and four norditerpenoid alkaloids (delphatine, lappaconitine, puberanine, and N-acetylsepaconitine) were isolated from 90% EtOH extract of defatted *A. leave* aerial part

Table 3. Correlations between the biological activity and total phenolic and flavonoid contents of *A. austrokoreense* aerial parts.

	Correlation R <sup>2</sup>	
	Total phenolic content	Total flavonoid content
DPPH radical scavenging activity	-0.773*	-0.418
Reducing power	0.579	-0.957**
Anti-inflammatory activity	0.562	-0.376
Anti-cancer activity	0.884**	-0.306

\*Significance at  $p < 0.05$ , \*\*Significance at  $p < 0.01$ . The MeOH extract and its fractions were used in the correlation.

(Shaheen *et al.*, 2005), various flavonoids (flavonol glycosides) identified in the MeOH extract or its BuOH fraction of aerial parts of *A. chiisanense*, *A. jaluense*, *A. napellus*, *A. anthora*, *A. burnatii*, and *A. variegatum* (Whang *et al.*, 1994; Jeong *et al.*, 1997; Fico *et al.*, 2001; Mariani *et al.*, 2008; Vitalini *et al.*, 2010) exhibited pharmacological activities including antioxidant activity. These findings indicate that polyphenolic compounds are major contributors to the antioxidant activity of the extract of the aerial part *A. austrokoreense* and support the importance of phenolic compounds in the anticancer properties of plant extracts as suggested by Roleira *et al.* (2015).

## Conclusions

To investigate the biological activities of the aerial part of *A. austrokoreense*, we analyzed the antioxidant, antibacterial, anti-inflammatory and anti-cancer activities. The overall results of the present study suggest that the aqueous fraction could be useful as a source of natural antibacterial, anti-inflammatory and anti-cancer agents, indicating that the aerial part of *A. austrokoreense* has potential as a crude drug and dietary health supplement. Further studies will be required to investigate the isolation and characterization of active compounds from the aerial part of *A. austrokoreense*.

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