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Antioxidant Capacity and Cytoprotective Effects of Gombo-Baechu (*Salvia plebeia* R. Br.) Extract Against H_2O_2-Induced Cellular Injury in NCI-H292 Human Bronchial Epithelial Cells

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ABSTRACT - *Salvia plebeia* R. Br. is a traditional medicinal herb used widely in Korea, China, and Japan, and has long been employed for the treatment of respiratory ailments, including bronchitis and coughing, in traditional medicine. In this study, the antioxidant capacity of *S. plebeia* R. Br. leaf extract and its cytoprotective effects against H_2O_2-induced cellular injury were investigated in NCI-H292 human bronchial epithelial cells. The total polyphenol and flavonoid contents of the extract were determined to be 715.2±34.3 (mg GAE/100 g DW) and 371.38±18.06 ppm (mg RE/100 g DW), respectively. The free-radical-scavenging capacity, evaluated using ABTS and DPPH assays, yielded inhibition rates of 80.83±2.14% and 56.18±1.43%, respectively. Cell viability was assessed using the MTT assay with a 24-h treatment, which remained above 90% at concentrations up to 20 μg/mL, with decreases observed at 50 and 100 μg/mL, but still reaching 70%. The H_2O_2-treated group exhibited a relative cellular injury value of 130.0%, whereas pretreatment with the extract significantly decreased this value to 88.1%, 82.3%, 69.7%, and 69.1% at 10, 20, 50, and 100 μg/mL, respectively, demonstrating a concentration-dependent cytoprotective effect. Our findings provide preliminary *in vitro* evidence of the antioxidant and cytoprotective properties of *S. plebeia* extract and suggest its potential as a functional food ingredient. However, further *in vivo* studies are required to confirm the physiological relevance and efficacy of *S. plebeian* extract.

Keywords: Rosmarinic acid, Hispidulin, Luteolin, MTT, NCI-H292

Oxidative stress occurs when the production of oxidative substances exceeds the capacity of cellular defense systems, causing damage to proteins, lipids, and DNA within cells[1]. Hydrogen peroxide (H_2O_2) is one of the most commonly used chemical agents to induce oxidative stress in cell-based studies, as it readily penetrates cell membranes and causes direct oxidative damage, leading to a reduction in cell viability[2]. The bronchial epithelium is particularly susceptible to oxidative damage because it is continuously exposed to air pollutants, pathogens, and other environmental stressors[3]. Oxidative damage in airway epithelial cells disrupts normal cell function and has been linked to the development and worsening of respiratory diseases such as asthma and chronic obstructive pulmonary disease[4].

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NCI-H292, a human bronchial epithelial cell line, has been widely used as an *in vitro* model for studying oxidative damage and inflammatory responses in the airway[3]. Since this cell line is sensitive to H_2O_2-induced cell damage, it is a suitable model for evaluating the cytoprotective effects of plant-derived antioxidant compounds.

Salvia plebeia R. Br. (gombo-baechu) is a member of the Lamiaceae family and is widely distributed in Korea, China, Japan, and other Asian countries[5]. It has long been used in traditional medicine to treat respiratory conditions such as bronchitis and cough, and is also known for its anti-inflammatory and liver-protective effects[5]. Several bioactive compounds have been identified in *S. plebeia*, including the phenolic acid rosmarinic acid and the flavonoids hispidulin and luteolin, which are known for their antioxidant and anti-inflammatory activities[6,7]. Previous studies on *S. plebeia* have primarily focused on its anti-inflammatory and anti-asthmatic effects. Jang et al.[8] demonstrated that ethanol extracts of *S. plebeia* suppressed the production of inflammatory mediators in LPS-stimulated RAW264.7 macrophages, reduced IL-6 and IL-8 production in stimulated BEAS-2B bronchial

epithelial cells, and alleviated airway inflammation in an ovalbumin-induced mouse model. In addition, the protective effects of *S. plebeia* against H_2O_2-induced cellular injury have been evaluated in non-airway cell models, including macrophage and neuronal cells[9,10], while other respiratory studies have examined fermented herbal mixtures containing *S. plebeia* rather than the leaf extract alone[11].

However, the direct cytoprotective effect of a chemically characterized *S. plebeia* leaf extract against H_2O_2-induced oxidative injury in human airway epithelial cells remains insufficiently investigated. Unlike previous studies focusing primarily on inflammatory responses, non-airway cell models, or herbal mixtures, the present study integrates high-performance liquid chromatography (HPLC)-based quantification of hispidulin, luteolin, and rosmarinic acid with chemical antioxidant assays and an H_2O_2-induced injury model using NCI-H292 cells. Therefore, this study aimed to characterize the major antioxidant compounds and antioxidant capacity of *S. plebeia* leaf extract and to evaluate its protective effect against H_2O_2-induced cellular damage in NCI-H292 human airway epithelial cells.

Materials and Methods

Plant material and extract preparation

Dried *S. plebeia* R. Br. leaves were milled before extraction. The extract was prepared by mixing 1 g of sample with 100 mL of 70% methanol, heating at 80°C for 3 h. After cooling to room temperature, the extract was sequentially filtered through No. 2 filter paper (Ø 70 mm) and a 0.22 μm membrane filter. The filtered extract was brought to a final volume of 100 mL with 70% MeOH and stored at -20°C until further analysis.

Total flavonoid content

Total flavonoids were quantified using an aluminum nitrate based colorimetric assay, adapted from the method reported by Kim et al.[12]. Briefly, 0.5 mL of the extract was combined with 1.5 mL of 80% ethanol, 0.1 mL of 10% (w/v) aluminum nitrate, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water in a test tube. After vigorous vortex mixing, the reaction mixture was incubated at room temperature for 30 min, and the absorbance was recorded at 415 nm using a UV–Vis spectrophotometer (Spectronic Genesys 5, Milton Roy, Ivyland, PA, USA). Rutin served as the reference standard; calibration solutions were prepared in 70% methanol at multiple concentrations and measured under identical conditions. Flavonoid content was calculated from the rutin calibration curve and expressed as milligrams of rutin equivalents per 100 g of dry weight (mg RE/100 g DW).

Total phenolic content

Total phenolic content was determined using a Folin–Denis colorimetric assay with minor modifications[13]. Briefly, 0.2 mL of the extract was combined with 0.2 mL of 1 M Folin–Denis reagent and 1.8 mL of distilled water in a test tube. After allowing the mixture to react for 3 min, 0.4 mL of 10% (w/v) Na_2CO_3 was added, and the final volume was brought to 4.0 mL with distilled water. The reaction was incubated for 1 h at room temperature in the dark, after which absorbance was measured at 725 nm using a UV–Vis spectrophotometer. Quantification was performed using a gallic acid calibration curve, and total phenolic content was expressed as milligrams of gallic acid equivalents per 100 g dry weight (mg GAE/100 g DW). All assays were conducted in triplicate.

DPPH radical scavenging activity

DPPH radical scavenging activity was assessed using a modified version of the protocol originally reported by Blois[14]. A 0.15 mM DPPH solution was prepared in methanol and kept protected from light until analysis. For each measurement, 1 mL of the sample extract was added to 3 mL of the DPPH solution in a test tube. The mixture was vortexed and incubated for 30 min at room temperature in the dark. The absorbance was then read at 517 nm with a UV–Vis spectrophotometer (Spectronic Genesys 5, Milton Roy). Radical scavenging activity was calculated as percent inhibition according to the following equation:

$$\text{DPPH radical scavenging activity}(\%) = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

ABTS radical scavenging activity

ABTS radical scavenging activity was evaluated following the method of Re et al.[15] with minor modifications. To generate the ABTS radical cation, an ABTS solution was combined with 2.45 mM potassium persulfate at a 35:65 (v/v) ratio and kept in the dark at room temperature for 16 h. The activated ABTS solution was then diluted with ethanol until the absorbance reached 0.70±0.02 at 734 nm. For the assay, 0.1 mL of the sample extract was mixed with 2.9 mL of the diluted ABTS working solution in a test tube, vortexed, and allowed to react for 6 min at room temperature. Absorbance was recorded at 734 nm using a UV–Vis spectrophotometer (Spectronic Genesys 5, Milton Roy). Radical scavenging activity was expressed as percent inhibition and calculated using the following formula:

$$\text{ABTS radical scavenging activity}(\%) = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

HPLC analysis

The HPLC conditions for hispidulin and luteolin were established according to Bardakci et al.[16] with modifications, whereas rosmarinic acid was analyzed using a modified method based on Liu et al.[17]. Separation was performed on a C18 column (150×4.6 mm, 5 μm) at 30°C with a flow rate of 1.0 mL/min and an injection volume of 10 μL. The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). A gradient elution program was applied as follows for hispidulin and luteolin: 0 min 15% B, 10 min 20% B, 15 min 25% B, 20 min 30% B, 30 min 50% B; and for rosmarinic acid: 0 min 12% B, 10 min 18% B, 15 min 22% B, 20 min 30% B, 27 min 12% B. Detection wavelengths were set at 340 nm for hispidulin and luteolin, and 330 nm for rosmarinic acid. Standard curves were prepared using authentic standards, and compound contents were expressed as mg/L.

Cell culture

NCI-H292 human mucoepidermoid carcinoma bronchial epithelial cells were obtained from Korean Cell Line Bank (Seoul, Korea). Cells were cultured and maintained in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum and 1% (v/v) penicillin (100 U/mL)/streptomycin (100 μg/mL). Cultures were maintained at 37°C in a humidified incubator with 5% CO_2.

Cell viability

Cell viability was assessed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cells were seeded at 1×10^4 cells per well in 96-well plates in 100 μL of medium and allowed to adhere for 4 h. The medium was then replaced with medium containing the extract at concentrations of 0, 1, 10, 20, 50, and 100 μg/mL and incubated for 24 h. After treatment, 20 μL of MTT solution (5 mg/mL in PBS) was added to each well and incubated at 37°C for 2 h. The formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 450 nm using a microplate reader using Epoch Microplate Spectrophotometer (Seoul, Korea). Cell viability was expressed as a percentage relative to the untreated control.

Cytoprotective effect against H_2O_2-induced cellular injury

To evaluate the cytoprotective effect of the extract against H_2O_2-induced cellular injury, NCI-H292 cells were seeded in 96-well plates at a density of 1×10^4 cells per well in 100 μL of culture medium and allowed to attach for 4 h. The medium was then replaced with fresh medium containing the extract at concentrations of 10, 20, 50, and 100 μg/mL, and the cells were pretreated for 1 h at 37°C. Based on a previously reported method with modifications[18], H_2O_2 was subsequently added to each well to achieve a final concentration of 10 μM, followed by incubation for 24 h at 37°C. After treatment, 20 μL of MTT solution (5 mg/mL in PBS) was added to each well, and the plates were incubated for 3-4 h at 37°C. The supernatant was carefully removed, and the resulting formazan crystals were dissolved in 100 μL of isopropyl alcohol with gentle shaking. Absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as follows:

$$\text{Cell viability}(\%) = \left(\frac{A_{\text{treated}}}{A_{\text{control}}}\right)\times 100$$

where A_{treated} and A_{control} represent the absorbance values of the treated and untreated control cells, respectively. The cytoprotective effect was evaluated by comparing the cell viability of the extract-pretreated groups with that of the H_2O_2-treated control group. All measurements were performed in triplicate.

Statistical analysis

All experiments were performed in triplicate, and the results are expressed as the mean±standard deviation. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). Differences among group means were evaluated by one-way analysis of variance (ANOVA). When a significant effect was detected, Duncan's multiple range test was performed to determine significant differences among the groups. A value of $P<0.05$ was considered statistically significant.

Results and Discussion

Total phenolic and flavonoid contents of *S. plebeia* extract

The total phenolic and total flavonoid contents of *S. plebeia* leaf extract are shown in Table 1. The extract yielded 715.2±34.3 (mg GAE/100 g DW) of phenolic content and 371.38±18.06 (mg RE/100 g DW) of flavonoid content. The higher phenolic content compared to flavonoid

Table 1. Total phenolic content and total flavonoid content of *S. plebeia* extract

Parameter	Value
Total phenolic content	715.2±34.3 (mg GAE/100 g DW)
Total flavonoid content	371.38±18.06 (mg RE/100 g DW)

Values are mean±SD (n=3). GAE, gallic acid equivalents; RE, rutin equivalents; DW, dry weight.

content indicates that phenolic acids such as rosmarinic acid account for a substantial proportion of the total phenolic content, which is consistent with the HPLC results above and with previous phytochemical reports on *S. plebeia*[7]. The high phenolic content of the extract suggests that it is a rich source of antioxidant compounds with potential for use as a functional food material.

ABTS and DPPH radical scavenging activities

The ABTS and DPPH radical scavenging activities of *S. plebeia* extract are shown in Table 2. ABTS and DPPH radical scavenging activities were 80.83±2.14% and 56.18±1.43%, respectively. The higher scavenging activity in the ABTS assay compared to DPPH is commonly observed in polyphenol-rich plant extracts, as the ABTS assay detects both hydrophilic and lipophilic antioxidants while DPPH mainly reacts with hydrogen donating compounds[15]. The strong radical scavenging activity is consistent with the high phenolic and flavonoid values and supports the antioxidant potential of the extract, which can be linked to the presence of rosmarinic acid, hispidulin, and luteolin identified by HPLC[7,19].

HPLC analysis

The HPLC-determined concentrations of the three marker compounds are summarized in Table 3. Hispidulin was the most abundant compound at 155.69±0.12 mg/L, followed by luteolin (128.59±3.45 mg/L) and rosmarinic acid (125.40±9.24 mg/L). These three compounds are well known bioactive constituents of *S. plebeia* and have been reported to contribute significantly to its antioxidant and anti-inflammatory activities[7]. Hispidulin and luteolin are flavones known to protect cells from oxidative damage and suppress inflammatory signaling[6], while rosmarinic acid is a hydroxycinnamic acid ester with potent free radical scavenging capacity[20]. The detection of these compounds in the extract provides a compositional basis for the antioxidant and cytoprotective activities observed in subsequent assays.

Table 2. Radical-scavenging activity of *S. plebeia* extract

Assay	Radical scavenging activity (%)
ABTS	80.83±2.14
DPPH	56.18±1.43

Values are mean±SD (n=3).

Table 3. Contents of hispidulin, luteolin, and rosmarinic acid in *S. plebeia* leaf extract determined by HPLC

Compound	Content (mg/L)
Hispidulin	155.69±0.12
Luteolin	128.59±3.45
Rosmarinic acid	125.40±9.24

Values are mean±SD (n=3).

Cell viability in NCI-H292 cells

The effect of *S. plebeia* extract on the viability of NCI-H292 cells is shown in Fig. 1. Cell viability was 121.0%, 109.0%, and 99.0% of the untreated control at 1, 10, and 20 μg/mL, respectively, indicating no apparent cytotoxicity within this concentration range. The increased MTT reduction observed at 1 and 10 μg/mL may reflect enhanced cellular metabolic activity[21]. However, this finding should not be interpreted as an increase in cell number without additional supporting assays. In contrast, cell viability decreased significantly to 78.0% and 70.0% at 50 and 100 μg/mL, respectively, indicating a concentration-dependent reduction in cellular metabolic activity at higher extract concentrations[2]. Because the MTT assay does not directly determine the mechanism of cell death, the reductions observed at these concentrations may represent cytotoxicity or metabolic suppression and should be interpreted cautiously. These findings indicate that concentrations up to 20 μg/mL were within the non-cytotoxic range, whereas the effects observed at 50 and 100 μg/mL require careful interpretation when determining an appropriate concentration range for further functional evaluation.

Cytoprotective effect of *S. plebeia* extract against H_2O_2-induced cellular injury

The cytoprotective effect of *S. plebeia* extract against H_2O_2-induced cellular injury in NCI-H292 cells, as assessed using the MTT assay, is presented in Fig. 2. H_2O_2 treatment

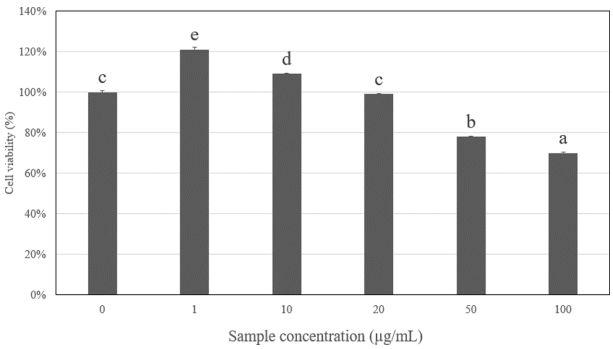

Fig. 1. Effect of *S. plebeia* extract on the viability of NCI-H292 cells. Cells were treated with the extract at concentrations of 0, 1, 10, 20, 50, and 100 μg/mL for 24 h, and cell viability was determined using the MTT assay. Cell viability was expressed as a percentage relative to the untreated control. Values are expressed as mean±SD (n=3). Different letters (a–e) above the bars indicate significant differences among groups according to Duncan's multiple range test (P<0.05).

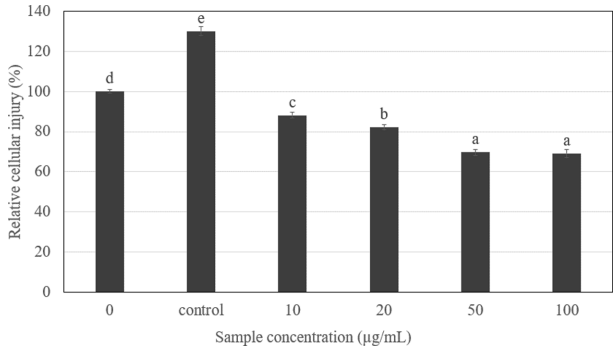

Fig. 2. Cytoprotective effect of *S. plebeia* extract against H_2O_2-induced cellular injury in NCI-H292 cells. Cells were pretreated with the extract at concentrations of 0, 10, 20, 50, and 100 μg/mL for 1 h and subsequently exposed to H_2O_2 at a final concentration of 10 μM for 24 h. Relative cellular injury was evaluated using the MTT assay and expressed as a percentage relative to the untreated control. Values are expressed as mean±SD (n=3). Different letters (a–e) above the bars indicate significant differences among groups according to Duncan's multiple range test (P<0.05).

significantly increased the relative cellular injury value to 130.0% compared with the untreated control (100%), indicating the induction of cellular injury. Pretreatment with *S. plebeia* extract reduced the relative cellular injury values to 88.1%, 82.3%, 69.7%, and 69.1% at 10, 20, 50, and 100 μg/mL, respectively, and all values were significantly lower than that of the H_2O_2-treated control (P<0.05). In particular, the reductions observed at 10 and 20 μg/mL, which were within the non-cytotoxic concentration range, indicate that the extract attenuated H_2O_2-induced cellular injury. However, because treatment with the extract alone reduced cell viability at 50 and 100 μg/mL, the results obtained at these higher concentrations should be interpreted cautiously. The responses at 50 and 100 μg/mL may reflect not only cytoprotection against H_2O_2-induced injury but also concentration-dependent changes in cellular metabolic activity caused by the extract itself.

The cytoprotective effect observed within the non-cytotoxic concentration range may be associated with bioactive constituents identified by HPLC, including hispidulin, luteolin, and rosmarinic acid, although their specific contributions and underlying mechanisms were not directly examined in the present study[20,22]. Jang et al.[8] reported that *S. plebeia* extract reduced inflammatory cytokine production in airway epithelial cells, while An et al.[11] demonstrated that a fermented herbal mixture containing *S. plebeia* attenuated airway inflammation in an asthma mouse model. These previous findings support the potential relevance of *S. plebeia* to respiratory health; however, direct comparisons should be made cautiously because of differences in experimental models and sample preparations. Taken together, the present findings provide preliminary *in vitro* evidence that *S. plebeia* leaf extract may have potential as a candidate functional food ingredient when used within an appropriate non-cytotoxic concentration range. Further studies using complementary cytotoxicity and mechanistic assays, as well as *in vivo* and clinical studies, are required to establish its optimal concentration, safety, physiological efficacy, and applicability.

국문요약

곰보배추(*Salvia plebeia* R. Br.) 잎 추출물의 지표성분 함량, 항산화 활성 및 NCI-H292 인간 기관지 상피세포에서 H_2O_2로 유도된 산화적 스트레스에 대한 세포 보호 효과를 평가하였다. HPLC 분석 결과, 히스피둘린, 루테올린, 로즈마린산의 함량은 각각 155.69±0.12, 128.59±3.45, 125.40±9.24 mg/L로 측정되었다. 총 폴리페놀 함량은 715.2±34.3 (mg GAE/100 g DW), 총 플라보노이드 함량은 371.38±18.06 (mg RE/100 g DW)이었으며, ABTS 및 DPPH 라디칼 소거 활성은 각각 80.83±2.14% 및 56.18±1.43%로 나타났다. MTT 분석 결과, 추출물은 20 μg/mL 이하 농도에서 90% 이상의 세포 생존율을 유지하였으나 50 및 100 μg/mL에서는 유의적인 감소가 관찰되었다. H_2O 처리군은 무처리 대조군 대비 상대적 세포 손상 지표가 130.0%로 증가하였으나, 곰보배추 추출물 전처리군에서는 10, 20, 50 및 100 μg/mL에서 각각 88.1%, 82.3%, 69.7% 및 69.1%로 감소하였다. 이러한 결과는 곰보배추 추출물이 H_2O_2로 유도된 세포 손상을 농도 의존적으로 완화하여 세포보호 효과를 나타냄을 보여준다. 이러한 결과는 곰보배추 추출물의 세포보호 효과와 기능성 식품 후보 소재로서의 가능성을 보여주는 *in vitro* 기초자료이며, 실제 생리적 효능을 확인하기 위해서는 추가적인 *in vivo* 연구가 필요하다.

Conflict of Interest

The authors declare no potential conflict of interest.

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