



Original Article

 Antiproliferative effect of *Momordica cochinchinensis* seeds on human lung cancer cells and isolation of the major constituents

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ABSTRACT

Gac, *Momordica cochinchinensis* (Lour.) Spreng., Cucurbitaceae, is an indigenous South Asian edible fruit and has been used therapeutically in Traditional Chinese Medicine. Previous studies have shown that *M. cochinchinensis* seed (*Momordicae Semen*) has various pharmaceutical properties such as antioxidant and anti-ulcer effects as well as contains secondary metabolites with potential anticancer activities such as triterpenoids and saponins. However, its biological activities in cancer have not yet been investigated. In this study, we found that its ethanol extract reduced cell proliferation in four human lung cancer cell lines, A549, H1264, H1299 and Calu-6. Phytochemical investigation of the ethanol extract was carried out, and resulted in isolation of two major saponins, which were identified as gypsogenin 3-O-β-D-galactopyranosyl(1 → 2)-[α-L-rhamnopyranosyl(1 → 3)]-β-D-glucuronopyranoside (**1**) and quillaic acid 3-O-β-D-galactopyranosyl(1 → 2)-[α-L-rhamnopyranosyl(1 → 3)]-β-D-glucuronopyranoside (**2**). Treatment with these isolated compounds (**1** and **2**) decreased cell proliferation in all human lung cancer cell lines tested. In addition, the compounds attenuated primary lung endothelial cell proliferation. Taken together, these findings suggest *M. cochinchinensis* seeds have antiproliferative activity on human lung cancer cells as well as angiostatic effect on lung endothelial cells.

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Introduction

Momordica cochinchinensis (Lour.) Spreng., Cucurbitaceae, is a dioecious vine that grows throughout Southeast Asia and small parts of Northern Australia. *M. cochinchinensis* fruits are known as baby jackfruit or “gac” in Vietnam. Outer shell of the fruit is not considered edible, but the seeds and the soft pulp are edible and they have been used in Traditional Chinese Medicine. *M. cochinchinensis* seeds (*Momordicae Semen*) have been applied for many therapeutic purposes including relieving muscle spasms, rheumatic pain, hemorrhoids, and bruises (Perry, 1980; Gao, 2005; Tran et al., 2016). Previous studies have shown that *Momordicae Semen* have pharmaceutical properties, such as antioxidant effects (Tsoi et al., 2005), immune response enhancement (Xiao et al., 2007), and anti-ulcer effects (Kang et al., 2010). In addition, the

seeds are mixed with glutinous rice to make a popular Vietnamese dish served at weddings and New Year celebrations. Ripe gac fruits contain high amounts of lycopene and β-carotene and are associated with various health benefits including stroke reduction and Vitamin A deficiency prevention (Vuong, 2000). Previous chemical investigations on this fruit have demonstrated the presence of major secondary metabolites including triterpenoids and saponins (Iwamoto et al., 1985; De Shan et al., 2001; Kan et al., 2006; Jung et al., 2013, 2016). Our recent study revealed that saponins from *M. cochinchinensis* seed have renoprotective effects against cisplatin-induced damage to LLC-PK1 kidney cells (Jung et al., 2016).

As an ongoing investigation of biological activities of *M. cochinchinensis* seeds, we explored effects of its EtOH extract on cell proliferation in human lung adenocarcinoma cell lines and found that the EtOH extract treatment decreased proliferation of all human lung cancer cell lines tested. To the best of our knowledge, these antiproliferative activities of *M. cochinchinensis* seed on human cancer cells have not been reported elsewhere. Additionally, phytochemical investigation was conducted to identify constituents of *M. cochinchinensis* seed contributing to its

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anti-proliferative activity against human lung cancer cells. This resulted in successful isolation of two major saponins. These were further evaluated for their antiproliferative activity against human lung cancer cells as well as primary lung endothelial cells.

Materials and methods

General experimental procedures

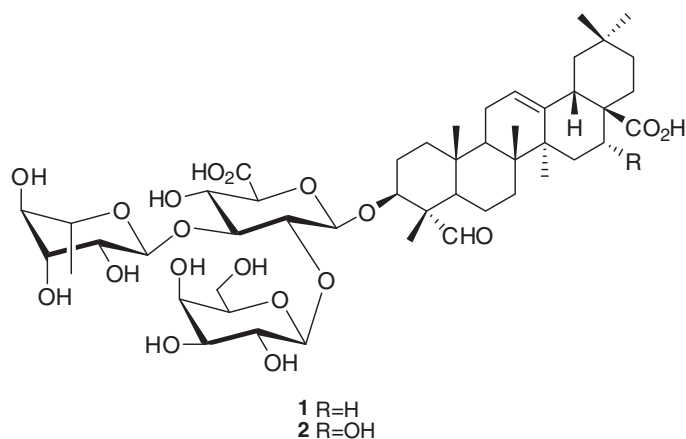
JASCO DIP-1000 digital polarimeter (Jasco, Tokyo, Japan) was used to measure optical rotations. IR spectra were collected using a JASCO FT/IR 300E spectrophotometer (Jasco). High resolution-FAB-MS was performed with a JEOL JMS-700 mass spectrometer (Akishima, Tokyo, Japan). With the Buchi B-540 apparatus (Buchi, Flawil, Switzerland) the melting point was recorded. Nuclear magnetic resonance (NMR) spectra such as, DEPT, HSQC, HMBC, TOCSY, and NOESY experiments, were performed under a Bruker AVANCE III 700 NMR spectrometer operating at 700 MHz (^1H) and 175 MHz (^{13}C) (Bruker), with chemical shifts labeled as ppm (δ). Preparative high performance liquid chromatography (HPLC) was performed using a Waters 1525 Binary HPLC pump with Waters 996 Photodiode Array Detector (Waters Corporation, Milford, CT, USA). Semi-preparative HPLC was conducted with a Shimadzu Prominence HPLC System with SPD-20A/20AV Series Prominence HPLC UV-vis Detectors (Shimadzu, Tokyo, Japan). Silica gel 60 (Merck, 230–400 mesh) and RP-C₁₈ silica gel (Merck, 40–63 μm) were used for column chromatography. Merck pre-coated Silica gel F₂₅₄ plates and RP-18 F_{254s} plates (Merck, Darmstadt, Germany) were used for TLC. Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde-sulfuric acid.

Plant material

Momordica cochinchinensis (Lour.) Spreng., Cucurbitaceae, seeds used in this study were obtained from a local medicinal herb store located near the Yunnan province, People's Republic of China in May 2010. The seeds were classified by one of the authors (K.H. Kim). A voucher (MBJ-2010-05) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation

Momordica cochinchinensis seeds (1.2 kg) were ground into soft powder and extracted with 50% ethanol under reflux twice for 4 h. The extract was followed by filtration and then concentrated *in vacuo*. The resulted residue (41 g) was stirred with distilled water then partitioned with hexane, ethyl acetate (EtOAc), and BuOH, yielding 2.1, 3.1, and 11.6 g, respectively. Before further isolation process, all of the partitioned fractions were processed with LC/MS analysis, and BuOH soluble fraction resulted as a main fraction and selected for isolation of chemical constituents. The BuOH soluble fraction (4 g) was purified using preparative HPLC of 10–70% MeOH gradient solvent system with Eclipse DBX-C18 column, Agilent (250 mm \times 21.2 mm i.d., 7 μm , at a 5 ml/min flow rate) to produce seven fractions (C1–C7). These following fractions were performed by LC/MS analysis for thorough selection process. Fraction C7 (150 mg) was selected for further purification. Fraction C7 (150 mg) was separated by a semi-preparative reversed phase HPLC using a column of Luna phenyl-hexyl (250 mm \times 10 mm i.d., 10 μm , at a 2 ml/min flow rate) with 30% MeCN isocratic system to yield compounds **1** (18 mg) and **2** (20 mg).



Cell culture

Human non-small cell lung cancer cell lines, A549, H1264, H1299 and Calu-6, were maintained in RPMI-1640 medium (WelGENE, Seoul, Korea) supplemented with 10% FBS (Hyclone, Logan, UT, USA), 2 mM L-glutamine (Gibco BRL, Grand Island, NY, USA), 50 U/ml penicillin, and 50 $\mu\text{g}/\text{ml}$ streptomycin (WelGENE). Primary lung endothelial cells (LEC) were isolated from wild-type C57BL/6 mice as previously described (Shin et al., 2014) and cultured in Advanced DMEM (Gibco BRL) supplemented with 15 mM HEPES (Sigma, St. Louis, MO, USA), 100 $\mu\text{g}/\text{ml}$ heparin (Sigma), 100 $\mu\text{g}/\text{ml}$ endothelial cell growth supplement (ECGS; Biomedical Technologies, Stoughton, MA, USA), 20% FBS, 2 mM L-glutamine, 50 U/ml penicillin and 50 $\mu\text{g}/\text{ml}$ streptomycin (Gibco BRL). The purity of LEC was assessed by staining for CD31, an endothelial cell marker, using anti-CD31 antibody (Abcam, Cambridge, UK) followed by examination under a confocal microscope (Carl Zeiss, Jena, Germany) as previously described (Shin et al., 2014).

Cell proliferation analysis

Human lung cancer cells (5×10^3) and 3×10^3 LEC were plated in triplicate in 96 well tissue culture plates (Thermo Scientific, Waltham, MA, USA) overnight and then treated with EtOH extract of *M. cochinchinensis* seeds or isolated compounds (**1** and **2**) for 48 h. Cell proliferation was then assessed by incubating with 1/10 volume of WST-1 reagent (Daeil Lab Service, Seoul, Korea) for 2 h followed by measuring absorbance at 450 nm with a scanning multi-well spectrophotometer (Molecular Device, Sunnyvale, CA, USA). Cell proliferation was then determined by percent of treated to untreated cell absorbance.

Statistical analysis

Unpaired student's two-tailed *t* test was used to assess statistical differences between cells treated with the EtOH extract of *M. cochinchinensis* seeds or isolated compounds (**1** and **2**) and untreated controls. Data are presented as mean \pm SEM and *p* values less than 0.05 were considered to be statistically significant.

Results and discussion

Cancer, a group of diseases involving unregulated cell growth, adjacent tissue invasion and metastasis to other body organs, is now the second leading cause of death worldwide (Hanahan and Weinberg, 2011; Fitzmaurice et al., 2015). Among all cancers, lung cancer has been the most common cancer death cause for both men and women worldwide for several decades (Jemal et al., 2008; Fitzmaurice et al., 2015; Ko et al., 2015; Park et al., 2015). Therefore,

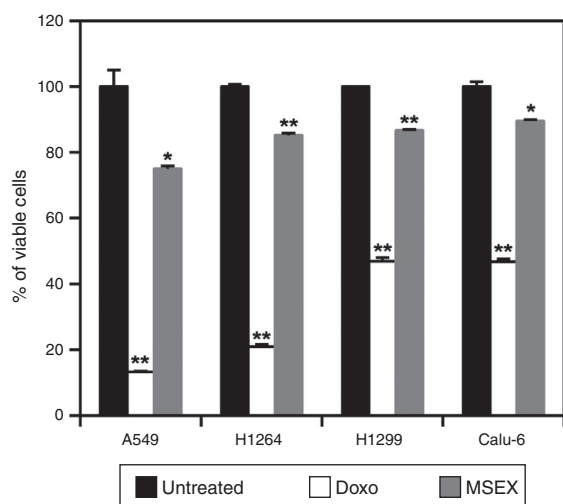


Fig. 1. The ethanolic extract of *Momordica cochinchinensis* seed reduces human lung cancer cell proliferation. Four human lung cancer cell lines, A549, H1264, H1299 and Calu-6, were treated with 60 $\mu\text{g/ml}$ of *M. cochinchinensis* seed EtOH extract (MSEX) or 1 μM doxorubicin (Doxo) as a positive control for 48 h. Cell proliferation was then assessed by WST-1 assay. Data are presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$.

to investigate novel biological activity of *M. cochinchinensis* seeds, we first prepared EtOH extract from *M. cochinchinensis* seeds and then examined the effects of its treatment on cell proliferation in four human lung cancer cell lines, A549, H1264, H1299 and Calu-6, using the WST-1 cell proliferation assay (Fig. 1). Treatment with EtOH extract at 60 $\mu\text{g/ml}$ for 48 h decreased A549, H1264, H1299 and Calu-6 cell proliferation up to 25, 15, 13 and 11%, respectively,

compared with untreated controls, indicating that EtOH extract of *M. cochinchinensis* seed possessed anti-proliferative activity against human lung cancer cells *in vitro*.

The EtOH extract of *M. cochinchinensis* seeds was subjected to fractionation with hexane, EtOAc, and BuOH to yield three fractions. Through thorough process of LC/MS analysis, BuOH soluble fraction was subjected to phytochemical investigation which resulted in isolation and identification of two triterpenoidal saponins by HPLC purification. Isolated compounds **1** and **2** were structurally resolved by spectroscopic methods including ^1H and ^{13}C NMR, and ESI-MS analysis. The purified compounds were classified as gypsogenin 3-*O*- β -D-galactopyranosyl(1 \rightarrow 2)-[α -L-rhamnopyranosyl(1 \rightarrow 3)]- β -D-glucuronopyranoside (**1**) (Jung et al., 2013, 2016) and quillaic acid 3-*O*- β -D-galactopyranosyl(1 \rightarrow 2)-[α -L-rhamnopyranosyl(1 \rightarrow 3)]- β -D-glucuronopyranoside (**2**) (Jung et al., 2013, 2016) after comparison to previously reported ^1H and ^{13}C NMR and MS data.

To determine whether the anti-proliferative activity of EtOH extract against human cancer cells is attributed to the isolated compounds **1** and **2**, we evaluated their effects on the proliferation of A549, H1264, H1299 and Calu-6 cells (Fig. 2). After treatment with compounds **1** and **2** at 20 and 60 $\mu\text{g/ml}$ for 48 h, WST-1 assay revealed a decrease in cell proliferation in all four human lung cancer cell lines in a dose-dependent manner, suggesting that anti-proliferative activity of the EtOH extract was due to these compounds.

Previously published studies have shown that triterpenoidal saponins attenuate tumor angiogenesis, the growth of new capillary blood vessels which is essential for tumors to become rapidly growing malignant tumors (Folkman, 2007). This is accomplished through inhibition of endothelial proliferation, leading to tumor growth suppression in tumor xenograft models of mouse (Arai et al.,

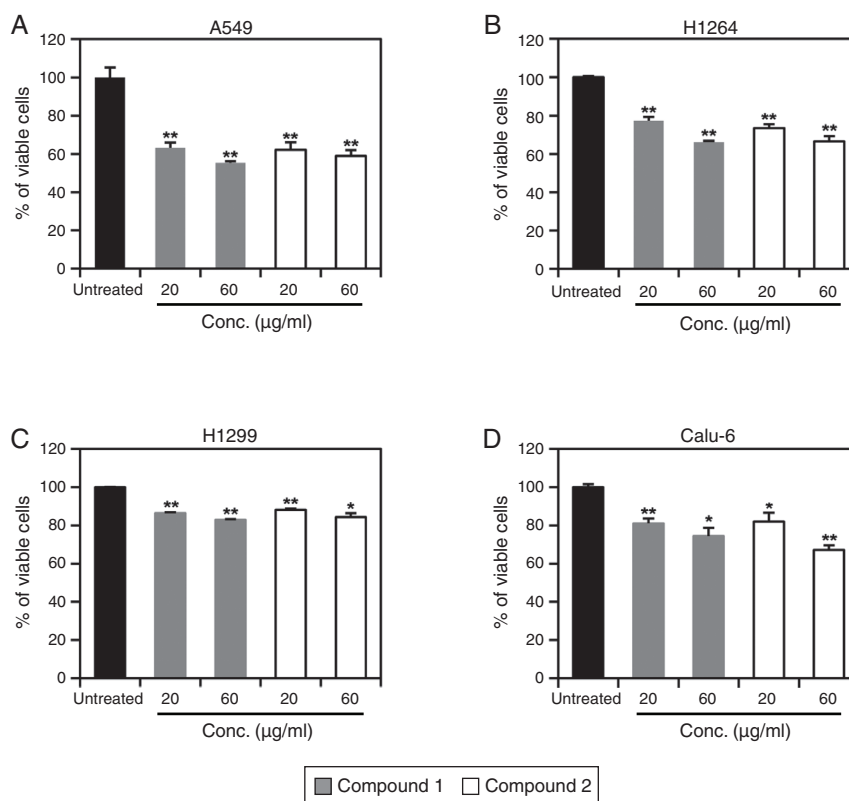


Fig. 2. Compounds **1** and **2** isolated from the ethanolic extract of *Momordica cochinchinensis* decrease human lung cancer cell proliferation. Antiproliferative activity of compounds **1** and **2** isolated from the EtOH extract (MSEX) in A549 (A), H1264 (B), H1299 (C) and Calu-6 (D) cells was assessed by WST-1 assay 48 h after treatment at the indicated concentrations. Data are presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$.

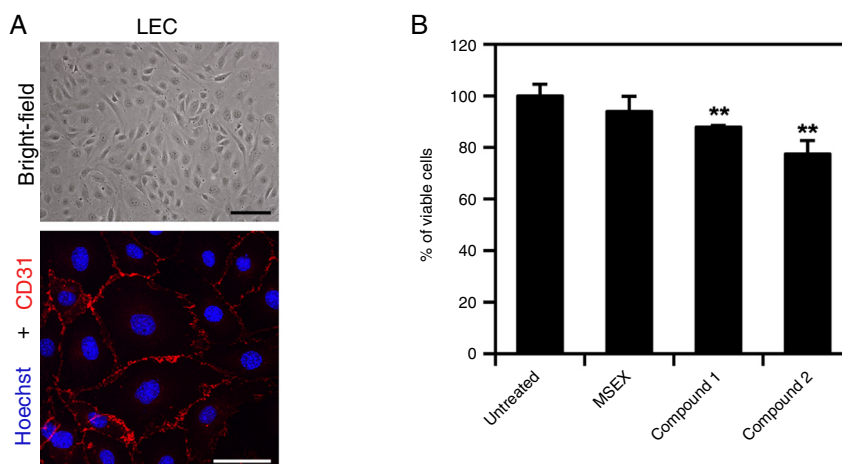


Fig. 3. Compounds **1** and **2** attenuate primary mouse lung endothelial cell proliferation. (A) Bright-field (upper) and immunofluorescence (lower) images of primary lung endothelial cells isolated from mice showing the typical cultured endothelial cell cobblestone morphology and CD31 expression, respectively. Scale bars: 50 μ m. (B) Antiproliferative effects of the ethanolic extract of *Mormodica cochinchinensis* seeds (MSEX) and its major constituents, compounds **1** and **2**, in primary mouse lung endothelial cells were assessed by WST-1 assay at 48 h after treatment with 60 μ g/ml of MSEX and 20 μ g/ml of compounds **1** and **2**. Data are presented as mean \pm SEM. ** $p < 0.01$.

2011; Guan et al., 2015). Therefore, we also examined whether the EtOH extract of *M. cochinchinensis* seed and its major constituents, compounds **1** and **2**, affected endothelial cell proliferation (Fig. 3). Since we focused on the inhibitory activity of *M. cochinchinensis* seeds against lung cancer cells, we used primary LEC isolated from mice (Fig. 3A). Although the EtOH extract failed to exhibit profound effects on endothelial proliferation, treatment with compounds **1** and **2** at 20 μ g/ml decreased cell proliferation in primary LEC up to 12 and 24%, respectively (Fig. 3B).

Taken together, our observations indicate that compounds **1** and **2** also possessed anti-proliferative activity in LEC, as well as in lung cancer cells.

Conclusions

Mormodica cochinchinensis is an indigenous South Asian fruit that has long been used as food and in Traditional Chinese medicine for many therapeutic purposes. This traditional Asian fruit is known to contain high levels of carotenoids, which partially explains its seeds' therapeutic effects. Yet, there has been no investigation of biological activities of *M. cochinchinensis* fruit in cancer and its components which may be responsible for the biological activities. This study identified the antiproliferative effects of EtOH extract of *M. cochinchinensis* seeds against human lung cancer cells. In addition, compounds **1** and **2**, identified as its major constituents, inhibited cell proliferation in human lung cancer cells as well as primary LEC, implying that the isolated compounds contribute to antiproliferative activity of the extract against human lung cancer cells as well as have angiostatic activity *in vitro*. These novel observations raise the possibility of its potential use as a therapeutic food for lung cancer intervention.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

Authors' contribution

JSY, HSR, and SL contributed the experiments. JSY, KHB, and KHK prepared and wrote the manuscript. KJ, KHB, and KHK contributed analysis and discussion. KHB and KHK contributed the design of the project and reviewed the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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References

- Arai, M., Hayashi, A., Sobou, M., Ishida, S., Kawachi, T., Kotoku, N., Kobayashi, M., 2011. Anti-angiogenic effect of triterpenoidal saponins from *Polygala senega*. *J. Nat. Med.* 65, 149–156.
- De Shan, M., Hu, L.H., Chen, Z.L., 2001. New multiflorence triterpenoids ester from *Momordica cochinchinensis* spring. *Nat. Prod. Lett.* 15, 139–145.
- Fitzmaurice, C., Dicker, D., Pain, A., Hamavid, H., Moradi-Lakeh, M., MacIntyre, M.F., Allen, C., Hansen, G., Woodbrook, R., Wolfe, C., Hamadeh, R.R., 2015. The global burden of cancer 2013. *JAMA Oncol.* 1, 505–527.
- Folkman, J., 2007. Angiogenesis: an organizing principle for drug discovery? *Nat. Rev. Drug Discov.* 6, 273–286.
- Gao, X.M., 2005. *Chinese Material Medica. Traditional Chinese. Materia Medica Press, Beijing.*
- Guan, Y.Y., Liu, H.J., Luan, X., Xu, J.R., Lu, Q., Liu, Y.R., Gao, Y.G., Zhao, M., Chen, H.Z., Fang, C., 2015. Raddeanin A, a triterpenoid saponin isolated from *Anemone raddeana*, suppresses the angiogenesis and growth of human colorectal tumor by inhibiting VEGFR2 signaling. *Phytomedicine* 22, 103–110.
- Hanahan, D., Weinberg, R.A., 2011. Hallmarks of cancer: the next generation. *Cell* 144, 646–674.
- Iwamoto, M., Okabe, H., Yamauchi, T., Tanaka, M., Rokutani, Y., Hara, S., Mihashi, K., Higuchi, R., 1985. Studies on the constituents of *Momordica cochinchinensis* Spreng. I. Isolation and characterization of the seed saponins, *Momordica* saponins I and II. *Chem. Pharm. Bull.* 33, 464–478.
- Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., Thun, M.J., 2008. Cancer statistics, 2008. *CA Cancer J. Clin.* 58, 71–96.

- Jung, K., Chin, Y.W., Yoon, K.D., Chae, H.S., Kim, C.Y., Yoo, H., Kim, J., 2013. Anti-inflammatory properties of a triterpenoidal glycoside from *Momordica cochinchinensis* in LPS-stimulated macrophages. *Immunopharmacol. Immunotoxicol.* 35, 126–132.
- Jung, K., Lee, D., Yu, J.S., Namgung, H., Kang, K.S., Kim, K.H., 2016. Protective effect and mechanism of action of saponins isolated from the seeds of gac (*Momordica cochinchinensis* Spreng.) against cisplatin-induced damage in LLC-PK1 kidney cells. *Bioorg. Med. Chem. Lett.* 26, 1466–1470.
- Kan, L.D., Hu, Q., Chao, Z.M., Song, X., Cao, X.L., 2006. Chemical constituents of unsaponifiable matter from seed oil of *Momordica cochinchinensis*. *Zhongguo Zhong Yao ZaZhi* 31, 1441–1444.
- Kang, J.M., Kim, N., Kim, B., Kim, J.H., Lee, B.Y., Park, J.H., Lee, M.K., Lee, H.S., Kim, J.S., Jung, H.C., Song, I.S., 2010. Enhancement of gastric ulcer healing and angiogenesis by Cochinchina *Momordica* seed extract in rats. *J. Korean Med. Sci.* 25, 875–881.
- Ko, H.R., Nguyen, T.L., Kim, C.K., Park, Y., Lee, K.H., Ahn, J.Y., 2015. P42 Ebp1 functions as a tumor suppressor in non-small cell lung cancer. *BMB Rep.* 48, 159–165.
- Park, J.H., Noh, T.H., Wang, H., Kim, N.D., Jung, J.H., 2015. Viriditoxin induces G2/M cell cycle arrest and apoptosis in A549 human lung cancer cells. *Nat. Prod. Sci.* 21, 282–288.
- Perry, L.M., 1980. *Medicinal Plants of East and Southeast Asia, Attributed Properties and Uses*. The MIT Press, Cambridge.
- Shin, J., Lee, J., Baek, K.H., 2014. A single extra copy of Dscr1 improves survival of mice developing spontaneous lung tumors through suppression of tumor angiogenesis. *Cancer Lett.* 342, 70–81.
- Tran, X.T., Parks, S.E., Roach, P.D., Golding, J.B., Nguyen, M.H., 2016. Effects of maturity on physicochemical properties of Gac fruit (*Momordica cochinchinensis* Spreng.). *Food Sci. Nutr.* 4, 305–312.
- Tsoi, A.Y.K., Ng, T.B., Fong, W.P., 2005. Antioxidative effect of a chymotrypsin inhibitor from *Momordica cochinchinensis* (Cucurbitaceae) seeds in a primary rat hepatocyte culture. *J. Pept. Sci.* 11, 665–668.
- Vuong, L.T., 2000. Underutilized β -carotene-rich crops of Vietnam. *Food Nutr. Bull.* 21, 173–181.
- Xiao, C., Rajput, Z.I., Liu, D., Hu, S., 2007. *Clin. Vaccine Immunol.* 14, 1634–1639.