TITLE PAGE
- Korean Journal for Food Science of Animal Resources -
Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Title	Antioxidant and Antibacterial Properties of Hovenia (*Hovenia dulcis*) Monofloral Honey Produced in South Korea
Running Title (within 10 words)	*Hovenia monofloral honey has antioxidant and antibacterial activities*
Author	Seung Hee Park[1], Young Ki Kim[2], Moon Sub Kim[2], Seung Ho Lee[1,*]
Affiliation	[1]Department of Nano-Bioengineering, Incheon National University, 119 Academy-ro, Incheon 22012, Korea [2]Division of Special Purpose Trees, Korea Forest Research Institute, Suwon 441-350, Korea
Special remarks – if authors have additional information to inform the editorial office	
ORCID (All authors must have ORCID) https://orcid.org	Seung Hee Park(https://orcid.org/0000-0003-4356-1660) Young Ki Kim (https://orcid.org/0000-0002-1456-3256) Moon Sub Kim (https://orcid.org/0000-0002-5344-4436) Seung Ho Lee (https://orcid.org/0000-0002-9941-4195)
Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This research was also supported by an Incheon National University research grant in 2017.
Author contributions (This field may be published.)	Conceptualization: Lee SH. Data curation: Lee SH. Formal analysis: Lee SH. Methodology: Park SH, Kim MS, Lee SH. Software: Park SH, Kim YK. Validation: Park SH, Kim YK. Investigation: Park SH, Lee SH. Writing - original draft: Lee SH Writing - review & editing: Park SH, Kim YK , Kim MS, Lee SH
Ethics approval (IRB/IACUC) (This field may be published.)	This manuscript does not require IRB/IACUC approval because there are no human and animal participants.

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Seung Ho, Lee
Email address – this is where your proofs will be sent	Lsh1602@gmail.com
Secondary Email address	seungho@inu.ac.kr
Postal address	22012
Cell phone number	010-8014-1614

Office phone number	032-835-8269
Fax number	032-835-0798

7

8

Antioxidant and Antibacterial Properties of Hovenia (*Hovenia dulcis*) Monofloral Honey Produced in South Korea

Abstract

The aim of this study was to evaluate the antioxidant and antibacterial activity of Hovenia (*Hovenia dulcis*) monofloral honey produced in Korea. To produce Hovenia monofloral honey, Hovenia trees were surrounded by a net house, and honeybees were breed there over a 20-day period. Hovenia monofloral honey contained more than 95% of Hovenia pollen and showed physicochemical properties in agreement with the international honey standard (Codex). The total phenolic and flavonoid contents of Hovenia monofloral honey ranged from a 24.82~27.0 mg gallic acid equivalent and a 0.43~0.45 mg quercetin equivalent, respectively. In addition, to evaluate the functional properties of Hovenia monofloral honey, the antioxidant activity of Hovenia monofloral honey was estimated by using the 1,1-diphenyl-2-picrylhydrazyl radical and the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay. Furthermore, Hovenia monofloral honey showed an antibacterial activity against foodborne gram positive (*Listeria monocytogenes* and *Staphylococcus aureus*) and gram negative bacteria (*Salmonella* Typhimurium and *Escherichia coli* O157:H7).

Key words Honvenia (*Hovenia dulcis*) honey, monofloral honey, antioxidant activity, anti-bacterial activity

Introduction

Honey is a well-known natural sweet food and has been considered an important source of traditional medicine (Eteraf-Oskouei and Najafi, 2013). Honey can be classified by the floral source because honeybees use nectar to produce honey. If a honeybee uses the nectar of many types of flowers to produce honey, it is classified as polyfloral honey, and it is also referred to as wildflower honey; however, if a honeybee uses the nectar of one type of flower to produce honey, it is classified as monofloral honey (Louveaux et al., 1978). Because pollen is a traceable floral source, a melissopalynological analysis is used to identify the types of plant sources used by honeybees for the production of honey. When the pollen of monofloral honey is analyzed in practice, many other types of pollen are often detected because honeybees have access to other types of honey plants even if the beehives are in a field where honeybees have access to only one type of honey plant. Therefore, generally, honey is recognized as monofloral honey when the content of the majority of the pollen is more than 45% of the total pollen (Soria et al., 2004; Olga et al., 2012).

There are several types of monofloral honey worldwide. Because each type of monofloral honey has distinct characteristics, such as flavor, taste, and physiochemical properties, which are derived from their botanical origins, there has been increased consumer demand for a better flavor and specific pharmacological attributes of monofloral honey, and thus the commercial value of monofloral honey has gradually increased (Pierce et al., 2009).

In South Korea, more than 70% of annual honey production is comprised of Acacia (*Robinia pseudo-acacia*) honey; however, recently, the total amount of honey production in South Korea has dramatically decreased because climate change has decreased the period of blooming as well as the growth of the acacia flower, resulting in the reduction of total honey

production in South Korea (Kohsaka et al., 2017). Therefore, developing a new candidate for a honey plant to compensate for the decrease in Acacia honey production is strongly required in South Korea. The Hovenia (*Hovenia dulcis*) tree is found in East Asian countries, such as China, Japan, and Korea, and is also reported to be found in the Himalayas up to altitudes of 2000 m (Hyun et al., 2010). The Hovenia tree prefers to grow in a sunny position, and the blooming period is about 20 days from June to July. The nectar production of the Hovenia flower is higher than that of the Acacia flower (Han et al., 2018; Song et al., 2014). Thus, Hovenia trees have been considered a candidate for honey plants in South Korea. There is a regional report that discusses the antioxidant activity of Hovenia honey produced in South Korea; however, the honey used for the study was harvested in open fields without a pollen analysis, indicating a low reliability regarding the purity of the Hovenia honey used (Paik et al., 2015). Therefore, for a more accurate and reliable evaluation of the value of Hovenia trees as honey plants, an investigation of the physiochemical, antioxidant, and antibacterial properties of Hovenia monofloral honey must be performed before increasing the number of Hovenia trees for the production of honey.

In this study, the physiochemical properties and antioxidant activity of Hovenia monofloral honey, which was prepared using a net house system, was investigated along with the antibacterial activity of Hovenia monofloral honey against foodborne bacteria.

Materials and Methods

Preparation of Hovenia (*Hovenia dulcis*) monofloral honey

Twenty-six Hovenia trees and honeybees (*Apis mellifera*) were cultured in a net house (23m

x 13m x 9m : W x D x H) constructed by the Korea Forest Research Institute (Suwon, Korea), and Hovenia monofloral honey-1 and Hovenia monofloral honey-2 were harvested on June 21, 2019, and July 2, 2019, respectively. Two types of acacia honey were obtained from the Korea Beekeeping Agricultural Cooperative and the National Institute of Forest Science, respectively, and were used as reference honey. Honey samples were stored at 4℃ under a dark condition until analysis.

Physiochemical analysis

To determine the moisture content, the honey sample was dried in a dry oven (Wiseven WOF-105, Daihan Scientific Co., Seoul, Korea) at 105℃ until a constant mass was obtained. Ash content was determined by calcinations in an Electric Muffle Furnace (JSMF-270T, JSR, Gongju, Korea) at 600℃ until the honey sample reached a constant weight. An electrical conductivity of 20% (w/v) of a honey solution was measured using an electrical conductivity (EC) meter. The hydroxymethylfurfural (HMF) and the carbon isotope ratio were measured using the standardized method listed in the Korean Food Code (2019).

Glucose, fructose, and sucrose content was determined using a high-performance liquid chromatograph (HPLC, Agilent Technologies, Palo Alto, CA, USA) equipped with an Ri-101 detector (Showa Denko K.K., Kawasaki, Japan). Briefly, the honey sample (5 g) was mixed with 25 mL of petroleum ether. Then, 25 mL of distilled water was added, and it was incubated in a water bath at 85℃ for 25 min. The sugar solution extracted from the honey was filtered using a 0.45 μm membrane filter. Finally, it was separated using a PhenoSphere NH2 80A column (250 mm × 4.6 mm, 5 μm, Phenomenex Inc., Torrance, CA, USA). The mobile phase

was composed of 80 % acetonitrile. The injection volume of the samples was 20 μL with a flow rate of 1.0 mL/min.

Mineral contents

The honey sample (1 g) was mixed with nitric acid (5 mL) and incubated for 1 h at room temperature. Then, the mixture was heated at 200℃ for 2 h and made 20 mL by adding distilled water. Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn content was measured using inductively coupled plasma optical emission spectroscopy (720 ICP-OES, Agilent Technologies, Palo Alto, CA, USA) equipped with a VistaChip II CCD detector (Agilent Technologies, Palo Alto, CA, USA). The detection limit of the mineral content was less than 0.1 mg/L.

Melissopalynological analysis

A melissopalynological analysis was performed according to the method of Louveaux (1978) with some modifications. The honey sample (10 g) was diluted in 10 mL of distilled water and incubated at 37℃ for 10 min. After the honey solution was centrifuged at 1500 x g for 10 min, the sediment of the honey solution was washed with 5 mL of distilled water and centrifuged again at 1000 x g for 5 min. Then, the sediment was resuspended in 50 μL of 50% (w/v) glycerin. The sediment of the solution was spread on a 22×22 mm area on a slide. More than 300 pollen grains were photographed using a microscope (Nikon Eclipse Ti-S, Tokyo, Japan) and counted.

Antioxidant activity

118 The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of Hovenia honey
119 was determined according to the method of Tuberoso (2003). 0.5 mL of an aqueous honey
120 solution (10%, w/w) was mixed with 2.5 mL of a 200 μM DPPH solution and incubated in the
121 dark for 1 h at room temperature. The absorbance of the solution was measured at 517 nm using
122 a UV/Vis spectrophotometer (Optizen POP, Mecasys, Daejeon, Korea). DPPH scavenging
123 activity was calculated by the following equation:

124 DPPH radical scavenging activity = [1-(A-B)/C]

125 Where A is the absorption of all reagents, B is the absorption of the honey solution, and C is
126 the absorption of all reagents without the honey solution. Trolox was used to determine the
127 standard curve (25-300 μM, R^2=0.995). The antioxidant capacity was expressed as the μmol of
128 Trolox equivalent (TE)/100g of honey.

129

130 The 2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging
131 activity of Hovenia honey was determined according to the methods of Tuberoso (2003). The
132 ABTS radical solution was prepared by reacting 10 mL of 2 mM ABTS in PBS with 0.1 mL of
133 70 mM potassium persulfate. After 16-24 h of incubation in the dark at room temperature, the
134 ABTS radical solution was diluted with PBS to obtain the absorbency of 0.7 ± 0.1 at 734 nm.
135 The 0.5 mL aqueous honey solution (5%, w/w) and the 1.8 mL ABTS radical solution was
136 mixed and incubated for 6 min in the dark at room temperature. Finally, the absorbance of the
137 solution was measured at 734 nm using a UV/Vis spectrophotometer (Optizen POP, Mecasys,
138 Daejeon, Korea). The ABTS radical scavenging activity was calculated by the following
139 equation:

ABTS scavenging activity = [1-(A-B)/C]

Where A is the absorption of all reagents, B is the absorption of the honey solution, and C is the absorption of all reagents without the honey solution. Trolox was used to determine the standard curve (100-500 μM, R^2=0.999). The antioxidant capacity was expressed as the μmol of Trolox equivalent (TE)/100g of honey.

Measurement of total phenolic content

The total phenolic content of Hovenia honey was determined using the Folin-Cioalteu method (Meda et al., 2005). The 0.5 mL aqueous honey solution (10%, w/w) was mixed with 2 mL of the 0.2 N Folin-Ciocalteu reagent (Sigma-Aldrich Co., St. Louis, MO, USA) and incubated at room temperature for 6 min. Then, a 1.5 mL of 7% (w/v) sodium carbonate solution was added and incubated for 2 h at room temperature. Finally, the absorbance of the solution was measured at 750 nm with a UV/Vis spectrophotometer (Optizen POP, Mecasys, Daejeon, Korea). Gallic acid (Sigma-Aldrich Co., St. Louis, MO, USA) was used to determine the standard curve (12.5-200 μg/mL, R^2=0.999). The total phenolic content was expressed as the mg of gallic acid equivalent (GAE)/100g of honey.

Measurement of total flavonoid content

The total flavonoid content of Hovenia honey was determined according to the methods of Kim et al. (2005). A 0.5mL aqueous honey solution (20%, w/w), 0.1 mL of 10% (w/v) aluminum nitrate solution, 0.1 mL of 1M potassium acetate solution, 0.5 mL of 80% (v/v) ethanol, and 2.8 mL of distilled water were mixed and incubated for 40 min at room

temperature. The absorbance of the solution was measured at 415 nm using a UV/Vis spectrophotometer (Optizen POP, Mecasys, Korea). Quercetin (Sigma-Aldrich Co., St. Louis, MO, USA) was used to determine the standard curve (10-80 μg/mL, R^2=0.999). The total flavonoid content was expressed as the mg quercetin equivalent (QE)/100g of honey.

Measuring the antibacterial activity

Four foodborne pathogens were used in the test. The gram negative bacteria, *Escherichia coli* O157:H7 (ATCC 35150) and *Salmonella* Typhimurium (KCTC 1925), were grown in Luria Bertani Broth (Difco, Michigan, USA) at 37℃ in an incubator. The gram positive bacteria, *Staphylococcus aureus* (ATCC 29213) and *Listria monocytogenes* (ACTC 3569), were grown in Brain Heart Infusion Broth (MB cell, Seoul, Korea) at 37℃ in an incubator. The minimum inhibitory concentration (MIC) of Hovenia honey was determined by the broth micro-dilution method in 96-well microplates (Bucekova et al., 2018). The 50% (w/v) honey stock solution in a broth medium was diluted at different concentrations ranging from 1.56 to 50% (w/v). 90 μL of the honey solution was dispensed into each well. The bacterial cultures were diluted to the 10^5 CFU/mL using a broth medium. Then, 10 μL of the diluted bacterial culture was mixed to test the honey solution and was incubated at 37℃ for 18 h. The absorbance of the culture medium was measured at 490 nm using a micro absorbance spectrophotometer (iMark™ Microplate Reader, Hercules, CA, USA).

Results and Discussion

Production of Hovenia monofloral honey

To produce Hovenia monofloral honey, honeybees and Hovenia trees were bred in a net house (Fig. 1), and honey was harvested on two different day. The purity was determined by a pollen analysis, and both honeys showed more than 95% of Hovenia pollen content, which indicated that the honey produced in the net house was Hovenia monofloral honey (Fig. 2). Because there is no dense, open area with Hovenia trees to produce Hovenia monofloral honey in Korea, even though the honey was harvested near Hovenia trees during the Hovenia blossom season, it may contain several other types of pollen, and the content of major pollen may be less than 45%. In general, when the major content of pollen is less than 45%, the honey is classified as multifloral honey. Thus, the net house, which can produce high-purity monofloral honey, could be an optimal small-scale system used to estimate the potential of the honey plant. Therefore, Hovenia monofloral honey harvested in a net house system is a good source to evaluate the potential of the Hovenia tree as a honey plant.

Physiochemical analysis of Hovenia monofloral honey

The physiochemical properties of Hovenia monofloral honey produced in the net house were investigated to determine the potential of the type of honey. As showed in table 1, Hovenia monofloral honey was composed of glucose (29.0±0.42%), moisture (18.9±0.28%), fructose (35.9±0.78%), reducing sugar (64.9±0.35%), sucrose (3.9±1.63%), and ash (0.1±0.00%). The contents of hydroxymethylfurfural (HMF) were not detected in Hovenia monofloral honey, and the carbon isotope ratio was -26.6±0.14 ‰. All contents of Hovenia monofloral honey were in the range of the international standards by Codex Alimentarius (2001) as well as the food code legislated by the Ministry of Food and Drug Safety of Korea (MFDSK, 2019). In addition, the mineral contents of Hovenia monofloral honey included potassium (407.5±3.11 mg/L),

magnesium (10.7±1.1 mg/L), sodium (1.8±0.28 mg/L), manganese (2.9±0.14 mg/L) phosphorus (20.55±1.77 mg/L), and zinc (13.9±015.13 mg/L) (Table 2). These data suggest that Hovenia monofloral honey produced in a net house system could be used as a primary honey source, and the net house used in this study could be used for the large-scale production of Hovenia monofloral honey as well as other monofloral blossom honeys.

Antioxidant activity of Hovenia monofloral honey

The DPPH and ABTS radical scavenger activity of Hovenia monofloral honey was then evaluated and compared with that of acacia honey. The two types of acacia honey used in this study were identified as monofloral honeys by a pollen analysis (data not shown). As shown in Fig. 3, both Hovenia monofloral honey-1 (24.8±2.53 μmol TE/100g honey) and -2 (27.0 ±0.28 μmol TE/100g honey) showed a significantly ($p<0.05$) higher DPPH radical scavenger activity than that of Acacia honey-1 (23.4 ±2.16 μmol TE/100g honey) and -2 (19.3 ±1.48 μmol TE/100g honey); however, although the ABTS radical scavenger activity of the Acacia honey-2 sample (110.4 ±6.63 μmol TE/100g honey) was slightly lower than that of Hovenia monofloral honeys, the Acacia honey 1 sample (110.4 ±6.63 μmol TE/100g honey) showed a similar ABTS radical scavenger activity as that of Hovenia monofloral honeys (Hovenia monofloral honey 1; 130.6 ±6.73 μmol TE/100g honey, Hovenia monofloral honey 2; 141.9 ±5.49 μmol TE/100g honey). These results indicate that Hovenia monofloral honey has a higher antioxidant activity than Acacia honey when tested with a DPPH radical but not with an ABTS radical. Interestingly, the amounts of total phenol and total flavonoid were not significantly different between Hovenia monofloral honey and Acacia honey (Fig. 3c and 3d). Therefore, as the DPPH assay was used to identify the role of hydrophobic antioxidants in the samples

(Arnao et al., 2000), the data suggested that the type of hydrophobic antioxidants may be different between Hovenia monofloral honey and Acacia honey. A detailed analysis of the single components of Hovenia monofloral honey will be performed in a future study.

Antibacterial activity of Hovenia monofloral honey

To estimate the antibacterial activity of Hovenia monofloral honey, the minimum inhibitory activity of Hovenia monofloral honey against four foodborne bacteria, including *E. coli* O157:H7, *S.* Typhimurium, *S. aureus* (ATCC 29213), and *L. monocytogenes*, was evaluated (Fig. 4 and Table 3). Hovenia monofloral honey showed MIC values of 25-50 % (w/v) against two gram positive foodborne bacteria, *E.coli* O157:H7 and *S.* Typhimurium, and MIC value of 25 % (w/v) against two gram negative foodborne bacterial, *S. aureus* and *L. monocytogenes.* The MIC values are similar to that of Acacia honey (Fig. 4 and Table 3), suggesting that Hovenia monofloral honey produced in a net house system has a strong antibacterial activity and can be used for food preservation against food pathogens. Furthermore, the MIC values of artificial honey which constituted with sugars (glucose:33.5g, fructose:40.5g, sucrose:1.5g, maltose:7.5g in DW:17mL) against foodborne bacteria were more than 50% (w/v) (data not shown) indicated that a part of antibacterial activity of Hovenia monofloral honey was derived from honey constituents other than sugar such as phenols and flavonoids.

Conclusion

In this study, high-purity Hovenia monofloral honey was produced using a net house system, and its physiochemical properties, such as the contents of sugar, minerals, total phenoic acid,

252 and total flavonoids, were evaluated. Hovenia monofloral honey showed DPPH and ABTS
253 radical scavenger activities and antibacterial activity against gram positive and gram negative
254 foodborne bacteria. To the best of our knowledge, this is the first evaluation of Hovenia
255 monofloral honey, and it can be used to evaluate the potential of the Hovenia tree as a honey
256 plant. Furthermore, because the amount of nectar per flower bud of the Hovenia tree is higher
257 than that of the Acacia (*Robinia pseudoacacia*) tree, the Hovenia tree could be a candidate to
258 compensate for the loss of the Acacia tree as a honey plant.

259

260 ## Conflicts of Interest

261 The authors declare no potential conflict of interest.

262 ## Acknowledgements

263 This research was also supported by an Incheon National University research grant in 2017.

264

265

References

Arnao MB. 2000. Some methodological problems in the determination of antioxidant activity using chromogen radicals: A practical case. Trends in Food Sci &Tec 11:419-421.

Bucekova M, Juricova V, Monton E, Martinotti S, Ranzato E, Majtan J. 2018. Microwave processing of honey negatively affects honey antibacterial activity by inactivation of bee-derived glucose oxidase and defensin-1. Food Chem 240:1131-1136.

Eteraf-Oskouei T, Najafi M. 2013. Traditional and modern uses of natural honey in human diseases: A review. Iran J Basic Med Sci 16:731-742.

Codex Alimentarius. 2001. Standard for honey. Codex Stan 12-1981.

Han J, Kim SH. 2008. Flowering and nectar secretion characteristics of honey plant, *Hovenia dulcis var. Koreana* Nakai. Korean J Apiculture 23:199-205.

Hyun TK, Eom SH, Yu CY, Roitsch T. 2010. Hovenia dulcis – An Asian traditional Herb. Planta Med 76: 943-949.

Kim EJ, Lee HJ, Kim HJ, Nam HS, Lee MK, Kim HY, Lee JH, Kang YS, Lee JO, Kim HY. 2005. Comparison of colorimetric methods for the determination of flavonoid in propolis extract products. Korean J Food Sci Technol 37:918-921.

Louveaux J, Maurizio A, Vorwohl G. 1978. Methods of melissopalynology. Bee World 51:125-131.

Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem 91:571-577.

MFDSK. 2019. Food code. Ministry of Food and Drug Safety of Korea http://www.mfds.go.kr/brd/m_211/list.do.

Olga E, María FG and Carmen SM. 2012. Differentiation of blossom honey and honeydew honey from northwest Spain. Agriculture 2:1-13.

Paik WK, Kwak AK, Lee ML, Sim HS. 2015. Studies on the chemical characteristics of Hovenia (*Hovenia dulcis*) honey produced in Korea. J. Apiculture 30:75-85.

Pires J, Estevinho ML, Fe´as X, Cantalapiedra J, Iglesias A. 2009. Pollen spectrum and physico-chemical attributes of heather (*Erica* sp.) honeys of north Portugal. J Sci Food Agric 89:1862-1870.

Soria AC, González M, de Lorenzo C, Martínez-Castro I, Sanz J. 2004. Characterization of artisanal honeys from Madrid (Central Spain) on the basis of their melissopalynological, physicochemical and volatile composition data. Food Chem 85:121-130.

Song JH, Kim SH, Kim H, Kim MS. 2014. New cultivars multiplication of oriental raisin tree (*Hovenia dulcis var. Koreana Nakai*) by veneer grafting. Korean J. Plant Res 27:183-187.

Tuberoso CI, Boban M, Bifulco E, Budimir D, Pirisi FM. 2013. Antioxidant capacity and vasodilatory properties of Mediterranean food: The case of Cannonau wine, myrtle berries liqueur and strawberry-tree honey. Food Chem 140:686-691.

Kohsaka R, Sun Park M, Uchiyama Y. 2017. Beekeeping and honey production in Japan and South Korea: Past and present. J Ethn Foods 4:72-79.

308 **Table 1. Physiochemical properties of Hovenia monofloral honey**

	Glucose (%)	Moisture (%)	Fructose (%)	Reducing sugar (%)	Sucrose (%)	HMF (mg/kg)	F/G ratio	Ash (%)	Electrical conductivity (μS/cm)	Carbon isotope ratio (‰)
*HMH-1	29.3±1.3	18.7±0.0	35.3±0.5	64.6±1.7	2.7±0.2	0.0±0.0	1.2±0.06	0.1±0.0	191	-26.7
HMH-2	28.7±0.8	19.1±0.0	36.4±1.5	65.8±3.5	5.0±0.7	0.0±0.0	1.27±0.06	0.1±0.0	172	-26.5
Ave.	29.0±0.4	18.9±0.28	35.9±0.78	64.9±0.35	3.9±1.6	0.0±0.0	1.24±0.05	0.1±0.0	181.5±13.4	-26.6±0.1

309 *HMH: Hovenia monofloral honey

310

311 **Table 2. The mineral contents of Hovenia monofloral honey**

312 (mg/L)

	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
*HMH-1	19.3±2.9	< 0.1	< 0.1	409.7±3.1	9.9±0.3	2.8±0.0	2±0.3	19.3±0.1	3.2±0.1
HMH-2	20.8±0.5	< 0.1	< 0.1	405.3±8.1	11.5±0.1	3.0±0.0	1.6±0.1	21.8±0.1	24.6±0.2
Ave.	20.05±1.06	< 0.1	< 0.1	407.5±3.11	10.7±1.13	2.9±0.14	1.8±0.28	20.55±1.77	13.9±15.13

313 *HMH: Hovenia monofloral honey

314

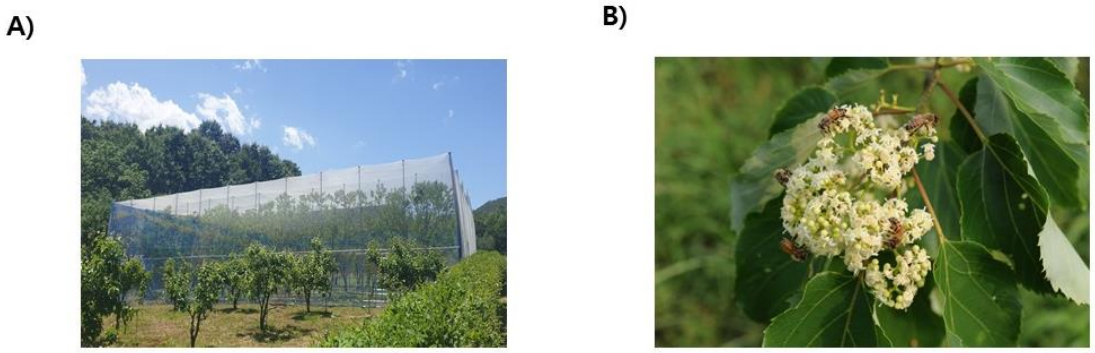
315 **Table 3. Antibacterial activity of Hovenia monofloral honey**

	MIC (%, w/v)			
	Gram negative		Gram positive	
	E.coli	***S.*Typhimurium**	***S.aureus***	***L.monocytogenes***
*HMH-1	50	50	25	25
HMH-2	25	25	25	25
Acacia honey-1	50	50	50	25
Acacia honey-2	50	25	25	25

316 *HMH: Hovenia monofloral honey

317

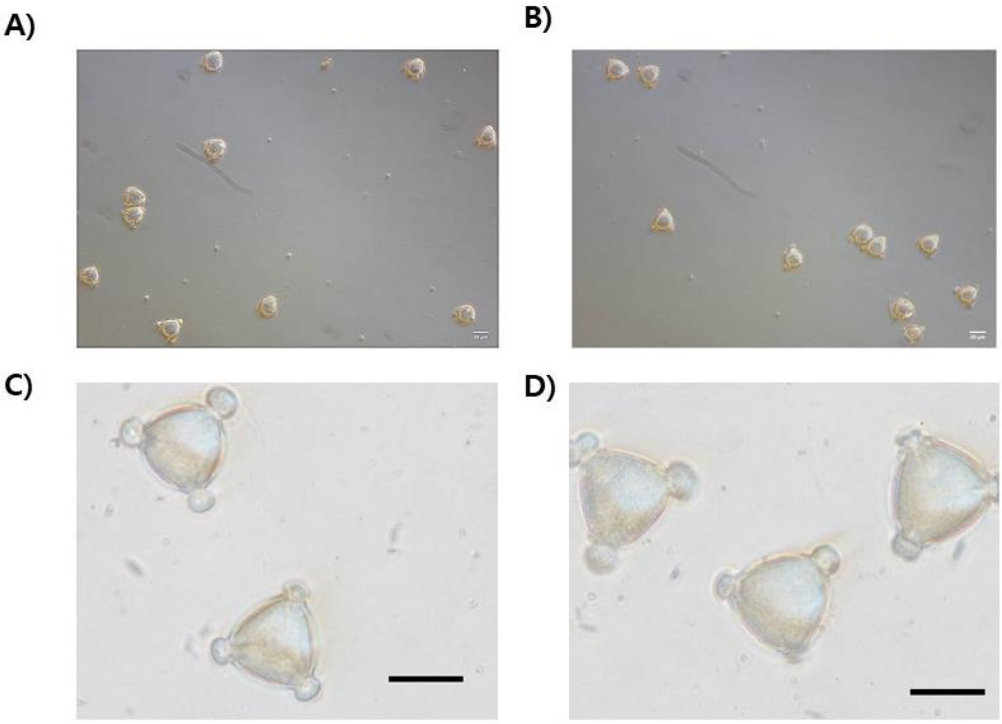
318 **Figure legends**



319

320 **Fig. 1. Net house system used to produce high-purity Hovenia monofloral honey.** Hovenia
321 trees were surrounded with a net house (A) and cultivated by honeybees (*Apis melifera*) (B).
322 Hovenia monofloral honey was harvested two times once per week and was used as Hovenia
323 monofloral honey-1 and -2.

324



325

326 **Fig. 2. Pollen analysis of Hovenia monofloral honey.** A pollen analysis was performed to
327 estimate the purity of Hovenia monofloral honey produced in a net house. Hovenia pollen was
328 isolated from Hovenia monofloral honey and photographed. Both Hovenia monofloral honey-
329 1 (A and C) and -2 (B and D) contained more than 95% Hovenia pollen. Representative pictures
330 of the Hovenia pollen of Hovenia monofloral honey -1 (A and C) and -2 (B and D) are shown.
331 The size of the scale bar is 20 μm.

332

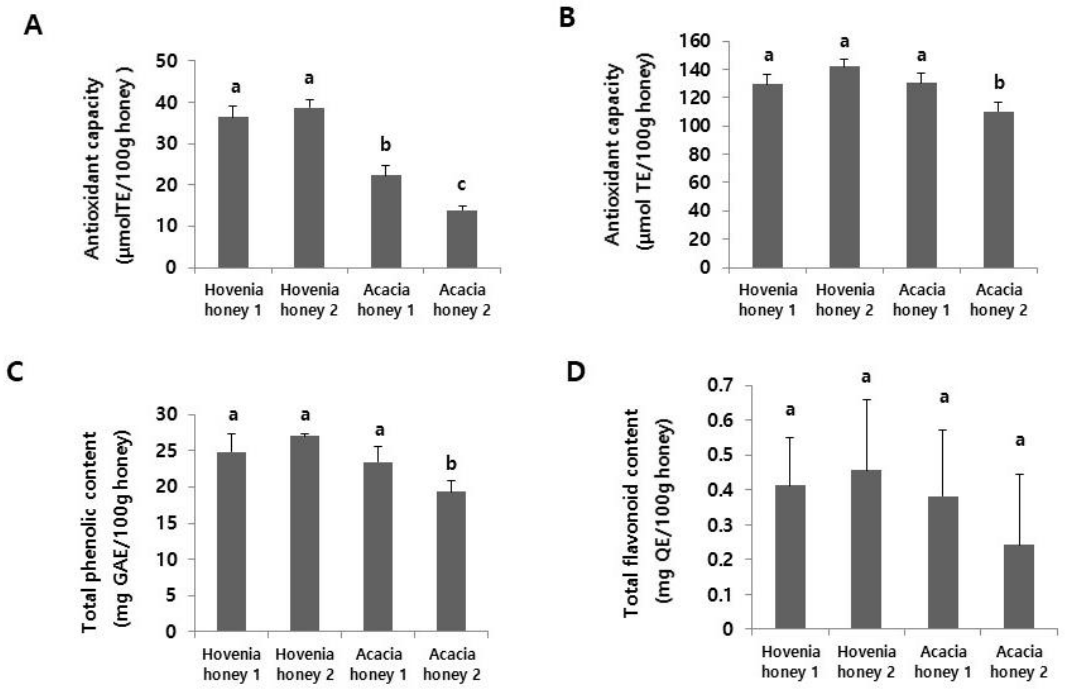
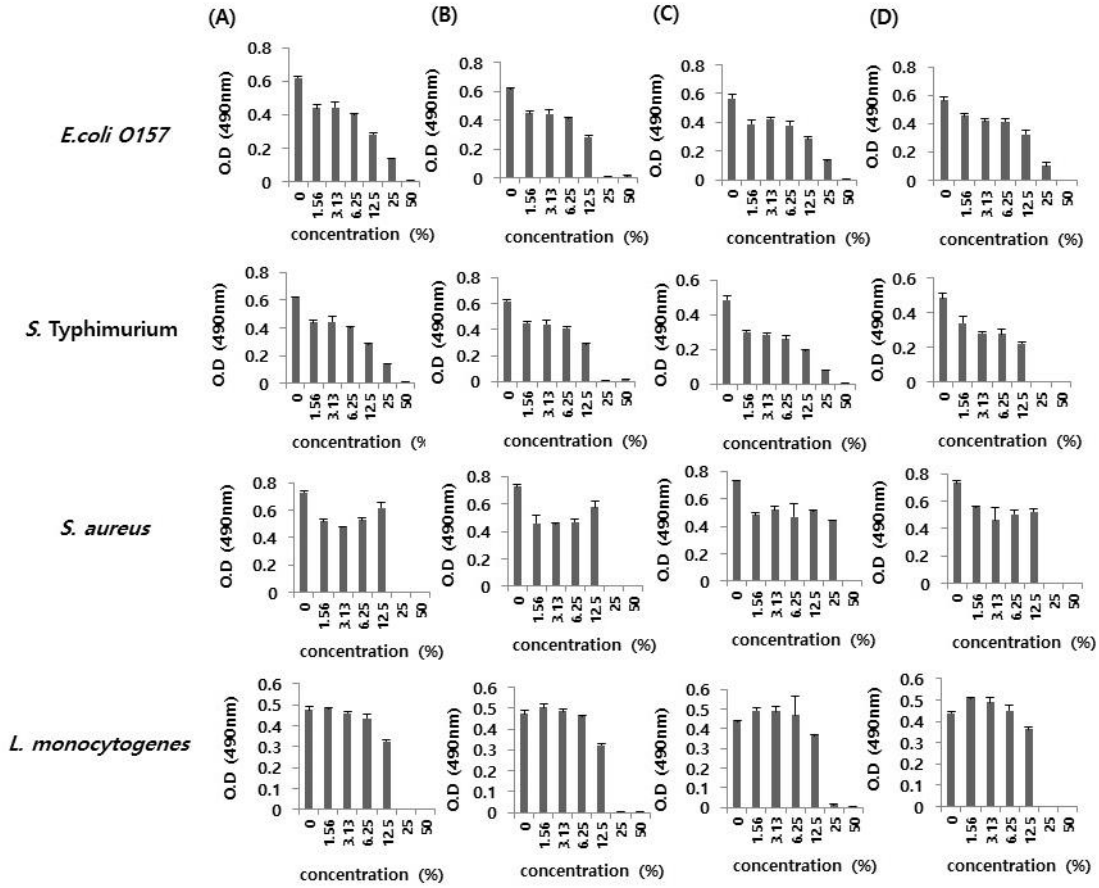


Fig. 3. Antioxidant property, total phenol, and total flavonoid contents of Hovenia monofloral honey. The DPPH radical scavenger activity (A) and ABTS radical scavenger activity (B) of Hovenia monofloral honey were estimated and compared with those of Acacia honey. The contents of the total phenol (C) and total flavonoid (D) of Hovenia monofloral honey were measured according to the method described in the Material and Methods section. Different letters indicate the significant differences between the groups ($p < 0.05$).



341

342 **Fig. 4. Hovenia monofloral honey inhibited the growth of foodborne bacteria.** The growth
343 of gram positive (*Staphylococcus aureus*: A, and *Listria monocytogenes Escherichia coli*: B)
344 and gram negative (*E. coli* O157:H7: C, and *Salmonella* Typhimurium*:* D) bacteria were
345 measured with and without Hovenia monofloral honey to determine the minimum inhibitory
346 concentration (MIC). The optimum density (OD) of each bacteria type was measured using a
347 UV spectrometer at 490 nm.

348