International Journal of Secondary Metabolite
2024, Vol. 11, No. 2, 191–199
https://doi.org/10.21448/ijsm.1353669
journal homepage: https://dergipark.org.tr/en/pub/ijsm
Research Article

In vitro antimicrobial and antioxidant activities of *Sambucus williamsii* and *Sambucus pendula*

Kyoung-Sun Seo[1], **Kyeong Won Yun**[2*]

[1]Jangheung Research Institute for Mushroom Industry, Jangheung 59338, Republic of Korea
[2]Department of Oriental Medicine Resources, Sunchon National University, Suncheon 57922, Republic of Korea

ARTICLE HISTORY
Received: Sep. 01, 2023
Accepted: Jan. 29, 2024

KEYWORDS
Sambucus williamsii,
Sambucus pendula,
Antimicrobial activity,
Antioxidant activity.

Abstract: The present study aimed to compare the *in vitro* antimicrobial and antioxidant activities of *Sambucus williamsii* and *Sambucus pendula*. The antimicrobial activity of the two plants was evaluated using the disc diffusion and minimal inhibition concentration (MIC) method against three Gram-positive bacteria (*Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 9327 and *Listeria monocytogenes* ATCC 15313), four Gram-negative bacteria (*Escherichia coli* ATCC 15489, *Psendomonas aeruginosa* ATCC 27853, *Pseudomonas fluorescens* KCCM 41443 and *Salmonella typhimurium* KCCM 11862) and one yeast (*Saccharomyces cerevisiae* IFO 1950). The tested ether and ethyl acetate fractions of ethanol extract from *Sambucus williamsii* showed significant antimicrobial activity against *Bacillus cereus* and *Pseudomonas fluorescens*; conversely, the ether fraction of *Sambucus pendula* leaf showed no clear zone formation against any tested bacteria. In general, the fractions of the two *Sambucus* species exhibited a lower MIC against tested Gram-negative bacteria than the tested Gram-positive bacteria. The antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay. The results showed that the half inhibitory concentration (IC_{50}) for the ethanol extract of *Sambucus williamsii* was the lower value, which means the greater antioxidant activity. On the other hand, the IC_{50} value of the hot water extract of *Sambucus pendula* was the lower value. The richness of the total polyphenol contents of the two *Sambucus* implies their potential as raw material sources for the pharmaceutical and cosmeceutical industries.

1. INTRODUCTION

Medicinal plants have been used for treating human diseases for thousands of years because they contain diverse organic compounds such as alkaloids, flavonoids, terpenoids, saponins, and phenolic compounds, which can exert physiological effect in the human body. A number of such compounds isolated from plants can be used to develop drugs to inhibit the growth of bacterial pathogens and quench reactive oxygen species (ROS) with low toxicity (Akhtar *et al*., 2018).

*CONTACT: Kyeong Won Yun ykw@scnu.ac.kr Department of Oriental Medicine Resources, Sunchon National University, Suncheon 57922, Republic of Korea

e-ISSN: 2148-6905

The genus *Sambucus* L. (elderberry) belongs to the Viburnaceae family. Plants of the genus *Sambucus* are widely distributed, perennial woody shrubs. *Sambucus* consists of 5-30 species and 6-11 subspecies, depending on the taxonomic system. Five of these species are naturally found in Korea (Lee, 1980; Młynarczyk *et al*., 2018). The most common species, *Sambucus nigra* L., does not occurr in Korea. Previous *in vitro* studies have indicated that *Sambucus* berry extracts possess anticarcinogenic, immune-stimulating, antibacterial, and anti-inflammatory properties (Dulf *et al*., 2015). The stem of *Sambucus williamsii* is an important folk medicine used for wound-healing and treating bone fractures. This plant is naturally in the valleys or forest edges of Korea. The leaf and stem extracts of *Sambucus williamsii* exhibit a higher antioxidant activity than butylated hydroxytolune, a positive control, mainly owing to its total polyphenol content (Chae & Cho, 2012). *Sambucus pendula* is a woody shrub that only grows on Ulleung-do Island of Korea. However, this plant has not yet been used for medicinal purposes in Korea.

The present research aimed to study the possible antimicrobial and antioxidant activities of *Sambucus williamsii* and *Sambucus pendula*. The activities of the extracts from the two plants are interest for their potential use in food and pharmaceutical industries.

2. MATERIAL and METHODS

2.1. Plant Material

The leaf and stem of *Sambucus williamsii* and *Sambucus pendula* was collected from Mt. Baegunsan in Gwangyang-si, Jellanam-do Province, and Ulleung-do Island, Gyeongsangbuk-do, Korea, respectively, in July 2020. The two provenances were air-dried at room temperature for 2 weeks. The air-dried sample was pulverized using an electric mill. The plants were authenticated by one of the authors, Prof. K.W. Yun and voucher specimens were deposited in the Herbarium of Sunchon National University (SCNUH 20200023 and 20200024), Korea.

2.2. Antimicrobial Activity

2.2.1. *Test bacteria*

The tested bacteria included three gram-positive bacteria (*Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 9327, and *Listeria monocytogenes* ATCC 15313), four gram-negative bacteria (*Escherichia coli* ATCC 15489, *Psendomonas aeruginosa* ATCC 27853, *Pseudomonas fluorescens* KCCM 41443, and *Salmonella typhimurium* KCCM 11862), and one yeast strain (*Saccharomyces cerevisiae* IFO 1950). These strains were obtained from American Type Culture Collection and Korean Culture Center of Microorganisms.

2.2.2. *Preparation of extract*

Air-dried and powdered plant materials (100 g each) were soaked in 1,000 mL of ethanol at room temperature for 24 h and then filtered through Whatman No.2 paper. The crude ethanol extract was partitioned with 500 mL hexane, and the top layer was concentrated (comprising the hexane fraction). The remaining layer was successively fractionated with 500 mL of diethyl ether and ethyl acetate (forming the ether and ethyl acetate fractions, respectively). The remaining residue was the aqueousfraction. Each fraction was concentrated with a rotary evaporator (30 °C) to 30 mL and stored at 5 °C until tested.

2.2.3. *Determination of antimicrobial activity*

Each bacterial strain was grown in a nutrient broth at 30 °C for 18-24 h prior to testing and subcultured three times for another 18-24 h. The turbidity of bacterial cell suspensions was brought to 0.3 optimal density at 660 nm by adding sterile broth and was then used for the tests. We poured 0.1 mL of the bacterial cell suspensions uniformly onto nutrient broth agar plates. The paper disks containing the extracts were carefully placed on the seeded Petri dishes. The diameters of the resulting inhibition zones were measured in mm after the cultures were incubated at 38 °C for 24 h. At the end of the incubation period the antimicrobial activity was

evaluated by measuring the inhibition zone. The minimal inhibition concentration (MIC) was determined as the lowest concentration that produced an inhibition zone. MIC was measured for the ether and ethyl acetate fractions.

2.3. Antioxidant Activity

2.3.1. *Preparation of extract*

The powdered leaf and stem (100 g) was macerated in 1000 mL of ethanol and hot water for 6 h, respectively. The percolates were then filtered (ADVANTEC No.2 filter paper). The extracts were then concentrated *in vacuo* to 100 mL at 30°C and tested for the DPPH and ABTS radical scavenging activities.

2.3.2. *DPPH radical scavenging activity*

The DPPH radical scavenging activity of the extracts was evaluated using a modified version of the method described by Blois (Blois, 1958). Briefly, 160 µL of each extracts at various concentrations (100 µM as the final concentration) were added to 40 µL of DPPH solution (1.5×10^{-4} M). The solutions were gently mixed and allowed to stand at room temperature for 30 min, after which the optical density was measured at 520 nm using a microplate spectrophotometer (EL800; Bioteck, Vinooski, VT, USA). The DPPH radical scavenging activity of each extract was expressed in terms of IC_{50} values (the concentration required to inhibit DPPH radical formation by 50%). L-Ascorbic acid was used as a positive control.

2.3.3. *ABTS radical scavenging activity*

The experiment was performed using a modified ABTS decolouration assay (Re *et al*., 1999). ABTS radical cation was produced by reacting the ABTS stock solution (7 mM in water) and 2.45 mM potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing the mixture to react at room temperature in the dark for 12 h. The solution was then diluted with ethanol to an absorbance of 0.70 ± (0.02) at 734 nm. The diluted ABTS (2.0 mL) was added to 50 µL of each extract and then the absorbance was measured on a microplate spectrophotometer reader (EL800; Bioteck, Vinooski, VT, USA) at 734 nm (Jaitak *et al*., 2010). The ABTS radical scavenging activity of each extract was expressed in terms of IC_{50} values (the concentration required to inhibit ABTS radical formation by 50%). L-Ascorbic acid was used as a positive control.

2.3.4. *Determination of total polyphenol content*

The total polyphenol content was determined using a modified version of the Folin-Denis method (Velioglu *et al*., 1998). Each 25 µL sample extract (1mg/mL) was mixed with 500 µL of Folin-Denis' reagent (diluted 10-fold with distilled water) was added. The mixtures were allowed to stand at room temperature for 5 min and then centrifuged at 1200 rpm for 10 min, following which the supernatant was collected. The clear supernatant (0.1 mL) of the samples was mixed with 0.75 mL of Folin-Denis' reagent. After 5 min, 500 µL of sodium bicarbonate (7.5 % in distilled water) was added and the solution was allowed to stand at 30 °C in darkness. Absorbance was measured at 765 nm using a microplate spectrophotometer (EL800; Bioteck). A standard curve prepared from gallic acid (100-1000 µg/mL) was used for quantification and the total polyphenol content was expressed as mg (of gallic acid)/g (dry weight).

2.3.5. *Determination of total flavonoid content*

The total flavonoid content was determined according to the method described by Moreno *et al*. (2000), with slight modifications. Each sample fraction (10 µL;1 mg/mL) was diluted with 80% aqueous ethanol (90 µL). An aliquot of 0.5 mL was added to test tube containing 2 µL of 10% aluminum nitrate, 2 µL of 1 M aqueous potassium acetate and 86 µL of 80% ethanol and the solution was allowed to stand at room temperature for 40 min. The absorbance was then measured at 415 nm using a UV-visible spectrophotometer (HP-8453; Agilent Technologies,

Santa Clara, CA, USA). A standard curve prepared from quercetin (100-1000 µg/mL) was used for quantification and the total flavonoid content was expressed as mg quercetin/g dry weight.

2.4. Statistical Analysis

Data with triplicate are analyzed using the SPSS software (version 24.0; IBM Corp., Armonk, NY, USA) and expressed as the mean ± SD. The statistical significance of the differences between means was determined using one-way analysis of variance. The level of significance was set at $p<0.05$.

3. RESULTS and DISCUSSION

3.1. Antimicrobial Activity

The antimicrobial activity of the ether and ethyl acetate fractions of the *Sambucus williamsii* extract was evaluated according to their MIC values against three gram-positive and four gram-negative bacteria and one yeast (Table 1). *Pseudomonas fluorescens* and *Escherichia coli* showed higher sensitivity and resistance to the two tested fractions of *Sambucus williamsii*. *Listeria monocytogenes*, a gram-positive bacterium, exhibited lower MIC value to the tested fractions. All the tested fractions of *Sambucus williamsii* affected *Bacillus cereus* and *Pseudomonas fluorescens* (MIC, 0.25-1.5 mg/mL). No inhibition against *Escherichia coli* was observed with any of the tested fractions of *Sambucus pendula* (Table 2). Stronger growth inhibitory effects on *Pseudomonas fluorescens* and *Salmonella typhimurium* were observed with the two fractions of the stem extract. The ethyl acetate fraction exhibited a higher antimicrobial activity than the ether fraction. In general, antimicrobial activity is associated with the outer layers of gram-positive and gram-negative bacteria. For gram-positive bacteria, antibacterial agents can easily destroy bacterial cell walls. Contrastingly, the outer membrane of gram-negative bacteria provides a hydrophilic surface to block external hydrophobic substances, resulting in stronger resistance to antibacterial compounds (Tian *et al*., 2018). In our experiments, both higher sensitivity and resistance to the tested fractions of the two plants were observed for the tested gram-negative bacteria. MIC against *Saccharomyces cerevisiae* was not detected for any tested fractions from the two plants. The antimicrobial activity of plant extracts forms the basis for many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies (Hammer *et al*., 1999).

Table 1. MIC of the two fractions of the ethanol extract of leaf and stem from *Sambucus williamsii* against tested microorganisms.

MIC (mg/mL)	Samples			
	Ether fraction		Ethyl acetate fraction	
	Leaf	Stem	Leaf	Stem
Gram(+) bacteria				
Bacillus cereus	1.5	0.25	1.0	0.5
Bacillus subtilis	-	1.5	-	-
Listeria monocytogenes	-	0.25	0.25	0.1
Gram(-) bacteria				
Escherichia coli	1.0	-	-	-
Psendomonas aeruginosa	-	-	1.0	0.5
Psendomonas fluorecens	1.0	1.5	0.5	0.25
Salmonella typhimurium	1.5	-	0.25	0.25
Yeast				
Saccharomyces cerevisiae	-	-	-	-

- : No clear zone was formed.

Table 2. MIC of the two fractions of ethanol extract of leaf and stem from *Sambucus pendula* against tested microorganisms.

MIC (mg/mL)	Samples			
	Ether fraction		Ethyl acetate fraction	
	Leaf	Stem	Leaf	Stem
Gram(+) bacteria				
Bacillus cereus	-	0.25	1.5	0.25
Bacillus subtilis	-	-	-	-
Listeria monocytogenes	-	0.1	-	0.1
Gram(-) bacteria				
Escherichia coli	-	-	-	-
Psendomonas aeruginosa	-	0.25	1.5	0.25
Psendomonas fluorecens	-	0.1	1.5	0.1
Salmonella typhimurium	-	0.1	1.5	0.1
Yeast				
Saccharomyces cerevisiae	-	-	-	-

- : No clear zone was formed.

3.2. Antioxidant Activity

The antioxidant activity of extracts is related to the compounds capable of protecting a biological system against the potentially harmful effects of oxidative processes (Fernandez-Agullo *et al*., 2013). The results are expressed as IC_{50}, which defined as concentration of plant extract with 50% DPPH and ABTS free radical scavenging potential (Akhtar *et al*., 2018). The lower the IC_{50} value, the higher the antioxidant activity. Free radicals are highly reactive and toxic substances that cause several health problems including cancer, diabetes, aging, cardiovascular disorders and liver cirrhosis (Khuda *et al*., 2021). The DPPH free radical scavenging activities of the extracts of the two *Sambucus* are shown in Table 3. The IC_{50} values of the leaf ethanol extracts from the two *Sambucus* were lower than those of ascorbic acid, the positive control. This result was in agreement with that reported in a previous study on the DPPH scavenging activity of *Sambucus williamsii* (Chae & Cho, 2012). In particular, DPPH scavenging activity was the highest in the leaf ethanol extract of *Sambucus williamsii* (IC_{50}, 0.18 ± 0.07 mg/mL).

Table 3. DPPH free radical scavenging activity of solvent extracts of leaf and stem from *Sambucus williamsii* and *Sambucus pendula*.

Samples			DPPH free radical scavenging activity (IC_{50}, mg/mL)
Sambucus williamsii	Leaf	Ethanol	0.18 ± 0.07
		Hot water	1.60 ± 0.11
	Stem	Ethanol	0.23 ± 0.05
		Hot water	1.16 ± 0.09
Sambucus pendula	Leaf	Ethanol	0.21 ± 0.06
		Hot water	1.02 ± 0.07
	Stem	Ethanol	2.25 ± 0.09
		Hot water	1.05 ± 0.05
Ascorbic acid			0.27 ± 0.01

Values are expressed as mean±SD (n=3).

Table 4 shows ABTS radical scavenging activities of *Sambucus williamsii* and *Sambucus pendula* extracts. In general, the IC_{50} values of hot water extracts of the two plants were lower than those of ethanol extracts; in particular, the ABTS scavenging activity was found to be highest in the stem ethanol extract of *Sambucus williamsii* (IC_{50}, 0.71 ± 0.01 mg/mL).

Rungruang *et al.* (2021) confirmed that the phenolic compound is the important molecule that can reduce ABTS via hydrogen atom transfer or electron donation. The IC_{50} values of ABTS in all the tested extracts displayed a different pattern of those of DPPH. The DPPH scavenging activities of all the tested extracts were better than those for the ABTS. It was shown that methanolic extracts of *Sambucus ebulus* had a better phenolic profile and this was reflected in their antioxidant activity (Barak *et al*., 2020).

Table 4. ABTS radical scavenging activity of solvent extracts of leaf and stem from *Sambucus williamsii* and *Sambucus pendula*.

Samples			ABTS radical scavenging activity (IC_{50}, mg/mL)
Sambucus williamsii	Leaf	Ethanol	2.27 ± 0.08
		Hot water	1.63 ± 0.04
	Stem	Ethanol	0.71 ± 0.01
		Hot water	1.23 ± 0.13
Sambucus pendula	Leaf	Ethanol	2.11 ± 0.04
		Hot water	1.08 ± 0.07
	Stem	Ethanol	6.24 ±0.12
		Hot water	1.99 ± 0.02
Ascorbic acid			0.25 ± 0.01

Values are expressed as mean±SD (n=3).

Plant are known to have antimicrobial and antioxidant activities owing to the presence of polyphenols and flavonoids (Kumar *et al*., 2018). Our results showed that the total polyphenol content (TPC), expressed as Gallic acid equivalents (GAE) per g dry weight (DW) of a sample, ranged from 681.6 ± 10.4 to 1,031.0 ± 11.5 mg Gallate/g DW in the ethanol extract of the two *Sambucus* and from 925.3 ± 19.6 to 1596.9 ± 24.7 mg Gallate/g DW in the hot water extract. The highest DPPH radical scavenging activity of the leaf ethanol and hot water extracts of *Sambucus williamsii* is associated with high TPC. The stem ethanol extract of *Sambucus pendula*, which had the lowest TPC, exhibited the weakest DPPH and ABTS scavenging activities (Table 5). Milena *et al.* (2019) showed that the 50% ethanol extract of *Sambucus nigra* had the highest content of bioactive compounds and exhibited very strong antioxidant activity in all applied assays.

Table 5. Total polyphenol content of solvent extracts of leaf and stem from *Sambucus williamsii* and *Sambucus pendula*.

Samples			Total polyphenol content (mg Gallate/g DW)
Sambucus williamsii	Leaf	Ethanol	1031.0±11.5
		Hot water	1596.9±24.7
	Stem	Ethanol	833.5±12.1
		Hot water	1114.0±24.2
Sambucus pendula	Leaf	Ethanol	904.5±18.5
		Hot water	1314.0±56.2
	Stem	Ethanol	681.6±10.4
		Hot water	925.3±19.6

Values are expressed as mean±SD (n=3).

Table 6. The total flavonoids content of solvent extracts of leaf and stem from *Sambucus williamsii* and *Sambucus pendula*.

Samples			Total flavonoid content (mg Quercetin/g DW)
Sambucus williamsii	Leaf	Ethanol	388.7±5.8
		Hot water	388.7±90.2
	Stem	Ethanol	42.0±0.0
		Hot water	42.0±5.1
Sambucus pendula	Leaf	Ethanol	902.0±10.0
		Hot water	902.0±15.3
	Stem	Ethanol	22.0±0.0
		Hot water	22.0±5.8

Values are expressed as mean±SD (n=3).

Some well-known compounds, such as flavonoids, have many important biological activities, including the ability to inhibit enzymes and have antioxidant, anti-inflammatory, antitumor, antithrombogenic, antiosteoporotic, and antimicrobial activities (Akhtar *et al*., 2018; Ding *et al*., 2011). Total flavonoid content (TFC) was calculated as quercetin equivalents ($y=0.0002x + 0.0458$, $R^2=0.9942$). The ethanol and hot leaf water extracts of *Sambucus pendula* showed the highest TFC, with 902.0 mg Quercetin/g DW. In contrast, the stem ethanol and hot water extracts of *Sambucus pendula* contained 22.0 mg quercetin/g DW, showing the lowest TFC (Table 6). It has been reported that flavonoid compounds have a wide range of chemical and biological activities, and variations in their phytochemical contents depend on the season, geography, nutrient content of the environment, light density, photoperiod, and temperature (Omar *et al*., 2018).

4. CONCLUSION

This is the first study to compare the antimicrobial and antioxidant activities of *Sambucus williamsii*, and *Sambucus pendula* extracts. The ethyl acetate fractions of the ethanol extracts from the two species showed more potent antibacterial activity than the ether fractions. Our results also showed that ethanol extracts of *Sambucus williamsii* exhibited remarkable antioxidant activity, as determined by DPPH and ABTS radical scavenging assays. Total phenolic and flavonoid content showed no clear association with antioxidant activity. The results obtained will be useful for further research, such as the identification of specific compounds responsible for the antibacterial or antioxidant activities. The extracts from the two *Sambucus* belonging to the Viburnaceae family have potential applications in the pharmaceutical and cosmeceutical industries.

Acknowledgments

This work was supported by a Research promotion program of SCNU.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Kyeong Won Yun: Conceptualization, methodology, and plant species identification. **Kyeong Won Yun** and **Kyoung-Sun Seo**: supervision, extraction, antimicrobial and antioxidant bioassays, data analysis, writing, original draft preparation, and editing.

Orcid

Kyoung-Sun Seo https://orcid.org/0000-0003-2397-5305
Kyeong Won Yun https://orcid.org/0000-0002-9167-8348

REFERENCES

Akhtar, N., Ihsan-ul-Haq, & Mirza, B. (2018). Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species *Arabian Journal of Chemistry, 11*(8), 1223-1235. https://doi.org/10.1016/j.arabjc.2015.01.013

Barak, T.H., Celep, E., İnan, Y., & Yeşilada, E. (2020) *In vitro* human digestion simulation of the bioavailability and antioxidant activity of phenolics from *Sambucus ebulus* L. fruit extracts. *Food Bioscience*, *37*, 100711. https://doi.org/10.1016/j.fbio.2020.100711

Blois, M.S. (1958). Antioxidant determination by the use of a stable free radical. *Nature*, 26, 1199-1202.

Chae, J.W., & Cho, Y.J. (2012). Antioxidant activity of extracts from *Sambucus williamsii* var. *coreana*. *Korean Journal of Plant Resources*, *25*(4), 363-371.

Ding, H.-Y., Chou, T.-H., Lin, R.-J., Chan, L.-P., Wang, G.-H., & Liang, C.-H. (2011). Antioxidant and antimelanogenic behaviors of *Paeonia suffruticosa*. *Plant Foods for Human Nutrition, 66*, 275–284. https://doi.org/10.1007/s11130-011-0235-3.

Dulf, F.V., Vodnar, D.C., Dulf, E.-H., & Toşa, M.I. (2015). Total Phenolic Contents, Antioxidant Activities, and Lipid Fractions from Berry Pomaces Obtained by Solid-State Fermentation of Two *Sambucus* Species with *Aspergillus niger*. *Journal of Agricultural and Food Chemistry, 63*(13), 3489-3500. https://doi.org/10.1021/acs.jafc.5b00520

Hammer, K.A., Carson, C.F., & Riley, T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology*, *86*, 985-990. https://doi.org/10.1046/j.1365-2672.1999.00780.x

Fernández-Agulló, E., Pereira, M., Freire, P., Valentao, P., Andrade, J., González-Álvarez, J., & Pereira, J.A. (2013). Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts. *Industrial Crops and Products*, *42*, 126–132. https://doi.org/10.1016/j.indcrop.2012.05.021

Hammer, K.A., Carson, C.F., & Riley, T.V. (1999) Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology*, *86*(6), 985-990. https://doi.org/10.1046/j.1365-2672.1999.00780.x

Jaitak, V., Sharma, K., Kalia, K., Kumar, N., Singh, H.P., Kaul, V., & Singh, B., (2010). Antioxidant activity of *Potentilla fulgens*: An alpine plant of western Himalaya. *Journal of Food Composition and Analysis*, *23*, 142-147. https://doi.org/10.1016/j.jfca.2009.02.013

Khuda, F., Haq, Z.U., Ilahi, I., Ullah, R., Khan, A., Fouad, H., Khalil, A.A.K., Ullah, Z., Sahibzada, M.U.K., Shah, Y., Abbas, M., Iftikhar, T., & Batiha, G.E.-S. (2021). Synthesis of gold nanoparticles using *Sambucus wightiana* extract and investigation of its antimicrobial, anti-inflammatory, antioxidant and analgesic activities. *Arabian Journal of Chemistry*, *14*(10), 103343. https://doi.org/10.1016/j.arabjc.2021.103343

Kumar, V., Sharma, N., Sourirajan, A., Khosla, P.K., & Dev, K., (2018). Comparative evaluation of antimicrobial and antioxidant potential of ethanolic extract and its fractions of bark and leaves of *Terminalia arjuna* from north-western Himalayas, India. *Journal of Traditional Complementary Medicine, 8*, 100-106. https://doi.org/10.1016/j.jtcme.2017.04.002

Lee, T.B. (1980). *Illustrated Flora of Korea*. HyangMoonSa, Seoul, Korea. pp 702-703.

Milena, V., Tatjana, M., Gökhan, Z., Ivana, B., Aleksandra, C., Mohammad, M.F., & Marija, R., (2019). Advantages of contemporary extraction techniques for the extraction of bioactive constituents from black elderberry (Sambucus nigra L.) flowers. *Industrial Crops and Products*, *136*, 93-101. https://doi.org/10.1016/j.indcrop.2019.04.058

Młynarczyk, K., Walkowiak-Tomczak, D., & Łysiak, G.P. (2018). Bioactive properties of *Sambucus nigra* L. as a functional ingredient for food and pharmaceutical industry. *Journal of Functional Foods*, *40*, 377-390.Omar, H., Al-Judaibiand, A., & El-Gendy, A. (2018). Antimicrobial, antioxidant, anticancer activity and phytochemical analysis of the red alga, *Laurencia papillosa*. *International Journal of Pharmacology*, *14*, 572-583. https://doi.org/10.3923/ijp.2018.572.583

Moreno, M.I.N., Isla, M.I., Sampietro, A.R., Vattuone, M.A. (2000). Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. *Journal of Ethnopharmacology*, *71*, 109-114. https://doi.org/10.1016/S0378-8741(99)00189-0

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*, 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rungruang, R., Ratanathavorn, W., Boohuad, N., Selamassakul, O., Kaisangsri, N. (2021). Antioxidant and anti-aging enzyme activities of bioactive compounds isolated from selected Zingiberaceae plants. *Agriculture and Natural Resources*, *55*, 153-160. https://doi.org/10.34044/j.anres.2021.55.1.20

Tian, Y., Puganen, A., Alakomi, H.-L., Uusitupa, A., Saarela, M., & Yang, B. (2018). Antioxidative and antibacterial activities of aqueous ethanol extracts of berries, leaves, and branches of berry plants. *Food Research International*, *106*, 291-303. https://doi.org/10.1016/j.foodres.2017.12.071

Velioglu, Y.S., Mazza, G., Gao, L., & Oomah, B.D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, *46*, 4113-4117.