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연구논문

Chemical Composition of *Pinus koraiensis* Seed and Its Biological Activity

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Abstract: This study was to investigate the chemical composition and biological activities of *Pinus Koraiensis* seed. The oil, moisture, ash, crude protein, and carbohydrate contents of *P. Koraiensis* seed were 58.21, 7.84, 1.56, 14.26, and 18.13%, respectively. The ratios of essential amino acid and non-essential amino acid against total amino acids were 36.6 and 60.3%, respectively. The saponification value of seed oil was 166.8 mg KOH/g oil. Among various fatty acids, the linoleic acid content was the highest, 35.5%, which was approximately 72.6% of polyunsaturated fatty acid. The ethylacetate extract of *P. Koraiensis* seed had the highest DPPH radical scavenging activity (62.8%) at 7.0 mg/mL, followed by hexane extract, methanol extract, and hot water extract. The maximum nitrite scavenging activity was obtained 59.3% at pH 1.2. The total phenolic concentration of ethylacetate extract was 98.7 mg/g, approximately 4.8 folds higher than that of the hot water extract. The maximum inhibition activities of elastase using ethylacetate extract and collagenase using hexane extract were 58.8 and 40.7%, respectively. These results indicate that *P. koraiensis* seed extract could be applied to present the possibilities of industrial applications for the developments of cosmetics.

Keywords: *P. Koraiensis* seed, Antioxidant activity, Nitrite scavenging activity, Elastase, Cosmetics

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1. Introduction

Pinus koraiensis, commonly called Korean nut pine, is an evergreen tree species in Korea, China, and Japan. *P. koraiensis* seeds, nuts and cones are enriched with essential oil with antibacterial and fungal [1] and antiplatelet aggregation activities [2]. Recently, Kim et al. evaluated the antihyperlipidemic activities of the essential oil from the leaves of *P. koraiensis* SIEB (EOPK) that has been used as a folk remedy for heart disease. The reverse transcription polymerase chain reaction revealed that EOPK up-regulated low density lipoprotein receptor at the mRNA level as well as negatively suppressed the expression of sterol regulatory element-binding protein (SREBP)-1c, SREBP-2, 3-hydroxy-3-methylglutaryl-CoA reductase, fatty acid synthase and glycerol-3-phosphate acyltransferase involved in lipid metabolism in HepG2 cells [3]. Lee and Rhee investigated the physicochemical properties of rice starch suspensions affected by interactions with pine nut oil fractions. Oil fractions (crude pine nut oil, glycolipid, nonpolar lipid, gums, and degummed oil) from *P. koraiensis* were prepared by solvent extraction and addition of boiling water. The glycolipid fraction had the highest complexing index with rice amylose. Rice amylose solutions containing the glycolipid fraction exhibited greater pseudoplastic fluid behaviour, higher colour difference values, and lower absorption ratios (1.64-1.48) than did those containing other fractions. Hydrated oil fraction (gums) complexed with amylose only poorly, if at all, but amylose solutions containing them had rheological properties similar to those containing the glycolipid fraction. The addition of oil fractions increased the swelling power value compared to the control [4]. Also Ferramosca et al. reported the influence of a diet enriched with pine nut oil on the body weight gain and on the level of some lipid classes in the liver and in the plasma of mice [5]. Furthermore, they have also studied a possible modulation of the fatty

acid synthesis in the liver of the pine nut oil-fed animals. Nonetheless, there was no information on the antioxidant activity, nitrite scavenging activity, and the inhibition activity of elastase and collagenase of *P. koraiensis* seed extract so far.

Thus, in the present study, firstly, to investigate chemical composition of *P. Koraiensis* seed, proximate composition and amino acid of seed and fatty acid and physicochemical properties of the oil were investigated. Secondly, DPPH radical scavenging activity and reducing power, nitrite scavenging activity, and the inhibitory activities of elastase and collagenase using various extracts of *P. Koraiensis* seed were investigated.

2. Material and Methods

2.1. Sample preparation

P. koraiensis seed grown in Hamra mountain at Jeonbuk, South Korea in 2011 were obtained. The dried seed was grinded by pulveriser and extracted with proportional hot water, methanol, ethylacetate, and hexane. The extract was centrifuged at 3,000 rpm for 5 min and the residue was extracted once again with new solvent under the same conditions. The supernatant was evaporated and the extract powder was obtained by freeze drying.

2.2. Proximate composition and physicochemical properties

Proximate composition of seed and physicochemical properties of the oil were determined by the official and tentative methods of the American oil chemists' society [6]. Especially, the protein concentration was determined as percent nitrogen × 6.25 using the micro Kjeldahl technique.

2.3. Amino acid composition

Sample (0.5 g) was added to 3 mL of HCl (6 N) and the hydrolysis was carried out for 24 hr at 121°C. The mixture was then pressure-concentrated and 10 mL of sodium phosphate buffer (pH 7.0) was added. An aliquot of 1 mL of solution was filtered by membrane filter (0.2 mM) and then analyzing by automatic amino acid analyzer (Biochrom 20, England).

2.4. Fatty acid composition

Sample (500 mg) was mixed with 2 mL of toluene and 3 mL of methanolic hydrogen chloride, and incubated at 70°C for 2 hr. Then, 5 mL of potassium carbonate (6%) and 2 mL of toluene were added to the same tube to hydrolyze the samples; the samples were then centrifuged at 1,500 rpm for 2 min. After transferring the upper layer to new tube, 1 g of anhydrous sodium sulphate and 1 g of activated charcoal were added to the samples, and fatty acid methyl esters were analyzed by GC (Varian CP-3800, 1200L Varian inc., Palo alto, CA, USA).

2.5. DPPH radical scavenging activity

The DPPH (1, 1-diphenyl-2-picry-hydrazil) radical scavenging activity was determined by spectrophotometer. A sample (1 mg/mL) was added to 1 mL of DPPH (10 mM) in methanol. The mixture was shaken and maintained at room temperature for 10 min. The absorbance was measured at 517 nm.

2.6. Reducing power

Sample (1 mg) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium hexacyanoferrate (1%). The mixture was placed in a 50°C water bath for 30 min. A 2.5 mL of trichloroacetic acid (10%) was added, and the mixture was centrifuged for 10 min at 3,000 rpm. The supernatant was recovered, and 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%) were added. The absorbance of the sample was measured at 700 nm.

2.7. Nitrite scavenging activity

An aliquot of 1 mL of $NaNO_2$ (1 mM) was added to 1 mL of the sample, and the pH of the resulting mixtures was adjusted to 1.2, 3.0, 5.0 and 7.0. The final sample volume was adjusted to 10 mL. The sample was allowed to react at 30°C for 1 hr, after which 1 mL of sample was obtained, mixed thoroughly with 5 mL of acetic acid (2%) and 0.4 mL of Griess reagent, and kept at room temperature for 15 min. A blank was prepared by adding 0.4 mL of distilled water instead of the Griess reagent. The nitrite scavenging activity was determined at 520 nm.

2.8. Total phenolic and flavonoid content

Total phenolic content were determined with Folin-Ciocalteu reagent with gallic acid as a standard. Sample (1 mL) was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated Na_2CO_3 (35%) was added to the mixture, which was brought to a volume of 10 mL by the addition of distilled water. The reaction was maintained in the dark for 90 min, and its absorbance was measured at 725 nm relative to a blank. The total flavonoid content was determined in terms of catechin equivalents. Catechin standard (1 mg) was dissolved in 1 mL of ethanol/water (3:7, v/v). A 500 mL of the sample was collected and mixed with 75 mL of sodium nitrite solution (5%), followed by thorough mixing, standing at room temperature for 5 min, adding 150 mL of aluminum chloride (10%), standing for an additional 5 min, adding 500 mL of sodium hydroxide (1 N), and measuring the absorbance at 510 nm.

2.9. Inhibitory activity of elastase

An aliquot of 20 mL of Human leukocyte elastase solution containing sodium acetate buffer solution (50 mM, pH 5.3) and 25 ~400 μg/mL of sample were reacted in 48-well plates and 200 mL of p-nitroanilide (400 mM) was added. After reaction at 37°C for 20 min, an aliquot of 120 mL of reaction was added into 96 well plates. The absorbance was measured at 410 nm using an ELISA reader. Elastase inhibition was calculated with the following formula:

$$\text{Elastase inhibition (\%)} = [1 - (\text{OD of sample}/\text{OD of control})] \times 100$$

2.10. Inhibitory activity of collagenase

An aliquot of 300 mL of collagen solution (0.25 mg/mL), 600 mL of sample, and 600 mL of collagenase solution (0.5 unit) were mixed with 1,500 mL of PBS (pH 6.0). After pre-incubation for 20 min in a dark room, the absorbance was measured at 280 nm and 200 nm with a fluorescence spectrophotometer (F-4500, Hitachi, Japan). Collagenase inhibition was calculated with the following formula:

$$\text{Collagenase inhibition (\%)} = [1 - (\text{OD of sample}/\text{OD of control})] \times 100.$$

3. Result and Discussion

3.1. Proximate composition of *P. Koraiensis* seed

To investigate the proximate composition of *P. Koraiensis* seed, moisture, oil, protein, ash, and carbohydrate contents were measured. The proximate compositions of *P. Koraiensis* seed are shown in Table 1. The oil content of *P. Koraiensis* seed was 58.21%, which was higher than those of cottonseed seed oil (19~24%), sesame oil (45~55%), corn oil (4~7%), linseed oil (35~42%), grape seed oil (13~17%), and poppyseed oil (44~50%) [7]. Moisture, ash, crude protein, and carbohydrate contents of *P. Koraiensis* seed were 7.84, 1.56, 14.26, and 18.13%, respectively. However, In the case of *P. Koraiensis* needles, oil content was 10.4%. Moisture, ash, crude protein, and carbohydrate contents were 10.6, 2.1, 6.8, and 70.1%, respectively [8].

Table 1. Proximate composition of *P. Koraiensis* seed

Proximate composition	Content (%)
Moisture	7.84
Ash	1.56
Crude protein	14.26
Crude oil	58.21
Carbohydrate	18.13

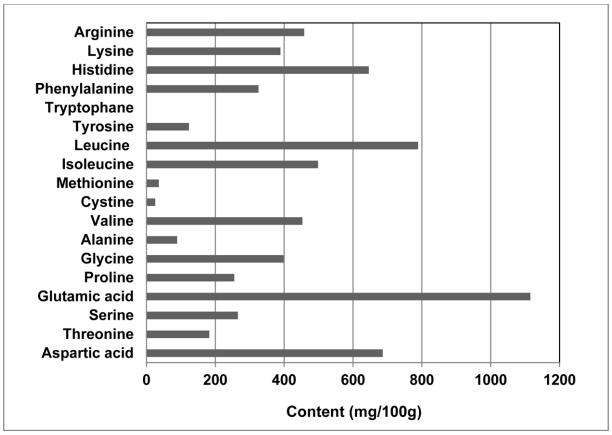

Fig. 1. Amino acid composition of *P. Koraiensis* seed.

3.2. Amino acid composition of *P. Koraiensis* seed

The composition and concentration of amino acid of *P. Koraiensis* seed were investigated and shown in Fig. 1. The most major components of essential amino acid showed leucine, isoleucine, and valine, respectively. On the other hand, in the case of non-essential amino acids, they were glutamic acid, aspartic acid, and histidine, respectively. The ratios of essential amino acid and non-essential amino acid against total amino acid were 36.6 and 60.3%, respectively. Total amino acid concentration was 6734.8 mg/100g. Of various essential amino acids, the concentrations of amino acid were order of leucine > isoleucine > valine > lysine > phenylalanine > threonine > methionine and their concentrations were 789.2, 498.3, 452.9, 389.2, 325.3, 182.6, and 35.6 mg/100g, respectively. In the case of tryptophane, it was not detectable in the seed. However, in the case of non-essential amino acids, the concentrations of amino acid were order of glutamic acid > aspartic acid > histidine > arginine > glycine > serine > proline > tyrosine > alanine > cystine and their concentrations were 1115.2, 686.2, 645.8, 458.2, 398.3, 265.7, 255.1, 123.2, 88.9, and 25.3 mg/100g, respectively. From the result of this study, it seems to be clear that *P. Koraiensis* seed has many amino acid components, and has the potential to be a resource for supplementation of essential amino acids.

3.3. Physicochemical properties of *P. Koraiensis* seed oil

The physicochemical properties of *P. Koraiensis* seed oil were examined by measuring the specific gravity, refractive index, acid value, peroxide value, iodine value, saponification value, and unsaponifiable matter. The results are shown in Table 2. The specific gravity of *P. Koraiensis* seed oil was 0.93. Refractive index (20°C) of *P. Koraiensis* seed oil was 1.56, which was higher than those of cottonseed (1.458~1.466), mustard seed (1.461~1.469), groundnut (1.460~1.465), almond kernel (1.462 ~1.465), kapok seed (1.460~1.466) oils, low-, and high-erucic

Table 2. Physicochemical properties of *P. Koraiensis* seed oil

Physicochemical parameter	
Specific gravity $[d]_{20}$	0.93
Refractive index (20°C)	1.56
Peroxide value (mEq/kg oil)	3.21
Iodine value (g I/g oil)	98.02
Saponification value (mg KOH/g oil)	166.8
Unsaponifiable matter (%)	0.23

Table 3. Fatty acid composition of *P. Koraiensis* seed oil

Fatty acid	Concentration (%)
Caprylic acid	0.26
Myristic acid	0.77
Palmitic acid	14.26
Heptadecanoic acid	0.35
Stearic acid	6.46
Arachidic acid	3.88
Lignoceric acid	0.04
Palmitoleic acid	1.13
Oleic acid	21.48
cis-11-Eicosenoic acid	0.57
Erucic acid	0.56
Nervonic acid	0.15
Linoleic acid	35.05
Linolenic acid	11.36
Eicosatrienoic acid	1.68

acid rapeseed (1.465~1.469), soybean (1.467~1.470), sunflower (1.467~1.469), safflower and grape seed (1.473~1.477) oils [9]. Peroxide and iodine values of *P. Koraiensis* seed oil were 3.21 mEq/kg oil and 98.02 g I/g oil, respectively. The saponification value was 166.8 mg KOH/g oil, which was lower than those of corn (187~195), cottonseed (189~198), olive (184~196), pumpkin (185~198), soybean (188~195) and rice bran (179~195) oils [9]. However, it was similar to that of baobab seed oil (162) and cassaia alaata oil (165). The unsaponifiable matter (0.23%) of *P. Koraiensis* seed oil were lower than those of cottonseed (0.5~1.5%), olive (0.7~2.5%), corn (0.5~2.8%) oils, cocoa butter (0.5%), coconut (0.5%), palm (kernel), (0.8%), tea seed (1.0%), groundnut (0.8%), safflower (1.5%), palm fruit (1.2%), high-erucic acid rapeseed (2.0%) and low erucic acid rapeseed oil (1.8%) [9].

3.4. Fatty acid and composition of *P. Koraiensis* seed oil

The fatty acid compositions of *P. Koraiensis* seed oil are shown in Table 3. The main fatty acids of *P. Koraiensis* seed oil were linoleic acid, oleic acid, palmitic acid, and linolenic acid which comprised approximately 82.15% of the total fatty acids. The unsaturated fatty acid can affect the physical properties of the membrane, such as fluidity and permeability [10]. Among various fatty acids, the linoleic acid content was the highest, 35.5%, which was approximately 72.6% of polyunsaturated fatty acid. However, linolenic acid (15.12%) was the major fatty acid, followed by palmitic acid (8.82%) in the *P. Koraiensis* neddle oil and oleic acid (4.45%). In the *P. Koraiensis* pollen oil, linoleic acid (24.30%) was the major fatty acid, followed by oleic acid (16.77%) and palmitic acid (15.49%) [11]. Among the monounsaturated fatty acids, the oleic acid content was the second highest, 21.48%, comprising approximately 89.9% of monounsaturated fatty acid content. The total saturated fatty acid is 27.82%, which makes it strongly resistant to oxidative rancidity. Among the saturated fatty acids, palmitic acid, stearic acid, and arachidic acid were the highest, 14.26, 6.46 and 3.88%, respectively, which was approximately 51.26, 23.2 and 13.95% of saturated fatty acid. The other saturated fatty acid concentrations were below 1.0%. The polyunsaturated fatty acid (PUFA)/saturated fatty acid (SFA) ratio is generally used to evaluate the nutritional value of oil.

3.5. Effect of *P. Koraiensis* seed extract on DPPH radical scavenging activity and reducing power

Free radical scavenging is very important due to the deleterious role of free radicals (FR) in biological systems. DPPH is a stable nitrogen-centered, lipophilic FR that is widely used in the evaluation of free radical scavenging activity as results can be obtained more quickly when compared with other methods. DPPH is usually used as a substrate to evaluate the activity of antioxidants [12]. To investigate the effect of the various extracts from *P. Koraiensis* seed on DPPH scavenging activity, 1, 3, 5, 7 and 9 mg/mL of each extract from *P. Koraiensis* seed were used. The DPPH radical scavenging activities are shown in Fig. 2. It was increased with concentrations of *P. Koraiensis* seed extract up to 7.0 mg/mL irrespective of the type of extract. The ethylacetate extract had the highest DPPH radical scavenging activity at 7.0 mg/mL, followed by hexane extract, methanol extract, and hot water extract. Especially, when the ethylacetate extract concentration was increased from 1.0 to 7.0 mg/

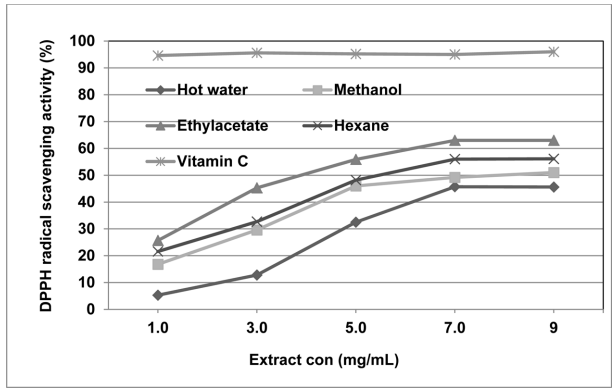

Fig. 2. Effect of *P. Koraiensis* seed extract on DPPH radical scavenging activity.

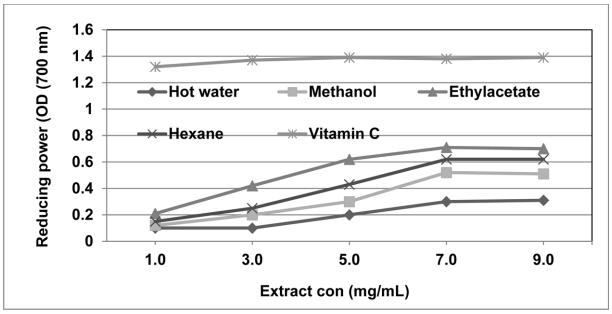

Fig. 3. Effect of *P. Koraiensis* seed extract on reducing power.

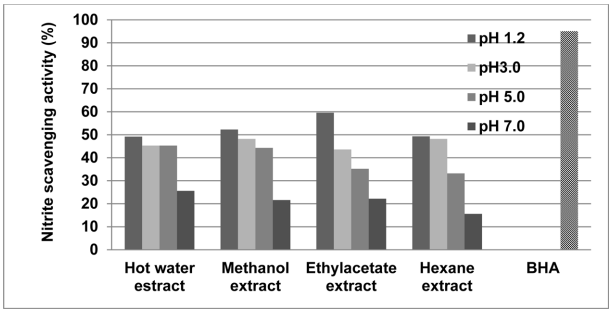

Fig. 4. Effect of *P. Koraiensis* seed extract on nitrite scavenging activity.

mL, DPPH radical scavenging activity increased from 25.7 to 62.8%. However, above 9.0 mg/mL of ethylacetate extract, no further increases were observed. In the case of ascorbic acid, the DPPH radical scavenging activity was 94.6% at 1.0 mg/mL. This finding indicates that active, water-insoluble components might exist in the seed extracts. Free radical scavenging is known to inhibit lipid oxidation, which otherwise can be deleterious to cells' components and functions [13].

The reducing power, which provides an estimate of the ability of a compound to reduce ferric iron (III) to ferrous iron (II), was determined by using a redox-linked colorimetric reaction. In addition, the reducing capacity of a compound may serve as a significant indicator of its potential for use as an antioxidant [14]. The results of *P. Koraiensis* seed extract on reducing power are shown in Fig. 4. When the hot water extract concentration increased from 1.0 to 7.0 mg/mL, the reducing power increased from 0.11 to 0.31 OD (700 nm). For methanol extract, it increased from 0.12 to 0.52 OD (700 nm). For hexane extract, it increased from 0.15 to 0.62 OD (700 nm). However, the reducing power increased from 0.21 to 0.71 OD (700 nm) when the ethylacetate extract concentration increased from 1.0 to 7.0 mg/mL. According to DPPH radical scavenging activity and reducing power, the reducing power of extracts from *P. Koraiensis* seed correlates with DPPH scavenging activity, indicating that reducing powers contribute to their antioxidant activities.

3.6. Effect of *P. Koraiensis* seed extract on nitrite scavenging activity

Nitrite reacts with amines in protein-rich foods, medicines, and residual pesticides to form nitrosamines and is present in large quantities in meat colors and both leaf and root vegetables. Nitrosamine is converted to diazoalkane, proteins, and intracellular components, which can increase the risks of cancer [15]. To investigate the effect of the various extracts from *P. Koraiensis* seed on nitrite scavenging activity, 9 mg/mL of *P. Koraiensis* seed extract was used. The results are shown in Fig. 4. The nitrite scavenging activities of the *P. Koraiensis* seed extracts were dependent on extracts pH. When pH of hexane extract decreased from 7.0 to 1.2, the nitrite scavenging activity increased from 15.6 to 49.1%. For methanol extract, it increased from 21.6 to 51.7%. However, when pH of ethylacetate extract decreased from 7.0 to 1.2, the nitrite scavenging activity was increased from 22.5 to 59.3%. The present results suggested that the ethylacetate extract of *P. Koraiensis* seed could be useful for preventing nitrosamine formation in foods.

3.7. Effect of *P. Koraiensis* seed extract on total polyphenol and flavonoid content

Phenolic compounds are commonly found in both edible and nonedible plants. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are of increasing interest to the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [16,17]. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers in a trend that is moving towards functional foods with specific health effects [17]. Thus, it is important to consider the effects of the various solvents used for extraction on the phenolic contents of the *P. Koraiensis* seed. Hot water, methanol, ethylacetate, and hexane were used to investigate the effect of solvents on the total concentrations of phenol and flavonoid. The results are shown in Fig. 5. The total phenolic concentrations of the *P. Koraiensis* seed were dependent on the solvent. The ethylacetate extract had the highest polyphenol content, followed by the extracts from ethanol, hexane, and hot water. Especially, when ethylacetate was used as extractant, the total phenolic concentration was 98.7 mg/g, approximately 4.8 folds higher than that of the hot water extract. For hexane and methanol as extractants, they were 72.1 and 61.4 mg/g, respectively. The ethylacetate extract also contained the highest total flavonoid content (25.1 mg/g), followed by hexane extract (17.9 mg/g), methanol extract (7.3 mg/g), and hot water extract (5.6 mg/g). These results indicate that the total phenol and flavonoid

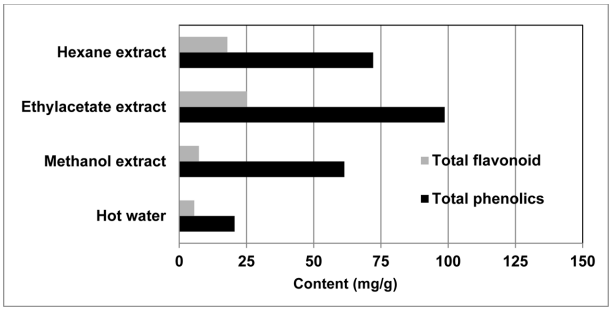

Fig. 5. Effect of *P. Koraiensis* seed extract on total polyphenol and flavonoid content.

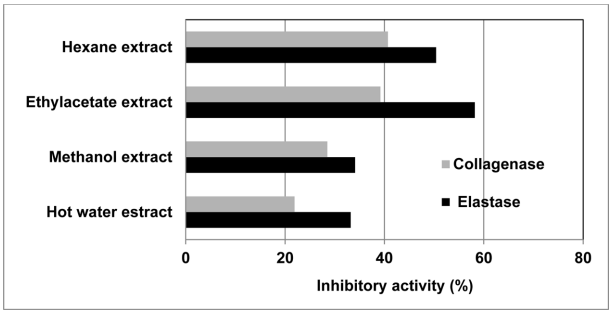

Fig. 6. Effect of *P. Koraiensis* seed extract on activity of elastase and collagenase.

concentrations of *P. Koraiensis* seed are strongly affected by extractants, with a similar pattern of efficacy by solvent to their antioxidants and radical scavenging activities.

3.8. Effect of *P. Koraiensis* seed extract on the inhibitory activity of elastase and collagenase

UV irradiation from sun produces free radicals and related reactive oxygen species (ROS) in human skin. These injure the DNA and extracellular matrix (ECM) in dermis of human skin. UV irradiation has been shown to stimulate fibroblast which secretes matrix metalloproteinases (MMPs) by cytokines. MMPs constitute more than 20 proteinase, and can degrade most components of ECM such as collagen, laminins, and elastins. Since collagen fibrils with elastin are responsible for the strength and resiliency of skin, their degradation causes wrinkles and skin aging [18]. To investigate the anti-wrinkle effect of *P. Koraiensis* seed, various extracts on the inhibition effects of elastase and collagenase were studied. The inhibition effects of elastase and collagenase are shown in Fig. 6. Among various extracts of *P. Koraiensis* seed, the ethylacetate extract had the highest inhibition effect of elastase, followed by hexane extract, methanol extract, and hot water extract. Especially, when ethylacetate extract (9.0 mg/mL) was used, the inhibition effect of elastase was 58.8%, approximately 1.8 folds higher than that of the methanol or hot water. For hexane extract, it was 50.3%. These results showed that the inhibition effect of elastase was strongly affected by the ethylacetate extract concentration of *P. Koraiensis* seed. In the case of inhibitory activity of collagenase of extracts of *P. Koraiensis* seed, the hexane extract had the highest inhibition activity of collagenase, followed by ethylacetate extract, methanol extract, and hot water extract. Especially, when hexane extract was used, the inhibition activity of collagenase was 40.7%, approximately 1.9 or 1.4 folds higher than that of the hot water or methanol. For ethylacetate extract, it was 39.2%. These results showed that the effects of collagenase were affected by the hexane and ethylacetate extract of *P. Koraiensis* seed.

4. Conclusion

Chemical composition of *P. koraiensis* seed and its biological activity were investigated. The most abundant component of essential amino acid and non-essential amino acid were leucine and glutamate, respectively. It seems to be clear that *P. Koraiensis* seed has many amino acid components, and has the potential to be a resource for supplementation of essential amino acids. Among fatty acids, *P. Koraiensis* seed oil had the highest linoleic acid, followed by oleic acid, palmitic acid, and linolenic acid. The reducing power increased from 0.21 to 0.71 OD (700 nm) when the ethylacetate extract concentration increased from 1.0 to 7.0 mg/mL. According to DPPH radical scavenging activity and reducing power, the reducing power of extracts from *P. Koraiensis* seed correlates with DPPH scavenging activity, indicating that reducing powers contribute to their antioxidant activities. The ethylacetate extract of *P. Koraiensis* seed gave the maximum the nitrite scavenging activity (59.3%). Among various extracts of *P. Koraiensis* seed, the ethylacetate extract had the highest inhibition effect of elastase, followed by hexane extract, methanol extract, and hot water extract. However, in the case of inhibitory activity of collagenase, the hexane extract had the highest inhibition activity, followed by ethylacetate extract, methanol extract, and hot water extract. Overall, these findings demonstrate that *P. Koraiensis* seed extract can be used as a a functional cosmetic agent.

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REFERENCES

1. Lee, J. H., H. Y. Yang, H. S. Lee, and S. K. Hong (2008) Chemical composition and antimicrobial activity of essential oil from cones of *Pinus koraiensis*. J. *Microbiol. Biotechnol.*18: 497-502.
2. Sugano, M., I. Ikeda, K. Wakamatsu, and T. Oka (1994) Influence of Korean pine (*Pinus koraiensis*)-seed oil containing cis-5,cis-9, cis-12-octadecatrienoic acid on polyunsaturated fatty acid metabolism, eicosanoid production and blood pressure of rats. *Br. J. Nutr*. 72: 775-783.
3. Kim, J. H., H. J. Lee, S. J. Jeong, M. H. Lee, and S. H. Kim (2012) Essential oil of *Pinus koraiensis* leaves exerts antihyperlipidemic effects via up-regulation of low-density lipoprotein receptor and inhibition of acyl-coenzyme A. *Phytother. Res.* 26: 1314-1319.
4. Lee, S. W. and C. R. Rhee (2003) Influence of pine nut (*Pinus koraiensis*) oil fractions on physicochemical properties of rice starch solutions. *Starch/Stärke.* 55: 87-93
5. Ferramosca1, A.., V. Savy1, A. W. C. Einerhand, and V. Zara1 (2008) *Pinus koraiensis* seed oil (PinnoThinTM) supplementation reduces body weight gain and lipid concentration in liver and plasma of mice. *J. Animal Feed Sci.* 17: 621-630.
6. A.O.C.S. (1990) *Official and tentative methods of american oil chemist's society*. pp. 37-48. 15th Ed., American Oil Chemists Society Press. Champaign. USA.
7. Fireston, D. (2006) *Physical and chemical characteristics of oil, fat, and waxes*. pp. 1-168. 2nd ed, American Oil Chemists Society Press, New York. USA.
8. Hwang, B. H., J. H. Cho, S. S. Ham, and H. Y. Kang (2000) Chemical analysis of pinus leaves. *J. Kor. Soc. Food Sci. Nutri*. 29: 6-9.
9. Rossell, J. B. (1991) Vegetable oil and fats. pp. 261-319. In: Rossell JB, Pritchard JLR (eds) *Analysis of oilseeds, fats and fatty foods*. Elsevier Applied Sciences, New York. USA.
10. Nasri, N., A. Khaldi, B. Fady, and S. Triki (2005) Fatty acids from seeds of *Pinus pinea* L. *Phytochem.* 66: 1729-1735.
11. Yoon, T. H. (1992) Fatty acid composition of total lipids from needles and pollen of Korean *Pinus densiflora* and *Pinus koraiensis*. *J. Kor. Oil Chem. Soci*. 9: 25-30.
12. Raes, M., C. Michiels, and J. Relale (1987) Comparative study of the enzymatic defense systems against oxygenderived free radicals: the key role of glutathione peroxidase. *Free Radical Biol. Med.* 3: 3-7.
13. Puttaraju. N. G, S. U. Venkateshaiah, S. M. Dharmesh, S. M. N. Urs, R. Somasundaram (2006) Antioxidant activity of indigenous edible mushrooms. *J. Agric. Food Chem*. 54: 9764-9769.
14. Barros, L., P. Baptista, and sabel C. F. R. Ferreira (2007) Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. *Food Chem. Toxi*. 45: 1731-1737.
15. Beckman, J. S. and W. H. Koppenol (1996) Nitroxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am. J. Physiol*. 217: 1421-1437.
16. Lee, H. J. and Y. W. Seo (2006) Antioxidant properties of erigeron annuus extract and its three phenolic constituents. *Biotechnol. Bioprocess Eng*. 11: 13-18.
17. Lo. I. J. (1997) The use of antioxidants in food, in: Free Radicals and Food Additives. p. 129-135. Taylor and Francis, London. UK.
18. Elsner, P. and H. I. Mailbach (2005) *Cosmeceuticals and active cosmetics*. pp. 234-239. 2*nd* Ed. Taylor & Francis, New York. USA.