Original Article

In Vitro Anti-oxidation, Anti-inflammatory and Anti-aging Activities of Oak Extract

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Abstract

Introduction: Intrinsic and extrinsic factors can influence skin aging. Intrinsic skin aging can occur due to the formation and accumulation of reactive oxygen species, furthermore UV radiation contributes up to 80% of extrinsic aging. Anti-oxidation bioactive components from natural sources have been recommended for skin aging prevention.

Objectives: We explored the anti-oxidation, anti-inflammatory and anti-aging activities of oak extract (freeze-dried) product.

Methods: Anti-oxidation activity was determined using superoxide dismutase (SOD) activity assay and radical scavenging activity assay (DPPH). Anti-inflammatory activity was determined by hyaluronidase activity inhibitory assay and hexosaminidase release inhibitory assay. Anti-aging activity was determined using inhibitory assay of human neutrophil elastase activity, inhibitory assay of matrix metalloproteinase-1 (MMP-1) and promoting assay of hyaluronic acid production.

Results: Anti-oxidation activity was observed for oak extract in both SOD activity assay and radical scavenging activity assay with IC_{50} of 2.27µg/mL and 3.25 µg/mL respectively. Anti-inflammatory activity was detected in hyaluronidase activity inhibitory assay with an IC_{50} of >400µg/ml and hexosaminidase release inhibitory effect was observed with IC_{50} of 223.4 µg/mL. The oak extract also exhibited anti-aging activity with human neutrophil elastase activity inhibitory at an IC_{50} of 20.87µg/mL and MMP-1 activity inhibitory effect at an IC_{50} of 125.7µg/mL. Oak extract did not promote any hyaluronic acid production in epidermal keratinocytes.

Conclusions: Oak extract exhibits a strong antioxidant effect comparable to control Baicalin and ascorbic acid. Together with the inhibitory effect on human neutrophil elastase and MMP-1, these results suggest that oak extract can be an ingredient or part of a formula of a product to be used as a topical anti-aging agent.

Keywords: Anti-aging, Oak extract, Anti-oxidation, Anti-inflammatory

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Introduction

Facial skin aging is a prevalent cosmetic concern that troubles all genders. Skin aging may manifest as wrinkles, skin sagging, dark spots, uneven skin tone and these could significantly affect an individual's self-esteem, confidence, and interpersonal relationships[1]. Both intrinsic and extrinsic factors can influence skin aging. Intrinsic factors can occur due to formation and accumulation of reactive oxygen species (ROS). Extrinsic factors such as UV radiation, alcohol usage, smoking, diet and pollution could exacerbate and accelerate skin aging. Furthermore, UV radiation can contribute up to 80% of extrinsic skin aging[2,3].

Judged to have fewer side effects, treatment with topical anti-oxidation natural bioactive components offers a noninvasive approach for skin aging prevention.

Since ancient times, the oak tree has intermingled with several fundamental aspects of human life. From providing shelter, transportation across the ocean, to the aging of fine whiskey, humans have uncovered multiple protective benefits of the esteemed oak tree. Oak trees and their parts or extracts have also been used extensively in folk medicine. The acorn has been used to treat colitis, stomatitis and diarrhea[4,5]. The leaves, bark and wood have also been investigated for their anti-microbial[6], anti-metastatic[7], anti-depressant, and anti-anxiolytic potential[8]. In this study, we set out to explore the anti-oxidation, anti-inflammatory and anti-aging activities of oak extract (freeze-dried) product to extend the protective function of oak extract as an anti-aging skin care ingredient.

Methods

- Oak extract (freeze-dried) product

Oak extract, extracted with 50% ethanol from Quercus robur Linne (Fagaceae) trees, was purchased from Maruzen Pharmaceuticals (Hiroshima, Japan) and used for all experiments in this study. Test samples were dissolved in DMSO solution and diluted in medium or buffer to test concentrations.

- Antioxidant (Suppression of Reactive Oxygen Species)

• Superoxide dismutase (SOD) Activity Assay[9]

2.4 mL of 0.05 mol/L sodium carbonate buffer (pH 10.2), 0.1 mL of 3 mmol/L xanthine, 0.1 mL of 3 mmol/L EDTA, 0.1 mL of 1.5 mg/mL bovine serum albumin and 0.1 mL of 0.75 mmol/L nitroblue tetrazolium were added to test tube, where 0.1 mL of test sample solution was added and left for 10 minutes at 25 °C. 0.1 mL of xanthine oxidase solution was added and stirred quickly, and the mixture was allowed to react at 25 °C for 20 minutes. Thereafter, 0.1 mL of 6 mmol/L copper chloride was added to stop the reaction. The absorbance at a wavelength of 560 nm was deter mined. A blank test was performed in the same manner for correction.

The method for calculating the superoxide elimination rate is as follows.

$$\text{Elimination rate (\%)} = \{1 - (A - B) / (C - D)\} \times 100$$

A: Absorbance of the test sample solution at a wavelength of 560 nm

B: Absorbance of the test sample solution (blank) at a wavelength of 560 nm

C: Absorbance at a wavelength of 560 nm in the control solution

D: Absorbance at a wavelength of 560 nm of the control solution (blank)

• Radical-Scavenging Activity Assay (DPPH)[10]

3 mL of test sample solution was added to 3 mL of 150 μmol/L diphenyl-*p*-picrylhydrazyl (DPPH) ethanol solution and incubate for 30 minutes. After that, absorbance at a wavelength of 520 nm was determined. A blank test was performed in the same manner for correction.

The calculation method of DPPH radical elimination rate is as follows.

$$\text{DPPH Elimination Rate (\%)} = \{A - (B - C)\} / A \times 100\}$$

A: Absorbance at a wavelength of 520 nm in the control solution

B: Absorbance of the test sample solution at a wavelength of 520 nm

C: Absorbance of the test sample solution (blank) at a wavelength of 520 nm

- Anti-inflammatory

• Hyaluronidase activity inhibitory assay[11]

0.1 mL of hyaluronidase solution (Type IV-S) (from bovine testis; SIGMA 400 NF units/mL))

and 0.2 mL of 0.1 mol/L acetic acid buffer (pH 3.5) was added to the test sample and incubated at 37 °C for 20 minutes. After that, 0.2 mL of 2.5 mmol/L calcium chloride was added as an activator and incubated at 37°C for 20 minutes. Then, 0.5 mL of 0.8 mg/mL sodium hyaluronate solution (from rooster comb) was added to the solution and incubated again at 37 °C for 40 minutes. Thereafter, 0.2 mL of 0.4 mol/L sodium hydroxide was added to stop the reaction, and then 0.2 mL of boric acid solution was added to each reaction solution and boiled for 3 minutes. After ice cooling, 6 mL of p-DABA reagent was added and the samples were incubated at 37 °C for 20 minutes. Thereafter, the absorbance at a wavelength of 585 nm was determined. A blank test was performed in the same manner for correction.

The calculation method of hyaluronidase activity inhibition rate is as follows.

Hyaluronidase activity inhibition rate (%)
= {1 - (A - B) / (C - D)} × 100

A: Absorbance of the test sample solution at a wavelength of 585 nm

B: Absorbance of the test sample solution (blank) at a wavelength of 585 nm

C: Absorbance at a wavelength of 585 nm in the control solution

D: Absorbance at wavelength 585 nm of control solution (blank)

- **Hexosaminidase Release Inhibitory Assay**[12]

Rat basophil leukemia cells (RBL-2H3) were cultured using 15% FBS-added S-MEM and collected by trypsinization. Recovered cells were diluted in medium to a concentration of 4.0×10^5 cells/mL and DNP-specific IgE was added such that the mixture will have a final concentration of 0.5 μg/mL of DNP-specific IgE. 100 μL mixture was plated onto each well of the 96-well plate and cultured overnight. The medium was next removed, and the cell was washed twice with 100 μL of Siraganian buffer. Next, 30 μL of the buffer and 10 μL of the test sample prepared in the Siraganian buffer were added and allowed to stand at 37 °C for 10 minutes. Thereafter, 10 μL of 400 ng/mL DNP-BSA solution was added and allowed incubate at 37 °C for 15 minutes to release hexosaminidase. Later, the reaction was stopped by placing the 96-well plate on ice. 10 μL of cell supernatant of each well and 10 μL of 1 mmol/L p-nitrophenyl N-acetyl-D-glucosaminide β (p-NAG) solution was added to a new 96-well plate and incubated at 37 °C for 1 hour. After completion of the reaction, 250 μL of 0.1 mol/L Na_2CO_3 / $NaHCO_3$ were added to each well, and the absorbance at wavelength 415 to 650 nm was determined. Blank tests include 10 μL of cell supernatant, 10 μL of citrate buffer and 250 μL of 0.1 mol/L Na_2CO_3/$NaHCO_3$ mixture, where absorbance at wavelength 415 to 650 nm was determined and corrected.

The method for calculating the hexosaminidase release inhibition rate is as follows.

Hexosaminidase release inhibition rate (%)
= {1 - (B / A)} × 100

A: Absorbance at wavelength 415 to 650 nm without test sample added

B: Absorbance at wavelength 415 to 650 nm with test sample added

- **Anti-aging**

- **Inhibitory Assay of Human Neutrophil Elastase Activity**[13]

50 μL test sample prepared in 0.1 mol/L HEPES buffer (pH 7.5) was mixed with 25 μL of 6 μg/mL elastase (Human Leukocyte) solution in a 96-well plate. Thereafter, 25 μL of 2 mmol/L N-METHOXYSUCCINYL-ALA-ALA-PRO-VAL-p-NITRO-ANILIDE solution prepared with the above buffer solution was added and incubated at 25 °C for 15 minutes. After completion of the reaction, absorbance at a wavelength of 415 nm was determined. A blank test was performed in the same manner for correction.

The method for calculating the elastase activity inhibitory rate is as follows.

Elastase activity inhibition rate (%)
= {1 -(C - D)/(A - B)} × 100

A: Absorbance at wavelength 415 nm without test sample added but enzymes added

B: Absorbance at wavelength 415 nm without test sample and enzymes added

C: Absorbance at wavelength 415 nm with test sample added and enzyme added

D: Absorbance at wavelength 415 nm with test sample added but no enzymes added

- **Inhibitory Assay of MMP-1**[14]

This test method is a partial modification of the Wunsch and Heidrich method. 50 μL of test

sample that was dissolved in 0.1 mol/L Tris-HCl buffer (pH 7.1) containing 20 mmol/L calcium chloride was mixed with MMP-1[(a)] solution 50 µL and 400 µL of Pz-peptide [(b)] solution. After incubating at 37°C for 30 minutes, the reaction was stopped with 1 mL of 25 mmol/L citric acid solution. Thereafter, 5 mL of ethyl acetate was added and vigorously shaken. This was centrifuged (1600 × g, 10 min) and the absorbance of the ethyl acetate layer at a wavelength of 320 nm was determined. A blank test was performed in the same manner for correction.

The calculation method of the MMP-1 activity inhibitory rate is as follows.

MMP-1 activity inhibition rate (%)
= {1 - (C - D) / (A - B)} × 100

A: Absorbance at wavelength of 320 nm without test sample but with enzymes added

B: Absorbance at a wavelength of 320 nm without both test sample and enzymes added

C: Absorbance at wavelength of 320 nm with both test sample and enzyme added

D: Absorbance at wavelength of 320 nm with test sample added but no enzymes added

(a) MMP-1: COLLAGENASE Type IV from Clostridium histolyticum (Sigma)

(b) Pz-peptide: Pz-Pro-Leu-Gly-Pro-D-Arg-OH (BACHEM Feinchemikalien AG)

- **Promoting Assay of Hyaluronic Acid Production (Epidermal Keratinocytes)**[15,16]

Normal human neonatal epidermal keratinocytes (NHEK) were cultured using normal human epidermal keratinocyte proliferation medium (KGM), and then cells were collected by trypsin treatment. The recovered cells were diluted with KGM to a concentration of 1×10^5 cells/mL and plated at a volume of 100 µL per well in a 96-well plate and cultured for 24 hours. After which 100 µL of test sample dissolved in KGM was added to each well and cultured for 3 days. The amount of hyaluronic acid in the medium of each well was determined by the sandwich method using hyaluronic acid-binding protein (HABP).

The calculation method of the hyaluronic acid production promotion rate is as follows.

Hyaluronic acid production promotion rate (%) = (A / B) × 100

A: Amount of hyaluronic acid with test sample added

B: Amount of hyaluronic acid without test sample added

- **MTT Assay**

Cell viability in this study was confirmed using the MTT assay. After 2 hours of incubation, the blue formazan produced in the cells was extracted with 100 µL of 2-propanol. After extraction, measured the absorbance at 570 nm and at 650 nm as turbidity, and counted the difference between the two as the amount of blue formazan produced. The difference between blue formazan production in the cells with and without the test sample was evaluated.

Statistical analysis

All statistical analyses were carried out using the paired t-test and compared with the control (no sample added). A *p-values* <0.05 were considered statistically significant. Data in the Tables are shown as mean ± SEM.

Results

- Antioxidant effects (Suppression of Reactive Oxygen Species)

- **Effect of Oak Extract on Superoxide dismutase (SOD) Activity Assay**

Oak extract exhibited a strong anti-oxidation effect in SOD activity assay. In the same experiment, compared to positive control Baicalin, Oak extract was more effective with a determined IC_{50} of 2.27 µg/mL compared to 3.94 µg/mL of the positive control. (Table 1)

Table 1 Effect of Oak Extract on SOD Activity

Concentration of tested sample (µg/mL)	Elimination rate (%) of Oak Extract Product
0	0.0 ± 3.3
1.56	**37.6 ± 0.8*****
6.25	**80.1 ± 0.2*****
25	**94.4 ± 0.1*****
IC_{50} (µg/mL)	**2.27**

Positive control Baicalin; IC_{50} = 3.94 µg/mL
Mean ± S.E., n = 3, ***: $p<0.001$

• **Effect of Oak Extract on Radical-Scavenging Activity Assay (DPPH)**

Oak extract exhibited a strong radical-scavenging activity in DPPH assay. Compared to positive control, ascorbic acid, a well-known anti-oxidant, the IC_{50} of Oak extract was determined to be 3.25 µg/mL comparable to the IC_{50} of 3.04 µg/mL ascorbic acid. (Table 2)

Table 2 Effect of Oak Extract on DPPH elimination

Concentration of Tested sample (µg/mL)	DPPH Elimination Rate (%) of Oak Extract Product
0	0.0 ± 1.2
3.125	**48.2 ± 1.1*****
6.25	**84.2 ± 0.1*****
12.5	**92.4 ± 0.0*****
IC_{50} (µg/mL)	**3.25**

Positive control ascorbic acid; IC_{50} = 3.04 µg/mL
Mean ± S.E., n = 3, ***: $p<0.001$

- **Anti-inflammatory effects**

• **Effect of Oak Extract in hyaluronidase activity inhibitory assay**

Oak extract exhibited a weak anti-inflammatory action on hyaluronidase activity inhibitory assay. Compared to indomethacin IC_{50} of 30.3 µg/mL, Oak extract has an IC_{50} of more than 400 µg/mL. (Table 3)

Table 3 Effect of Oak Extract on hyaluronidase activity inhibitory assay

Concentration of Tested sample (µg/mL)	Hyaluronidase Activity Inhibition Rate (%) of Oak Extract Product
0	0.0 ± 0.2
25	-12.6 ± 3.5
100	1.8 ± 1.6
400	**24.7 ± 0.7*****
IC_{50} (µg/mL)	**>400**

Control indomethacin; IC_{50} = 30.3 µg/mL
Mean ± S.E., n = 3, ***: $p<0.001$

• **Effect of Oak Extract on Hexosaminidase Release Inhibitory Assay**

Oak extract exhibited a weak anti-inflammatory action on hexosaminidase release inhibitory Assay. Compared to indomethacin IC_{50} of 101.8 µg/mL, Oak extract has an IC_{50} of 223.4 µg/mL. (Table 4)

Table 4 Effect of Oak Extract on Hyaluronidase activity inhibitory assay

Concentration of Tested sample (µg/mL)	Hexosaminidase Release Inhibition Rate (%) of Oak Extract Product
0	0.0 ± 6.0
100	8.5 ± 3.2
200	**43.6 ± 1.0****
400	**75.5 ± 1.3*****
IC_{50} (µg/mL)	**223.4**

Control indomethacin; IC_{50} = 101.8 µg/mL
Mean ± S.E., n = 3, **: p<0.01, ***: p<0.001

- Anti-aging effects

• **Effect of Oak Extract on Inhibitory Assay of Human Neutrophil Elastase Activity**

Anti-aging effect was observed for Oak extract on inhibitory assay of human Neutrophil Elastase activity with an IC_{50} of 20.87 µg/mL. This is effect is stronger compared to *Hypericum Ascyron* flowers an ingredient with established IC_{50} of 150 µg/mL[17]. (Table 5)

Table 5 Effect of Oak Extract on Inhibitory Assay of Human Neutrophil Elastase Activity

Concentration of Tested sample (µg/mL)	Elastase Activity Inhibition Rate (%) of Oak Extract Product
0	0.0 ± 8.2
6.25	23.7 ± 6.0
25	**57.4 ± 0.3****
100	**79.9 ± 0.1*****
IC_{50} (µg/mL)	**20.87**

Mean ± S.E., n = 3, **: p<0.01, ***: p<0.001

• **Effect of Oak Extract on Inhibitory Assay of MMP-1**

Inhibitory assay of MMP-1 was carried out to determine the IC_{50} of Oak Extract. A moderate inhibitory effect could be detected with an IC_{50} of 125.7 µg/mL. (Table 6)

Table 6 Effect of Oak Extract on Inhibitory Assay of MMP-1

Concentration of Tested sample (µg/mL)	MMP-1 Activity Inhibition Rate (%) of Oak Extract Product
0	0.0 ± 0.2
25	**8.7 ± 1.5***
100	**40.9 ± 0.7*****
400	**77.4 ± 0.2*****
IC_{50} (µg/mL)	**125.7**

Mean ± S.E., n = 3, *: p<0.05, ***: p<0.001

- Effect of Oak Extract on Promoting Assay of Hyaluronic Acid Production (Epidermal Keratinocytes)

Finally, we tried to detect if Oak extract has any effect in promoting hyaluronic acid production in epidermal keratinocyte, however we were not able to detect any activity. The lowest hyaluronic acid production rate at the highest concentration (100 μg/mL) was due to a decrease in cell viability to 10% (confirmed by MTT assay). (Table 7)

Table 7 Effect of Oak Extract on Promoting Assay of Hyaluronic Acid Production (Epidermal Keratinocytes)

Concentration of Tested sample (μg/mL)	Hyaluronic Acid Production Promotion Rate (%) of Oak Extract Product
0	100.0 ± 2.1
1.56	98.3 ± 1.2
6.25	82.1 ± 1.2
25	82.1 ± 1.9
100	67.1 ± 1.4

Discussion

Facial skin aging is a prevalent cosmetic concern that troubles all genders. Skin aging may manifest as wrinkles, skin sagging, dark spots, uneven skin tone and these could significantly affect an individual's self-esteem. UV radiation, ambient pollutant and smoking are the most prominent and prevalent external stressor affecting skin aging, the continuous exposure to these environmental stressors stimulate the production of reactive oxygen species, leading to oxidative stress[18-20]. In this study we set out to investigate if oak extract can confer any *in vitro* anti-oxidation, anti-inflammatory and anti-aging activities.

In our anti-oxidation study (Table 1 and 2) we found that oak extract was able to confer anti-oxidation effect at a level that is comparable or stronger than the control. This is consistent with other studies which had demonstrated oak genus possess antioxidant capacity[21,22] which suggests that oak extract has a great potential to be used to counter oxidative stress dealt to the skin by the environment.

Skin inflammation can manifest as redness, pain, itchiness and dryness which can be unsightly and a challenge to manage[23]. We evaluated the anti-inflammatory action in Oak extract through Hyaluronidase activity inhibitory assay (Table 3) and Hexosaminidase Release Inhibitory Assay (Table 4). Hyaluronidase is an enzyme that depolymerizes the hyaluronic acid involved in inflammation, and it has been shown that the inhibition of hyaluronidase could reduce inflammation[24]. Hexosaminidase is released by mast cells via IgE-mediated degranulation and the release mediates inflammation and acute allergy reaction[25]. While oak extract weakly inhibited hyaluronidase activity, we were able to detect hexosaminidase release inhibitory activity, therefore suggesting that oak extract product might play a role in attenuation of inflammation and acute allergy reaction.

Intrinsic skin aging can be classified as natural aging and is caused by changes in skin elasticity. Collagen, the most abundant protein in the extracellular matrix (ECM) of connective tissue in the human dermis, can be cleaved by elastase which can then cleave elastin, fibronectin and other ECM proteins, leading to a deterioration of the skin structure[26]. The degradation of elastin and collagen further induce the production of MMPs, which activates a series of destructive processes such as inflammation and aging of the skin[27]. Compared to Hypericum ascyron flowers, an ingredient with established IC_{50} of 150 μg/mL[17], oak extract product has a more potent IC_{50} (Table 5) and at the same time we were able to detect its inhibitory effect on MMP-1 (Table 6). Hyaluronic acid is another key component of the ECM and it's free-radical scavenging function makes it an attractive molecule to study for anti-aging skin care. Unfortunately, we were not able to detect any effect of oak extract on the production of hyaluronic acid in epidermal keratinocytes (Table 7), the reduction in hyaluronic acid observed was due to reduction of cell viability, which we validated with an MTT assay.

In conclusion, we have investigated and reported on the *in vitro* anti-oxidation, anti-inflam matory and anti-aging activities of oak extract through multiple experiments. Oak extract exhibits a strong antioxidant effect comparable to control Baicalin and ascorbic acid. It also exhibited an inhibitory effect on human neutrophil elastase and MMP-1. In addition to being able to confer protection on extrinsic aging factors, oak extract has the potential to inhibit intrinsic factors responsible for skin aging. These results suggest that oak extract can be an ingredient or part of a formula of a product to be used as a topical anti-aging agent.

This study has shed light on the *in vitro* activity of oak extract, in order to further establish the functionality, an *in vivo* clinical study should be considered. This study also warrants further investigation to identify the key active ingredient in oak extract.

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References

1. Gupta MA, Gilchrest BA. Psychosocial Aspects Of Aging Skin. *Dermatol Clin.* 2005;23(4):643-648. doi:10.1016/J.DET.2005.05.012.
2. Yasui H, Sakurai H. Chemiluminescent Detection and Imaging of Reactive Oxygen Species in Live Mouse Skin Exposed to UVA. *Biochem Biophys Res Commun.* 2000;269(1):131-136. doi:10.1006/BBRC.2000.2254.
3. Jin HC, Jin YS, Hai RC, et al. Modulation of skin collagen metabolism in aged and photoaged human skin in vivo. *Journal of Investigative Dermatology.* 2001;117(5):1218-1224. doi:10.1046/j.0022-202X.2001.01544.x.
4. Kim H, Song MJ, Potter D. Medicinal efficacy of plants utilized as temple food in traditional Korean Buddhism. *J Ethnopharmacol.* 2006;104(1-2):32-46. doi:10.1016/j.jep.2005.08.041.
5. MOON HR, CHUNG MJ, PARK JW, et al. ANTIASTHMA EFFECTS THROUGH ANTI-INFLAMMATORY ACTION OF ACORN (*QUERCUS ACUTISSIMA* CARR.) *IN VITRO* AND *IN VIVO*. *J Food Biochem.* 2013;37(1):108-118. doi:10.1111/j.1745-4514.2012.00652.x.
6. Sarwar R, Farooq U, Khan A, et al. Evaluation of Antioxidant, Free Radical Scavenging, and Antimicrobial Activity of Quercus incana Roxb. *Front Pharmacol.* 2015;6. doi:10.3389/fphar.2015.00277.
7. Zehra B, Ahmed A, Sarwar R, et al. Apoptotic and antimetastatic activities of betulin isolated from Quercus incana against non-small cell lung cancer cells. *Cancer Manag Res.* 2019;11:1667-1683. doi:10.2147/CMAR.S186956.
8. Sarwar R, Farooq U, Naz S, et al. Isolation and Characterization of Two New Secondary Metabolites From Quercus incana and Their Antidepressant- and Anxiolytic-Like Potential. *Front Pharmacol*. 2018;9. doi:10.3389/fphar.2018.00298.
9. Durak I, Yurtarslanl Z, Canbolat O, Akyol Ö. A methodological approach to superoxide dismutase (SOD) activity assay based on inhibition of nitroblue tetrazolium (NBT) reduction. *Clinica Chimica Acta.* 1993;214(1):103-104. doi:10.1016/0009-8981(93)90307-P.
10. Rahman MdM, Islam MdB, Biswas M, Khurshid Alam AHM. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. *BMC Res Notes.* 2015;8(1):621. doi:10.1186/s13104-015-1618-6.
11. Katsunari lppo, Ushi Yyhkia and Hhigashi. Evaluation of Inhibitory Effects of Vegetables and Herbs on Hyaluronidase and ldentification of Rosmarinic Acid as a Hyaluronidase Inhibitor in Lemon Balm (Melissa officinalis L.). *Katsunari lppoUSHI, Yuichi YAMAGUCHI, Hidekazu ITOU, Keiko AZUMA and Hisao HIGASHI.* 2006;6 (1):74-77.
12. Tanaka Y, Takagaki Y, Nishimune T. Effects of Metal Elements on beta-Hexosaminidase

Release from Rat Basophilic Leukemia Cells (RBL-2H3). *Chem Pharm Bull (Tokyo)*. 1991;39(8):2072-2076. doi:10.1248/cpb.39.2072.

13. Dou D, He G, Kuang R, Fu Q, Venkataraman R, Groutas WC. Effects of structure on inhibitory activity in a series of mechanism-based inhibitors of human neutrophil elastase. *Bioorg Med Chem*. 2010;18(18):6646-6650. doi:10.1016/j.bmc.2010.07.071.
14. Florin Barla. Potential Use of Bischofia javanica as an Active Ingredient of Functional Foods and Cosmeceutical Products Possessing Hyaluronidase, Collagenase, Tyrosinase and Urease Inhibitory Effects. *Japanese Journal of Complementary and Alternative Medicine.* 2010;(2): 129-133.
15. Pogrel MA, Low MA, Stern R. Hyaluronan (hyaluronic acid) and its regulation in human saliva by hyaluronidase and its inhibitors. *J Oral Sci.* 2003;45(2):85-91. doi:10.2334/josnusd.45.85.
16. Martins JRM, Passerotti CC, Maciel RMB, Sampaio LO, Dietrich CP, Nader HB. Practical determination of hyaluronan by a new noncompetitive fluorescence-based assay on serum of normal and cirrhotic patients. *Anal Biochem*. 2003;319(1):65-72. doi:10.1016/S0003-2697(03)00251-3.
17. Li ZP, Kim JY, Ban YJ, Park KH. Human neutrophil elastase (HNE) inhibitory polyprenylated acylphloroglucinols from the flowers of Hypericum ascyron. *Bioorg Chem.* 2019;90. doi:10.1016/j.bioorg.2019.103075.
18. Kammeyer A, Luiten RM. Oxidation events and skin aging. *Ageing Res Rev.* 2015;21:16-29. doi:10.1016/J.ARR.2015.01.001.
19. Parrado C, Mercado-Saenz S, Perez-Davo A, Gilaberte Y, Gonzalez S, Juarranz A. Environmental Stressors on Skin Aging. Mechanistic Insights. *Front Pharmacol.* 2019;10:759. doi:10.3389/FPHAR.2019.00759/BIBTEX.
20. Rittié L, Fisher GJ. UV-light-induced signal cascades and skin aging. *Ageing Res Rev.* 2002;1(4):705-720. doi:10.1016/S1568-1637(02)00024-7.
21. Rakić S, Petrović S, Kukić J, et al. Influence of thermal treatment on phenolic compounds and antioxidant properties of oak acorns from Serbia. *Food Chem.* 2007;104(2):830-834. doi:10.1016/j.foodchem.2007.01.025.
22. Rakić S, Povrenović D, Tešević V, Simić M, Maletić R. Oak acorn, polyphenols and antioxidant activity in functional food. *J Food Eng*. 2006;74(3):416-423. doi:10.1016/j.jfoodeng.2005.03.057.
23. Pullar JM, Carr AC, Vissers MCM. The Roles of Vitamin C in Skin Health. *Nutrients.* 2017;9(8). doi:10.3390/NU9080866.
24. Esser PR, Wölfle U, Dürr C, et al. Contact sensitizers induce skin inflammation via ROS production and hyaluronic acid degradation. *PLoS One.* 2012;7(7). doi:10.1371/JOURNAL.PONE.0041340.
25. Williams CMM, Galli SJ. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. *Journal of Allergy and Clinical Immunology.* 2000;105(5):847-859. doi:10.1067/MAI.2000.106485.
26. Laga AC, Murphy GF. The translational basis of human cutaneous photoaging: On models, methods, and meaning. *American Journal of Pathology.* 2009;174(2):357-360. doi:10.2353/ajpath.2009.081029.
27. Hadler-Olsen E, Fadnes B, Sylte I, Uhlin-Hansen L, Winberg JO. Regulation of matrix metalloproteinase activity in health and disease. *FEBS Journal.* 2011;278(1):28-45. doi:10.1111/J.1742-4658.2010.07920.X.