Pharmacogn. Mag.
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ORIGINAL ARTICLE

Evaluation on the Skin Anti-Aging Potential of an Aqueous Extract from *Oenanthe javanica* (Blume) DC

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Submitted: 08-Aug-2021 Revised: 04-Sep-2021 Accepted: 16-Nov-2021 Published: 28-Mar-2022

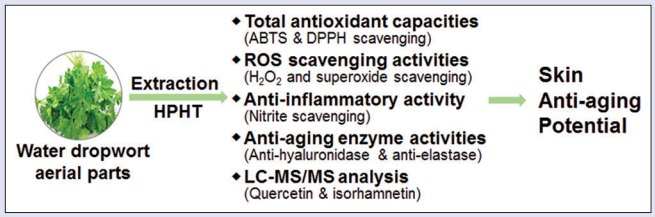
ABSTRACT

Background: Water dropwort (*Oenanthe javanica* [Blume] DC.) has been used for the improvement of diverse health disorders in the traditional folk medicine. **Objectives:** This work aimed to primarily assess the skin anti-aging potential of its aerial parts. **Materials and Methods:** The dried aerial parts of water dropwort were extracted with water under high pressure and high temperature, which produced the resultant powdered extract, named OJW. The antiradical activity of OJW was quantitated using $ABTS^{\bullet+}$ and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assays. The *in vitro* scavenging activities of OJW against superoxide anion radical, hydrogen peroxide and nitrite ions were determined based upon spectrophotometric protocols. The anti-elastase and anti-hyaluronidase activities of OJW were spectrophotometrically determined. **Results:** The contents of total polyphenols and total flavonoids in OJW were determined to be 13.5 ± 0.3 mg gallic acid equivalent/gram OJW and 16.9 ± 0.1 mg naringin equivalent/gram OJW, respectively. OJW exhibited total antiradical activities in both $ABTS^{\bullet+}$ and DPPH scavenging assays. The scavenging activities of OJW on individual Reactive oxygen species, such as hydrogen peroxide and superoxide anion radical, and nitrite ions were convinced. OJW exerted inhibitory activities on elastase and hyaluronidase. Liquid chromatography-tandem mass spectrometry analysis indicated the presence of two flavonoids, quercetin, and isorhamnetin, in OJW. **Conclusion:** *O. javanica* aerial parts possess skin anti-aging potential through the antioxidant, anti-inflammatory, and anti-wrinkle activities.

Key words: Anti-elastase, anti-hyaluronidase, anti-inflammatory, antioxidant, anti-wrinkle, *Oenanthe javanica*

SUMMARY

- *Oenanthe javanica* aqueous extract (OJW) contains total antiradical activity
- OJW scavenges superoxide anion radical and hydrogen peroxide
- OJW has a scavenging activity against nitrite ions
- OJW possesses anti-elastase and anti-hyaluronidase activities
- Collectively, OJW possesses skin anti-aging capability through antioxidant, anti-inflammatory and anti-wrinkle activities.



Abbreviations used: OJW: An aqueous extract of *O. javanica* aerial parts, ROS: Reactive oxygen species, NO: Nitric oxide, DPPH: 2,2-Diphenyl-1-picrylhydrazyl, $ABTS^{\bullet+}$: ABTS radical cation, AA: Ascorbic acid, SC_{50}: 50% scavenging concentration, IC_{50}: 50% inhibitory concentration, RT: Room temperature, HA: Hyaluronic acid, UVR: Ultraviolet radiation, MMP: Matrix metalloproteinase, SOD: Superoxide dismutase.

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DOI: 10.4103/pm.pm_366_21

Access this article online
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INTRODUCTION

Like all other organs in the humans, the skin undergoes aging with increasing age and various environmental factors. Skin aging is divided into two overlapping and synergic categories, intrinsic or chronological aging and extrinsic aging or photoaging. Intrinsic aging, as an inevitable physiological and functional alteration affecting the skin, is caused by natural internal factors such as genetic predisposition, hormones and metabolic processes over time.[1] However, extrinsic aging is prematurely developed by external environmental factors, such as severe physical and psychological stress, poor nutrition, alcohol intake, overeating, smoking, environmental pollution, ultraviolet radiation (UVR), and so on.[2,3] Intrinsically aged skin looks dry and has fine wrinkles, but is still smooth and light, whereas extrinsically aged skin has thick layers, deep and coarse wrinkles, irregular pigmentation, elastosis, capillary telangiectasia, actinic keratosis, and malignant tumors.[4]

Reactive oxygen species (ROS) are known to play triggering roles in both intrinsic and extrinsic aging, but the exact mechanisms remain to be elusive.[5] ROS are continuously generated as byproducts through the mitochondrial respiratory chain in keratinocytes and dermal fibroblasts. Excess ROS production and reduced antioxidant activity with advancing age are mainly regarded as the principal cause of intrinsic aging.[6] ROS are excessively generated by environmental factors, for example, UVR impairing skin structure and function, which leads to the phenotypic features of extrinsic aging.[6]

In both extrinsic and intrinsic aging, the elevated levels of cutaneous matrix metalloproteinases (MMPs) have been demonstrated.[7] MMPs are largely responsible for breakdown of extracellular matrix (ECM)

Cite this article as: Lim HW, Huang YH, Park M, Lim CJ. Evaluation on the Skin Anti-Aging Potential of an Aqueous Extract from *Oenanthe javanica* (Blume) DC. Phcog Mag 2022;18:75-81.

proteins such as elastin, fibronectin, collagen, and proteoglycans.[8] The augmented ROS plays a major enhancing role in the MMP levels of aged skin.[9] Oxidative damage is more evident in extrinsically aged skin than in intrinsically aged skin, and this supposedly explains more prominent aging-related features like deep wrinkles. While the primary source of MMPs in intrinsic aging are dermal fibroblasts, MMPs in extrinsic aging are produced also by epidermal keratinocytes.[10]

During the aging process, ECM is subjected to the degradation of its components, such as collagen, elastin, laminin, fibronectin, and hyaluronic acid (HA), and together with the decreased ECM synthesis, eventually leads to ECM alterations and subsequent aging symptoms, including wrinkles, freckles, and laxity. Skin fibroblast elastase, an MMP member for the degradation of elastic fiber, has been shown to play a decisive role in wrinkling formation.[11] HA is degraded by hyaluronidase present in the aged dermis, which causes skin to become dry and wrinkled.[12] Diverse enzymes involved in skin aging through the degradation of ECM, like elastase and hyaluronidase, were found to be up-regulated by ROS induced during the aging process.[13] Thus, the inhibitory activities on some dermal enzymes, such as elastase and hyaluronidase, have been considered to be desirable in preventing skin aging.

Oenanthe javanica (Blume) DC.(family Apiaceae), an aquatic perennial herb commonly known as water dropwort, has been cultivated for a long time in several East Asian countries, including Korea, China, and Japan. In traditional folk medicine, it has been utilized for the treatment of abdominal pain, fever, hypertension, jaundice, mumps, polydipsia, urinary infections, and difficult urination.[14,15] From the pharmacological standpoint, various extracts and ingredients of *O. javanica* have been assessed to possess wide biological properties such as antioxidant, hepatoprotective, hypotensive, antiarrhythmic, anti-anaphylactic, anti-inflammatory, antidiabetic, antiviral, anti-cancer, hypoglycemic, analgesic, insecticidal and antigenotoxic activities.[16-19] Nonetheless, the skin beneficial properties of *O. javanica* remain to be clearly ascertained.

In this work, an aqueous extract of *O. javanica* has been evaluated on its skin anti-aging potential through the antioxidant, anti-inflammatory, and anti-wrinkle activities and can be utilized as a plausible resource in the manufacture of novel anti-aging cosmetics.

MATERIALS AND METHODS

Chemicals

Bovine serum albumin, ascorbic acid (AA), diethylene glycol, sodium carbonate, naringin, gallic acid (GA), HA, epigallocatechin gallate (EGCG), apigenin, Folin-Ciocalteu reagent, Griess reagent, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), NADH, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ammonium persulfate, nitroblue tetrazolium, phenazine methosulfate, hydrogen peroxide, ferrous ammonium sulfate, 1,10-phenanthroline, sodium nitrite, porcine pancreatic elastase, bovine testes hyaluronidase, and *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide (STANA) were from Sigma-Aldrich Chemical Co.(St Louis, MO, USA). Other chemicals in this work were obtained also from Sigma-Aldrich Chemical Co.

Plant material

The aerial parts of *Oenanthe javanica*, obtained from a local market in Chuncheon, Korea, on September 2019, were authenticated by Prof. Ki-Oug Yoo, Department of Biological Sciences, Kangwon National University, Chuncheon, Korea, and deposited under the accession number KWNU90983 in the herbarium of the same department.

Preparation of an aqueous extract

The aerial parts were dried out using a drying oven (50°C, 24 h) prior to the extraction. The dried plant samples were mixed with 20-fold distilled water, and extracted in an autoclave for 30 min under a pressure of 2.1 atmospheres and a temperature of 121°C. Freeze drying was performed to generate the dried extract (OJW; yield, 23.6%). For the experiments, OJW was solubilized in distilled water.

Determination of total phenolic compounds

Total phenolic compounds in OJW were quantitated according to Folin-Ciocalteu method.[20] The 100 µL of 1.0 mg/mL OJW was mixed with 10 µL of 1 N Folin-Ciocalteu reagent, and the mixture was stood for 5 min at RT. The 100 µL of 7% sodium carbonate was then added to the mixture, and the total mixture was further incubated at RT for 10 min. The absorbance at 750 nm was measured at a microplate reader. The total phenolic content was formulated as mg GA equivalent per g OJW.

Quantitation of total flavonoids content

Total flavonoids content in OJW was determined according to a previously described procedure.[15] The 0.05 mL of diethylene glycol and 0.05 mL of 1.0 mg/mL OJW were added to 0.05 mL of 1 N NaOH and the mixture was incubated for 1 h at 37°C. The absorbance at 420 nm was measured at a microplate reader. Total flavonoid content was formulated as mg naringin equivalent/gram OJW.

Antioxidant activities

ABTS scavenging assay

The scavenging activity of OJW against ABTS radical was determined using ABTS radical scavenging assay.[21] ABTS radical cations (ABTS$^{\bullet+}$), generated by reacting 0.07 mM ABTS stock solution with 0.12 mM ammonium persulfate, were incubated in the dark at RT for 16 h before use. After OJW (0.01 mL) at varying concentrations (0.5, 1, 2, and 4 mg/mL) was mixed with 0.29 mL of ABTS$^{\bullet+}$ solution, the mixture was incubated at RT for 15 min under the darkness. AA was employed as a positive control. The absorbance at 745 nm was measured. The percent scavenging by OJW was calculated using the formula, scavenging (%) = ([Control − Test]/Control) × 100.

2,2-diphenyl-1-picrylhydrazyl scavenging assay

The scavenging activity of OJW against DPPH radical was determined using the method described earlier.[22] The reaction mixture containing 30 µL of OJW at varying concentrations (0.5, 1, 2, 4, and 8 mg/mL) and 270 µL of DPPH at 0.1 mM was kept at RT for 30 min under the darkness. The absorbance was detected at 517 nm. AA was employed as a positive control. The percent scavenging by OJW was calculated as described above.

Superoxide radical scavenging assay

As previously described by[23] the superoxide radical scavenging activity of OJW was determined. OJW (40 µL) at varying concentrations (0.5, 2 and 4 mg/mL) was mixed with 40 µL of 50 mM Tris buffer (pH 8.0), 40 µL of 78 mM NADH, 40 µL of 50 mM nitroblue tetrazolium and 40 µL of 10 mM phenazine methosulfate. The reaction mixture was stood for 5 min at RT. The absorbance at 560 nm was detected at a microplate reader. AA was employed as a positive control.

Hydrogen peroxide scavenging assay

As previously described,[24] OJW (10 µL) at varying concentrations (0.5, 2, and 4 mg/mL), 40 µL of 5 mM hydrogen peroxide (freshly prepared daily), and 35 µL of 1 mM ferrous ammonium sulfate. After the

115 µL of 1,10-phenanthroline (1 mM) was added to the mixture, the reaction mixture was stood for 10 min at RT. The absorbance at 510 nm was measured at a microplate reader.

Anti-inflammatory activity

Nitrite scavenging assay

The nitrite scavenging activity of OJW was carried out as previously described.[25] OJW (60 µL) at varying concentrations (0.5, 1, 2, and 4 mg/mL) was mixed with 30 µL of 0.1 mM citrate buffer (pH 3.0) and 6 µL of 50 µg/mL sodium nitrite. After the mixture was kept for 60 min at 37°C, it was mixed with the equal volume of Griess reagent [equal volumes of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) naphtylethylenediamine-HCl]. After the incubation for 10 min, the absorbance at 538 nm was measured at a microplate reader.

Skin aging-related enzyme activities

Anti-elastase activity assay

The inhibitory activity of OJW on elastase activity was examined by determining a decrease in elastase activity under the presence of OJW. Elastase activity was detected according to the release of *p*-nitroaniline from STANA used as a substrate.[26] The mixture contained 100 µL of 0.2 M Tris buffer (pH 8.0), 100 µL of STANA at 0.8 mM and OJW (50 µL) at varying concentrations (0.5, 1, 4 and 8 mg/mL). After the mixture was preincubated for 20 min at 37°C, the reaction was begun by adding 50 µL of 0.1 U/mL elastase solution. The absorbance at 410 nm was monitored at a microplate reader.

Anti-hyaluronidase activity assay

The inhibitory activity of OJW on hyaluronidase activity was assessed by measuring a diminishment in hyaluronidase activity under the presence of OJW. The hyaluronidase activity was determined as previously described.[27] The mixture containing OJW (10 µL) at varying concentrations (0.5, 1, 4 and 8 mg/mL), and 20 µL of hyaluronidase solution (585 U/mL) was kept for 10 min at 37°C and added to the equal volume of HA solution (0.2 mg/mL). After 45 min-incubation, the 240 µL of acidic albumin solution (sodium acetate at 24 mM, acetic acid at 79 mM with bovine serum albumin at 0.1%, pH 3.8 at RT) was added to the reaction mixture and was kept for 10 min at RT. The absorbance at 600 nm was measured at a microplate reader.

Liquid chromatography-tandem mass spectrometry quantitative analysis

Some flavonoids in OJW were identified using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (TSQ Quantum Ultra Triple Quadrupole Mass Spectrometer] at the Korea Basic Science Institute (Seoul). The high-performance liquid chromatography separation was performed on a ROC C18 column (3.0 × 150 mm, 5 µm, RESTEK) with two mobile phases containing 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) under the flow rate of 200 µL/min and the injection volume of 10 µL. The overall chromatographic run time was approximately 20 min, and the gradient elution profile was 10% B (2 min), 10%–100% B (10 min), 100% B (2 min), and equilibration (5 min). The MS detection was operated with the electrospray ionization source in negative mode (spray voltage 3000 V, sheath gas pressure 40 [arbitrary unit], aux gas pressure 10 [arbitrary unit], capillary temperature 270°C) and with selected reaction monitoring mode.

Statistical analyses

The results were represented as mean ± standard deviation The differences between experimental groups were analyzed utilizing one-way analysis of variance accompanied with *post hoc* Tukey HSD test for multiple comparisons. In case of a $P < 0.05$, the difference was thought to be statistically significant.

RESULTS

Total phenolic and total flavonoid contents

Phenolic compounds, a heterogeneous group of phytochemicals with a phenol ring containing at least one hydroxyl substituent, have been reported to act as antioxidants, structural polymers, attractants, UV screens, signal compounds, and defense response chemicals. The total phenolic content of OJW was 13.6 ± 0.3 mg GA equivalent per g OJW.

Flavonoids, a large group of phenolic compounds with antioxidant activity and a class of the most important plant pigments, consist of the subgroups, including anthoxanthins, flavanones, flavanonols, flavans, and anthocyanidins. OJW was determined to contain 16.8 ± 0.1 mg naringin equivalent per gram OJW.

Total anti-radical activity

OJW, at 0.5, 1, 2, and 4 mg/mL, displayed ABTS + radical scavenging activity, giving rise to the percentage inhibition of 9.4, 16.0, 27.5, and 64.0%, respectively [Figure 1a]. Its SC_{50} value was 3.2 mg/mL. AA, a positive control, exhibited an SC_{50} value of 0.02 mg/mL. Likewise, OJW gave rise to an SC_{50} value of 7.1 mg/mL in DPPH radical scavenging assay [Figure 1b]. Collectively, OJW possesses total antiradical activity.

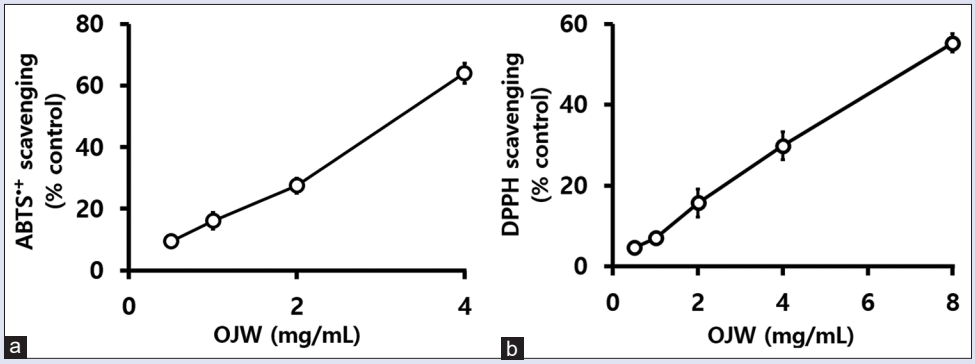

Figure 1: The ABTS•+ (a) and 2,2-diphenyl-1-picrylhydrazyl (b) scavenging activities of an aqueous extract (OJW) of *Oenanthe javanica* aerial parts. The SC_{50} values of ascorbic acid, a positive control, were 0.02 and 0.02 mg/mL in ABTS•+ and 2,2-diphenyl-1-picrylhydrazyl scavenging assays, respectively

Superoxide radical and hydrogen peroxide scavenging activities

Excessive superoxide anion radical, generated beyond the balancing capacities, is involved in several harmful biological processes, including protein denaturation and lipid peroxidation.[28] OJW, at 0.5, 2, and 4 mg/mL, was capable of scavenging the superoxide anion radical, giving rise to the percentage inhibition of 17.5, 34.2, and 63.7%, respectively [Figure 2]. Its SC_{50} value was 3.0 mg/mL. AA, as a positive control, gave rise to an SC_{50} value of 0.47 mg/mL.

Hydrogen peroxide is a major contributor to oxidative stress which disturbs normal mechanisms of cellular signaling, ultimately leading to various disorders. OJW, at 0.5, 2, and 4 mg/mL, was found to scavenge hydrogen peroxide molecules, exhibiting the percentage inhibition of 9.8, 33.1, and 67.7%, respectively [Figure 3], which gave rise to an SC_{50} value of 3.0 mg/mL. AA, used as a positive control, exhibited an SC_{50} value of 0.5 mg/mL.

In brief, OJW is capable of scavenging the two main ROS species, superoxide anion radical and hydrogen peroxide.

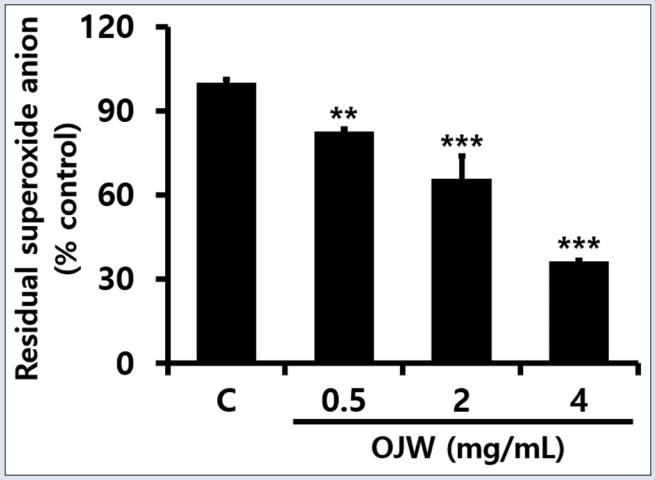

Figure 2: The superoxide radical scavenging activity of an aqueous extract (OJW) of *Oenanthe javanica* aerial parts. The SC_{50} value of ascorbic acid, a positive control, was 0.47 mg/mL. $^{**}P < 0.01$; $^{***}P < 0.001$ versus control (c)

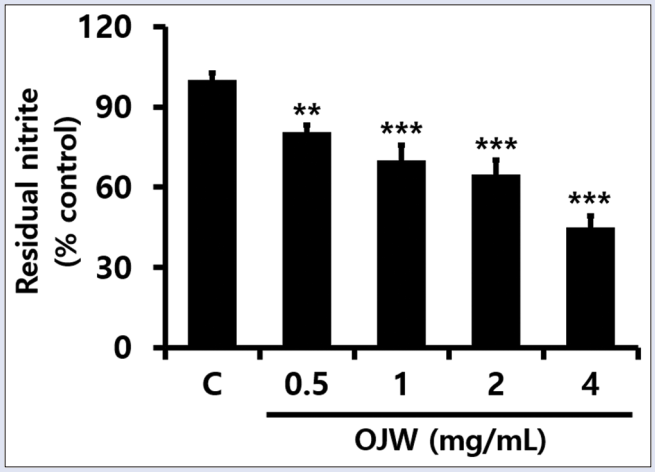

Figure 4: The nitrite scavenging activity of an aqueous extract (OJW) of *Oenanthe javanica* aerial parts. The SC_{50} value of ascorbic acid, a positive control, was 0.06 mg/mL. $^{**}P < 0.01$; $^{***}P < 0.001$ versus control (c)

Nitrite scavenging activity

Since NO, acting as a major pro-inflammatory mediator, reacts with oxygen to form nitrite ions, nitrite level is used as an index of NO. When OJW, at the varying concentrations of 0.5, 1, 2, and 4 mg/mL, was mixed with nitrite ion, it could scavenge nitrite, giving the percentage inhibition of 20.0, 28.9, 37.0, and 54.9%, respectively [Figure 4]. It gave rise to an SC_{50} value of 3.5 mg/mL. AA, a positive control, gave rise to an SC_{50} value of 0.06 mg/mL. The nitrite scavenging activity of OJW hints an anti-inflammatory activity in living cells.

Anti-elastase activity

Elastase activity in the dermis is elevated during the aging, which is known to contribute to the diminishment of skin elastic properties. When OJW, at 0.5, 1, 4, and 8 mg/mL, was utilized in the inhibition assay, it was capable of inhibiting the porcine pancreas elastase, showing the percentage inhibition of 8.5, 11.5, 14.9, and 29.9%, respectively [Figure 5].

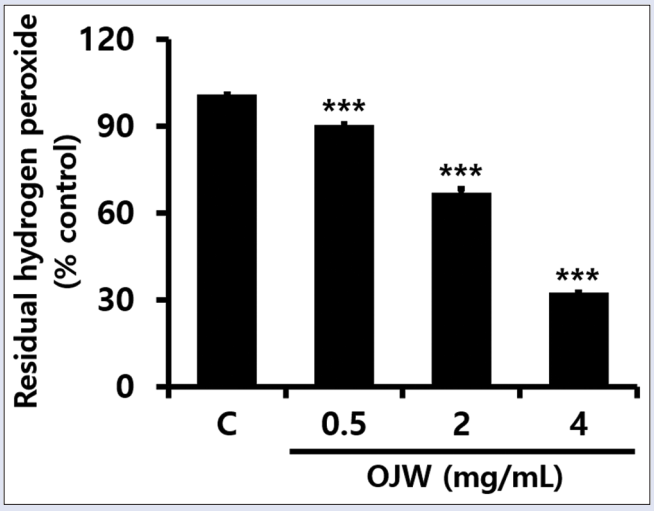

Figure 3: The hydrogen peroxide (H_2O_2) scavenging activity of an aqueous extract (OJW) of *Oenanthe javanica* aerial parts. The SC_{50} value of ascorbic acid, a positive control, was 0.5 mg/mL. $^{***}P < 0.001$ versus control (c)

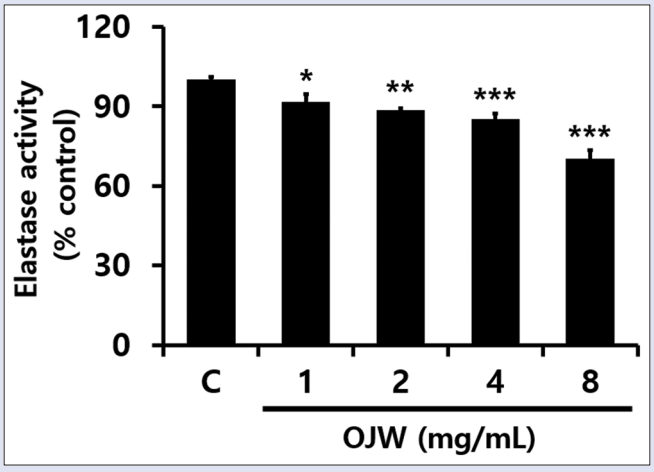

Figure 5: The inhibitory activity of an aqueous extract (OJW) of *Oenanthe javanica* aerial parts on elastase activity. Porcine pancreas elastase was used as an enzyme source, and a synthetic peptide STANA was used as a chromogenic substrate. The IC_{50} value of EGCG, a positive control, was 1.2 mg/mL. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus control (c)

The IC_{50} value of OJW was estimated to be higher than 4.0 mg/mL. EGCG, a positive control, gave rise to an IC_{50} value of 1.2 mg/mL.

Anti-hyaluronidase activity

Since hyaluronidase causes the hydrolysis of HA, the inhibition of hyaluronidase is expected to hinder skin aging. OJW, at 0.5, 1, 4, and 8 mg/mL, exhibited an inhibitory activity on hyaluronidase activity, exerting the percentage inhibition of 10.5%, 18.1%, 36.6%, and 61.6%, respectively [Figure 6]. Its IC_{50} value was determined to be 6.2 mg/mL. Apigenin, a positive control, gave rise to an IC_{50} value of 3.5 mg/mL.

The inhibitory activities of OJW on elastase and hyaluronidase activities imply its anti-wrinkle activity.

Flavonoids identification

As shown in Figure 7, two flavonoids, isorhamnetin and quercetin, were identified and quantitated in OJW. Isorhamnetin (119.8 μg/g OJW) was shown to be 100-fold richer than quercetin (1.2 μg/g OJW) in OJW. However, afzelin, a flavonol glycoside previously identified in other types of extracts from *O. javanica*, was not detected in OJW (data not shown).

DISCUSSION

Diverse extracts and purified ingredients of plant origin are known to protect the skin in various ways, such as absorbing the UVR, scavenging ROS, reducing ROS reactivity, inhibiting oxidation, and suppressing the appropriate enzymes, which then reduces the risk of wrinkle formation and prevents the skin from aging.[29] A cherry blossom extract was suggested to contain a skin anti-aging potential by protecting HaCaT keratinocytes from UV-B-induced oxidative stress and apoptosis.[30] Red ginseng NaturalGEL, prepared from the microgranulation of red ginseng, was proposed as a skin anti-aging agent which attenuates UV-induced MMPs and augments type 1 collagen in fibroblasts and increases hyaluronan synthetase 2 and filaggrin expressions, and ceramide in dermal keratinocytes.[31] Genetically modified rice, enriched with the anti-aging resveratrol, enhances the anti-aging potential of resveratrol via the down-regulation of the three major pathways, such as MMP-mediated aging, inflammaging and apoptosis-induced aging, resulting from the UV-B-induced ROS accumulation and responsible for skin wrinkle formation and photoaging.[32] A polyphenol mixture of Kuding tea, commonly used as a health drink in southwest China, exerts a protective effect on UV-B-induced skin aging in mice through the up-regulation of some antioxidant components, such as superoxide dismutase and catalase, and the down-regulation of MMP-2 and-9.[33] Polysaccharide from *Laminaria japonica* exerts an inhibitory effect on MMP-1 expression through preventing oxidative stress and c-Jun N-terminal kinase phosphorylation, sequentially delaying collagen breakdown during skin aging.[34] Standardized extract of *Tagetes erecta* flower, traditionally claimed to treat skin diseases, show inhibitory effects on hyaluronidase, elastase, and MMP-1, which supports its anti-wrinkle property.[35] Likewise, the present work could demonstrate the anti-elastase and anti-hyaluronidase activities of OJW, which suggests its skin anti-aging potential.

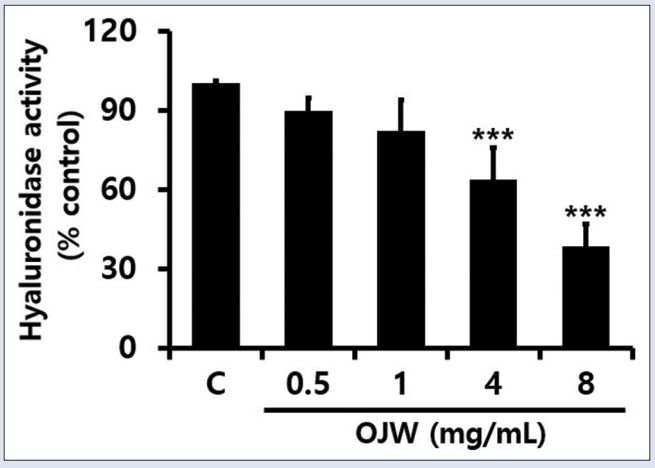

Figure 6: The inhibitory activity of an aqueous extract (OJW) of *Oenanthe javanica* aerial parts on hyaluronidase activity. Hyaluronidase from bovine testes was used as an enzyme source. The IC_{50} value of apigenin, a positive control, was 3.5 mg/mL. ***$P < 0.001$ versus control (c)

Until lately, the antioxidant, anti-inflammatory, and anti-aging properties of *O. javanica* have been assessed using its extracts and purified ingredients. An ethanol extract of *O. javanica* protects neurons against experimentally induced ischemic neuronal damage via its up-regulating capacity intracellular antioxidant enzymes, including catalase, Cu/Zn-SOD, Mn-SOD, and glutathione peroxidase, in gerbils.[36] An ethanol extract of *O. javanica* was found to augment expressions of catalase, Mn-SOD, Cu/Zn-SOD, and glutathione peroxidase in rat livers.[37] A recent work has verified that an *O. javanica* extract was found to enhance the productions of collagen types I and III, and decrease the MMP-1 and-3 expressions, tumor necrosis factor-α, and cyclooxygenase-2 against UV-B-induced skin damage in mice, suggesting the protective effects against UV-B-induced collagen disruption and inflammation.[38] A chlorophyll-rich methanol extract of *O. javanica* was shown to possess an antioxidant activity by measuring ferric reducing/antioxidant activity, oxygen radical absorbance capability, and Fe^{2+}/H_2O_2-induced DNA nicking.[17] An *O. javanica* cultivar was found to contain an antioxidant defense system in the form of osmolyte (proline), antioxidants (polyphenol and flavonoids), and antioxidant enzymes (ascorbate peroxidase and catalase), then resulting in its tolerance to salt stress.[39] Isorhamnetin sulfate, hyperin, and persicarin were previously isolated as major flavonoids from the leaves and stems of *O. javanica*.[40] Among them, persicarin was found to exert a hepatoprotective activity against hepatic lipid peroxidation in bromobenzene-treated rats.[41,42] Persicarin also protects against liver damage by diminishing oxidative stress and inflammation through the suppression of enhanced oxidative stress parameters, such as ROS and peroxynitrite, NADP oxidase subunits, such as Nox-4 and $P47^{phox}$, and inflammatory-related markers, such as COX-2, iNOS, NF-κB, AP-1, and TGF-β.[18] This work verifies that an aqueous extract from *O. javanica* aerial parts possesses total antiradical activity and scavenging activities against individual ROS species, such as superoxide anion and hydrogen peroxide. These findings might suggest that the previously identified antioxidant activities of *O. javanica* would be at least partly on its scavenging properties against reactive oxygen radicals. Since OJW has been shown to scavenge NO, a pro-inflammatory mediator but measured as a form of nitrite, the plausible anti-inflammatory activity of *O. javanica* is considered to be also based upon its scavenging activity.

Two flavonoids, isorhamnetin and quercetin, identified as ingredients of OJW, are well known to contain antioxidant and anti-inflammatory activities.[43-46] Their pharmacological activities, such as antioxidant and anti-inflammatory activities, would be partly responsible for the skin anti-aging potentials of OJW assessed in this work, although the related detailed mechanism (s) require further approaches. If additional ingredients of OJW, such as coumarins, other flavonoids and flavonoid glycosides, volatile oils, and polyphenols, can be ascertained in the future, the applicability of OJW will be gradually widened.

Throughout this work, it is demonstrated that an aqueous extract of *O. javanica* aerial parts possesses antioxidant, anti-inflammatory,

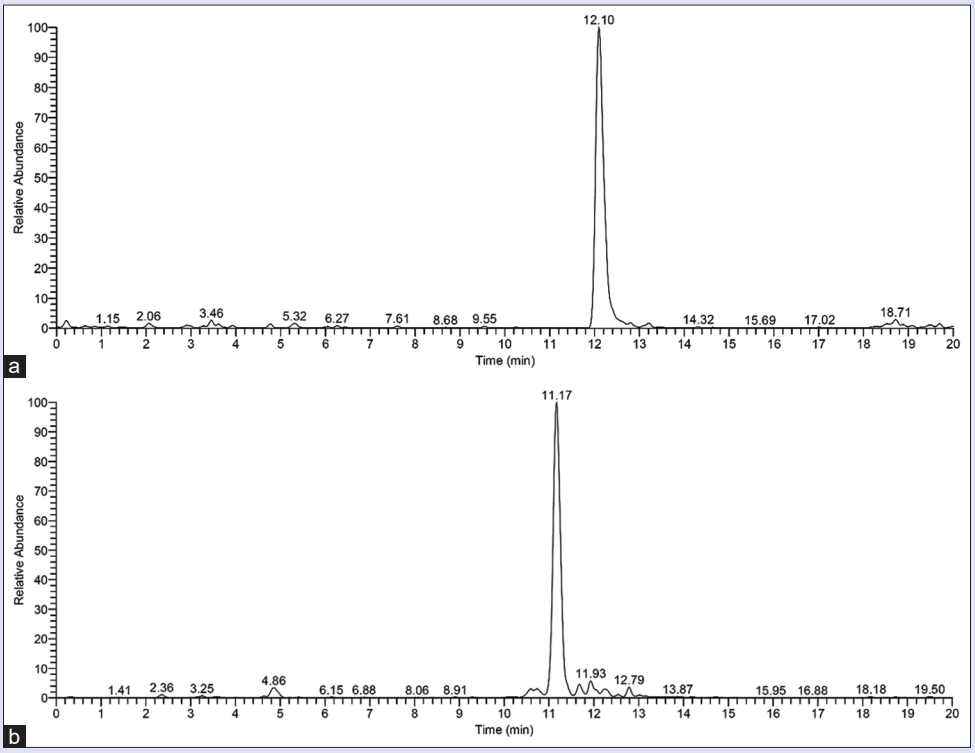

Figure 7: The LC-MS/MS chromatograms of isorhamnetin (a) and quercetin (b) in an aqueous extract (OJW) of *Oenanthe javanica* aerial parts. In (a), the major peak corresponds to isorhamnetin standard (12.12 min), and in (b), the major peak corresponds to quercetin standard (11.20 min)

and anti-wrinkle potentials, suggesting its skin anti-aging properties, although further studies are required prior to practical application. This finding would broaden the usefulness of *O. javanica* in its diverse industrial application, including cosmetic manufacture.

CONCLUSION

Some *in vitro* scavenging and enzyme inhibitory capacities of *O. javanica* aerial parts have been assessed using its high-pressure and high-temperature extract (OJW). OJW exhibited scavenging properties on ABTS•+, DPPH, superoxide anion radical and hydrogen peroxide, implying its antioxidant activity. OJW was also able to scavenge nitrite ion, suggesting its anti-inflammatory activity. OJW was supposed to possess an anti-wrinkle activity through ant-elastase and anti-hyaluronidase activities. In this work, it is demonstrated that *O. javanica* aerial parts possess plausible antioxidant, anti-inflammatory and anti-wrinkle properties, which would propose its possible application as a natural resource in the manufacture of anti-aging cosmetics.

Acknowledgements

The authors are very grateful to Dr. Joo Hee Chung for her help at the Korea Basic Science Institute, Seoul, Korea.

Financial support and sponsorship

This work was supported by the Industrial Complex Cluster Program (Grant No. PGW18002R1) from Korea Industrial Complex Corporation (KICOX), the Ministry of Trade, Industry and Energy (MOTIE), the Republic of Korea.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Sanches Silveira JEP, Myaki Pedroso DM. UV light and skin aging. Rev Environ Health 2014;29:243-54.
2. Zhang S, Duan E. Fighting against skin aging: The way from bench to bedside. Cell Transplant 2018;27:729-38.
3. Poljšak B, Dahmane R. Free radicals and extrinsic skin aging. Dermatol Res Pract 2012;2012:135206.
4. Gerasymchuk M, Cherkasova V, Kovalchuk O, Kovalchuk I. The role of microRNAs in organismal and skin aging. Int J Mol Sci 2020;21:5281.
5. Papakonstantinou E, Roth M, Karakiulakis G. Hyaluronic acid: A key molecule in skin aging. Dermatoendocrinol 2012;4:253-58.
6. Poljšak B, Dahmane RG, Godić A. Intrinsic skin aging: The role of oxidative stress. Acta Dermatovenerol Alp Pannonica Adriat 2012;21:33-6.
7. Sárdy M. Role of matrix metalloproteinases in skin ageing. Connect Tissue Res 2009;50:132-8.
8. Pittayapruek P, Meephansan J, Prapapan O, Komine M, Ohtsuki M. Role of matrix metalloproteinases in photoaging and photocarcinogenesis. Int J Mol Sci 2016;17:868.
9. Shin JW, Kwon SH, Choi JY, Na JI, Huh CH, Choi HR, *et al.* Molecular mechanisms of dermal aging and antiaging approaches. Int J Mol Sci 2019;20:2126.
10. Qin Z, Balimunkwe RM, Quan T. Age-related reduction of dermal fibroblast size up-regulates multiple matrix metalloproteinases as observed in aged human skin *in vivo*. Br J Dermatol 2017;177:1337-48.
11. Tsuji N, Moriwaki S, Suzuki Y, Takema Y, Imokawa G. The role of elastases secreted by fibroblasts in wrinkle formation: Implication through selective inhibition of elastase activity. Photochem Photobiol 2001;74:283-90.
12. Hetta MH. Hyaluronidase inhibitors as skin rejuvenating agents from natural source. Int J Phytocos Nat Ingred 2020;7:4.
13. Lee JH, Moon SH, Hong Y, Ahn DU, Paik HD. Anti-elastase and anti-hyaluronidase activity of phosvitin isolated from hen egg yolk. Br Poult Sci 2020;61:17-21.
14. Huang ZM, Yang XB, Cao WB. Modern study and clinic application of *Oenanthe javanica*. Pharm J Chin 2001;17:266-9.

15. Kim EJ, Choi JY, Yu M, Kim MY, Lee S, Lee BH. Total polyphenols, total flavonoid contents, and antioxidant activity of Korean natural and medicinal plants. Korean J Food Sci 2012;44:337-42.
16. Ji G, Yao X, Zang Z, Huang Z. Antiarrhythmic effect of *Oenanthe javanica* (Bl.) DC. injection. Zhongguo Zhong Yao Za Zhi 1990;15:429-31.
17. Kwon D, Yoon S, Carter O, Bailey GS, Dashwood RH. Antioxidant and antigenotoxic activities of *Angelica keiskei*, *Oenanthe javanica* and *Brassica oleracea* in the Salmonella mutagenicity assay and in HCT116 human colon cancer cells. Biofactors 2006;26:231-44.
18. Lee JY, Kim MY, Shin SH, Shin MR, Kwon OJ, Kim TH, *et al.* Persicarin isolated from *Oenanthe javanica* protects against diabetes-induced oxidative stress and inflammation in the liver of streptozotocin-induced Type 1 diabetic mice. Exp Ther Med 2017;13:1194-202.
19. Lu CL, Li XF. A review of *Oenanthe javanica* (Blume) DC. As traditional medicinal plant and its therapeutic potential. Evid Based Complement Alternat Med 2019;2019:6495819.
20. Afshari A, Sayyed-Alangi SZ. Antioxidant effect of leaf extracts from *Cressa cretica* against oxidation process in soybean oil. Food Sci Nutr 2017;5:324-33.
21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231-7.
22. Chen YS, Liou HC, Chan CF. Tyrosinase inhibitory effect and antioxidative activities of fermented and ethanol extracts of *Rhodiola rosea* and *Lonicera japonica*. Sci World J 2013;2013:612739.
23. Mandal S, Hazra B, Sarkar R, Biswas S, Mandal N. Assessment of the antioxidant and reactive oxygen species scavenging activity of methanolic extract of *Caesalpinia crista* leaf. Evid Based Complement Alternat Med 2011;2011:173768.
24. Mukhopadhyay D, Dasgupta P, Roy DS, Palchoudhuri S, Chatterjee I, Ali S, *et al.* A sensitive *in vitro* spectrophotometric hydrogen peroxide scavenging assay using 1,10-phenanthroline. Free Radic Antioxid 2016;6:124-32.
25. Gharibzahedi SM, Razavi SH, Mousavi M. Characterizing the natural canthaxanthin/2-hydroxy propyl-β-cyclodextrin inclusion complex. Carbohyd Polym 2014;101:1147-53.
26. Thring TS, Hili P, Naughton DP. Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. BMC Complem Altern Med 2009;9:27.
27. Dorfman A, Ott ML. A turbidometric method for the assay of hyaluronidase. J Biol Chem 1948;172:367-75.
28. Hayyan M, Hashim MA, AlNashef IM. Superoxide ion: Generation and chemical implications. Chem Rev 2016;116:3029-85.
29. Karim AA, Azlan A, Ismail A, Hashim P, Abd Gani SS, Zainudin BH, *et al.* Phenolic composition, antioxidant, anti-wrinkles and tyrosinase inhibitory activities of cocoa pod extract. BMC Complement Altern Med 2014;14:381.
30. Wang Y, Li W, Xu S, Hu R, Zeng Q, Liu Q, *et al.* Protective skin aging effects of cherry blossom extract (*Prunus Yedoensis*) on oxidative stress and apoptosis in UVB-irradiated HaCaT cells. Cytotechnology 2019;71:475-87.
31. Kim YH, Park HR, Cha SY, Lee SH, Jo JW, Go JN, *et al.* Effect of red ginseng NaturalGEL on skin aging. J Ginseng Res 2020;44:115-22.
32. Subedi L, Lee TH, Wahedi HM, Baek SH, Kim SY. Resveratrol-enriched rice attenuates UVB-ROS-induced skin aging via downregulation of inflammatory cascades. Oxid Med Cell Longev 2017;2017:8379539.
33. Yi R, Zhang J, Sun P, Qian Y, Zhao X. Protective effects of Kuding tea (*Ilex kudingcha* C. J. Tseng) polyphenols on UVB-induced skin aging in SKH1 hairless mice. Molecules 2019;24:1016.
34. Hu L, Tan J, Yang X, Tan H, Xu X, You M, *et al.* Polysaccharide extracted from *Laminaria japonica* delays intrinsic skin aging in mice. Evid Based Complement Alternat Med 2016;2016:5137386.
35. Maity N, Nema NK, Abedy MK, Sarkar BK, Mukherjee PK. Exploring *Tagetes erecta* Linn flower for the elastase, hyaluronidase and MMP-1 inhibitory activity. J Ethnopharmacol 2011;137:1300-5.
36. Park JH, Cho JH, Kim IH, Ahn JH, Lee JC, Chen BH, *et al. Oenanthe javanica* extract protects against experimentally induced ischemic neuronal damage via its antioxidant effects. Chin Med J (Engl) 2015;128:2932-7.
37. Lee CH, Park JH, Cho JH, Kim IH, Ahn JH, Lee JC, *et al.* Effect of *Oenanthe javanica* extract on antioxidant enzyme in the rat liver. Chin Med J (Engl) 2015;128:1649-54.
38. Her Y, Shin BN, Lee YL, Park JH, Kim DW, Kim KS, *et al. Oenanthe javanica* extract protects mouse skin from UVB radiation via attenuating collagen disruption and inflammation. Int J Mol Sci 2019;20:1435.
39. Kumar S, Li G, Yang J, Huang X, Ji Q, Zhou K, *et al.* Investigation of an antioxidative system for salinity tolerance in *Oenanthe javanica*. Antioxidants (Basel) 2020;9:940.
40. Park JC, Young HS, Yu YB, Lee JH. Isorhamnetin sulphate from the leaves and stems of *Oenanthe javanica* in Korea. Planta Med 1995;61:377-8.
41. Park JC, Yu YB, Lee JH, Hattori M, Lee CK, Choi JW. Protective effect of *Oenanthe javanica* on the hepatic lipid peroxidation in bromobenzene-treated rats and its bioactive component. Planta Med 1996;62:488-90.
42. Park JC, Kim JY, Lee YJ, Lee JS, Kim BG, Lee SH, *et al.* Protective effect of *Oenanthe javanica* extract on acetaminophen-induced hepatotoxicity in rats. Yakhak Hoeji 2008;52:316-21.
43. Lesjak M, Beara I, Simin N, Pintać D, Majkić T, Bekvalac K, *et al.* Antioxidant and anti-inflammatory activities of quercetin and its derivatives. J Funct Food 2018;40:68-75.
44. Lee HN, Shin SA, Choo GS, Kim HJ, Park YS, Kim BS, *et al.* Anti-inflammatory effect of quercetin and galangin in LPS-stimulated RAW264.7 macrophages and DNCB-induced atopic dermatitis animal models. Int J Mol Med 2018;41:888-98.
45. Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E. Anti-inflammatory effects of flavonoids: Genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-kappaB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-kappaB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. Mediators Inflamm 2007;2007:45673.
46. Zhou F, Mei J, Yuan K, Han X, Qiao H, Tang T. Isorhamnetin attenuates osteoarthritis by inhibiting osteoclastogenesis and protecting chondrocytes through modulating reactive oxygen species homeostasis. J Cell Mol Med 2019;23:4395-407.