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# In vitro screening of elastase, collagenase, hyaluronidase, and tyrosinase inhibitory and antioxidant activities of 22 halophyte plant extracts for novel cosmeceuticals

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## Abstract

**Background:** Halophyte plant (HPs), a salt-resistant flora, has been reported to provide several health benefits, but the knowledge of its cosmeceutical potential is still ambiguous. Here, 70% ethanol extracts of 22 HPs collected from along the coast of South Korea were investigated for their potentials of antioxidant, anti-aging, and whitening properties for use as materials in novel cosmeceuticals.

**Methods:** Antioxidant activities were determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical and hydrogen peroxide scavenging assays, and skin aging-related enzyme activities (anti-elastase, anti-collagenase, anti-hyaluronidase, and anti-tyrosinase) were evaluated by using the spectrophotometric method.

**Results:** Among the 22 HPs, we found that *Ischaemum antephoroides f. coreana* and *Atriplex gmelinii* extracts presented the strongest scavenging effects against DPPH free radical and hydrogen peroxide, respectively. Our finding additionally suggested that *Salicornia europaea* extract might provide a major source of anti-elastase and anti-hyaluronidase; meanwhile, *Rosa rugosa* extract showed the highest anti-collagenase effect. Furthermore, the highest tyrosinase inhibitory activity was possessed by *Spartina anglica* extract.

**Conclusion:** These findings may suggest that halophyte plants showing biological activities may be potent inhibitors of tyrosinase, elastase, collagenase, and hyaluronidase and could be useful for application in cosmeceuticals.

**Keywords:** Halophyte plants, Antioxidant, Skin aging-related enzyme activities, Cosmeceuticals

## Introduction

Skin is the most visible part in the human body which plays an essential role as a barrier protecting an internal organ against physical, chemical, and biological detractors (Kendall and Nicolaou 2013). In recent decades, an awareness of the aging skin has become one of the most highlighting issues for scientists, and the number of skin aging studies are continually increasing. Skin aging can be identified into intrinsic and extrinsic aging processes; intrinsic aging is an inevitable process or natural aging

undergone by the passage of time (Thring et al. 2009), whereas extrinsic aging is an event resulting from the exposure to external factors, predominantly by ultraviolet (UV) radiation known as photoaging (Yang et al. 2016). Solar radiation, or UV radiation, is the major stimulator that accelerates the overproduction of reactive oxygen species (ROS) which leads the endogenous oxidative stress in the epidermis (Kim et al. 2016). Moreover, the excess oxidative stress is so harmful that it induces unhealthy and aging skin contributing as wrinkle, roughness, dryness, elasticity loss, and uneven pigmentation due to the degradation of extracellular matrix (ECM) (Hornig et al. 2017). In addition, the exalted levels of ROS can cause not only the senescence and damage

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of biological functions such as lipid membrane peroxidation, DNA deterioration, cell death but also human's disease, namely, cancer, stroke, Parkinson's disease, heart disease, arteriosclerosis, infection, and autoimmune disease (Popoola et al. 2015). It has been carried out that an antioxidant provided a great potential to defend ROS and deplete the oxidative stress, thus, a compound with the strong antioxidant activity additionally facilitates the skin protecting against the oxidative damages along with delaying the skin aging process (Chanda et al. 2015; Palmer and Kitchin 2010).

ECM, the outermost part of the skin, consists of fibroblasts and proteins including collagen and elastin (Fulop et al. 2012; Ndlovu et al. 2013). Collagen is the most abundant protein structure in the human dermis layer providing the tensile strength of the skin; meanwhile, elastin, a fiber network located in the connective tissue, is responsible for the elastic recoil property. Indeed, collagen and elastin are necessary to the skin which play major role for the plumpness, flexibility, integrity, and elasticity keeping skin youthful and healthy (Hornig et al. 2017; Siedle et al. 2002; Thring et al. 2009; Varani et al. 1998). However, the accumulated ROS after skin exposure to photoaging stressors can indirectly activate dermal enzymes such as collagenase and elastase which basically break down and degrade collagen as well as elastin, respectively (Chatatikun and Chiabchalard 2017; Popoola et al. 2015; Sahasrabudhe and Deodhar 2010). Thereby, the synthesis of elastase and collagenase promotes premature skin aging as evidenced by signs such as wrinkles, freckles, sallowness, deep furrows or severe atrophy, laxity, and leathery appearance (Ding et al. 2018; Peres et al. 2011).

Hyaluronic acid or hyaluronan (HA), a glucose-based polymer, can be commonly found in tissues and fluids of the body, but it is most bountiful in the dermal compartment of skin and the epidermal layer. HA mainly promotes skin rejuvenation, contains moisture, increases viscosity, and reduces the permeability of extracellular fluid (Leach et al. 2003). Owing to the excellence of water-holding capability, the HA-rich area contributes emollience, smoothness, and youngness together with wrinkle diminution of the skin (Jegasothy et al. 2014). Unfortunately, HA is naturally decreased during the aging process, whereas hyaluronidase is synthesized. Hyaluronidase is an HA-destructive enzyme which leads to loss of strength, flexibility and moisture, and subsequently, skin aging (Ndlovu et al. 2013). Accordingly, one of the anti-wrinkle approaches is to prolong skin moisture by preserving HA contents underneath the skin.

Melanin, the black or brown pigment, is a major component of the skin, hair, and eye color which is synthesized by melanogenesis, a mechanism of the melanocyte. Melanin pigmentation mainly enhances the skin's protective barrier against various environmental factors especially

the UV radiation and hormonal factors, like the cytokines (Asanka et al. 2018; Chatatikun et al. 2019; Tu and Tawata 2015). However, uncontrolled and overproduction of melanin may cause skin disorders, including freckles, melasma, age spots, senile lentigines, and post-inflammatory hyperpigmentation leading to flaw and premature aging appearance. Tyrosinase is a melanogenic enzyme that plays a crucial role as a rate-limiting step during melanin pigmentation (Chatatikun et al. 2019). Therefore, the downregulation or inhibition of tyrosinase activity is a common approach that is recommended to deal with disorder pigmentation and used as a whitening agent in aesthetic purpose (Kang et al. 2018).

Since the lesser adverse reactions were mentioned by a natural product, the use of natural cosmetic ingredient including botanic plants has received attention and become target for investigation (Liyanaarachchi et al. 2018). Halophyte is a saline-tolerant plant that can grow under the extreme environment; the recent data mentioned that it might provide a good biological potential for human health due to its great resistance surviving in stressful conditions (oxidative stress, UV radiation, salinity, and extreme temperature; Jdey et al. 2017). Moreover, several investigations have been carried out, its health benefits used as folk drug such as antioxidant, anti-inflammatory, antinociceptive, anti-cancer, and antimicrobial properties (Dudonné et al. 2011; Küpeli et al. 2006; Meot-Duros et al. 2008). However, there have rarely been many previous reports of HP application in terms of cosmetic material. Here, biological activities on skin health of 22 halophytic plants located from South Korea were challenged by screening their antioxidant activity and anti-elastase, anti-collagenase, anti-hyaluronidase, and anti-tyrosinase effects as candidates for cosmeceutical applications.

## Materials and methods

### Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), peroxidase, 2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS), peroxidase from horseradish, tyrosinase from mushroom, L-tyrosine, collagenase from *Clostridium histolyticum*, Azo dye-impregnated collagen, N-succinyl-Ala-Ala-Ala-p-nitroanilide (AAAPN), elastase from porcine pancreas, 4-(dimethylamino)benzaldehyde (DMAB), potassium tetraborate tetra-hydrate ( $K_2B_4O_7 \cdot 4H_2O$ ), phosphate buffered saline (PBS) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All chemicals and reagents used in this experiment were analytical grade.

### Sample preparation

Seventy percent ethanol extract of 22 HPs used in the present study were kindly provided by the National Marine Biodiversity Institute of Korea (MABIK) as shown in Table 1.

### DPPH free radical scavenging activity

The DPPH scavenging activity was performed as described by Nanjo et al. (1996). Briefly, DPPH reagent was dissolved in methanol for a solution concentration of  $1.5 \times 10^{-4}$  M. One hundred microliters of DPPH reagent was mixed with 100  $\mu$ l sample in 96-well plates. After incubation at room temperature for 30 min, the absorbance was measured 517 nm using a microplate reader (Sunrise TW, Tecan Trading AG, Männedorf, Switzerland).

### Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was examined according to Müller (1985) with slight modification. A 100  $\mu$ l of 0.1 M PBS buffer (pH 5) was added into a 96-well plate. Each 20  $\mu$ l of sample and 20 mM hydrogen peroxide ( $H_2O_2$ ) were added to mix with the buffer, and then incubate 37 °C for 5 min. After the incubation, a 30  $\mu$ l of 1.25 mM ABTS and peroxidase (1 unit/ml) were added to the mixture and then incubated at 37 °C for 10 min. The absorbance was read with a microplate reader at 405 nm (Sunrise TW, Tecan Trading AG, Männedorf, Switzerland).

### Collagenase inhibitory assay

Collagenase inhibitory activity was evaluated based on the method described by Wang et al. (2018) with some modifications. A fixed weight of 1 mg of Azo dye-impregnated collagen was measured in the test tubes and then the homogenization was proceeded after the addition of an 800  $\mu$ l of 0.1 M Tris-HCl (pH 7) and a 100  $\mu$ l of sample into each of test tubes. A 100  $\mu$ l collagenase (200 units/ml) was immediately mixed into the mixture and incubated at 43 °C for 1 h. Afterward, the test tubes were centrifuged at 3000 rpm for 10 min. The supernatant section of each test tube was transferred into 96-well plates and the absorbance of each supernatant was measured at 550 nm (Sunrise TW, Tecan Trading AG, Männedorf, Switzerland).

### Elastase inhibitory effect assay

Elastase inhibition was determined by measuring the intensity of the solution color assayed using the method of Tu and Tawata (2015) with slight modifications. The mixture of AAAPVN elastase substrate in 0.1232 M Tris-HCl buffer solution (pH 8) was prepared to obtain a concentration of 1.015 mM. The elastase substrate was mixed with the 10  $\mu$ l of sample in the 96-well plates, and preincubated at 25 °C for 10 min. After the preincubation, the reaction was initiated by adding 10  $\mu$ l of elastase from porcine pancreas (7.5 units/ml) in Tris solution buffer to the preincubated mixtures. Finally, the absorbance was measured at 410 nm using a microplate reader (Sunrise TW, Tecan Trading AG, Männedorf, Switzerland).

### Hyaluronidase inhibitory assay

Hyaluronidase inhibitory effect was evaluated as the method described by Sahasrabudhe and Deodhar (2010) with few modifications. A 0.1 M of acetate buffer at pH 3.6 was prepared and used as the buffer solution in this assay. Firstly, each 10  $\mu$ l of 8 mg/ml hyaluronidase in buffer solution and sample were mixed in the test tube, and incubate at 37 °C for 20 min. A 20  $\mu$ l of 12.5 mM calcium chloride was, then, treated to the mixture, and incubated again at 37 °C for 20 min. After the incubation, the activated  $Ca^{2+}$  mixture was treated with a 50  $\mu$ l of 2.4  $\mu$ g/ml hyaluronic acid in buffer and incubated at 37 °C for 40 min. Next, the mixture reaction developed the color by adding 2  $\mu$ l of 0.4 N sodium hydroxide (NaOH) and 20  $\mu$ l of 0.4 N potassium tetraborate tetra-hydrate and then incubated in the water bath at 100 °C for 3 min. The DMAB solution containing 0.4 g of DMAB dissolved in 35 ml of 100% acetic acid and 5 ml of 10 N hydrochloric acid (HCl) were prepared. Lastly, 600  $\mu$ l of DMAB was added into the mixture solution after cooling to room temperature and incubated at 37 °C for 20 min. The absorbance was measured at a wavelength of 585 nm (Sunrise TW, Tecan Trading AG, Männedorf, Switzerland).

**Table 1** Scientific name of HPs used in this study

Halophyte plants	Scientific name (Korean name)
HP. 1	<i>Salsola komarovii</i> (수송나물)
HP. 2	<i>Salicornia europaea</i> (통통마디)
HP. 3	<i>Sonchus brachyotus</i> (사태풀)
HP. 4	<i>Triglochin maritimum</i> (지채)
HP. 5	<i>Suaeda japonica</i> (칠면초)
HP. 6	<i>Argusia sibirica</i> (모래지치)
HP. 7	<i>Artemisia scoparia</i> (비쭉)
HP. 8	<i>Vitex rotundifolia</i> (순비기나무)
HP. 9	<i>Spartina anglica</i> (갯끈풀)
HP. 10	<i>Artemisia princeps</i> (쭉)
HP. 11	<i>Elymus mollis</i> (갯그렁)
HP. 12	<i>Calystegia soldanella</i> (갯메꽃)
HP. 13	<i>Rosa rugosa</i> (해당화)
HP. 14	<i>Asparagus oligoclonus</i> (방울비짜루)
HP. 15	<i>Glehnia littoralis</i> (갯방풍)
HP. 16	<i>Suaeda maritima</i> (해홍나물)
HP. 17	<i>Suaeda glauca</i> (나문재)
HP. 18	<i>Spergularia marina</i> (갯개미자리)
HP. 19	<i>Chenopodium glaucum</i> (취명아주)
HP. 20	<i>Atriplex gmelinii</i> (가는갯능쟁이)
HP. 21	<i>Peucedanum japonicum</i> (갯기름나무)
HP. 22	<i>Ischaemum antephoroides f. coreana</i> (갯쇠보리)

**Tyrosinase inhibitory assay**

Tyrosinase inhibitory effect of mushroom tyrosinase assay was determined as the previous reported by No et al. (1999). In brief, the mixture solution contained the following reagents: 110 µl of 0.1 M sodium phosphate buffer (pH 6.8), 10 µl of sample, 10 µl of tyrosinase in phosphate buffer (1500 units/ml), and 20 µl of 1.5 mM L-tyrosinase were added into the 96-well plates. The reaction mixtures were incubated at 37 °C for 12 min and the reaction was stopped afterward by incubating on ice for 1 min. The microplate reader at 490 nm was used to measure the absorbance of the mixture.

**Results**

**Antioxidant capacities of HPs extracts**

In this study, there are two antioxidant methods to assess antioxidant activities which are DPPH free radical and hydrogen peroxide scavenging assays. As depicted in Table 2, the antioxidant activities were performed and diluted to achieve the final concentration at 0.1 mg/ml for both assays. All 22 HP extracts were possessed antioxidant activities to a varying degree ranging from 11.39 ± 5.99% to 93.32 ± 0.46% and 3.95 ± 2.65% to 79.28 ± 1.40% of DPPH free radical and hydrogen peroxide assays, respectively. Among 22 HPs extracts, *I. coreana* extract exhibited the highest DPPH free radical scavenging effect (93.32 ± 0.46%) whereas of which 51.14 ± 3.86% hydrogen peroxide scavenging activity was observed. Furthermore, the next strongest antioxidant capacities (> 80%) expressed by DPPH free radical assay were 82.22 ± 0.23% of *S. komarovii* extract, 82.90 ± 0.23% of *C. soldanella* extract, 84.52 ± 1.39% of *R. rugosa* extract, and 84.84 ± 1.05% of *A. princeps* extract. Meanwhile, the highest hydrogen peroxide scavenging effect was found by *A. gmelinii* extract at 79.28 ± 1.40% and the comparative scavenging effect (> 60%) were found by *S. marina* and *C. glaucum* extracts showing 65.91 ± 3.91% and 64.32 ± 8.30%, respectively. The percentages against hydrogen peroxide of all HP extracts showed the lower scavenging effects when compared with that of DPPH free radical except *S. marina*, *C. glaucum*, and *A. gmelinii* (Table 2).

**Anti-collagenase and anti-elastase activities of HP extracts**

The collagenase and elastase inhibition effects of all HP extracts at the final concentration of 1 mg/ml were determined and elucidated as shown in Table 3. Firstly, it was notable that the highest collagenase inhibitory effect was possessed by *R. rugosa* extract (90.31 ± 0.05%) and its potential also employed good effect in anti-elastase activity (60.76 ± 3.58%). Apart from *R. rugosa* extract, there were other two of all examined HP extracts that exhibited fairly high activities in both collagenase and elastase inhibitory effects

with 62.24 ± 0.55% and 74.47 ± 0.18% of *A. sibirica* extract together with 52.86 ± 0.50% and 52.86 ± 0.50% of *C. glaucum* extract, respectively. Among 22 HPs, *S. europaea* extract performed the highest elastase inhibitory effect with 74.88 ± 4.84% whereas anti-collagenase activity could not be found in the concentration at 1 mg/ml. Notably, a good inhibition of elastase activity was illustrated by *S. anglica* extract (71.63 ± 2.2%) with the minor anti-collagenase effect observation (34.99 ± 0.55%).

**Anti-hyaluronidase activity of HP extracts**

The inhibitory effects of 22 HPs on hyaluronidase were evaluated as illustrated in Table 3. Among examined HPs, three of 22 extracts (*V. rotundifolia*, *A. oligoclonos*, and *S. glauca*) did not show any inhibition at 1 mg/ml. In contrast, *S. europaea* extract possessed the highest hyaluronidase inhibition up to 72.70 ± 1.24% and the significant inhibitions were demonstrated by *A. princeps*, *S. marina*,

**Table 2** DPPH free radical and hydrogen peroxide scavenging activities of 22 HP extracts

Sample	DPPH radical scavenging activity (%)	Hydrogen peroxide scavenging activity (%)
<i>Salsola komarovii</i>	82.90 ± 0.23	33.46 ± 0.32
<i>Salicornia europaea</i>	60.02 ± 5.39	50.56 ± 2.71
<i>Sonchus brachyotus</i>	76.30 ± 2.09	34.62 ± 2.80
<i>Triglochin maritimum</i>	24.58 ± 6.64	32.47 ± 1.04
<i>Suaeda japonica</i>	20.97 ± 0.34	9.24 ± 3.15
<i>Argusia sibirica</i>	32.92 ± 0.76	6.98 ± 3.53
<i>Artemisia scoparia</i>	39.16 ± 0.79	22.76 ± 2.83
<i>Vitex rotundifolia</i>	51.64 ± 0.68	23.04 ± 4.71
<i>Spartina anglica</i>	21.60 ± 0.79	21.17 ± 4.20
<i>Artemisia princeps</i>	84.84 ± 1.05	30.56 ± 4.76
<i>Elymus mollis</i>	28.65 ± 0.46	15.43 ± 1.33
<i>Calystegia soldanella</i>	82.22 ± 0.23	29.78 ± 3.97
<i>Rosa rugosa</i>	84.52 ± 1.39	37.38 ± 2.48
<i>Asparagus oligoclonos</i>	33.20 ± 0.77	11.97 ± 3.88
<i>Glehnia littoralis</i>	56.94 ± 1.88	9.18 ± 2.03
<i>Suaeda maritima</i>	11.39 ± 5.99	3.95 ± 2.65
<i>Suaeda glauca</i>	24.62 ± 0.97	21.90 ± 7.05
<i>Spergularia marina</i>	34.28 ± 1.05	65.91 ± 3.91
<i>Chenopodium glaucum</i>	44.30 ± 1.52	64.32 ± 8.30
<i>Atriplex gmelinii</i>	30.41 ± 2.50	79.28 ± 1.40
<i>Peucedanum japonicum</i>	38.68 ± 1.39	19.76 ± 0.99
<i>Ischaemum antephoroides f. coreana</i>	93.32 ± 0.46	51.14 ± 3.86

The values are expressed as the mean ± SD in triplicate experiments. The final concentration of tested samples was 0.1 mg/ml.

and *C. glaucum* extracts represented at  $51.71 \pm 0.33\%$ ,  $57.73 \pm 4.09\%$ , and  $57.73 \pm 2.36\%$ , respectively.

#### Anti-tyrosinase activity of HP extracts

The summarized inhibitory potential of HPs against mushroom tyrosinase at the final concentration of 1 mg/ml was demonstrated in Table 4. *S. anglica* extract presented the highest tyrosinase inhibitory as  $58.62 \pm 6.08\%$  in the comparison with the other HP extracts at 1 mg/ml. Moreover, there were 5 HPs that exhibited good inhibitory effects (> 50%) noticed by *A. sibirica* ( $58.16 \pm 7.24\%$ ), followed by *P. japonicum* ( $54.74 \pm 0.54\%$ ), *S. glauca* ( $52.85 \pm 0.00\%$ ), *S. maritima* ( $52.03 \pm 3.45\%$ ), and *A. gmelinii* extract ( $50.77 \pm 0.00\%$ ).

#### Discussion

Skin aging is one of the most concerned issues to all of us especially facial skin due to its unavoidable process. In daily life, the skin exposure to sunlight is considered to be the most significant factor that accelerates premature skin aging appearance called photoaging. Photoaging can trigger the extrinsic mechanism of ROS synthesis in the cells, and the overproduction of ROS may cause lipid peroxidation,

which is deleterious to the DNA, followed by cell damage and cell death. Therefore, the depletion of ROS generations defended by the antioxidant activity may integrate the postponement of skin aging problems (Thring et al. 2009; Wittenauer et al. 2015).

Halophyte is a plant with unique adaptive mechanisms that naturally survives in saline environments such as tidal flats, sand dunes, and coasts (Lee et al. 2018a, 2018b). Regarding the successful tolerance in hostile conditions, for example, salinity, UV radiation, and extreme temperature, HP is believed to allow valuable secondary metabolites including polyphenol, carotenoid, and vitamins to cope with those stress-overwhelmed environments (Jdey et al. 2017). It is also suggested that HP may offer a great unexplored source of bioactivity for the medicine or cosmetic development with economic benefit (Kim et al. 2016). In our present study, 22 HPs harvested along the coast of South Korea were screened and evaluated whether to provide antioxidant, anti-aging, as well as whitening potentials for cosmeceutical applications.

First of all, antioxidant capabilities of HPs were challenged, performed by DPPH free radical and hydrogen

**Table 3** Skin aging-related enzyme inhibitory effects of 22 HP extracts

Sample	Collagenase inhibition (%)	Elastase inhibition (%)	Hyaluronidase inhibition (%)
<i>Salsola komarovii</i>	$29.69 \pm 0.17$	$17.68 \pm 2.10$	$45.68 \pm 5.85$
<i>Salicornia europaea</i>	-	$74.88 \pm 4.84$	$72.70 \pm 1.24$
<i>Sonchus brachyotus</i>	-	$32.65 \pm 11.63$	$41.62 \pm 1.62$
<i>Triglochin maritimum</i>	$11.94 \pm 1.84$	$6.00 \pm 2.62$	$39.74 \pm 1.24$
<i>Suaeda japonica</i>	$42.61 \pm 1.22$	-	$12.52 \pm 5.00$
<i>Argusia sibirica</i>	$62.24 \pm 0.55$	$74.47 \pm 0.18$	$11.13 \pm 2.60$
<i>Artemisia scoparia</i>	$15.21 \pm 0.55$	$10.38 \pm 7.87$	$11.74 \pm 2.44$
<i>Vitex rotundifolia</i>	$31.28 \pm 1.79$	$3.96 \pm 10.38$	-
<i>Spartina anglica</i>	$34.99 \pm 0.55$	$71.63 \pm 2.21$	$18.00 \pm 2.90$
<i>Artemisia princeps</i>	$23.45 \pm 0.05$	$5.41 \pm 2.15$	$51.71 \pm 0.33$
<i>Elymus mollis</i>	$25.05 \pm 3.34$	-	$16.12 \pm 1.66$
<i>Calystegia soldanella</i>	$19.60 \pm 3.08$	-	$48.01 \pm 1.66$
<i>Rosa rugosa</i>	$90.31 \pm 0.05$	$60.76 \pm 3.58$	$48.67 \pm 1.51$
<i>Asparagus oligoclonos</i>	$24.89 \pm 0.05$	$0.09 \pm 7.45$	-
<i>Glehnia littoralis</i>	$13.06 \pm 0.05$	-	$1.70 \pm 1.99$
<i>Suaeda maritima</i>	$36.53 \pm 0.05$	$6.19 \pm 1.95$	$9.43 \pm 1.50$
<i>Suaeda glauca</i>	$42.90 \pm 0.05$	$15.49 \pm 3.44$	-
<i>Spergularia marina</i>	$31.60 \pm 2.81$	$31.60 \pm 2.81$	$57.73 \pm 4.09$
<i>Chenopodium glaucum</i>	$52.86 \pm 0.50$	$52.86 \pm 0.50$	$57.73 \pm 2.36$
<i>Atriplex gmelinii</i>	$7.73 \pm 0.67$	$7.73 \pm 0.67$	$40.91 \pm 3.36$
<i>Peucedanum japonicum</i>	$36.89 \pm 1.86$	$36.89 \pm 1.86$	$30.45 \pm 0.68$
<i>Ischaemum antephoroides f. coreana</i>	$24.87 \pm 1.10$	$24.87 \pm 1.10$	$36.14 \pm 3.15$

The values are expressed as the mean  $\pm$  SD in triplicate experiments. The final concentration of tested samples was 1 mg/ml.

**Table 4** Tyrosinase inhibitory effect of 22 HP extracts

Sample	Tyrosinase inhibition (%)
<i>Salsola komarovii</i>	16.70 ± 1.84
<i>Salicornia europaea</i>	21.04 ± 2.04
<i>Sonchus brachyotus</i>	14.31 ± 4.83
<i>Triglochin maritimum</i>	20.02 ± 4.68
<i>Suaeda japonica</i>	21.03 ± 13.17
<i>Argusia sibirica</i>	58.16 ± 7.24
<i>Artemisia scoparia</i>	39.85 ± 2.65
<i>Vitex rotundifolia</i>	49.81 ± 4.65
<i>Spartina anglica</i>	58.62 ± 6.08
<i>Artemisia princeps</i>	43.64 ± 2.77
<i>Elymus mollis</i>	31.18 ± 2.94
<i>Calystegia soldanella</i>	12.16 ± 7.99
<i>Rosa rugosa</i>	38.07 ± 5.53
<i>Asparagus oligoclonus</i>	19.37 ± 4.98
<i>Glehnia littoralis</i>	46.75 ± 3.45
<i>Suaeda maritima</i>	52.03 ± 3.45
<i>Suaeda glauca</i>	52.85 ± 0.00
<i>Spergularia marina</i>	18.08 ± 13.60
<i>Chenopodium glaucum</i>	20.90 ± 2.72
<i>Atriplex gmelinii</i>	50.77 ± 0.00
<i>Peucedanum japonicum</i>	54.74 ± 0.54
<i>Ischaemum antephoroides f. coreana</i>	21.54 ± 9.79

The values are expressed as the mean ± SD in triplicate experiments. The final concentration of tested samples was 1 mg/ml.

peroxide scavenging assays (Table 2). DPPH is a relatively stable free radical compound which allows a short and simple method of screening anti-radical or hydrogen donor capacity of the suspicious unknown (Kim et al. 2016; Lee et al. 2004). Meanwhile, hydrogen peroxide is an unstable substance which can be provoked from almost all of the various oxidative stressors. It is also associated to form hydroxyl and singlet oxygen radicals causing lipid peroxidation, cell damage, as well as cell senescence (Ko et al. 2015; Heo et al. 2005). Both DPPH and hydrogen peroxide scavenging assays are the commonly used methods to evaluate the antioxidant activity of natural extracts and their compounds (Kim et al. 2010).

In our results, 5 of 22 HPs (*S. komarovii*, *A. princeps*, *I. coreana*, *C. soldanella*, and *R. rugosa*, extracts) showed comparatively strong antioxidant activities between 82.90 ± 0.23% and 93.32 ± 0.46% inhibition measured by DPPH assay. Among them, *I. coreana* extract possessed the highest DPPH free radical scavenging effect up to 93.32 ± 0.46% at the concentration of 0.1 mg/ml (Table 2). Likewise, the previous study of Kim et al. (2010)

found that the ethanol extract of *Artemisia princeps* (HP. 10) demonstrated the effective scavenging activities by DPPH and ABTS assays along with a good anti-obesity effect on 3T3-L1 preadipocyte cells. Notably, in the investigation of Lee et al., *R. rugosa* (HP. 13) was found to provide a strong DPPH scavenging activity, and another study by Zheng et al. also indicated that *R. rugosa* possessed the most powerful antioxidant potential evaluated by DPPH and ABTS assays among 65 edible flowers (Kim et al. 2016; Zheng et al. 2018). Moreover, there have been few investigations referring to the antioxidant activities of *S. komarovii*, *A. princeps*, and *R. rugosa* (Carvalho et al. 2011; Kim et al. 2018; Lee et al. 2012; Youwei and Yonghong 2007). On the other hand, *A. gmelinii* extract would be recommended as hydrogen peroxide free radical defense. However, there has been no previous study related to the hydrogen peroxide scavenging effect of *A. gmelinii*.

ECM is the infrastructure foundation of skin which consists of various components such as microfibril, proteoglycan, collagen, elastin fiber, as well as HA. During the maturation process, the transformations of those ECM structural components (the exiguous distribution and decrease of collagen, the shortening and absence of elastin including loss of HA content) are represented by atrophied skin, dryness, wrinkling, and sagging skin appearance (Bauman 2004; Paliwal et al. 2014). Tyrosinase, an enzyme in melanogenesis, plays a key role in melanin production. The overproduction of melanin is responsible for the hyperpigmentation events such as melasma, freckles, ephelides, and senile lentigines. Consequently, the suppression of skin-related enzymes (elastase, collagenase, hyaluronidase, and tyrosinase) which predominantly integrate the degradation of those ECM components including the melanin overproduction is believed to be a key strategy in providing good skin integrity and youthful and magnificent skin. In this present study, the inhibitory effects of photoaging, like anti-collagenase, anti-elastase, anti-hyaluronidase, and anti-tyrosinase, of 22 HPs were explored at the final concentration of 1 mg/ml as summarized in Tables 3 and 4.

Our screening found that the strongest anti-hyaluronidase (72.70 ± 1.24%) along with anti-elastase (74.88 ± 4.84%) activities among evaluated HPs were occupied by *S. europaea* extract. The results presumably recommended that *S. europaea* may contribute an excellent anti-wrinkle activity by interrupting the degradation of elastin and HA underneath the skin. *S. europaea* is one of the succulent euhalophyte plants that is used as a folk remedy for obesity, diabetes, and cancer (Aghaleh et al. 2010; Won et al. 2017). Recently, some investigation revealed its health benefits as anti-inflammatory, anti-bacterial, and anticoagulating agents (Kim et al.

2016; Lee et al. 2018a, 2018b). However, the inhibitory effect screening of the Jeju plant by Moon et al. (2010) unlikely found a slight inhibition of elastase, while no inhibitory effect of tyrosinase was detected at the concentration of 500 µg/ml.

Aside from a good DPPH scavenging effect as described above, *R. rugosa* also presented the highest anti-collagenase (90.31 ± 0.05%) and high anti-elastase activities (60.76 ± 3.58%) as well as the moderate inhibitions against hyaluronidase and tyrosinase as 48.67 ± 1.51% and 38.07 ± 5.5%, respectively. There were several previous studies mentioned about the splendid antioxidant activities by *R. rugosa* and the other results by Olech et al. that revealed the effective hyaluronidase inhibition of *R. rugosa* extracts is probably due to its the richness in polyphenol content (Ng et al. 2004; Olech et al. 2017). Nonetheless, another finding suggested that the ethanol extraction of *Rosa hybrida*, a plant species belonging to the same family with *R. rugosa*, provided the anti-elastase and anti-tyrosinase activity as a candidate for the improvement of skin aging (Choi et al. 2015).

Among the 22 HP extracts, *S. anglica* revealed the best tyrosinase inhibition activity (58.62 ± 6.08%) together with a comparably high elastase inhibitory effect (71.63 ± 2.21%) at 1 mg/ml. *S. anglica* was firstly discovered in 1963; it provides various ecological and economic benefits; for example, accretion for reclamation, amelioration of saline soils, animal fodder, and especially, seashore stabilization (Qin et al. 1998). However, our present study gives the first evidence for the screening of anti-aging potential in *S. anglica*.

Additionally, *A. sibirica*, Siberian sea rosemary, offered an acceptable inhibitory effect of both ECM-deteriorated enzymes (62.24 ± 0.55% of collagenase and 74.47 ± 0.18% of elastase). According to this, we implied that *A. sibirica* may have potential as an anti-wrinkle candidate. Nevertheless, *A. sibirica* extract indeed manifested high anti-tyrosinase detected by mushroom tyrosinase assay (58.16 ± 7.24%) compared with other HPs (Table 4). To the authors' knowledge, its effect on anti-aging potential has not yet been clarified.

It is vital to note that our screening results are the preliminary demonstration of antioxidant and anti-aging activities. However, to introduce it as a candidate in cosmeceutical application, a future study is needed. Further investigations including 50% inhibitory concentration (IC<sub>50</sub>) and in vitro cell-based experiment, chemically, biologically, and pharmaceutically are required.

## Conclusion

Our present study revealed the anti-aging activity explorations of 70% ethanol extracts of 22 HPs collected along the coast of South Korea. All HPs were assessed by

DPPH and hydrogen peroxide scavenging, anti-elastase, anti-collagenase, anti-hyaluronidase, and anti-tyrosinase assays. From our findings, the highest scavenging effects of *I. coreana* and *A. gmelinii* extracts would be introduced as antioxidant agents against DPPH free radical and hydrogen peroxide, respectively. On the other hand, the strongest anti-wrinkle potentials of examined HPs are provided by *S. europaea* (anti-elastase and anti-hyaluronidase effects) and *R. rugosa* (anti-collagenase effect), whereas *S. anglica* extract may be an available source of tyrosinase inhibition. In conclusion, all of these results may recommend the guidance of conceivable HPs in terms of antioxidant, anti-wrinkle, and whitening agent for cosmeceutical development. It is noteworthy that the supplementary experiments of examined HPs such as cell-based experiments and the adverse effects should be more investigated.

## Abbreviations

AAAPN: N-Succinyl-Ala-Ala-Ala-p-nitroanilide; ABTS: 2,2-Azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid; DMAB: 4-(dimethyl amino)benzaldehyde; DMSO: Dimethyl sulfoxide; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; ECM: Extracellular matrix; HA: Hyaluronic acid; HP: Halophyte plant; PBS: phosphate buffered saline; IC<sub>50</sub>: 50% Inhibitory concentration; ROS: Reactive oxygen species

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## Authors' contributions

CJ, YD, JML, HSK, SCK, and SHL constituted and designed the experimental plan. CJ, OH, STI, YJ, and SWM conducted the experimental work. CJ wrote the manuscript. YD and SHL edited the paper. All authors discussed and approved the final manuscript.

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## Competing interests

The authors declare that they have no competing interests.

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