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Effect of antioxidant and anti-inflammatory on bioactive components of carrot (*Daucus carota* L.) leaves from Jeju Island

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Abstract

The present study evaluated the potential of carrot (*Daucus carota* L.) leaf, a non-edible part of carrots, which are among the most consumed vegetables worldwide. The antioxidant activities of 70% ethanol extract (EEC) and hot water extract (HEC) of carrot leaves were compared. The results revealed that the total polyphenol content, total flavonoid content, and DPPH and ABTS radical scavenging activities were higher in EEC than in HEC. Both extracts protected the cells against H_2O_2-induced toxicity and markedly reduced the levels of reactive oxygen species in RAW 264.7 cells. Moreover, pretreatment of RAW 264.7 cells with EEC and HEC prior to H_2O_2 (500 μM) exposure increased superoxide dismutase and glutathione peroxidase activities in these cells. Notably, EEC and HEC increased intracellular catalase activity by 36.77 and 6.39 times, respectively. Compared to HEC, EEC remarkably inhibited the production of lipopolysaccharide-induced nitric oxide and reduced the gene expression of IL-6, IL-1β, iNOS, COX-2, and TNF-α. Comparative analysis of the composition of the extracts using HPLC–UV suggested notably higher contents of catechin, chlorogenic acid, caffeic acid, rutin, quercetin, and cynaroside in EEC than in HEC. Collectively, these results imply that carrot leaves are a potentially beneficial natural source of antioxidants and anti-inflammatory compounds in functional foods.

Keywords Carrot leaves, *Daucus carota* L. leaves, Antioxidant, Anti-inflammatory, Phenolic compounds, Cynaroside

Introduction

As a substantial relationship exists between diet and chronic diseases, the propensity to pursue a healthy diet is steadily increasing [1, 2]. Fruits and vegetables are a source of bioactive substances that are beneficial to health and lead to protection against various chronic diseases [2]. Previous studies have shown that increased fruit and vegetable intake is associated with reduced mortality [3, 4]. Furthermore, aging, resulting from oxidation and inflammation, progressively increases the risk of death from chronic diseases. Vegetables are well-known natural medicines that prevent aging [5, 6]. Particularly, root vegetables such as radishes, carrots, sweet potatoes, burdocks, onions, and ginger are rich in vitamin C, vitamin A, and carotenoids, which contribute to their potential bioactive effects that reduce mortality rates [7]. Importantly, root vegetables can supply and accumulate water and nutrients from the leaves to the roots in the ground [8]. Additionally, several benefits of the aerial parts of root vegetables have been previously reported. The leaves and stems of discarded radishes contain various phenolic constituents with high radical scavenging activity [9], and the leaves of sweet potato, a root vegetable, have high protein and polyphenol content [10]. Moreover, the iron chelate content, which acts as an

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antioxidant by scavenging reactive oxygen species (ROS), is higher in ginger leaves than in ginger roots [11].

Carrot (*Daucus carota* L.) is a popular root vegetable cultivated worldwide and an economically important vegetable crop in terms of production area and market size. [12, 13]. Several studies have revealed that carrots, owing to their bioactive compounds, have a variety of antioxidant, anticancer, and anti-inflammatory properties that prevent adverse health effects [14–17]. The roots of commonly eaten carrots contain large amounts of carotenoids and various other bioactive components [18, 19]. Moreover, similar to other root vegetables, the less useful aerial parts of carrots contain as many bioactive substances as the roots [20–24]. Carrot leaves contain the polyunsaturated fatty acid α-linolenic acid, which has anti-inflammatory effects [21, 22, 25]. Furthermore, the essential oil obtained from carrot leaves is remarkably rich in α-pinene, sabinene, and germacrene, which are known to have antimicrobial effects [23, 26, 27]. Luteolin is also present in subcritically extracted carrot leaves and can contribute to various effects such as antioxidant, anti-inflammatory, and anticancer effects [24, 28, 29]. As research on carrot leaves has increased, carrot leaves and stems, which are relatively discarded compared to the roots, can become a potential source of nutrients.

Jeju Island, Korea, which is made of volcanic soil with good drainage, is suitable for producing high-quality carrots; 60% of the carrots grown in Korea are grown on Jeju Island [30]. If the above-ground parts of the carrots, such as the leaves and stems, which are discarded after harvesting on Jeju Island, can be used in proportion to the number of carrots cultivated, the production value of carrots can be improved. In this context, an investigation was conducted to evaluate the antioxidant and anti-inflammatory effects of the above-ground parts of carrots grown on Jeju Island, Korea, using ethanol and water, which are the most accessible solvents for extracting natural products and identifying a wide range of compounds [31, 32].

Results and discussion

The extraction yields, total polyphenol and flavonoid content

The yield of carrot (*Daucus carota* L.) leaves treated with 70% ethanol and hot water is shown in Table 1. The yields of 70% ethanol extract (EEC) and hot water extract (HEC) of carrot leaves were calculated to be 25.80% and 29.48%, respectively, and the yield of the HEC was slightly higher. Previous studies have shown that water, which is polar, has a dielectric constant similar to that of organic solvents when heated, resulting in higher extraction yields than ethanol [33].

Table 1 Extraction yields and total polyphenol and flavonoid contents of extracts from carrot leaves

Extract	Yield (%)	TPC (mg GAE/g)	TFC (mg RE/g)
EEC	25.80	3.26 ± 0.20	1.80 ± 0.17
HEC	29.48	1.20 ± 0.01	0.80 ± 0.06

Values are mean ± SD ($n = 3$)

GAE gallic acid equivalent, *RE* rutin equivalent, *SD* standard deviation

The various phytochemicals found in plants and fruits are related to their antioxidant capacities. In particular, phenolic compounds, including flavonoids, are known to exhibit many physiological activities along with antioxidant effects [34]. As result of measuring the total polyphenol content (TPC), the EEC value was 3.26 ± 0.20 mg GAE/g, which was higher than HEC with 1.20 ± 0.01 mg GAE/g (Table 1). Also, HEC (0.80 ± 0.06 mg RE/g) showed a lower total flavonoid content (TFC) than EEC (1.80 ± 0.17 mg RE/g). These results indicate that ethanol extraction of carrot leaves can ensure a higher of phenol content than hot water extraction.

DPPH and ABTS radical scavenging activity

In vitro assays, such as DPPH and ABTS scavenging, are antioxidant experiments used to evaluate free radical scavenging activities [35]. As presented in Fig. 1A, the DPPH radical scavenging activity of both carrot leaf extracts increased in a concentration-dependent manner; however, the DPPH radical scavenging activity of EEC was the highest. The EEC also showed greater antioxidant ability than HEC in ABTS radical scavenging activity (Fig. 1B). EC_{50} values obtained for each assay are listed in Table 2. Through the EC_{50} values of EEC (1.35 ± 0.13 mg/mL for DPPH, 0.80 ± 0.10 mg/mL for ABTS), it can be seen that EEC has stronger radical scavenging effect than HEC (7.39 ± 0.93 mg/mL for DPPH, 1.98 ± 0.01 mg/mL for ABTS).

Cytotoxicity protection and reactive oxygen species inhibition effect of carrot leaves in H_2O_2-induced RAW 264.7 Cells

As a reactive oxygen species (ROS), H_2O_2 is one of the major cause of oxidative damage as well as causing various pathological processes [36]. H_2O_2 has high soluble in lipid and aqueous environments, therefore it is free to diffuse into cells and can regulate cell functions or induce cell death [37]. Exposure of RAW 264.7 cells to H_2O_2 resulted in decreased cell viability in a dose-dependent and time-dependent manner (Additional file 1: Fig. S1). However, the cytotoxicity induced by H_2O_2 was restored by carrot leaf extract (Fig. 2A). In particular, EEC exerted a greater protective

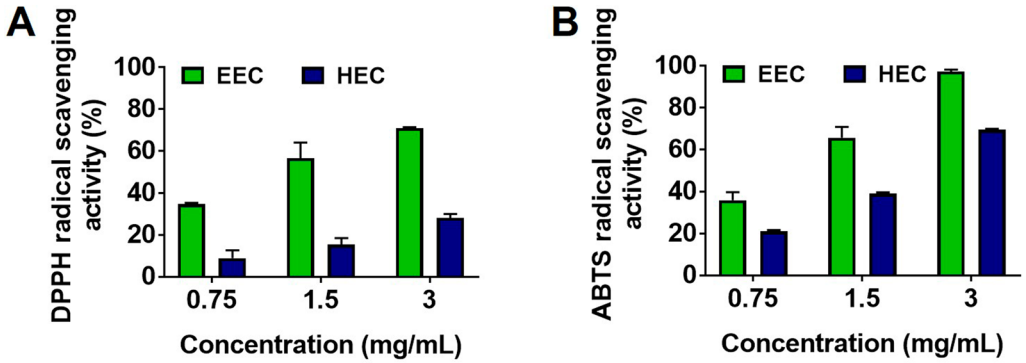

Fig. 1 Antioxidant activity of the carrot leaf extracts, **A** DPPH radical scavenging activity and **B** ABTS radical scavenging activity. Values are mean ± SD ($n = 3$)

Table 2 EC_{50} values of extracts from carrot leaves

Extract	DPPH (mg/mL)	ABTS (mg/mL)
EEC	1.35 ± 0.13	0.80 ± 0.10
HEC	7.39 ± 0.93	1.98 ± 0.01

Values are mean ± SD ($n = 3$)

effect on protecting RAW 264.7 cells than HEC. As cell death can be caused by oxidative stress accumulated due to H_2O_2 [38], we examined whether the two carrot leaf extracts that restored cell viability could affect intracellular ROS levels. As shown in Fig. 2B, H2O2 treatment alone increased intracellular ROS production

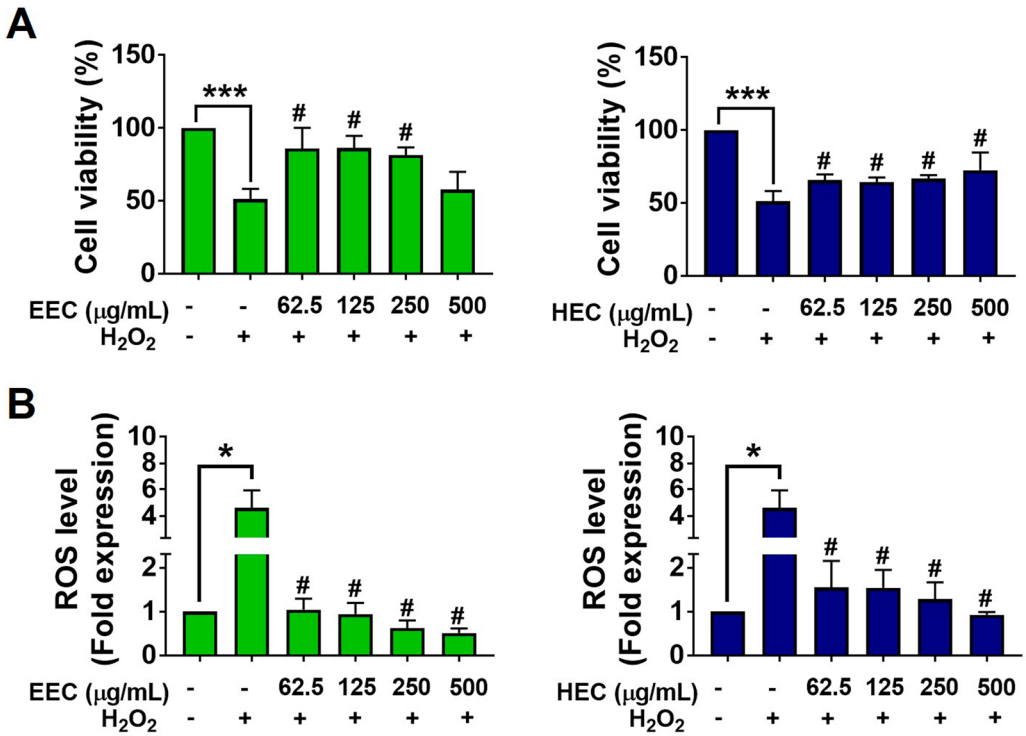

Fig. 2 Cytotoxic effects and intracellular reactive oxygen species (ROS) production levels in H_2O_2-induced RAW 264.7 cells. The cells were pretreated with carrot leaf extract at each concentration prior to exposure to 500 μM H_2O_2. **A** Cytotoxicity was assessed by MTT assay. **B** Intracellular ROS levels were measured using FACS. Values are presented as mean ± SD ($n = 3$). $^{*}p < 0.05$, $^{***}p < 0.001$ vs. untreated control group; $^{\#}p < 0.05$ vs. H_2O_2 only treated group

by 4.60 times compared to that in the control group, whereas in the experimental group pretreated with carrot leaf extract, H_2O_2-induced ROS production was remarkably reduced in a concentration-dependent manner. In addition, when the intracellular ROS production inhibition or ROS scavenging ability of the carrot leaf extract itself was measured, it significantly decreased rapidly from an extract concentration of 62.5 μg/mL treatment group (Additional file 1: Fig. S2). These observations suggest that carrot leaf extract exhibits a protective effect against H_2O_2-induced cytotoxicity and an excellent inhibitory effect on intracellular ROS, and that the antioxidant capacity of EEC is superior to that of HEC.

Effect of carrot leaf extract on cellular antioxidant enzyme activity

Catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) are the most important enzymes in the cellular oxidative stress defense system and are antioxidants that protect against oxidative damage caused by free radicals [39]. Catalase relieves oxidative stress by degrading H_2O_2 into oxygen and water molecules [40]. SOD is a front-line antioxidant enzyme that catalyzes the dismutation of superoxide anion free radical (O_2^-) into molecular oxygen (O_2) and H_2O_2, preventing the production of more reactive compounds, such as peroxynitrite ($ONOO^-$) or hydroxyl radicals (·OH) [41, 42]. GPxs are a family of oxidoreductases that convert H_2O_2 to water and catalyze the reduction of peroxide radicals to alcohols and oxygen [43]. Catalase, SOD, and Gpx enzyme activities were measured to confirm the effect of ROS-reducing carrot leaf extract on antioxidant enzyme activity. EEC exponentially increased catalase activity by more than 36.77 times in RAW 264.7 cells treated with 500 μM H_2O_2 (Fig. 3A). HEC also increased catalase activity 6.39 times in a dose-dependent manner in the same concentration range as EEC. In contrast, SOD activity after carrot leaf extract treatment did not increase as much as catalase activity. EEC increased SOD activity in H_2O_2-induced RAW 264.7 cells up to a concentration of 125 μg/mL, but slightly decreased at higher concentrations, and HEC induced an increase in SOD activity in a dose-dependent manner (Fig. 3B). The increase in GPx activity in cells pretreated with the extract was not remarkable, probably because intracellular GPx activity was not significantly reduced by H_2O_2 treatment (Fig. 3C). Among the antioxidant enzymes, catalase activity was most markedly increased by carrot leaf extract treatment, suggesting that leaf metabolites may act as catalase activators or affect enzyme protein levels [44]. These data provide strong evidence of antioxidant efficacy and support the antioxidant potential of carrot leaves reported in previous studies [45].

Restoration of NRF2 and HO-1 by extract of carrot leaves

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor responsible for regulating cellular redox balance by inducing the expression of heme oxygenase-1 (HO-1), SOD, and GPx in mammals [46]. HO-1, a gene regulated by Nrf2, participates in heme degradation to produce biliverdin and free iron as metabolites that can neutralize its pro-oxidative effects [47]. In many studies, increased Nrf2 and HO-1 expression is used to verify antioxidant activity [48–50], so to evaluate the antioxidant pathway by carrot leaf extract, the expression levels of Nrf2 and HO-1 were confirmed by Western blot. As shown in Fig. 4 and Additional file 1: Fig. S3, Western blot analysis revealed that treatment with carrot leaf extract increased the expression of Nrf2 in total and cytosolic fractions despite H_2O_2 pretreatment. In the nuclear fraction, induced oxidative stress caused by H_2O_2 promoted Nrf2 translocation, and EEC and HEC treatment showed that Nrf2 expression was maintained. Moreover, HO-1 expression significantly increased in EEC and HEC. Taken together, these results demonstrated that carrot leaves induce potent antioxidant effects by upregulating the Nrf2/HO-1 pathway.

Anti-inflammatory effect of carrot leaves in LPS-induced RAW 264.7 cells

Nitric oxide (NO) is an inflammatory mediator that causes inflammation owing to its overproduction under abnormal circumstances, causing cell damage along with ROS, resulting in various diseases [51]. Lipopolysaccharide (LPS) is an activator that mediates the production of pro-inflammatory cytokines, and its efficacy in inhibiting NO production in LPS-induced RAW 264.7 cells is generally a criterion for its anti-inflammatory effect [52]. When NO production was induced by exposing RAW 264.7 cells to 1 μg/mL LPS, the NO production was significantly decreased in a concentration-dependent manner by EEC (Fig. 5A). However, NO production in RAW 264.7, cells treated with HEC, was marginally reduced compared to EEC. A previous study comparing ethanol and water extracts demonstrated that NO production was further inhibited by ethanol extracts [53]. Similarly, it can be assumed that EEC contains more components than HEC, which can maximize the anti-inflammatory efficacy. Additionally, Fig. 5B showed that EEC significantly reduced the expression of several marker genes involved in anti-inflammatory. However, at

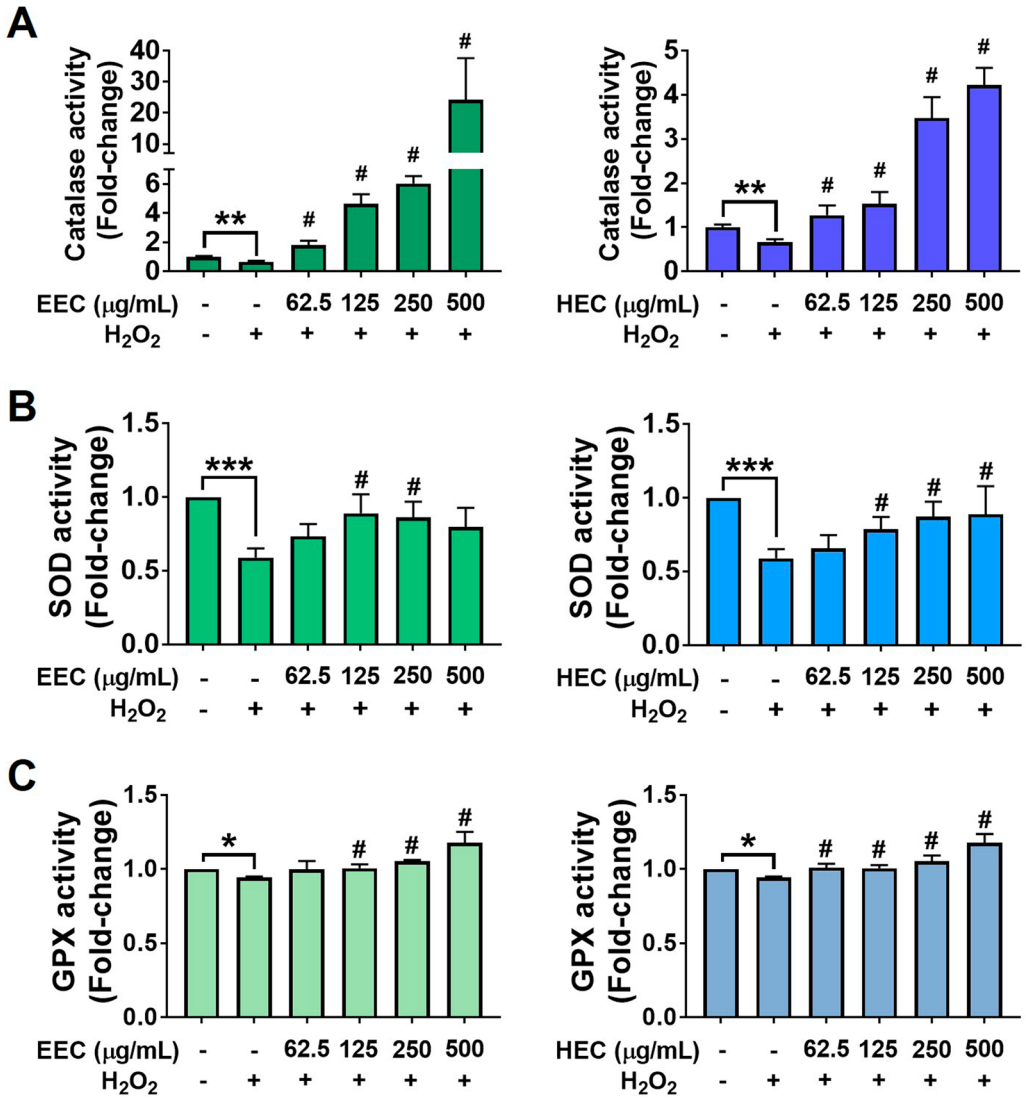

Fig. 3 Antioxidant enzyme activity in H_2O_2-induced RAW 264.7 cells. The cells were pretreated with extracts of carrot leaves at each concentration prior to exposure to 500 μM H_2O_2. **A** Catalase, **B** superoxide dismutase, and **C** glutathione peroxidase. Values are presented as mean ± SD (n = 3). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. untreated control group; $^{\#}p < 0.05$ vs. H_2O_2 only treated group

the same concentration range, HEC did not affect the expression levels of these marker genes (Data not shown).

HPLC analysis of extracts from carrot leaves

According to previous studies on carrot leaves, the quercetin content was very abundant in methanol extraction, and the chlorogenic acid content was overwhelming in the 50% ethanol extract [45, 54]. As the content of biological compounds can vary depending on the extraction conditions as well as the environment, such as soil and climate [55, 56], the content of representative phytochemicals contained in Jeju carrot leaf extract grown in volcanic soil was analyzed, and the components of EEC and HEC were compared. HPLC analysis using ten phenolic and flavonoid compounds, catechin, rutin, quercetin, and cymaroside, were found in both extracts (Table 3). Especially, the rutin content in EEC (3738.50 ± 74.90 μg/g DW) was remarkably higher than that in HEC (301.56 ± 42.22 μg/g DW). The quercetin content of EEC (236.53 ± 34.33 μg/g DW) was very high compared to HEC (56.01 ± 7.96 μg/g DW). Rutin and quercetin are commonly found in many fruits and vegetables, including broccoli, apples, and tomatoes [57]. The abundant content of the compounds found in

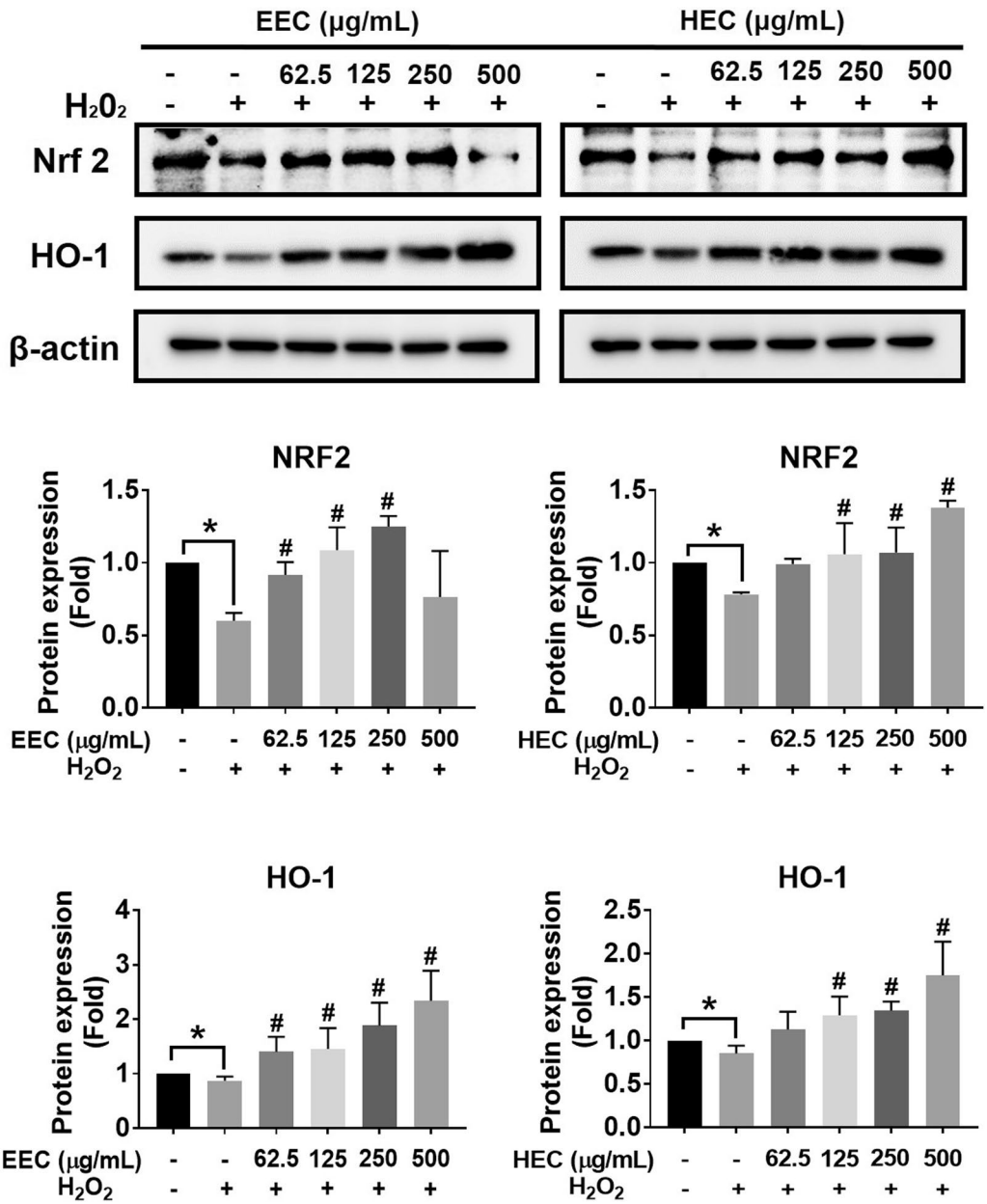

Fig. 4 Effect of carrot leaf extract on Nrf2/HO-1 pathways in H_2O_2-induced RAW 264.7 cells. The cells were pretreated with extracts of carrot leaves at each concentration prior to exposure to 500 μM H_2O_2. All protein levels were normalized compared to β-actin as the control using image J. Values are presented as mean ± SD (n = 3). $^{*}p < 0.05$ vs. untreated control group; $^{\#}p < 0.05$ vs. H_2O_2 only treated group

EEC is likely to contribute to its superior antioxidant effect compared to that of HEC. Actually, a Pearson product-moment correlation analysis indicated a strong positive relationship rutin and quercetin with the TPC ($R^2 = 0.990$ and $R^2 = 0.949$), TFC ($R^2 = 0.979$ and $R^2 = 0.966$), DPPH· ($R^2 = 0.990$ and $R^2 = 0.978$), ABTS· radical scavenging activity ($R^2 = 0.993$ and $R^2 = 0.977$), Catalase activity ($R^2 = 0.930$ and $R^2 = 0.906$) and NO production ($R^2 = 0.998$ and $R^2 = 0.973$) (Additional file 1: Table S1). Chlorogenic acid was only detected in EEC, whereas caffeic acid was detected only in HEC. Chlorogenic acid is known to exhibit anti-inflammatory and antioxidants [58]. The anti-inflammatory effect of carrot leaves may have been exerted by the chlorogenic acid uniquely present in the EEC.

Previous studies have shown that cynaroside, a compound isolated from the ethyl acetate extract of carrot leaves, exhibits anti-inflammatory activity

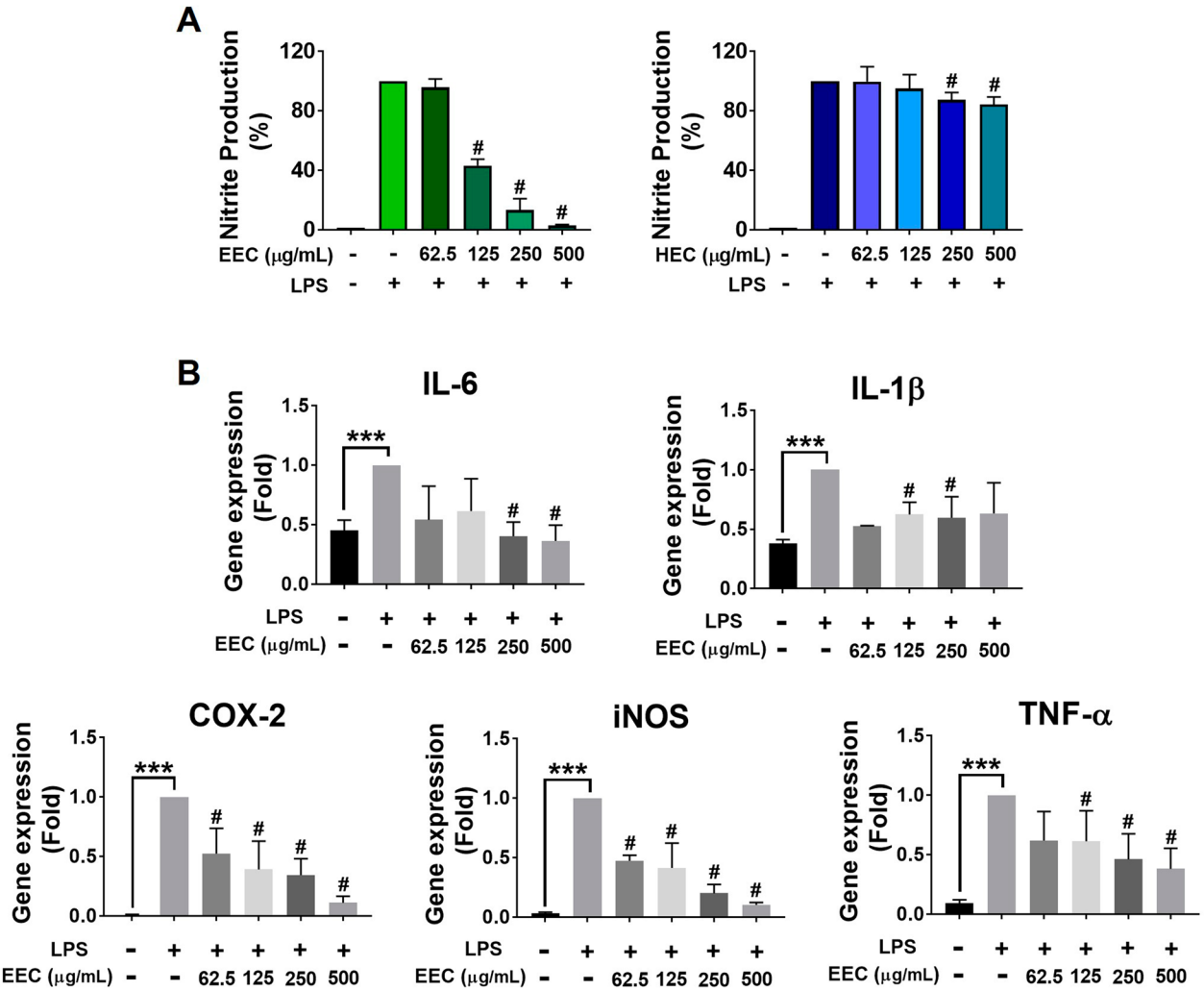

Fig. 5 Anti-inflammatory effects of carrot leaves on LPS-induced RAW 264.7 cells. Cells were pretreated with carrot leaf extract at each concentration prior to exposure to 1 μg/mL LPS. **A** Nitric oxide (NO) production levels were measured using the NO assay. **B** qRT-PCR was used to determine gene expression of anti-inflammatory markers after EEC treatment. Values are presented as the mean ± SD (n = 3). ***$p < 0.001$ vs. untreated control group; $^{\#}p < 0.05$ vs. LPS only treated group

Table 3 Phenolic and flavonoid compounds content of extracts from carrot leaves

Compound (μg/g DW)	EEC	HEC
Gallic acid	ND	ND
4-Hydroxybenzoic acid	ND	ND
Catechin	377.63 ± 43.59	353.14 ± 35.86
Chlorogenic acid	3208.68 ± 53.46	ND
Caffeic acid	ND	65.85 ± 11.22
p-Coumaric acid	ND	ND
Ferulic acid	ND	ND
Rutin	3738.50 ± 74.90	301.56 ± 42.22
Quercetin	236.53 ± 34.33	56.01 ± 7.96
Cynaroside	1150.13 ± 46.86	135.22 ± 13.64

Values are mean ± SD ($n = 3$)

DW dry weight, *ND* no detection

[59]. Cynaroside was analyzed for extracts of carrot leaves, and these compounds were detected at a higher level in EEC than HEC (1150.13 ± 46.86 μg/g DW and 135.22 ± 13.64 μg/g DW, respectively), which had excellent anti-inflammatory efficacy (Table 3 and Additional file 1: Fig. S4). Also, as shown in the Additional file 1: Table S1, there was a significant correlation between cynaroside and NO production ($R^2 = 0.996$). These results suggest that the compounds of carrot leaves from Jeju are different from those of previously reported carrot leaves, and may differ depending on the extraction solvent and conditions. The compounds identified through HPLC analysis provided a solid basis for supporting the antioxidant and anti-inflammatory effects of Jeju carrot leaves.

Materials and methods

Sample preparation and extraction

Aerial parts of carrots (*Daucus carota* L.) harvested in February–March 2022 were obtained from Gujwa-eup, Jeju Island, South Korea. Carrot leaves were used after being freeze-dried. Ground carrot leaf powder (20 g) was extracted with 1 L of 70% ethanol and sonicated thrice for 30 min. In another experiment, ground carrot leaf powder (20 g) was mixed with 1 L distilled water (DW) and heated for 10 min at 121 °C in an autoclave. Then, both mixtures were filtered using Whatman No.2 filter paper, evaporated in a vacuum rotary evaporator at 40 °C, and lyophilized to obtain 70% ethanol extract of carrot leaves (EEC) and hot water extract of carrot leaves (HEC), respectively.

Determination of total polyphenol content

The samples (125 µL) were added to 1.375 mL of DW, and 500 µL of Folin-Ciocalteu's reagent was added. After incubation for 3 min in the dark, 1 mL of 10% Na_2Co_3 was added and the mixture was allowed to react for 30 min in the dark. Absorbance was measured at 700 nm using a microplate reader. The results are presented as gallic acid equivalents (mg GAE/g).

Determination of total flavonoid content

The samples (40 µL) were added to 80 µL of DW and 6 µL of 5% $NaNO_2$ was added. After incubation for 5 min, 12 µL of 10% $AlCl_3$ was added and the mixture was allowed to react for 6 min. Then, 40 µL of 1N NaOH and 42 µL of DW were added to the reaction mixture, and the absorbance was measured at 510 nm using a microplate reader. The results are expressed as rutin equivalents (mg RE/g).

DPPH radical scavenging assay

Prior to the assay, a DPPH solution was prepared using 3 mM DPPH in 100% ethanol. Samples (40 µL) were added to 160 µL of 200 µM DPPH solution and incubated at 37 °C for 30 min. The absorbance was measured at 510 nm using a microplate reader. Catechin was used as a positive control. EC_{50} values were calculated using the GraphPad Prism 7.0 software (GraphPad Software, Inc., La Jolla, CA, USA).

ABTS radical scavenging assay

Before the assay, 7 mM ABTS solution in potassium persulfate was prepared and shaken in the dark for 20 h. Samples (100 µL) were added to 900 µL ABTS solution and allowed to react for 2 min. Absorbance was measured at 734 nm using a UV-1800 spectrophotometer (Shimadzu Co., Kyoto, Japan). α-tocopherol was used as the positive control. EC_{50} values were calculated using GraphPad Prism 7.0 software (GraphPad Software, Inc., La Jolla, CA, USA).

MTT assay

An MTT assay was performed to measure cell viability. Raw 264.7 cells, which are macrophages, were seeded (1×10^4 cell/well) in 96 well plate and incubated for 24 h. The cells were pre-treated with the samples for 6 h and exposed to 500 µM H_2O_2 for 3 h. The supernatant was removed, 100 µL of 1 mg/mL MTT solution was added to each well, and the cells were incubated at 37 °C for 3 h. After incubation, the MTT solution was discarded and 150 µL of dimethyl sulfoxide was added to each well for 1 h at room temperature in the dark. Absorbance was measured at 570 nm using a microplate reader.

Flow cytometry

Intracellular reactive oxygen species (ROS) levels were determined using H_2DCFDA. Raw 264.7 cells were seeded (20×10^4 cells/dish) in a 60 mm dish and incubated for 24 h. The cells were pre-treated with the samples for 6 h and exposed to 500 µM H_2O_2 for 1 h. Then, 10 µM H_2DCFDA was added to each dish for 20 min, and the collected cells were analyzed using a flow cytometer (BD Biosciences Co., Franklin, NJ, USA) in Bio-Health Materials Core-Facility, Jeju National University.

Catalase, superoxide dismutase and glutathione peroxidase activity

RAW 264.7 cells were seeded (20×10^4 cells/dish) in a 60 mm dish and incubated for 24 h. The cells were pre-treated with the samples for 6 h and exposed to 500 µM H_2O_2 for 1 h. Cells were collected using a buffer solution, and Catalase, Superoxide dismutase (SOD), and glutathione peroxidase (GPx) activity kits (Cayman Chemical Co., Ann Arbor, MI, USA) were used according to the manufacturer's instructions.

Nitric oxide assay

RAW 264.7 cells were seeded (10×10^4 cells/well) in 24 well plate and incubated for 18 h. Following incubation, cells were pre-treated with samples for 1 h, and 1 µg/mL LPS was added to each well, except for the control well, and incubated at 37 °C for 24 h. Next, 100 µL of the supernatant was transferred to a new 96 well plate, and 100 µL of Griess reagent was added. The mixture was incubated for 10 min in the dark and the absorbance was measured at 545 nm using a microplate reader.

Western blot

Following exposure to EEC and HEC for 24 h, RAW 264.7 cells were collected with RIPA buffer before sonication, and centrifuged for 25 min at 4 °C. Protein concentration was quantified using a BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA), and the lysates were subjected to 10% sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Following that, the resolved proteins were transferred to polyvinylidene diffuoride (PVD) membranes. After blocking with skim milk in Tris-buffered salin containing 0.1% Tween (TBST), the membranes were treated with the primary antibody for 24 h at 4 °C and then with a secondary antibody after washing with TBST. Protein bands were visualized using a ChemiDoc (Bio-Rad, Inc., Hercules, CA, USA) and quantified with β-actin as a control using an Image J software (National Institutes of Health, Bethesda, MD, USA). Primary antibodies were used at 1:1,000 dilutions (Nrf2 and HO-1 Cell Signaling Technology, Danvers, MA, USA), except β-actin (1:10,000, Cell Signaling Technology, Danvers, MA, USA), and secondary antibodies were used at 1:5,000 dilutions.

Quantitative real time PCR

RNA from RAW 264.7 cells treated with EEC and HEC was extracted using TRIzol reagent and used for complementary DNA synthesis. Real time PCR was performed using a thermal cycler (Takara Inc., Shiga, Japan). The results were quantified using the $2-\Delta\Delta Cq$ method [60] and GAPDH used as control. Primer sequences used were as follows: F-5′ATTCACCTCTTCAGAACGAATTG3′R-5′CCATCTTTGGAAGGTTCAGCTTG3′ for IL-6, F-5′AAGATGGCTTATTACAGTGGCAA3′R-5′GTCGGAGATTCGTAGCTCGA3′ for IL-β, F-5′TGATCTACCCTCCCCACGTC3′R-5′ACACACTCTGTTGTGCTCCC3′ for COX2, F-5′AGCAACTACTGCTGGTGGTG3′R-5′TCTTCAGAGTCTGCCCATTG3′ for iNOS, F-5′CTCGAGTGACAAGCCCGTAG3′R-5′ATCTGCTGGTACCACCAGTT3′ for TNF-α.

High performance liquid chromatography analysis

The high performance liquid chromatography (HPLC) was determined using a HPLC-ultraviolet detector (Shimadzu co., Kyoto, Japan) as previously described [61, 62]. EEC and HEC was diluted in 70% ethanol and DW, respectively. Phenolic compounds (gallic acid, 4-hydroxybenzoic aicd, catechin, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, rutin and quercetin) and cynaroside were diluted in methanol. (A) Acetic acid: methanol: water (2.5%: 2.5%: 95%, v/v/v) and (B) acetic acid: water: methanol (2.5%: 5%: 92.5%, v/v/v) used as mobile phase for phenolic compound analysis. The binary gradient elution mode was as follows: 0–10 min, 0–10% B; 10–48 min, 10–80% B, 48–58 min, 80–0% B. The column temperature and flow rate was maintained at 30 °C and 1.0 mL/min, respectively. The 20 µL of samples were injected, and detection wavelength was set to 280 nm. The analysis condition for cynaroside was indicated in Table 4.

Table 4 The HPLC condition for the quantitative analysis for cynaroside

Mobile phase	**(A)0.1% trifluoroacetic acid in water (B)0.1% trifluoroacetic acid in acetonitrile**	
Time (min)	**A (%)**	**B (%)**
0.0	90	10
5.0	90	10
25.0	50	50
35.0	70	30
40.0	5	95
45.0	5	95
55.0	90	10
Flow rate	1.0 mL/min	
Column temp	40 °C	
Detection length	190–600 nm@345 nm	
Injection vol	10 µL	

Statistical analysis

All experiments were performed in triplicate and analyzed using the GraphPad Prism software (version 7.0). Differences among groups were compared by one-way analysis of variance (ANOVA) using Tukey's method, or multiple t-tests using the Holm-Sidak method. Data are expressed as means ± standard deviation (SD) and statistical significance at $p < 0.05$.

Abbreviations

ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid
DPPH	2,2-Diphenyl-1-picryhydrazyl
EEC	70% Ethanol extract of carrot leaves
EC_{50}	The effective concentration of drug that causes 50% of the maximum response
GPx	Glutathione peroxidase
HEC	Hot water extract of carrot leaves
HPLC	High performance liquid chromatography
ROS	Reactive oxygen species
LPS	Lipopolysaccharide
NO	Nitric oxide

MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
TFC	Total flavonoid content
TPC	Total polyphenol content
SOD	Superoxide dismutase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-023-00786-2.

Additional file 1: Figure S1. Cytotoxicity of H_2O_2 induced Raw 264.7 cells. Cell viability was measured by MTT assay. Values are mean ± SD ($n = 3$). **Figure S2.** Levels of reactive oxygen species (ROS) production in Raw 264.7 cells. Intracellular ROS levels were measured using FACS. Values are mean ± SD ($n = 3$). *$p < 0.05$ vs. untreated control group. **Figure S3.** Cell fractionation of Nrf2 in H_2O_2-induced RAW 264.7 cells. The cells were pretreated with extracts of carrot leaves at each concentration prior to exposure to 500 μM H_2O_2. α-tubulin and Histone H3 were used as controls for the protein in cytoplasmic and nuclear fractions, respectively. Values are mean ± SD ($n = 3$). **Figure S4.** HPLC-UVD chromatogram of cynaroside composition. (A) cynaroside, (B) EEC and (C) HEC. **Table S1.** Correlations between the compounds and activities of carrot leaf extract.

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Author contributions
JSK: formal analysis; investigation; methodology; software; writing—original draft; writing—review and editing. JHL: investigation; funding acquisition. SKC: conceptualization; data curation; funding acquisition; project administration; resources; supervision; validation; visualization; writing—review and editing. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Competing interests
There are no competing interests to declare.

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