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Characterization of antioxidant and anti-inflammatory activities in the grains of collected *Sorghum bicolor* varieties

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Abstract The current study evaluated the presence and concentration of health-promoting phenolic antioxidants in sorghum seed accessions from seven regions in three different countries (Uzbekistan, Myanmar, and the United States). The free radical scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl assay was 13.10±2.37 μg/mL in the Uzbekistan sorghum seed extracts, demonstrating the highest antioxidant activity. Additionally, the total phenol content was the highest in the Uzbekistan sorghum seed extracts [157.87±4.04 mg GAE/g (gallic acid equivalents per gram)]. The total flavonoid content was 12.69±0.72 mg QE/g (quercetin equivalents per gram) and represents the highest value in the Myanmar collection. The Uzbekistan collection demonstrated the greatest potential for whitening activity, with the tyrosinase inhibition rate of 79.20±4.15%. The wrinkle improvement function using elastase also showed the highest anti-aging activity of 55.85±4.78% in the same seed extract. The Uzbekistan seed extract had the highest NO production inhibitory activity of 144.35±4.55%. We conclude from these results that the Uzbekistan sorghum seed has excellent antioxidant activity, content of various functional ingredients, and anti-inflammatory activity.

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Keywords Anti-aging activity · Anti-inflammatory activity · Antioxidant activity · NO production inhibitory activity · Sorghum collection

Introduction

Sorghum is an annual human and animal food crop from the grass family Poaceae, which thrives in the semi-arid and arid regions of Africa and Asia. In addition to food use, sorghum may also be used in brewing, building materials, and bioenergy. Sorghum is used as a staple food by approximately 500 million people across 30 different countries [1]. In developing countries, such as West Africa, the demand for sorghum is increasing both due to population growth and the effect of Government policy to stimulate industrial development [2]. Moreover, agricultural scientists are increasingly interested in plants that provide good quality food products without harming human health or the environment [3]. Sorghum contains various nutrients and antioxidant compounds, such as phenolic acids (ferulic acid, tannic, p-coumaric acid), flavonoids (luteolin, apigenin, catechin gallate, epigallocatechin), tannic acid, and lipids [4]. Previously published papers have reported sorghum to have excellent LDL-inhibitory, antioxidant, antimicrobial, and anticancer activities [5-7]. The nutritive profile of sorghum may assist in the prevention of serious diseases, such as obesity, diabetes, cardiovascular disease, and high blood pressure. The protective effects of sorghum have been ascribed to plant compounds that reduce oxidative stress and inflammation, particularly the antioxidant-type phenolic compounds [8,9].

Phenolic compounds from sorghum have shown protective effects against oxidative damage to deoxyribonucleic acid (DNA), which may assist in the prevention of cardiovascular diseases that are caused by oxidative stress [10]. More than 90% of the phenolic compounds are derivatives of benzoic and cinnamic acids, which are found in the outer layers of the sorghum seed (the

aleurone, the testa, and the pericarp) [11,12]. The pericarp contains high concentrations of flavonoids; the black pericarp is rich in 3-deoxyanthocyanidins, and the brown pericarp is an excellent source of flavones and flavanone [13]. Tannins are another key phenolic compound found within the pigmented testa of sorghum seeds, with concentrations rates reported between 10-69 mg/g [14-16]. Sorghum-based tannins exist in the form of oligomers or polymers, which are capable of chemically interacting with proteins, carbohydrates, lipids, and metals [17,18].

When the human body is under a lot of stress due to the external environment, reactive oxygen species (ROS) are overproduced, which can lead to aging-related diseases such as diabetes and Alzheimer's disease. A lot of research is being conducted to increase human viability by using plant extracts as natural antioxidants that can scavenge these ROS [19]. These natural antioxidants have been studied not only as health functional foods, but also anti-inflammatory and whitening functions, and are spreading to the bio-cosmetics industry that increases human skin immunity [20].

This study aims to provide foundational data on the use of sorghum seed extracts as a bio-cosmetic material. The experiments evaluated the presence and concentration of health-promoting phenolic compounds in sorghum seeds (specifically the presence of phenols, tannins, flavonoids and anthocyanins), the inhibitory activity from tyrosinase and elastase, the general viability of cells treated with sorghum seed extract, and an assessment of the anti-inflammatory activity of the sorghum seed extract.

Materials and Methods

Plant extraction and concentration

Sorghum seed accessions (A to G) were collated from seven regions in three different countries (Uzbekistan, Myanmar, and the United States) (Fig. 1). Whole sorghum seeds were placed in a dehydrator at 2 ºC for 48 h, then extracted with 100% methanol in the dark for 48 h. The seed extractions were filtered through filter paper, and then reduced by volume using a reduced pressure concentrator at 45 ºC. The resulting seed extracts were used in each of the experiments as described below.

DPPH analysis

2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to measure the free radical scavenging activity with some modifications from the original method described by Blois (1958) [21]. DPPH was diluted with methanol to 0.15 mM for further use in the experiment. Seed extraction concentrations of 10, 25, 50, and 100 ppm were formulated by diluting seed extractions with diluted DPPH solution. Each concentration of the seed extraction was plated in a 96-well plate, and then incubated at room temperature for 30 min under dark conditions. An ultraviolet (UV) spectrophotometer (Multiskan FC Microplate Photometer, Thermo Fisher Scientific Inc., Waltham, MA, USA) at 519 nm was used to read the absorbance of each seed extraction. The measured ability to scavenge free radicals was expressed as the RC_{50} value.

Total phenol content

To measure the total phenol content, we modified the Folin-Ciocalteu method as described in Sato et al. (1996) [22]. A total of 100 µL of seed extraction (at 1,000 ppm) was mixed with 50 µL of Folin-Ciocalteu's phenol reagent, and allowed 5 min for the reaction to occur at room temperature. 300 µL of 20% sodium carbonate (Na_2CO_3) solution (diluted with distilled water) was added and allowed 15 min for the reaction to occur at room temperature. The seed extraction was then mixed with 1 mL of distilled water and centrifuged (Hettich Zentrifugen, Tuttlingen, Germany) for 2 min at 13,000 rpm at room temperature. Thereafter, 200 µL of the supernatant was placed into a 96-well plate. The absorbance of the seed extraction was measured with a UV spectrophotometer at 740 nm. Total phenol content was recorded

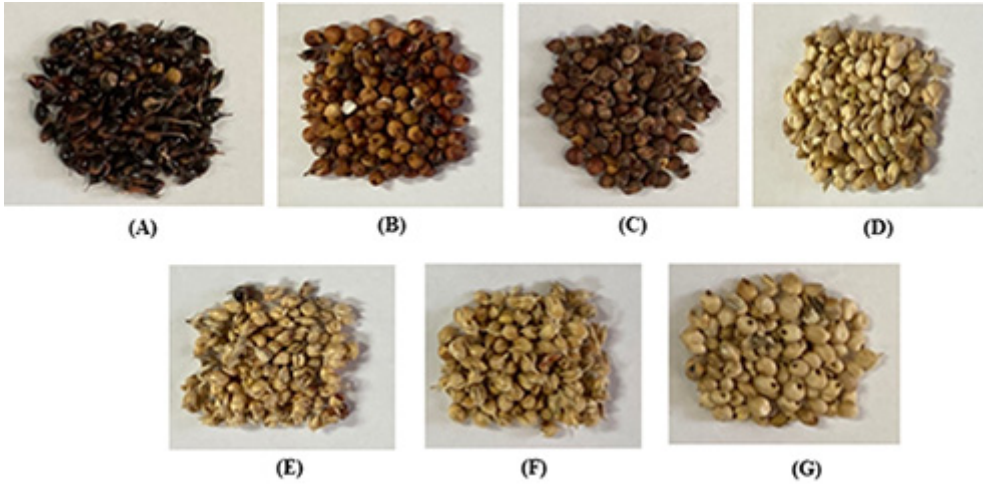

Fig. 1 Morphology for 7 kinds of sorghum collected from three regions. Uzbekistan (A), Myanmar (B, C, D, F, and G), and United States (E)

as gallic acid equivalents (GAE), using gallic acid as the standard substance.

Total tannin content

Total tannin content was assayed based on modified method described by Duval and Shetty (2001) [23]. 95% ethanol and 1 mL of distilled water were combined with 1 mL of each seed extraction (at 1,000 ppm). Then, 1 mL of 5% Na_2CO_3 solution and 0.5 mL of Folin-Ciocalteau's phenol were added and allowed 60 min for the reaction to occur at room temperature. Seed extracts were centrifuged for 2 min at 13,000×g at room temperature. Thereafter, 200 μL of each of the supernatants were placed into a 96-well plate and the absorbance was measured at 740 nm using a UV spectrophotometer. The total tannin content was calculated as tannic acid equivalents (TAE), using tannic acid as the standard substance.

Total flavonoid content

We measured the total flavonoid content using the method of Moreno et al. (2000) [24]. We mixed 200 μL 1 M potassium acetate and 10% aluminum nitrate diluted with distilled water in a 1:1 ratio. This solution was then mixed with 500 μL of each seed extraction (at 1,000 ppm), then plated into a 96-well plate and reacted. The absorbance was measured with a UV spectrophotometer at 414 nm, and the total flavonoid content as quercetin equivalents (QE).

Total anthocyanin content

To measure the total anthocyanin content, we modified the method described by Lako et al. (2007), Different pH were used using the two buffers (0.025 M, pH 1.0 and 0.4 M, pH 4.5) [25]. 100 μL of seed extraction (at 1,000 ppm) was mixed with 900 μL of each buffer. The samples in a 96-well plate were measured the absorbance at both 51 and 740 nm using a UV spectrophotometer. We calculated absorbance using the following equation:

$$A = (A_{519\ nm} - A_{740\ nm})_{pH=1.0} - (A_{519\ nm} - A_{740\ nm})_{pH=4.5}$$

Total anthocyanin content was expressed based on the cyanidin-3-glucoside content per 100 g of seed extraction using the following formula:

$$\text{Total anthocyanin content (mg/L)} = A \times MW \times DF \times 1{,}000/(\varepsilon \times 1),$$

A = absorbance,
MW = molecular weight (449.2 g/mol),
DF = dilution factor, and
ε = extinction molar coefficient (26,900 $L/^{cm}$ mol).

Tyrosinase inhibitory activity

Tyrosinase inhibitory activity was checked with the method of Bermard and Berthon (2000) [26]. The substrate (L-DOPA) and enzyme (tyrosinase) were prepared at 10 mM and 123 unit/8 mL, respectively, using 67 mM phosphate buffered saline (PBS) as a buffer solution. The 40 μL of each of the seed extractions (at 1,000, 2,500, and 5,000 ppm, respectively) was placed on a 96-well plate. Thereafter we added 40 μL of tyrosinase enzyme and 120 μL of the L-dopa substrate, and allowed for 30 min for the reaction to occur at 37 °C in the dark. The absorbance of each sample was measured at 519 nm using a UV spectrophotometer, and koijc acid was used as the positive control.

Elastase inhibitory activity

100 mM tris hydrochloride (Tris-HCl) buffer, the substrate (N-succinyl-Ala-Ala-p-nitroanilide) and the enzyme (elastase) were prepared at 1 mM and 0.25 unit/10 mL, respectively. Thereafter, 100 μL of buffer, 30 μL of substrate and enzyme, 40 μL of seed extraction diluted to 250, 500, and 1,000 ppm were added to a 96-well plate, and allowed 30 min for the reaction to occur at 37 °C. The absorbance of each sample was measured at 414 nm using a UV spectrophotometer. The elastase inhibitory activity was expressed as the absorbance reduction rate of the seed extraction-treated and control groups [27]. Ascorbic acid was used as the positive control measure. Calculation formula is $\{(A_{414\,nm}$ Control-$A_{414\ nm}$ Sample)/$A_{414\ nm}$ Control$\}\times 100$.

Cell metabolic activity (MTT assay)

MTT assay was tested using RAW 264.7 cells (mouse murine macrophage cells from a cell line bank in Korea). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum sourced from Hyclone Laboratories Inc., Logan, UT, USA, and 1% penicillin sourced from Lonza Walkersville Inc., USA. (100 U/mL) at 37 °C in a 5% carbon dioxide (CO_2) incubator (Sanyo Co. Ltd., MCO-19AIC, Fukuyama Japan). Raw 264.7 cells were placed at a density of 1×10^5 cells/well in a 96-well plate, then cultured the cells for 24 h at 37 °C in a 5% CO_2 incubator (Sanyo Co., Ltd., MCO-19AIC), then the supernatant was removed. Seed extract (100 μL) diluted with DMEM medium was plated in a 96-well plate at each concentration (50, 100, 200 ppm), and then the culture was incubated for 24 h at 37 °C in a 5% CO_2 incubator. To each cell, MTT reagent (0.5 μg/mL dissolved in PBS) was added, and the mixture was incubated for 4 h. The supernatant was removed, treated each of the cells with 100 μL of dimethyl sulfoxide, then incubated for 20 min at room temperature. Absorbance of each sample was measured at 620 nm using a UV spectrophotometer [28].

Anti-inflammatory activity assay of lipopolysaccharide (LPS) induced RAW 264.7 cell

Raw 264.7 cells were dispensed at a density of 1×10^5 cells/well in a 96-well plate and cultured for 24 h at 37 °C in a 5% CO_2 incubator (Sanyo Co., Ltd., MCO-19AIC). The supernatant was removed after culturing for 24 h. Lipopolysaccharide (LPS) and seed extractions at each concentration (10, 50, and 100 μg/mL) were mixed at a ratio of 1:1, and then allowed to incubate for

24 h. 50 μL of the supernatant was removed, and 50 μL of 1% sulfanilamide (Sigma Aldrich Co. Ltd., St. Louis, MO, USA) diluted in Griess reagent (5% phosphoric acid (Wako Chemicals Inc., Richmond, VA, USA) + 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride (Sigma Aldrich Co. Ltd., USA) was added for 20 min, and absorbance was measured at 519 nm using a UV Spectrophotometer [29].

Statistical analysis

IBM SPSS Statistics v24 program (SPSS, International Business Machines Co. Ltd., Armonk, NY, USA) was used to analyze the significance of the values that was repeated three times per each experiment. The significance level was represented at 0.05 level of significance (p <0.05).

Results

Assessments for DPPH scavenging activities in sorghum collection varieties

The highest level of free radical scavenging activity was measured in the Uzbekistan (A) sorghum seed extract (13.10±2.37 μg/mL). The five sorghum seed extracts collected from Myanmar (B, C, D, F, G) showed a numerical interval of 108.00±1.86 μg/mL to 1662.54±59.43 μg/mL (Table 1). American (A) sorghum seed extracts measured at 1435.79±32.88 μg/mL. However, the results were not significantly different from the low radical scavenging activity among the Myanmar seed extractions. We measured and compared the total phenol, tannin, flavonoid, and anthocyanin contents of seven foreign-collected sorghum seeds (Table 2). The total phenol content of Uzbekistan sorghum seeds (A) was 157.84 ±4.04 mgGAE/g, showing the highest amount among the 7 seed extractions. The value (concentration) of the Myanmar collection (B, C, D, F, G) was in the range of 7.90±0.27 mgGAE/g to 54.25 ±4.53 mgGAE/g, and the American collection (E) was 12.61± 0.15 mgGAE/g. The Uzbekistan sorghum seed showed the highest value of total tannin content at 362.96±5.05 mgTAE/g, while the Myanmar-collected species (F) showed the lowest amount of total tannin at 5.53±0.50 mgTAE/g. Results demonstrating that seeds with a high phenol content also had a high tannin content. The total flavonoid content was the highest in Myanmar collected species (F) at 12.69±0.72 mgQE/g, and the total anthocyanin content was also highest in the sorghum seed (F) of the same collected species (0.43±0.01 mgC3G/100g).

Analysis of whitening and anti-wrinkle activities by assay of tyrosinase and elastase in sorghum collection varieties

To measure the degree of whitening activity of the collected sorghum species, the tyrosinase inhibitory activity was analyzed (Fig. 2). The concentration of the extract was 1000, 2500, and 5000 μg/mL, and tyrosinase inhibitory activity was measured at each concentration. The results showed a difference in the tyrosinase activity for each seed extraction at a concentration of 5000 μg/mL. In particular, the highest inhibitory activity in sorghum seed extract collected in Uzbekistan (A) of 79.20± 4.15%. Analysis of the collected species (F) from Myanmar revealed that it had the lowest whitening activity (33.80±1.61%). The effect of wrinkle inhibition was analyzed based on elastase activity. The elastase inhibitory activity of sorghum seeds (A) collected from Uzbekistan was 55.85±4.78%, followed by seed extraction B collected from Myanmar of 46.65±2.10%. The United States collection (E) seed extraction had 23.35±4.98%, which we consider as the lowest wrinkle improvement ability (Table 3).

Evaluation of the anti-inflammatory activity in sorghum collection varieties

We measured the effect on cell viability in the seed extractions from seven foreign species using the MTT assay (Fig. 3). The seed extraction concentrations were set at 50, 100, and 200 μg/mL and applied to RAW264.7 cells. All RAW264.7 cells treated with a seed extraction at 50 μg/mL showed a cell viability of 73.96± 6.50% or more. When the same cells were treated with a seed extraction at 100 μg/mL, results showed a cell viability of 89.28±

Table 1 DPPH scavenging activities in 7 types of sorghum seeds collected from three regions

Sample	Accession No.[1)]	Country[2)]	RC_{50}[3)] (μg/mL)
A	K261774	UZB	13.10±2.37[a]
B	K167100	MMR	108.00±1.86[b]
C	K172258	MMR	134.17±3.30[c]
D	K167116	MMR	1662.54±59.43[g]
E	K269323	USA	1435.79±32.38[e]
F	K167034	MMR	1556.80±22.08[f]
G	K167197	MMR	862.89±16.59[d]
Ascorbic acid			8.18±0.30

Accession number obtained from an Agricultural Genetic Resource Center in South Korea
Collection region: UZB (Uzbekistan), MMR (Myanmar), USA (America)
RC_{50}; Amount of sample that reduces oxidizing power by half. Values are represented as mean ± SD (n =3) and means are significantly different (p <0.05) by Duncan's multiple range test. Mean values in each column with different letter are significantly different

Table 2 Total phenol, tannin, flavonoid, and anthocyanin content in 7 kinds of sorghum collected from three regions

Sample[1)]	Total phenolic (mgGAE[2)]/g)	Total tannin (mgTAE[3)]/g)	Total flavonoid (mgQE[4)]/g)	Total anthocyanin (mgC3G[5)]/g)
A	157.84±4.04[a]	362.96±5.05[a]	10.13±0.42[b]	0.20±0.0[b]
B	54.25±4.53[b]	76.21±0.87[c]	9.74±0.22[b]	0.08±0.02[b]
C	39.95±0.79[c]	83.74±3.05[b]	8.69±0.15[c]	0.30±0.09[ab]
D	7.90±0.27[e]	6.42±0.78[de]	4.62±0.35[d]	0.19±0.07[b]
E	12.61±0.15[d]	10.39±0.37[d]	2.34±0.07[d]	0.24±0.12[ab]
F	8.98±0.24[e]	5.53±0.50[e]	12.69±0.72[a]	0.43±0.01[a]
G	14.43±0.42[d]	8.98±0.40[de]	8.79±0.2[c]	0.29±0.01[ab]

[1)]All sample concentration used to measure is 1 mg/mL
[2)]Gallic acid equivalent
[3)]Tannic acid equivalent
[4)]Quercetin equivalent
[5)]Cyanidin-3-Glucoside
Values are represented as mean ± SD (n =3) and means are significantly different (p <0.05) by Duncan's multiple range test. Mean values in each column with different letter are significantly different

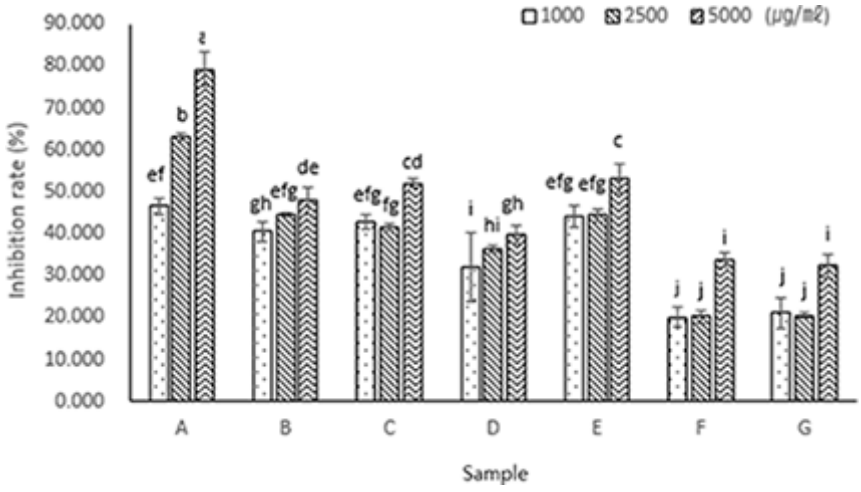

Fig. 2 Effect of whitening activity in 7 kinds of sorghum collected from three regions by tyrosinase analysis in 1000, 2500 and 5000 μg/mL seed extracts. Uzbekistan (A), Myanmar (B, C, D, F, and G), and United States (E). Values are represented as mean ± SD (n =3) and means are significantly different (p <0.05) by Duncan's multiple range test. Mean values in each column with different letter are significantly different (a-j)

Table 3 Effect of anti-wrinkle activity in 7 kinds of sorghum collected from three regions by elastase analysis

Sample[1)]	Inhibition rate (%)[1)]
A	55.85±4.78[a]
B	46.65±2.10[b]
C	34.96±1.17[c]
D	36.96±8.03[c]
E	23.35±4.98[d]
F	29.71±2.83[cd]
G	36.92±2.38[c]

[1)]All sample concentration used to measure is 1 mg/mL. Values are represented as mean ± SD (n =3) and means are significantly different (p <0.05) by Duncan's multiple range test. Mean values in each column with different letter are significantly different

15.30% or more, which verified that cell vitality was good in all seed extractions. We identified that cell activity appeared to be reduced when treated with 100 μg/mL or higher (A of Fig. 3), therefore the titer of the seed extraction was set at 100 μg/mL or less during the activity test for nitric oxide (NO) production inhibition in LPS-induced RAW264.7 cells (Fig. 4). When the seed extractions at concentrations of 10, 50, and 100 μg/mL were treated in LPS-induced RAW264.7 cells, it was observed that the inhibitory activity for NO production rate at all concentrations of the Uzbekistan seed extraction (A) was significantly higher than that of the other six species. The sorghum seed of Uzbekistan has significant inflammation-related functionality, demonstrated by a high NO production inhibitory activity of 144.35±4.55% at a concentration of 100 μg/mL.

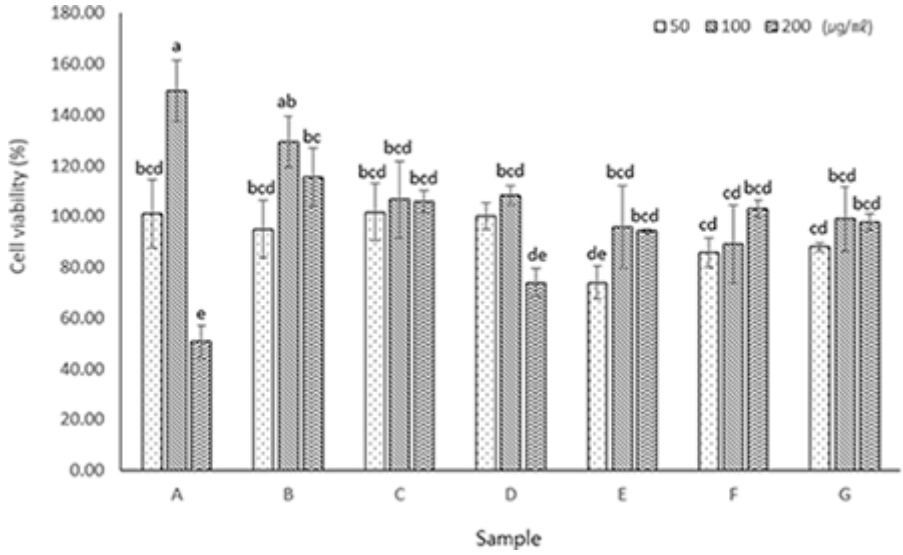

Fig. 3 Cytotoxicity comparison with 7 kinds of sorghum collected from three regions using RAW 264.7 cells. Each of samples was treated with different concentrations in RAW 264.7 cells for 48 hr. Values are represented as mean ± SD (n=3) and means are significantly different (p <0.05) by Duncan's multiple range test. Mean values in each column with different letter are significantly different

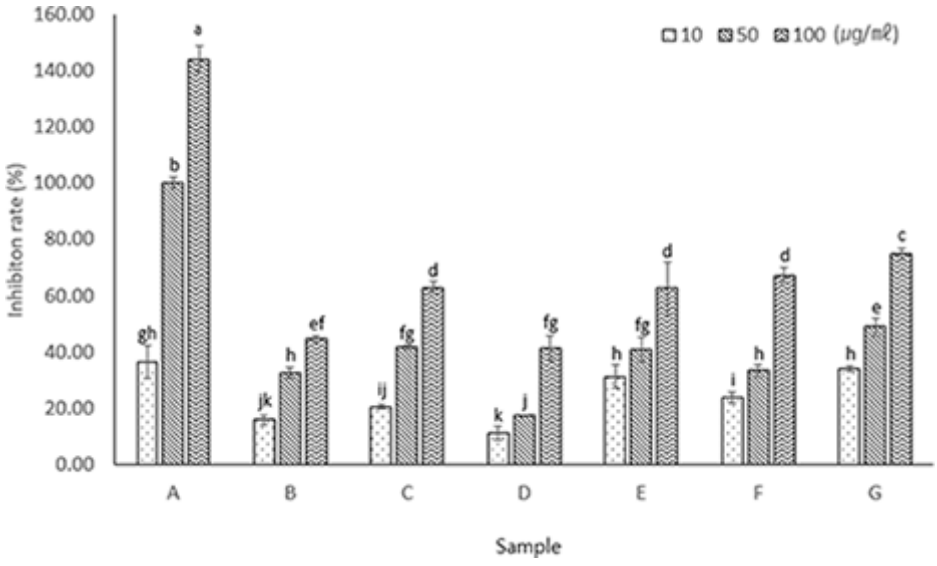

Fig. 4 Nitric oxide (NO) production comparison with 7 kinds of sorghum collected from three regions using LPS-induced RAW 264.7 cells. Each of samples was treated with different concentrations in RAW 264.7 cells for 48 h. Values are represented as mean ± SD (n =3) and means are significantly different (p <0.05) by Duncan's multiple range test. Mean values in each column with different letter are significantly different

Discussion

The antioxidant activity of natural products donates electrons to active radicals and inhibits lipid oxidation in food. The scavenging function of free radicals plays a very important role [30]. The DPPH radical scavenging activity is a measure of inhibiting oxidation by donating electrons to oxidative active free radicals involved in the chain reaction of lipid peroxidation [31]. Among the various methods for measuring antioxidant activity, DPPH radical scavenging ability has the advantage of easy comparison of results using a control group, such as ascorbic acid, and is therefore used often for measuring the antioxidant activity of medicinal or functional plant extract seed extractions [32]. The antioxidant activity of natural products plays a significant role in preventing diseases and aging by inhibiting the oxidation of fat in food and active radicals in the human body [33]. In our study, even in extracts without crushed sorghum seeds, the radical scavenging ability was found to some extent in most of the collected species, and in particular, the Uzbekistan collection species was found to have excellent radical scavenging ability.

The DPPH radical scavenging activity according to the variety (Hwanggeumchal, Nampungchal, and Sodamchal) and harvest period of sorghum was 9.94-16.54 μg/mL [34]. This was interpreted as showing antioxidant activity which was like the value obtained through DPPH analysis of the species collected in Uzbekistan in this experiment. Roasting of sorghum flour has significant effects on antioxidant properties, changing the DPPH activities from 92.50 to 83.95% [35]. DPPH analysis of sorghum has shown significant differences in antioxidant activity depending on the collection area, variety, cultivation method, and genotype. A report of a similar study with more sorghum collections showed a wide range of DPPH scavenging activity ranging from 11.91± 4.83 to 1343.90±81.02 μg/mL [4]. This, like our study, proves that the antioxidant activity can be varied by climatic conditions and genetic variation in sorghum growing regions.

Among the antioxidant substances contained in grains, polyphenols have excellent antioxidant activity. Flavonoids are primarily composed of anthocyanidins, flavanols, flavones, catechins, and flavanones, depending on their structure, and it is generally understood that these flavonoids provide antioxidant and antibacterial activities [36]. In the investigation of the total polyphenol content by sorghum variety, Kim et al., (2018) reported that the sorghum seeds that were harvested 40 days after planting had a content of 22.37-24.67 mgGAE/g [34]. In sorghum species, the black had 11.50±1.81 mgGAE/g GAE and the brown had 3.58±1.63 mgGAE/g on total phenolic content depending on pericarp colors [37]. Tannins are widely found in plants, such as fruits, vegetables, and plant seeds, and have pharmacological properties like astringency and hemostatic properties, as well as the ability of binding to proteins or alkaloids. Tannins have been reported to have useful physiological activities, such as antibacterial, antioxidant, antitumor, and heavy metal removal properties, and are expected to help improve the diet of modern people [38]. It has been reported earlier that the total polyphenol and tannin contents in plants are correlated. Published studies have suggested that when the total polyphenol content is elevated, the total tannin content will similarly be elevated [39,40]. In this work, the total phenol content and the tannin content were investigated with correlation in extracts of all seeds.

The flavonoid content with extractions of sorghum seed harvested over two years was represented as 13.24-13.50 mgCE/g of ‘Hwanggeumchal’, 13.85-13.25 mgCE/g of ‘Nampungchal’, and 12.46-11.35 mgCE/g of ‘Sodamchal’, respectively [34]. This showed a similar content to 12.69±0.72 mgQE/g of the highest sorghum collection (F) among our results. Anthocyanin is an excellent natural antioxidant, cyanidin-3-glucoside and peonidin-3-glucoside among the representative anthocyanin pigments are known to inhibit DNA damage caused by peroxyl and hydroxyl radicals, and inhibit the oxidation of low-density lipoprotein [41]. The total anthocyanin content was obtained with 11.42±0.42 mg/g in red-brown sorghum variety IS686 bran [4]. We found that the total anthocyanin content was significantly lower than the results of Ghimire et al. (2021). In our study, the anthocyanin content was thought to vary depending on the difference in material preparation due to solvent extraction from the seeds without breaking the sorghum seed.

Tyrosinase is an enzyme that acts as a key enzyme in the biosynthesis of melanin, a pigment component of the skin, using tyrosine or dopa as a substrate in the melanosome of melanocytes in the basal layer of the skin. Melanin is a pigment that is widely distributed in living organisms and is a polymer of quinone and hydroquinone monomer units, it is dark or reddish brown, and is synthesized by polymerization with amino acids or proteins after the production of quinone by the action of tyrosinase. Tyrosinase inhibitory activity is not only clinically useful because of its ability to treat skin cancer and skin diseases caused by melanin pigmentation, but is also particularly important in the cosmetic industry because of its ability to depigment and whiten the skin after sunburn [42]. The tyrosinase inhibitory activity of the ethanol extract of each sorghum variety was measured at 61.64-62.16% of Dongan Mega [43]. The results of their study are lower than our results, which showed that the tyrosinase inhibitory activity of Uzbekistan sorghum seed extract was 79.20±4.15%. These results are correlated with the fact that the extract with high phenolic compound content increases as well as the whitening activity. According to some reports, it was confirmed that phenolic compounds such as flavonoids are substances related to tyrosinase inhibitory activity [44]. Elastin exists in the dermal tissue of the skin, combined with collagen in a network structure. Elastase is a non-specific enzyme that decomposes elastin, fibronectin, and collagen, which are matrix proteins that control skin elasticity and cause wrinkles by breaking the bond between the mesh structures [45]. The elastase inhibitory activity of a seed extraction extracted by mixing native plants from Jeju Island was measured in the range of 11.2-37.2%. Similar results were reported by Lee et al. (2018) [46]. The anti-wrinkle effect of the Uzbekistan sorghum seed extract was 55.85±4.78%, confirming that the anti-wrinkle activity of the sorghum seed extract was much higher.

In order to find the appropriate concentration of non-toxic cells in the sorghum methanol extract treatment, an MTT assay was used in this study. There are many examples of using an assay like ours in cell activity experiments using plant extracts. LPS-induced cellular models play an important role in studying the gene expression networks involved in inflammatory regulation, such as iNOS (inducible nitric oxide synthase), COX (cyclooxygenase), and TNF (tumor necrosis factor). In nuclear tissue, NF-κB is involved in the transcriptional regulation of inflammatory genes such as iNOS and COX-2. iNOS converts L-arginine to L-citrulline and produces amount of NO, and COX-2 catalyzes arachidonic acid to prostaglandins. Prostaglandins cause vasodilation, resulting in inflammatory edema in the blood vessels [47]. To examine the anti-inflammatory effect of *Hovenia dulcis* extract, 10-40 μg/mL was treated in LPS-induced RAW264.7 cells and the effect on NO production was analyzed in the same way as this

study. Woo et al. (2018) reported that anti-inflammatory effect was reduced by 53.1% at 10 μg/mL and 94.7% at 40 μg/mL, respectively [48]. In our results, the Uzbekistan sorghum seed extract showed more than 100% inhibition of NO production at a seed extraction concentration of 50 μg/mL, demonstrating a higher anti-inflammatory activity than the results published by Woo et al. (2018).

Amongst the foreign sorghum seeds, the Uzbekistan sorghum seed extract has the highest total polyphenol content, antioxidant activity, and inhibition of NO production in RAW 264.7 macrophages, when compared with the seed extracts of the United States and Myanmar regions. In addition, because sorghum has excellent whitening and antiwrinkle functions, it is necessary to continuously explore and cultivate new varieties of sorghum as an antioxidant and anti-inflammatory material. These results provide basic information that the highly active ingredient of sorghum has applications as a functional material in pharmaceuticals, food, and cosmetics.

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