Enhancement of Phenolics Content and Biological Activities of Longan (*Dimocarpus Longan* Lour.) Treated with Thermal and Aging Process

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Research Article

Keywords: Dimocarpus longan, antioxidant, anti-hyaluronidase, gallic acid, corilagin, ellagic acid

Posted Date: April 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-378198/v1

Version of Record: A version of this preprint was published at Scientific Reports on August 5th, 2021. See the published version at https://doi.org/10.1038/s41598-021-95605-3.

Abstract

This study is the first to successfully produce novel black *Dimocarpus longan* by undergoing thermal and ageing process. Pericarp, aril, and seed of black *D. longan* were macerated in 95% v/v ethanol. Their chemical compositions were investigated by Folin–Ciocalteu assay, aluminium chloride assay, and high-performance liquid chromatography. Antioxidant activities were evaluated in terms of radical scavenging and iron (III) reducing capacity. Enzyme inhibitory assay was used to evaluate the hyaluronidase inhibition. Black *D. longan* seed extract contained the significantly highest content of flavonoids and phenolic compounds ($p < 0.05$). Each gram of the extract contained 53.6 ± 0.9 mg of gallic acid, 19.8 ± 2.9 mg of corilagin, and 24.5 ± 0.7 mg of ellagic acid. This extract was the most potent anti-hyaluronidase and antioxidant with the strongest free radical scavenging activity and reducing power. Therefore, it was proposed as functional food and further used in the pharmaceutical or cosmeceutical industries.

Introduction

Dimocarpus longan Lour., a subtropical evergreen plant in the family of Sapindaceae, is widely known as longan. *D. longan* can be cultivated in several countries in East Asia, South-East Asia, Australia, and some subtropical regions in the US.[1] China and Thailand are the largest area of commercial *D. longan* cultivation.[2] The succulent and edible aril with delicious flavor and health benefits lead *D. longan* to be more and more popular.[1] Since the aril part of *D. longan* contains several polyphenols, flavonoids, organic acids, and polysaccharides, it possessed various beneficial biological activities, including antioxidant, antiglycation, anticancer, immunomodulatory, prebiotic, anti-osteoporotic, anxiolytic, and memory-enhancing effects.[3] A decoction of the dried aril has been taken as a tonic for insomnia and neurasthenic neurosis treatment since an ancient time.[4] Not only the aril part of *D. longan*, which is the only edible portion, that has been reported for the health-beneficial effects, the pericarp has also been reported to contain abundant polyphenols, flavonoids, and polysaccharides, which possessed antioxidant, anti-tyrosinase, and anti-hyperglycaemic activities.[1] On the other hand, *D. longan* seed, which is a waste from the food and canning industry, contains antioxidative polyphenols and possesses anti-tyrosinase, antibacterial, and anti-fungal activity.[1,5,6,7] Besides, *D. longan* seed has been administered to counteract heavy sweating, whereas, the pulverized kernel was used for astyptic treatment according to the components of saponin, tannin, and fat .[8] Although *D. longan* have been reported to contain variety of biological active components and have a potential to be used for the treatment of various conditions, the fruit of *D. longan* has a short storage life since its pericarp rapidly turns brown and harden at ambient temperature.[1,9] Even if low temperature (1–5°C) could preserve *D. longan* from pathological decay, the fruit deteriorate easily after remove from the cold storage.[3] Therefore, dried flesh and the canned product of *D. longan* are widely consumed and can be distributed worldwide. Generally, dried foods can be kept for a longer period, but their sensory and nutritional characteristics are often lost along with the water removal during the drying processes.[10] Production of intermediate moisture food (IMF) is another technique to overwhelm this problem since the properties of IMF are close to fresh foods yet having

extended the shelf life.[10,11] A reduction of the moisture content and a water activity below 0.6 will not support the microbial growth and leads to shelf-stable products.[11,12] Various types of food have been preserved as IMF, such as meat and several fruits, e.g. grapes, tomatoes, peaches, prunes, apricots, strawberries, etc.[10,13] However, some additives are required in the production of IMF for antimicrobial (e.g. preservatives, sugar, and salt), along with the agents for water activity reducing and plasticizing, e.g. glycols and sorbitol.[11,12] Beside IMF, the heating and ageing process could also prolong the shelf life without refrigeration. A well-known food undergone this process is black garlic (*Allium sativum* L.), a processed garlic produced by thermal treatment on raw garlic at high temperature and high relative humidity for 60–90 days without using additives.[14,15] During the production process, raw garlic also undergoes Maillard reaction, which occurs between amine groups and carbonyl compounds, finally resulting in brownish melanoidin.[16] A variety of biological activities of melanoidin has been demonstrated, including antioxidant, antibacterial, anti-inflammatory, hypoglycemic, hypotensive, antitumor effects, prevention of obesity, lowering of serum lipopolysaccharide levels, and modulation of the composition of the gut microbiota.[16,17] Additionally, inhibition against oxidation and angiotensin I converting enzyme were enhanced in black garlic comparing to raw garlic.[15] Therefore, the heating and ageing process not only preserved the food but also enhanced their biological activities. Since the production of black *D. longan* undergone heating and ageing process has not been reported before, this study is the first to produce a novel black *D. longan* and investigate the chemical compositions, as well as its health benefit potential in antioxidant, anti-inflammatory, and anti-hyaluronidase activities.

Results And Discussion

DriedD. longanand blackD. longanextracts

The external appreance of dried *D. longan* were totally different from black *D. longan* as shown in Fig. 1. The color of pericarp, aril, and seed of black *D. longan* was obviously darker than that from dried *D. longan*, especially the aril part which turn from dark brownish color to black color. The pericarp and seed of black *D. longan* were substantially more moist than dried *D. longan*. The outer part of dried *D. longan* seed was shriveled, whereas, the dried *D. longan* pericarp was dry and brittle. All *D. longan* extracts were semisolid mass with different color as shown in Fig. 1. The color of black *D. longan* extracts were darker than the dried *D. longan* extracts. The color of pericarp extracts were the darkest, followed by the extract from seed and aril, respectively. Yields of each *D. longan* extracts are shown in Fig. 2. The aril part yielded the highest extract content, followed by pericarp and seed, respectively. The highest yield of the extract was obtained from black *D. longan* aril (21.6% w/w), followed by dried *D. longan* aril (17.6% w/w), dried *D. longan* pericarp (13.8% w/w), black *D. longan* aril (11.0% w/w), black *D. longan* seed (6.6% w/w), and dried *D. longan* seed (3.6% w/w), respectively.

Chemical compositions of driedD. longanand blackD. longanextracts

D. longan extracts were investigated for the content of total phenolic compounds, total flavonoid, gallic acid, corilagin, and ellagic acid. Gallic acid and corilagin are natural polyphenolic compounds which

belong to hydrolyzable tannin, whereas, ellagic acid belongs to a flavonoid group.[18] Among different parts of dried *D. longan*, pericarp extracts contained the significantly highest total phenolic content ($p < 0.05$) and the highest total flavonoid as shown in Fig. 3. The results were in a great agreement with the previous study reported that polyphenolic compounds are abundant in pericarp and seed of *D. longan* compared to the *D. longan* aril.[1] The total phenolic content of pericarp, seed, and aril extracts from dried *D. longan*, which were 967.6 ± 31.5, 739.3 ± 62.3, and 229.5 ± 2.6 µg GAE per g extracts, were found in agreement with the previous study reported that the total phenolic content of *D. longan* were in the range of 22.09–132.47 mg of gallic acid equivalents (GAE/100 g), which were equivalent to 220.9–1,324.7 µg GAE per g extracts.[1] Interestingly, the dramatically enhanced in total phenolic content was observed in black *D. longan* seed extract. The ethanolic extract from black *D. longan* seed contained as high as 1827.1 ± 73.1 µg GAE per g extracts, which was much higher that the previous reported.[1] On the other hand, there was no significantly difference between the total phenolic content of dried and black *D. longan* extract from pericarp and aril ($p > 0.05$).

In addition to the total phenolic content, black *D. longan* seed extract also contained the significantly highest flavonoids content ($p < 0.05$). Among various dried *D. longan* extracts, the pericarp part contained the significantly highest flavonoid content of 2.8 ± 2.4 µg QE per g extract as shown in Fig. 3 ($p < 0.05$). The results were in a great accordance with the previous study reported that the quercetin content of *D. longan* pericarp were 3.12 ± 0.76 mg/kg, which was equivalent to 3.12 ± 0.76 µg per g extract.[19] Besides, *D. longan* pericarp has been reported to contain slightly higher content of flavonoids than *D. longan* seed and aril.[20] Obviously, the flavonoid content of black *D. longan* seed extract, which was as high as 13.6 ± 2.5 µg QE per g extract, was dramatically enhanced, which was about four times higher than previously reported.

Although the thermal and ageing process of *D. longan* did not affect the phenolic and flavonoid content of *D. longan* pericarp and aril, the total phenolic and flavonoid content of *D. longan* seed were obviously enhanced after the production process of black *D. longan*. The likely explanations might be due to the formation of biological compounds, which were not originally present in the *D. longan* seed, during the thermal and ageing process.[21]

Gallic acid, corilagin, and ellagic acid have been identified as major polyphenolic components of *D. longan* pericarp and seed in several previous studies.[22,23,24] Therefore, the content of these polyphenolic components were also investigated in black *D. longan* extract, in a comparison with dried *D. longan* extracts. The HPLC chromatograms of gallic acid, corilagin, and ellagic acid are shown in Fig. 4. The peak of gallic acid, corilagin, and ellagic acid were detected at around 3.7, 9.9, and 19.2 min, respectively. The content of gallic acid, corilagin, and ellagic acid of each *D. longan* extracts are shown in Fig. 5. The results were in a good agreement with their total phenolic and total flavonoid content since black *D. longan* seed extract contained the significantly highest polyphenolic compounds and flavonoids content of ($p < 0.05$).

Among various parts of dried *D. longan* fruit, seeds contained the significantly highest content of gallic acid, corilagin, and ellagic acid ($p < 0.05$) with the amount of 5.3 ± 0.0, 8.9 ± 0.1 and 1.9 ± 0.2 mg/g extract, respectively. Interestingly, the content of these phenolic and flavonoid were significantly enhnaced after the production process of black *D. longan* ($p < 0.05$). The gallic acid, corilagin, and ellagic acid content of black *D. longan* seed extract were as high as 53.6 ± 0.9, 19.8 ± 2.9, and 24.5 ± 0.7 mg/g extract, respectively. The content of gallic acid and ellagic acid were about ten times increased, whereas, the content of corilagin was double when the *D. longan* undergone thermal and ageing process. The reason might be due to the liberation of free polyphenolic and flavonoid from the bound forms (i.e. esterified and glycosylate) or the decline in enzymatic oxidation involving in the antioxidant compounds in the raw fruit.[25] The results of black *D. longan* were in accordance with the black garlic as the total phenolic and total flavonoid content of the garlic subjected to the thermal processing steps were significantly higher than those of fresh garlic.[21,25] The previous study reported that the phenolic content was increased by about 4–10-fold in the black garlic cloves compared with the fresh garlic.[21]

Antioxidant activities of driedD. longanand blackD. longanextracts

The antioxidant activities of dried and black *D. longan* extracts were investigated by two assays with different mechanisms of action. The ABTS assays referred to the electron transfer reaction and represented the radical scavenging activity of the tested samples, while the FRAP assay referred to the ion reduction process, which represented the ability of the tested compound to convert ferric ions ($Fe3^{+}$) to ferrous ions ($Fe2^{+}$).[26,27,28] The ferric reducing antioxidant power (EC_1) and TEAC value of dried and black *D. longan* extracts are shown in Fig. 6.

The TEAC value of black *D. longan* extracts were not significantly different from dried *D. longan* extracts, except in the aril part. The dried *D. longan* aril extract had no antioxidant activity, whereas, the black *D. longan* aril extract possessed some antioxidant activity with the TEAC value of 4.1 ± 1.4 µg Trolox/mg extract. The probable explanation lies in the greater Maillard reaction which occurs in the aril part as compared with the others. As Maillard reactions refer to a chemical reaction between an amino acid and a reducing sugar in the presence of heat[29], *D. longan* aril, which contained glucose, fructose, and various types of amino acids, e.g. γ-aminobutyric acid, tended to undergone Maillard reaction.[2] Besides, this non-enzymatic browning reactions led black *D. longan* a darker color and resulting in the formation of some antioxidant compounds.[25]

On the other hand, black *D. longan* pericarp and seed extracts possessed the same radical scavenging activity as that from dried *D. longan*. The likely possibility might be because of the degradation of some oxidative compounds during the heating process although some free polyphenolic and flavonoid were liberated from the bound forms.[21] Interestingly, the TEAC values of pericarp and seed extracts from both dried and black *D. longan* were comparable to ascorbic acid, gallic acid, and corilagin ($p > 0.05$). Ellagic acid was remarked as the most potent radical scavenger (TEAC = 23.4 ± 0.3 µg Trolox/mg), followed by ascorbic acid (TEAC = 12.3 ± 0.0 µg Trolox/mg), gallic acid (TEAC = 12.8 ± 0.2 µg Trolox/mg), and

corilagin (TEAC = 12.7 ± 0.1 µg Trolox/mg). Thereby, ellagic acid was found to be the main compound responsible for the free radical scavenging activity of *D. longan* extracts together with gallic acid and corilagin.[30] Although the previous study reported that among various polyphenolic compounds, tannins demonstrated the strongest ABTS•+ radical scavenging activity[31], the present study remarked that ellagic acid, which belongs to a flavonoid group, was more potent than gallic acid and corilagin, which belong to hydrolyzable tannin.[1] Furthermore, *D. longan* extracts from both pericarp and seed part could be hence remarked as the natural extracts with potent radical scavenging activity.

Aside from radical scavenging activity, *D. longan* extracts also possessed a reducing ability as shown in Fig. 6. The reducing ability of *D. longan* extracts were in a very well accordance with their phenolic and flavonoid content. Gallic acid possessed the significantly highest EC_1 value of 237.0 ± 1.6 mM $FeSO_4$/mg, which was comparable to that of ascorbic acid (238.3 ± 0.2 mM $FeSO_4$/mg), followed by corilagin (226.2 ± 2.9 mM $FeSO_4$/mg) and ellagic acid (192.3 ± 0.7 mM $FeSO_4$/mg), respectively. However, both phenolic and flavonoid were responsible for their reducing capacity.[28] The black *D. longan* seed extract, which contained the highest content of total phenolic, total flavonoid, gallic acid, corilagin, and ellagic acid, thus possessed the significantly highest reducing ability with the EC_1 value of 150.0 ± 1.0 mM $FeSO_4$/mg extract ($p < 0.05$). Consequently, the black *D. longan* seed extract was suggested as the most potent antioxidant extract with the strongest free radical scavenging and reducing ability.

Anti-inflammatory activities of driedD. longanand blackD. longanextracts

The inhibitory activities against the secretion of IL-6 and TNF-α, which are key players involved in the age-related inflammatory process[32], of dried and black *D. longan* extracts were investigated and the results are shown in Fig. 7. TNF-α is known as an indicator of chronic inflammatory processes related to ageing, whereas, IL-6 has been noted as the cytokine for geriatricians, which are associated with poor physical performance, muscle weakness, and could predict the onset of disability.[33,34] Among various parts of *D. longan* fruit, aril of both dried and black *D. longan* were predominant in the IL-6 and TNF-α inhibition. Gallic acid was suggested to be the main compound responsible for both IL-6 and TNF-α inhibitory activities. In contrast, corilagin was responsible only for TNF-α inhibition. Although *D. longan* extracts and their major chemical components exhibited only low to moderate anti-inflammatory activities comparing to dexamethasone, a corticosteroid used in the treatment of inflammations, they were suggested as natural anti-inflammatory products with no steroidal side effect.

Anti-hyaluronidase activities of driedD. longanand blackD. longanextracts

Hyaluronidase, a homologous enzyme that hydrolysis or depolymerise hyaluronan, plays an important role in the modulating activity of many pathological processes.[35] Hyaluronan plays a pivotal role in the maintenance of the elastoviscosity of liquid connective tissues and control the water transportation that was related to the tissue hydration.[36] The degradation of hyaluronan resulting in the production of breakdown products, which are the signal that injury has occurred and initiates an inflammatory

response.[35] Additionally, hyaluronan has been known an lubricant and shock-absorber in joints and connective tissues.[37] Its degradation hence leads to the deterioration of viscoelastic properties of the synovial fluid.[38]

The inhibitory activities against hyaluronidase of dried and black *D. longan* extracts are shown in Fig. 8. Although *D. longan* extracts exhibited low anti-hyaluronidase actvitiy, the inhibitory effect of black *D. longan* seed was significantly enhanced compared to the dried *D. longan* seed extract. Since the anti-hyaluronidase activity of black *D. longan* seed extract (18.4 ± 2.0%) was the most significantly potent ($p < 0.05$), black *D. longan* seed extract could be suggested for the anti-hyaluronidase apart from its antioxidant activities.

In conclusion, Black *D. longan* was successfully produced after undergone thermal and ageing process in the controlled temperature of 70° C and controlled humidity of 75% RH. The external appearance of black *D. longan* was totally different from that of dried *D. longan.* The ethanolic extract of black *D. longan* seed contained the most significantly abundant of total phenolic, total flavonoid, gallic acid, corilagin, and ellagic acid content ($p < 0.05$). Furthermore, the black *D. longan* seed extract possessed the most significantly potent antioxidant and anti-hyaluronidase activities. The radical scavenging activity, reducing power, and anti-hyaluronidase activities of black *D. longan* seed extract were obviously higher than that of dried *D. longan* seed extract and the previously reports. Therefore, black *D. longan* seed extract was suggested for further used topically for the promotion of various health benefits, such as anti-skin ageing, joint pain reliever, etc. On the other hand, the aril part of both dried and black *D. longan* possessed significant anti-inflammatory activities. They were suggested as natural edible anti-inflammatory agents.

Material And Methods

Chemical material

L-Ascorbic acid, aluminium chloride ($AlCl_3$), 2,2'-azino-bis3-ethylbenzothiazoline-6-sulfonic acid (ABTS), calcium chloride ($CaCl_2$), corilagin, dexamethasone, disodium phosphate (Na_2HPO_4), ferric chloride ($FeCl_3$), ferrous chloride ($FeCl_2$), ferrous sulfate ($FeSO_4$), formic acid, Folin-ciocalteu reagent, 4,4',5,5',6,6'-hexahydroxy-diphenic acid 2,6,2',6'-dilactone (ellagic acid), hyaluronic acid, hydrochloric acid (HCl), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), bovine testicular hyaluronidase (E.C.3.2.1.3.5), potassium acetate (CH_3COOK), potassium persulphate ($K_2S_2O_8$), sodium acetate ($C_2H_3NaO_2$), sodium carbonate (Na_2CO_3), sodium chloride (NaCl), sodium dihydrogen phosphate (NaH_2PO_4), sodium phosphate (Na_3PO_4), 2,4,6-tripyridyl-striazine (TPTZ), and 3,4,5-trihydroxybenzoic acid (gallic acid) were analytical grade bought from Sigma-Aldrich (St. Louis, MO, USA). Amphotericin B, Dulbecco modified eagle medium (DMEM), L-glutamine, penicillin/streptomycin, trypan blue, and secondary antibody conjugated with HRP were bought from Invitrogen™ (Carlsbad, CA, USA). Lipopolysaccharide (LPS) was bought from Cell Signaling Technology® (Danvers, MA, USA). GlutaMAX™-

I supplement was bought from Thermo Fisher Scientific, Inc. (ThermoFisher Scientific, Waltham, MA, USA). Newborn bovine calf serum, fetal bovine serum (FBS), bovine serum albumin (BSA), and antibiotic-antimycotic solution (100 ×) was bought from Gibco™ (Thermo Fisher Scientific, Waltham, MA, USA). Acetic acid, ethanol, and dimethyl sulfoxide (DMSO), were purchased in analytical grade from Labscan, Ltd. (Dublin, Ireland). Acetonitrile was purchased in HPLC-grade from Labscan, Ltd. (Dublin, Ireland).

Plant Material

The *D. longan* fruits were collected from Chiang Mai Province in the Northern Thailand during 2020 by the gardeners according to WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants.[39] The preparation of conventional dried and black *D. longan* was performed by Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand.

Conventional dried *D. longan* preparation

Conventional dried *D. longan* was obtained after the whole fruit of fresh *D. longan* was incubated in an oven set the temperature of 50 ºC until dryness. The sample of dried *D. longan* was kept in sealed plastic bag to prevent contact with air and humidity in the room temperature until further experiments.

Black *D. longan* preparation by thermal and aging process

Black *D. longan* was obtained after the whole fruit of dried *D. longan* was incubated for 20 days in a controlled temperature of 70 ºC and controlled humidity of 75% relative humidity. The sample of black *D. longan* was kept in sealed plastic bag to prevent contact with air and humidity in the room temperature until further experiments.

Preparation of dried *D. longan* and black *D. longan* extracts

Seed, aril, and pericarp were separated from each other. Each part of *D. longan* fruit was ground into fine powder using 20-inch herbal medicine grinder tub with a powerful motor (Thai Pradith Industry Co., Ltd., Bangkok, Thailand). Dried *D. longan* powder was subsequently macerated in 95% v/v ethanol for 3 cycles of 24 h. The extracting solvent from 3 cycles were combined and removed using a rotary evaporator (Buchi Labortechnik GmbH, Essen, Germany). All extracts were stored in the refrigerator (4 ºC) until further experiments.

Chemical compositions determination of dried *D. longan* and black *D. longan* extracts

Total phenolic content determination

Each *D. longan* extract was analyzed for total phenolic content using the Folin-Ciocalteu method according to the previously described method.[40] The results were presented in the form of gallic acid equivalent values (GAE) representing an amount of gallic acid (µg) per g of the *D. longan* extracts. GAE was calculated following the equation; X = (Y - 0.0075)/0.3812, where X is GAE or µg of gallic acid per g

of the *D. longan* extracts and Y is an absorbance of each sample tested in Folin–Ciocalteu assay. The experiments were triplicately performed.

Total flavonoid content determination

Total flavonoid content of each *D. longan* extracts was investigated using aluminium chloride method which has been previously described with some modifications.[41] Firstly, 20 µl of the ethanolic solution of *D. longan* extracts was mixed with 80 µl of 10% w/v $AlCl_3$ aqueous solution and 100 µl of 1 M CH_3COOK aqueous solution. After the resulting mixtures were incubated for 30 min in the dark, they were measured for an absorbance at 415 nm using multimode detector (SPECTROstar Nano, BMG Labtech, Offenburg, Germany). Quercetin was applied as a standard compound to construct a calibration curve. Finally, the results were presented as quercetin equivalent (QE) values which represented a µg of quercetin per g of the *D. longan* extracts. QE was calculated following the equation; X = (Y + 0.033)/0.107, where X is QE or µg of quercetin per g of the *D. longan* extracts and Y is an absorbance of each sample tested in aluminium chloride assay. The experiments were triplicately performed.

Determination of gallic acid, corilagin, and ellagic acid content by high performance liquid chromatography (HPLC)

The quantitative analysis of gallic acid, corilagin, and ellagic acid were performed using an HP 1100 chromatographic system (Hewlett-Packard, Waldbronn, Germany). A gradient mobile phase system composed of two phases, including phase A (0.05% formic acid in acetonitrile) and phase B (0.05% formic acid aqueous solution). The program was set for gradient elution of 10% A (0–8 min), 20% A (8–28 min), 30% A (28-30 min), and 10% 10 (30-35 min), eluting the sample at a flow rate of 1.0 ml/min. The UV detector was set at 280 nm with a Eurospher II 100-5 C18 (250 ´ 4.6 mm, i.d. 5 µm, Knauer, Berlin, Germany). All samples, standard solution, and mobile phase had been filtrated through a 0.45 mm millipore filter, type GV (Millipore, Bedford, MA) before injection to the HPLC system. The injected volume was set at 20 mL. The sample of *D. longan* extracts was prepared at the concentration of 1 mg/ml. Various concentrations of standard gallic acid (10-150 µg/ml), ellagic acid (5-100 µg/ml), and corilagin (2-80 µg/ml) solution were used for the construction of standard curves for quantitative determination. Subsequently, the content of gallic acid, corilagin, and ellagic acid was then calculated.

Gallic acid content was calculated following the equation; *X1* = (100A + 1,296)/26.8C (R2 = 0.9964), where *X1* is the gallic acid concentration, A is the area under the curve (AUC) of the gallic acid peak detected around 4 min, and C is the concentration of the respective sample solution.

Corilagin content was calculated following the equation; *X2* = (100A + 2,325)/17.8C (R2 = 0.9996), where *X2* is the concentration of corilagin, A is the AUC of the corilagin peak detected around 10 min, and C is the concentration of the respective sample solution.

Ellagic acid was calculated following the equation; *X3* = (100A + 13,372)/33.7C (R2 = 0.9957), where *X3* is the concentration of ellagic acid, A is the AUC of the ellagic acid peak detected around 20 min, and C is

the concentration of the respective sample solution.

Antioxidant activities determination of dried *D. longan* and black *D. longan* extracts

2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The radical scavenging effects against $ABTS^{\bullet+}$ of *D. longan* extracts, gallic acid, corilagin, and ellagic acid were evaluated by ABTS assay and reported in the term of Trolox equivalent antioxidant capacity (TEAC) which represented the quantity of Trolox that equivalent to 1 mg of the *D. longa*n extracts.[42] TEAC values were calculated following the equation; X = (Y - 1.2028)/7.9964, where X is TEAC value and Y is an absorbance of each sample tested in ABTS assay. L-Ascorbic acid was used as a positive control. The experiments were triplicately performed.

Ferric reducing/antioxidant power (FRAP) assay

The reducing capacity of *D. longan* extracts, gallic acid, corilagin, and ellagic acid were investigated by means of ferric ion reducing activities.[40] The ferric reducing/antioxidant power of each *D. longan* extracts were expressed in the form of equivalent concentrations (EC_1) representing the amount of ferric-TPTZ reducing capacity, which is equivalent to 1 mg of the *D. longan* extract. EC_1 values were calculated following the equation; X = (Y - 0.0287)/0.01405, where X is EC_1 value and Y is an absorbance of each sample tested in FRAP assay. L-Ascorbic acid was used as a positive control. The experiments were triplicately performed.

Anti-inflammatory activities determination of dried *D. longan* and black *D. longan* extracts

Murine monocyte-macrophage (RAW 264.7) cells (American Type Culture Collection, ATCCTIB-71) treated with LPS were used to investigate the effect of *D. longan* extracts and their chemical compositions on the pro-inflammatory cytokine secretion (IL-6 and TNF-). The cell culture was performed according to a method previously described with some modifications.[43,44] The treated cells along with its supernatant were divided into two parts. The first withdrawal part was undergone centrifugation for 10 min at 13,500´ g and the supernatant was investigated for the cytokine secretion by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's protocol (R&D Systems). On the other hand, the second part, which was leftover in the wells, was investigated for the cell viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. To reduce variation due to cell density differences, secretion of IL-6 and TNF-α from RAW 264.7 cells were normalized to MTT levels.[44] RAW 264.7 cells without LPS treatment served as a negative control, while 100% of cytokine secretion was from positive control of which RAW 264.7 cells treated with LPS. The inhibitory activities of each samples were was calculated following the equation; %inhibition = 100 – A, where A is the amount of cytokines secretion. Dexamethasone was served as a positive control for both IL-6 and TNF-α secretory inhibition. The experiments were triplicately performed.

Anti-hyaluronidase activity determination of dried *D. longan* and black *D. longan* extracts

The hyaluronidase inhibitory activity of *D. longan* extracts, gallic acid, corilagin, and ellagic acid was evaluated by measuring a product from the cleavage of sodium hyaluronate by hyaluronidase.[45] Prior to the experiment, the enzyme activity of hyaluronidase was determined. Only more than 90% enzyme activity was used in the anti-hyaluronidase activity determination. The hyaluronidase inhibitory activity were calculated according to the following equation; % Inhibition = [1 – X/Y)] ´ 100, where X is the absorbance of the mixtures with sample; Y is the absorbance of the mixtures without sample. Oleanolic acid was used as a positive control. The experiments were triplicately performed.

Statistical analysis

All values were given as means ± standard and analyzed. The statistical analysis was used t-test and ANOVA by using SPSS software (SPSS Statistics 21.0, IBM Corporations, New York, NY, USA). A value of $p < 0.05$ were accepted as significant.

Declarations

Acknowledgements

This research was funded by Agricultural Research Development Agency (ARDA), grant number PRP6305032210. The APC was funded by Research Center of Pharmaceutical Nanotechnology, Faculty of Pharmacy, Chiang Mai University and Innovation Center for Holistic Health, Nutraceuticals, and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University. Sriyab is grateful to CMU Presidential Scholarship 2020 for Post-Doctoral researcher.

Author contributions statement

W.C. conceived and designated the experiments. P.H. conducted the experiments. P.H., C.P., W.N., and S.S. collected and analyzed the data. P.H. and W.C. wrote the initial manuscript and revised the manuscript.

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Figures

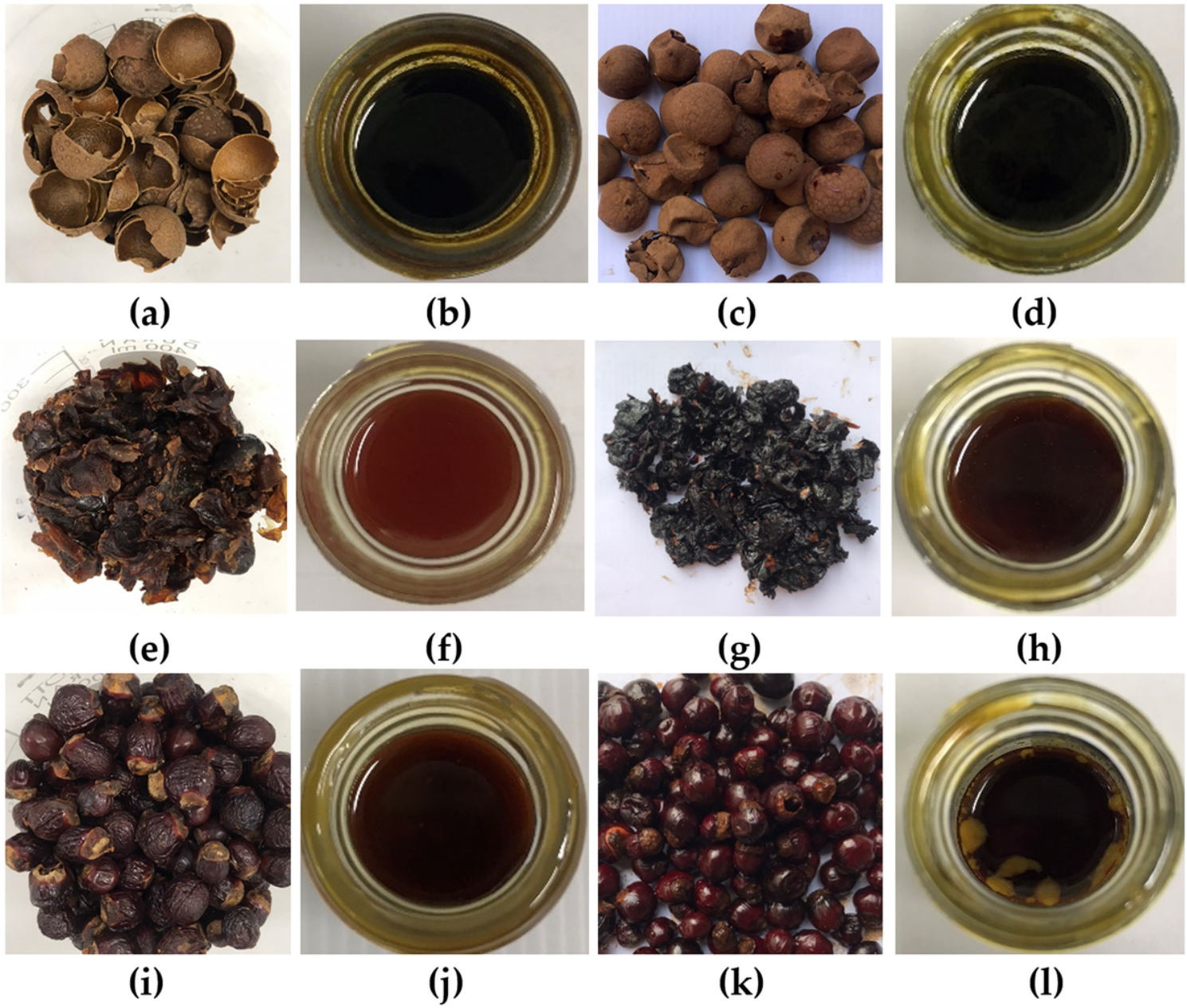

Figure 1

External appearance of dried D. longan pericarp (a), dried D. longan pericarp extract (b), black D. longan pericarp (c), black D. longan pericarp extract (d), dried D. longan aril (e), dried D. longan aril extract (f), black D. longan aril (g), black D. longan aril extract (h), dried D. longan seed (i), dried D. longan seed extract (j), black D. longan seed (k), black D. longan seed extract (l).

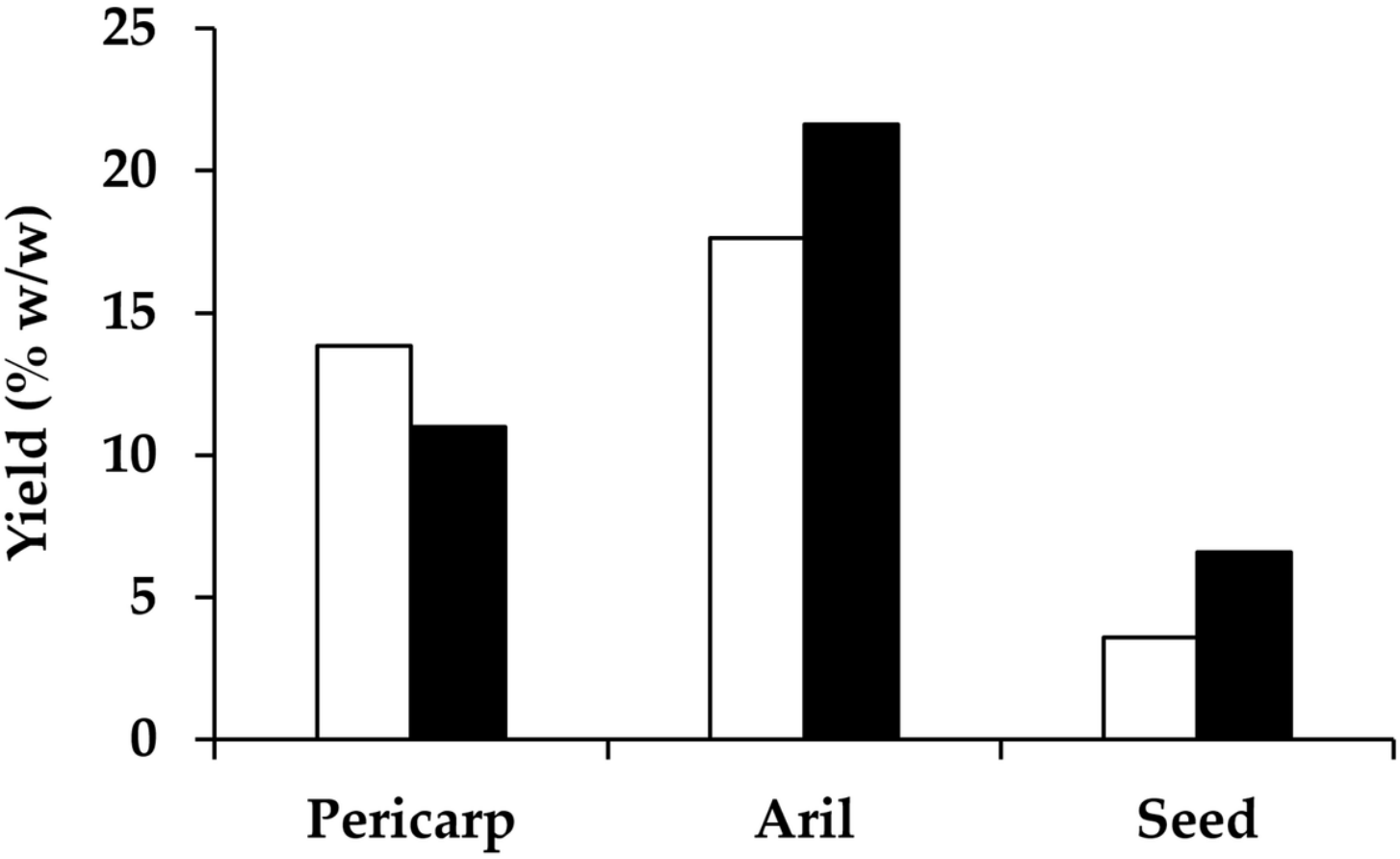

Figure 2

Yield of the ethanolic extracts from pericarp, aril, and seed part of dried (□) and black (■) D. longan.

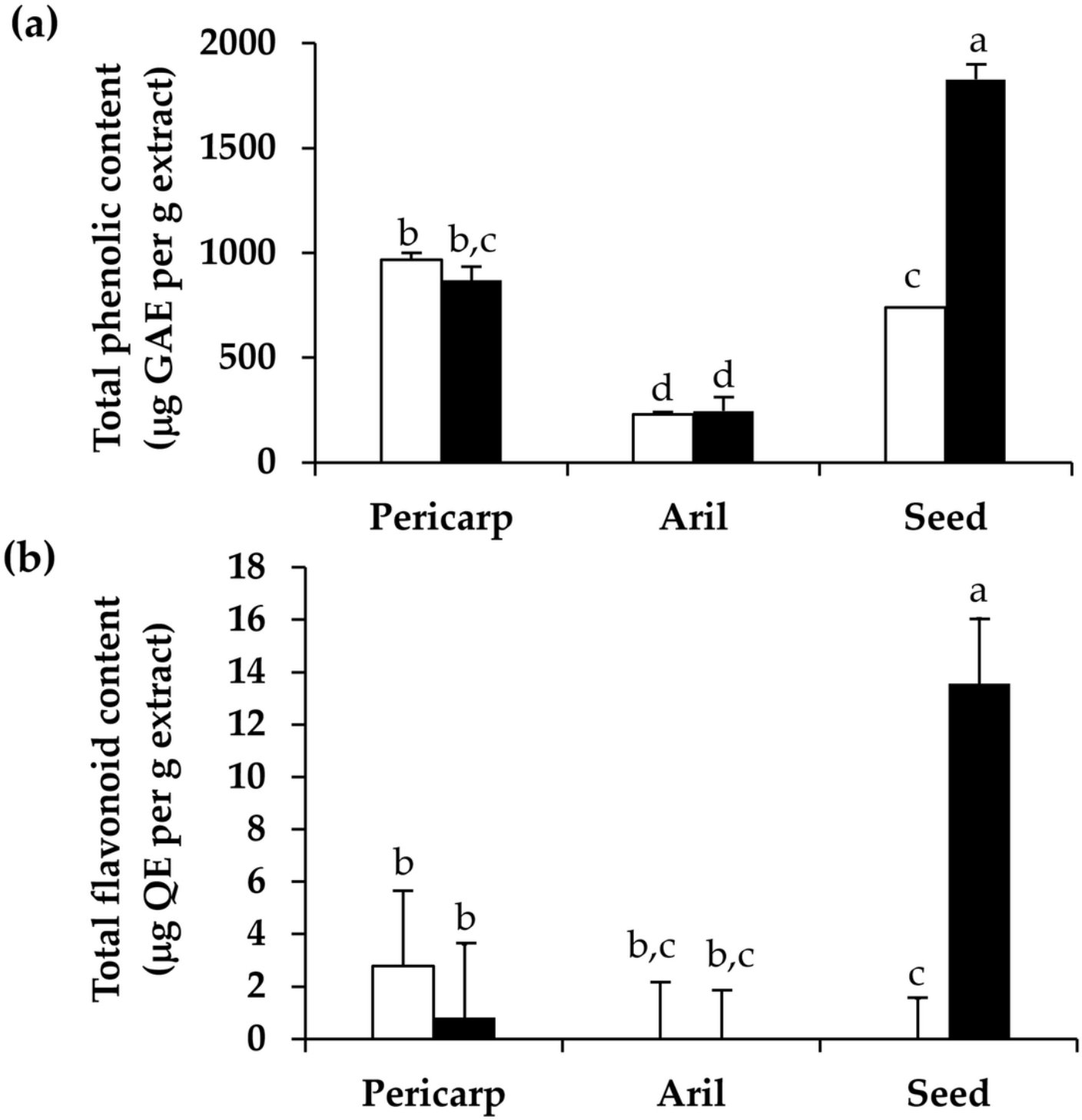

Figure 3

Total phenolic content (A) and total flavonoid content (B) of the ethanolic extracts from pericarp, aril, and seed part of dried (□) and black (■) D. longan. The letters (a, b, c, and d) denote significantly differences in total phenolic content or total flavonoid content among various D. longan extracts ($p < 0.05$).

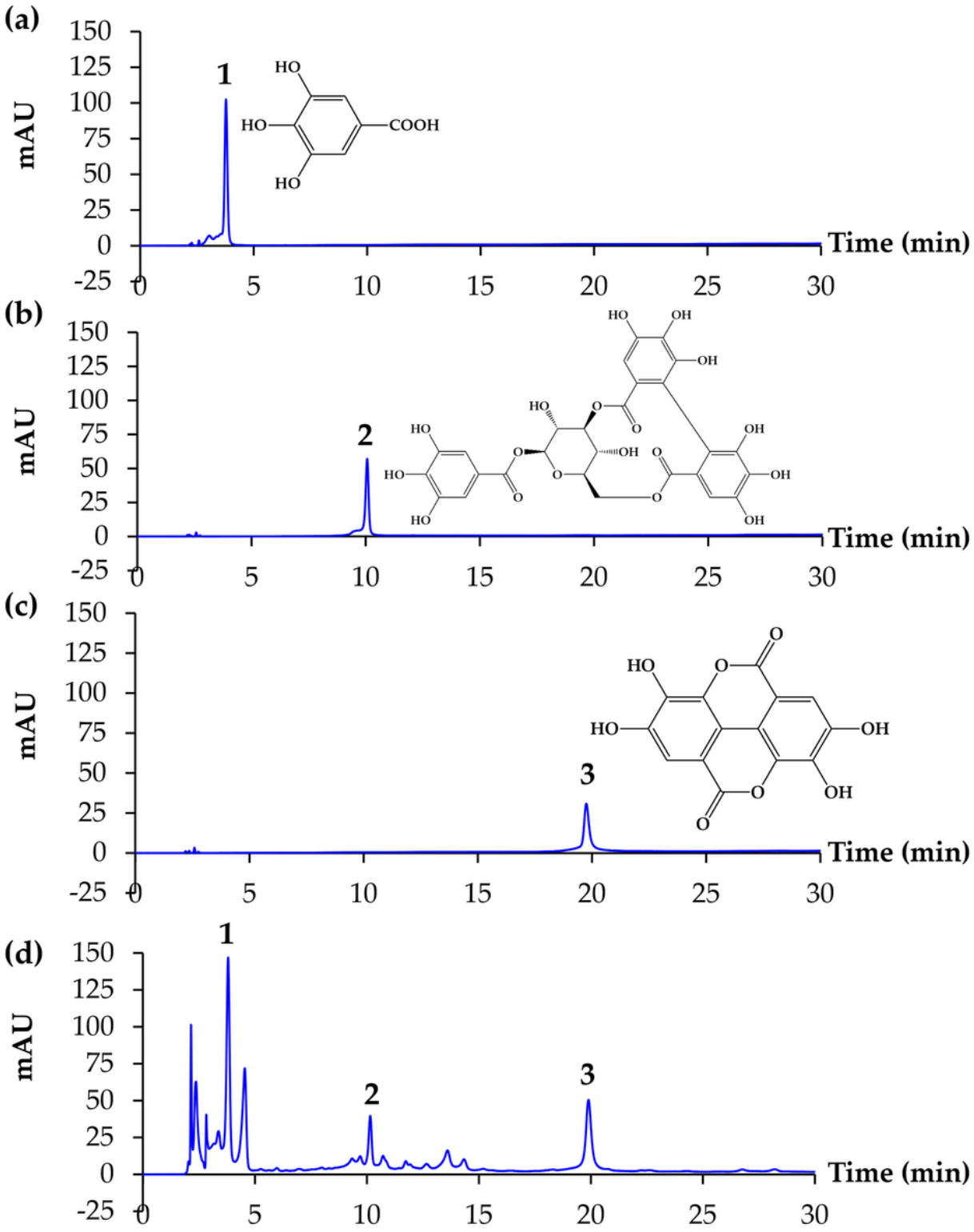

Figure 4

HPLC chromatograms of gallic acid (a), corilagin (b), ellagic acid (c), and black D. longan seed extract (d).

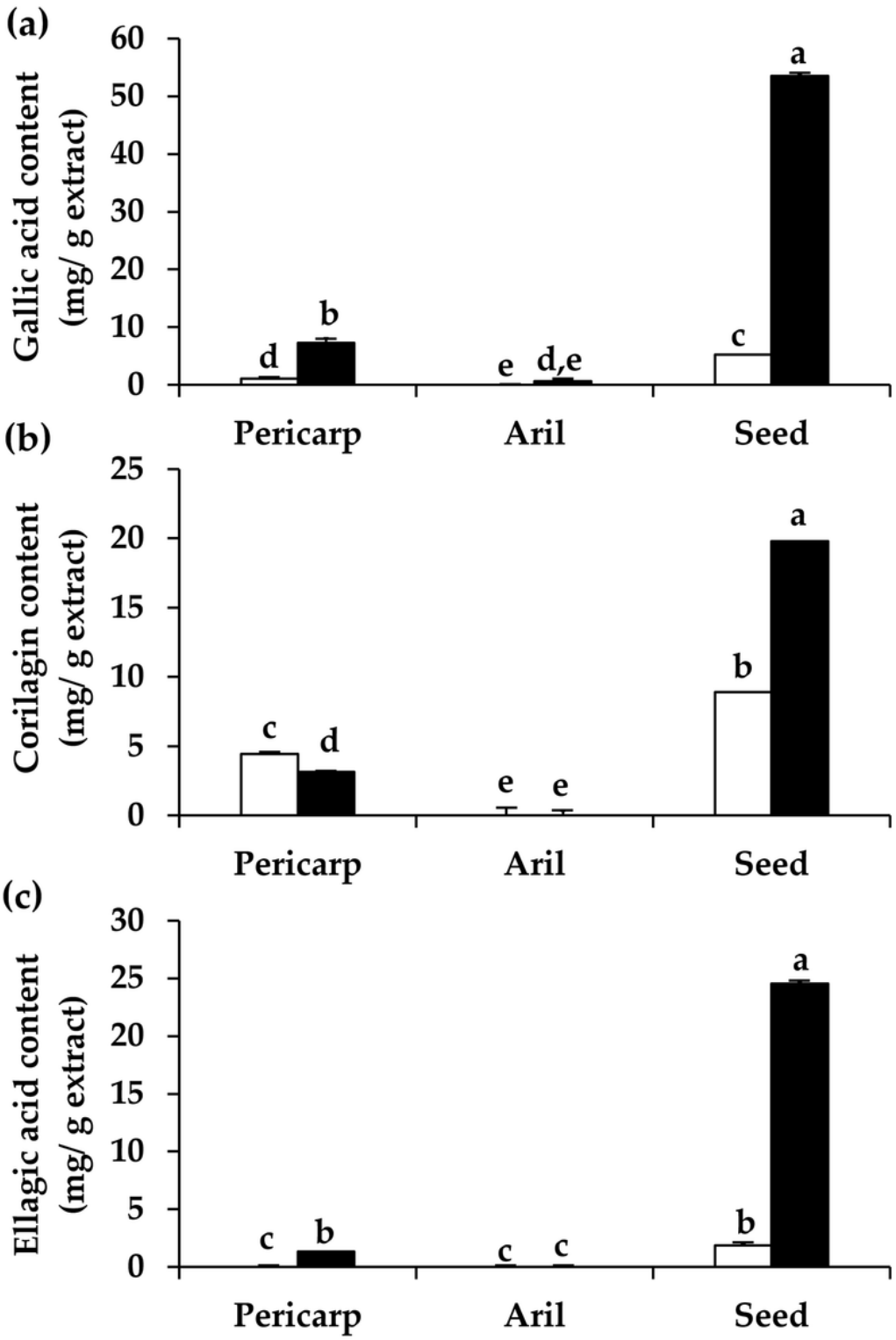

Figure 5

Gallic acid content (a), corilagin content (b), and ellagic acid content (c) of the ethanolic extracts from pericarp, aril, and seed part of dried (□) and black (■) D. longan. The letters (a, b, c, d, and e) denote significantly differences in the content of gallic acid, corilagin, or ellagic acid among various D. longan extracts ($p < 0.05$).

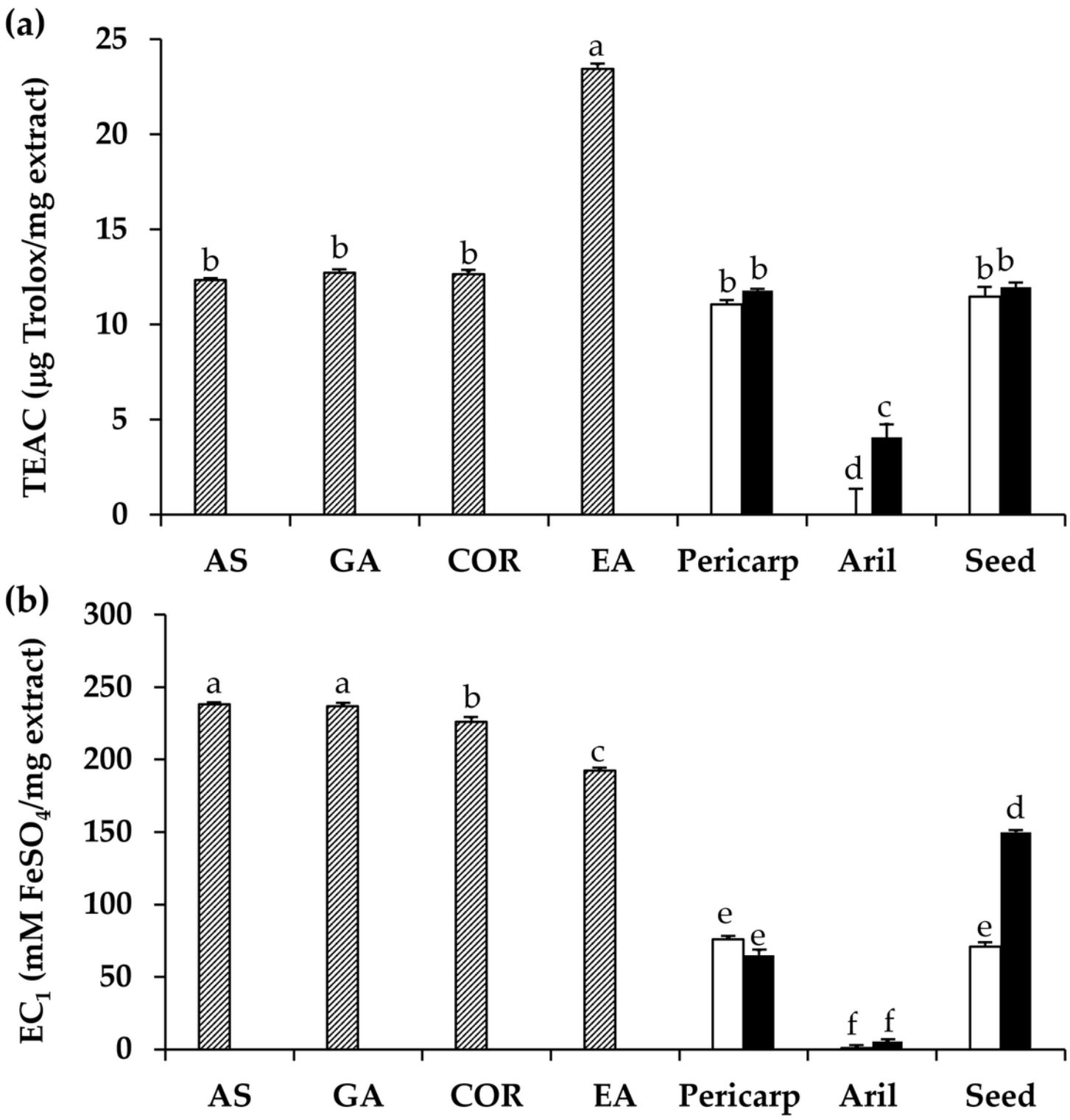

Figure 6

Trolox equivalent antioxidant capacity (TEAC) (a) and equivalent concentration (EC1) (b) of ascorbic acid (AS), gallic acid (GA), corilagin (CO), ellagic acid (EA), and the ethanolic extracts from pericarp, aril, and seed part of dried (□) and black (■) D. longan. The letters (a, b, c, d, e, and f) denote significantly differences in TEAC or EC1 values among various tested samples ($p < 0.05$).

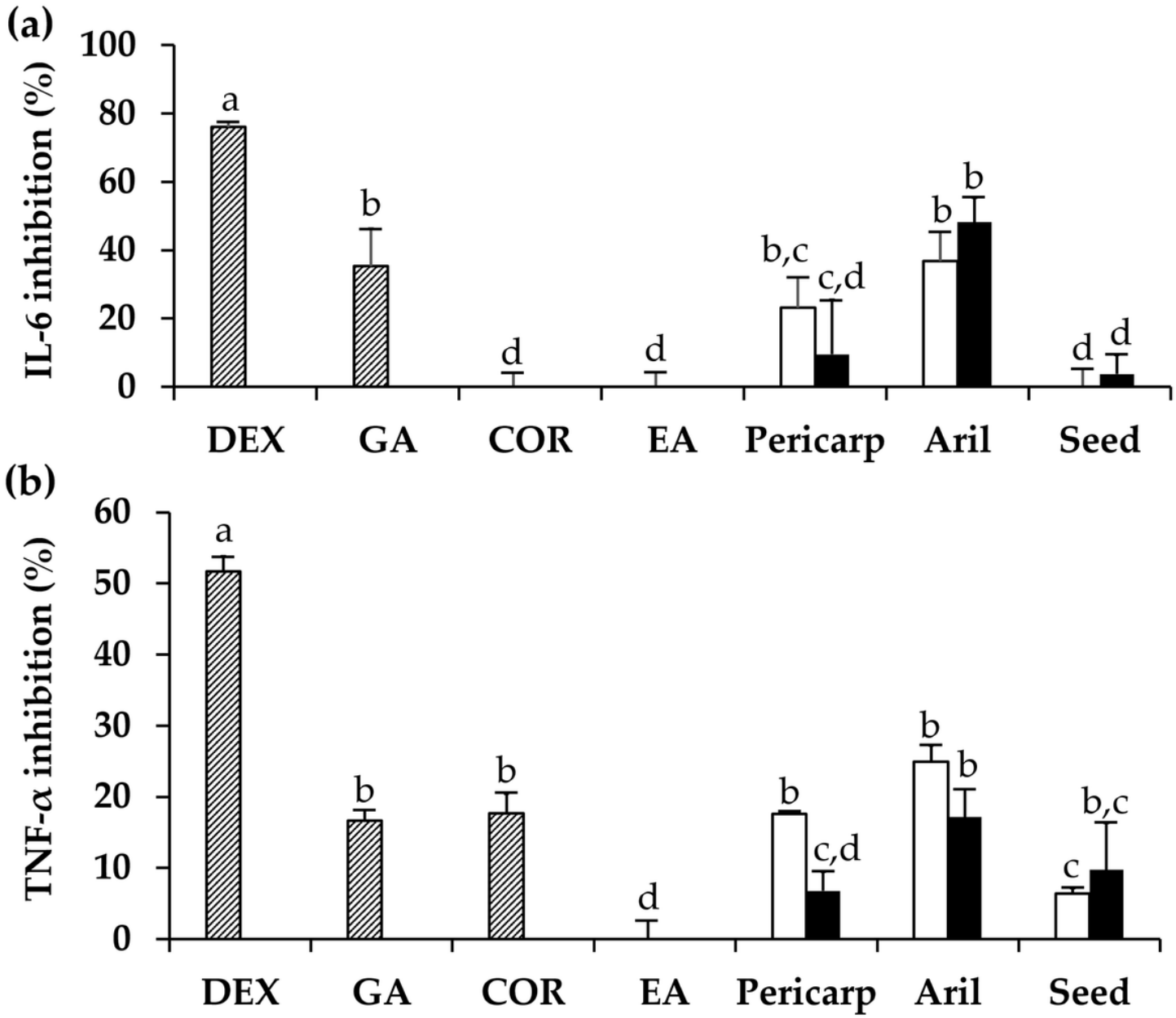

Figure 7

Inhibitory activities against the secretion of interleukin-6 (IL-6) (a) and tumor necrosis factor-α (TNF- α) of dexamethasone (DEX), gallic acid (GA), corilagin (CO), ellagic acid (EA), and the ethanolic extracts from pericarp, aril, and seed part of dried (□) and black (■) D. longan. The letters (a, b, c, and d) denote significantly differences in IL-6 or TNF- α inhibition among various tested samples ($p < 0.05$).

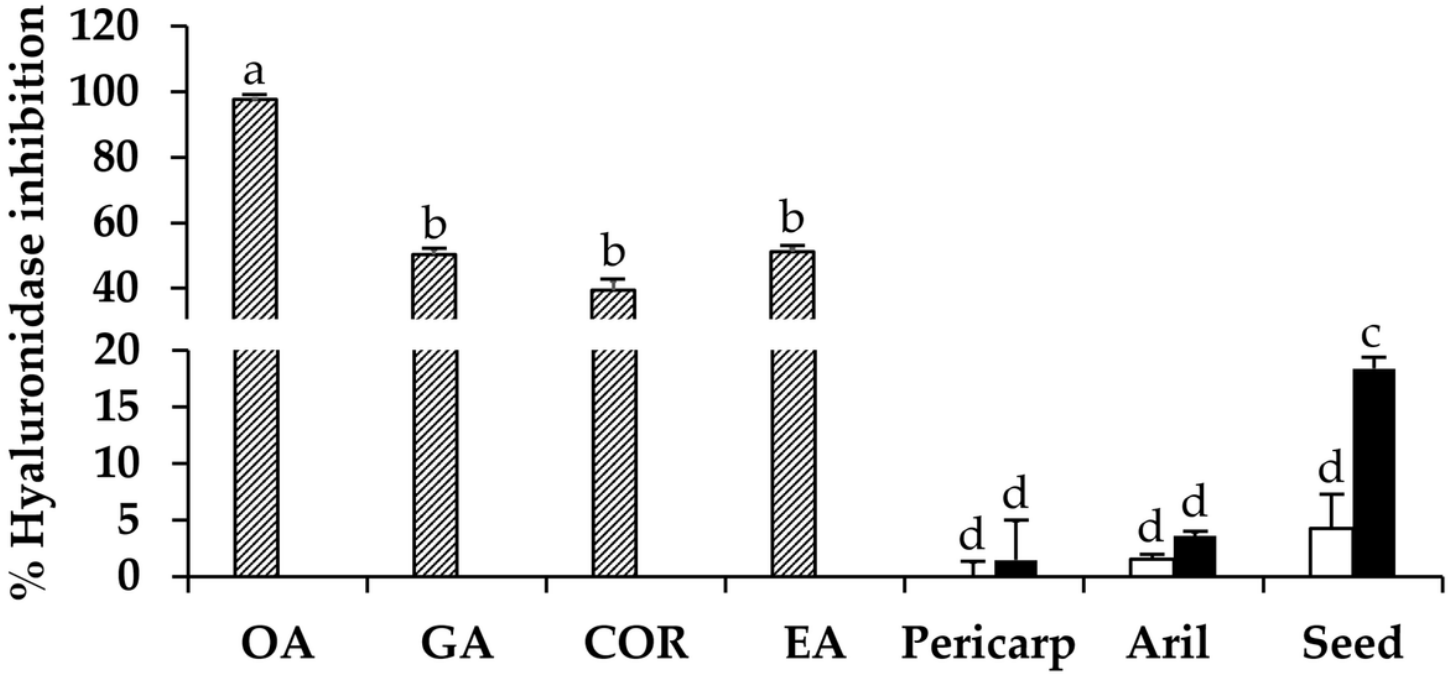

Figure 8

Inhibitory activities against hyaluronidase activity of oleanolic acid (OA), gallic acid (GA), corilagin (CO), ellagic acid (EA), and the ethanolic extracts from pericarp, aril, and seed part of dried (□) and black (■) D. longan. The letters (a, b, c, and d) denote significantly differences in hyaluronidase inhibition among various tested samples ($p < 0.05$).