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Original research

The effects of *Coleus scutellarioides* extract on physicochemical and antioxidant properties of fish gelatin active films

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A B S T R A C T

Active films are the new generation of packaging in the food industry that can maintain and develop the shelf life of food products. The aim of this study was to fabricate and characterize an antioxidant film using fish gelatin and *Coleus scutellarioides* extract (CSE). The CSE was added to the gelatin film solutions in three levels (10, 20, and 30 mL from the extract to 100 mL film solution). The results showed that increasing the extract level increased the thickness of films, and water vapor permeability decreased significantly ($p < 0.05$). The addition of CSE had no significant effect on the moisture content of films. Based on SEM images and FTIR spectra, the fabricated active films have a uniform and uneven structure and fracture were observed in the film structure at high levels of CSE. Chemical bonds were established between the protein-polymer and the phenolic compounds of extract. The incorporation of the CSE also increased the total phenol content, and the antioxidant activity of films and the highest amounts of them were observed in the sample containing the highest level of extract (28.73 mg GA/g of film and 83.24%, respectively). In summary, fish gelatin films containing CSE have the potential to be used as active packaging to extend the shelf life of food products.

Keywords: Active film; Fish Gelatin; *Coleus scutellarioides*; Antioxidant activity

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1. Introduction

Plastic packaging materials are often used in the food industry due to their ease of use and their good mechanical and barrier properties (Chamas et al., 2020). However, researchers have recently been looking for a way to replace these synthetic packages with natural biopolymer packaging materials because plastic synthetic isn't environmentally friendly and has poor recyclability and low biodegradability (Bayazidi et al., 2021; Velásquez et al., 2021). One of the most important biopolymers for food packaging is proteins. Various proteins from different herbal and animal sources are used for packaging. One of the most notable proteins is gelatin, which is obtained by partial hydrolysis of collagen in the skin and bones and connective tissues of animals and fish (Abedinia et al., 2020; Gómez-Guillén et al., 2009). Major source of gelatin is from mammalian animals and in recent decades researchers tried to find other sources for mammalian gelatin. Fish wastes and fish by-products are major and remarkable sources of gelatin. This gelatin forms a three-dimensional network and has an excellent film-forming ability, and thus is very suitable for manufacturing food packaging films (Mahmood et al., 2022). Fish gelatin-based films have moderate hydration capability and good gas barrier properties (Benbettaïeb et al., 2020; Mirzakhani et al., 2018).

Food spoilage is a major global issue that depending on the type of food, oxidation and activity of microorganisms can be the main cause of food products (Haghighatpanah et al., 2022). On the other hand, consumers are looking for more nutritional and functional bioactive compounds in their foods to enhance the health benefits of food products. The use of active packaging with functional characteristics is a noticeable solution to overcome these two issues (Tkaczewska et al., 2021). Biopolymer films have the ability to carry bioactive agents such as antioxidants and antimicrobial compounds and release them gradually into packaged food products (Oladzadabbasabadi et al., 2022). These active packages are able to extend the shelf life and maintain the quality and integrity of food products during handling, distribution and

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storage (Chen et al., 2019). The use of plant extracts and essential oils as the rich sources of functional compounds in the development of active packaging films has been considered by various researchers around the world (Adilah et al., 2018; Didar, 2019; Kadam et al., 2021; Rambabu et al., 2019).

Coleus (*Coleus scutellarioides* or *Solenostemon scutellarioides*) is a flowering plant that belongs to *Lamiaceae* family. This herb is native to Southeast Asia and Malaysia and often grows 0.5-1 m tall. The leaves of coleus are a combination of different colors, including rust, red, cream, chartreuse and purple-black. The leaves of this plant have various applications in traditional medicine, including digestive disorders, diarrhea, pain, bronchitis, etc. (Suva et al., 2015). These leaves contain different bioactive compounds such as essential oils, flavonoids, polyphenols, alkaloids (Ahmad & Massi, 2014), and red anthocyanins (Logan et al., 2015). Therefore, this study was performed to develop active and antioxidant fish gelatin-based packaging film using *Coleus scutellarioides* extract.

2. Material and Methods

2.1. Materials

Fish gelatin was purchased from SIM supply company (Penang, Malaysia). *Coleus scutellarioides* were obtained from local market (Damghan, Iran). All chemicals used were of analytical grade and were used as received without any further purification.

2.2. Preparation of Coleus scutellarioides extract (CSE)

Coleus scutellarioides leaves were dried for three days, milled and sieved. The 200-mesh powder was collected for the preparation of the extract. Five grams of the powder was mixed with 100 mL of the ethanol-water mixture (in a volume ratio of 1:1) and was stirred for 1 h at 500 rpm. The solution was then filtered through Whatman No.4 filter paper, and the obtained extract (CSE) was kept in the dark place at 4°C until further use (Luchese et al., 2018).

2.3. Preparation of fish gelatin/CSE active films

Firstly, 40% of glycerol as a plasticizer (w/w, gelatin basis) was mixed 100 mL of distilled water under stirring of 350 rpm until its temperature reached to 50°C. 8.0 g of fish gelatin was then slowly incorporated into the solution and stirred at 60°C for 40 minutes. The total anthocyanin content in the CSE, determined by the pH differential method, was calculated as 39.27 ± 5.78 mg L^{-1}. CSE was added to the film solution by 10 mL, 20 mL, 30 mL of CSE when the solution was cooled down to 40°C and stirred for 10 min. The film solution was cast into 16 cm × 16 cm and was dried for 24 h at room temperature (23 ± 2°C) and continued dried in the oven drying for 9 h at 40°C. The films were prepared with 10 mL, 20 mL, and 30 mL of CSE were expressed as CSG1, CSG2 and CSG3, respectively, while the control film without CSE added is CSG0 (Chi et al., 2020).

2.4. Film Characterization

2.4.1. Thickness and moisture content of films

The thickness of the film was measured at 5 randomly selected points with an ID-C112XBS micrometer (Mitutoyo Corp., Tokyo, Japan), and the average thickness of the film was recorded. The moisture content of the films was measured by Pourjavaher et al. (2017) method with slight modification. The films were cut into three different sizes (5 mm × 15 mm; 5 mm × 20 mm; 5 mm × 30 mm), and the films were stored at room temperature of 25 ˚C and 55% RH for 2 days. After two days, the weight of the films was measured and recorded as the initial weight. Then, the films were stored in a desiccator containing P_2O_5 (0% RH) at room temperature and were weighted after 7 days. The moisture content was then determined using the Eq. (1).

$$\text{Moisture content (\%)} = \frac{W_i - W_t}{W_i} \times 100 \quad (1)$$

where W_t is the weight of the film at a certain time; W_i is the initial weight.

2.4.2. Water vapor permeability

The water vapor permeability of the films was determined through a slightly modified method by ASTM E96-16 (2016) with some modification (Chang et al., 2021). A circular test cup (No. 318 Water Permeability Cup) was used to determine the water vapor permeability of the film. Silica gel as a desiccant with 0% relative humidity was placed inside the cup. The film was cut into a circular shape larger than the cup's inner diameter, and the thickness was measured with a micrometer (Mitutoyo Corp., Tokyo, Japan) at 5 randomly selected points. After that, the film was sealed to the top of the cup with parafilm, and the cup was weighed to calculate the initial weight. The cup was then located inside a desiccator containing distilled water (100% RH). The rate of the water vapor movement through the film into the silica gel was determined by periodic daily weighing over 7 days period.

The weight gain of the film determined the water vapor transmission rate (WVTR). A graph of weight gain as a function of time was plotted, and WVTR was calculated from the slope of the graph and divided by the permeation area. Water vapor permeability (WVP) was calculated by Eq. (2).

$$\text{WVP} = \frac{\text{WVTR} \times d}{S \times (RH_1 - RH_2)} \quad (2)$$

where S is the saturated water vapor pressure at test temperature (Pa); RH_1 is relative humidity of desiccator; RH_2 is relative humidity of permeation cell; d is film thickness (mm).

2.4.3. Fourier transform infrared (FTIR) spectroscopy

The IR spectra of the films were determined using a Nicolet iS10, Smart OMNI Transmission FTIR spectrometer (Thermo Scientific). Each sample was cut into a square shape with a 3 cm × 3 cm dimension and placed onto a magnetic sample holder. The measurements of FTIR spectra were recorded in the range of 4000-600 cm^{-1} with a resolution of 4 cm^{-1}.

2.4.4. Scanning electron microscopy (SEM)

The cross-section of the films was captured by scanning electron microscopy (Model Leo Sipra So vp Field Emission, CaH-

Zeiss SMT, Oberkochen, Germany) with an accelerating voltage of 10 kV. The SEM images were obtained by collecting the samples on an aluminum SEM disk, then coated with platinum. The samples were viewed perpendicular to the fracture surface for studying the cross-section.

2.4.5. Total phenol content and antioxidant activity

Briefly, each film sample (with a weight of 25 mg) was dissolved entirely in distilled water (5 mL). After that, a mixture containing 0.1 mL film solution, 0.5 mL Folin-Ciocalteu's reagent and 7 mL distilled water were prepared in a shaker at 100 rpm, and 23°C for 8 min under dark condition and then 1.5 mL sodium carbonate solution (2% w/v) was added to it and reached to a final volume of 10 mL with distilled water. After stirring for 15 s and standing for 30 min at 23°C for development of color at dark, the absorbance of the resulting mixture was recorded at 765 nm. The total phenol content of film samples was calculated according to the Eq. (3) and expressed as mg GAE (gallic acid equivalents) per g of film mass.

$$\mathrm{TPC} = \frac{\mathrm{C} \times \mathrm{V}}{\mathrm{M}} \quad (3)$$

where C is GA concentration obtained from the calibration curve (mg mL^{-1}), V is the volume of testing solution (mL), and M is the mass of testing film (g) (Slinkard & Singleton, 1977).

To determine the antioxidant activity of the film samples, the samples (20 mm × 20 mm) were first placed in a tube containing methyl alcohol (4 mL). The resulting mixture was stirred at room temperature for 2 h, after which the supernatant solution (3 mL) was mixed with 150 µM DPPH methanol solution (l mL). The absorbance of the resulting mixture was recorded at 517 nm (A1). In another test tube, 3 mL of the supernatant was mixed with 150 µM methanol solution (1 mL) and the absorbance was recorded at 517 nm (A0). Finally, the percentage of DPPH scavenging activity was determined according to the Eq. (4).

$$\text{Scavenging activity (\%)} = \left(1 - \frac{\mathrm{A1}}{\mathrm{A0}}\right) \times 100 \quad (4)$$

where: A1 and A0 are the absorbances of sample solution and control solution, respectively (Peng et al., 2013).

2.5. Statistical analysis

The statistical analyses were performed using one-way analysis of the variance (ANOVA), which showed the statistically different values. Meanwhile, the mean comparison was carried out by Duncan's test. The SPSS 26.0 software (IBM SPSS Inc, Chicago, IL) was used with differences at $p < 0.05$ considered significant.

3. Results and Discussion

3.1. Film thickness and moisture content

The thickness of films was measured using a micrometer at five randomly selected points, and the average of the film thickness was recorded as shown in Table 1. Based on the results shown in Table 1, the thickness film of all the samples increased significantly ($p < 0.05$) as the concentration CSE was added. CSE0, which is the control film without CSE added, has the lowest value, 0.10 ± 0.01 mm and CSE3 has the highest values, 0.17 ± 0.02 mm of thickness, which is the film with the highest amount of CSE added. The thickness of the films is directly proportional to the concentration of CSE added to the film. Since CSE is made up of both soluble and insoluble fibers, the extract cannot fully solubilize, which increases the film-forming solution (Hanani et al., 2019). Therefore, the increased thickness of films was observed in the presence of anthocyanins. According to Kaewprachu and Rawdkuen (2014), the film thickness will affect film properties such as mechanical properties, water vapor permeability, light transmission and film transparency. Due to the incorporation of phycocyanin and black soybean seed coat extract, increased film thickness has also been reported by Chentir et al. (2019) and Wang et al. (2019), respectively.

Table 1 also shows the moisture content of all the films. The moisture content obtained are 0.23 ± 0.01%, 0.23 ± 0.01%, 0.25 ± 0.02% and 0.25 ± 0.01% for the film CSG0, CSG1, CSG2 and CSG3 respectively. The results showed no significant ($p > 0.05$) change in the moisture content after incorporating CSE into the films regardless of the concentration of CSE. The study from Nur Hanani et al. (2018) also obtained no significant moisture content changes between the fish gelatin film without and with the incorporation of pomegranate peel. According to their study, the hydrophilic and hydrophobic components in the pomegranate peel could balance the films' hygroscopic properties, which results in no effect on the moisture content of the films. Hu et al. (2019) also found that adding Ginkgo biloba extract to a level of 3% had no significant effect on the moisture content of gelatin-based films.

Table 1. Thickness and moisture content of gelatin/*Coleus scutellarioides* extract films.

Film Sample	Thickness, (mm)	Moisture content, (%)
CSG0	0.10 ± 0.01^{a}	13.23 ± 0.41^{a}
CSG1	0.14 ± 0.01^{b}	13.37 ± 0.28^{a}
CSG2	0.15 ± 0.01^{bc}	13.45 ± 0.39^{a}
CSG3	0.17 ± 0.02^{c}	13.42 ± 0.34^{a}

Data points with same letters within same column indicate no significant different ($p > 0.05$) by Duncan's mean comparison test. CSG0, CSG1, CSG2, and CSG3 represent fish gelatin films with 0, 10, 20, and 30 mL of *Coleus scutellarioides* extract.

3.2. Water vapor permeability (WVP)

WVP is a measure of the ease of the passage of water vapor to penetrate a material, and this attribute has a great influence on food shelf life. Fig. 1 shows the WVP values of the CSG0, CSG1, CSG2 and CSG3 film. The WVP values showed an insignificant ($p > 0.05$) decrease as the concentration of CSE added in film increased. CSG0 film has the highest WVP values (8.09×10^{-11} g m/Pa s m^2), while CSG3 has the lowest (4.03×10^{-11} g m/Pa s m^2). CSG0 film might have a dense protein network with low polarity, which could resist water molecule transfer through the film. According to Otoni et al. (2017), WVP is affected by the mobility and free volume of the macromolecules and the integrity and hydrophilic–hydrophobic and crystalline–amorphous ratios of the films. CSG3 has the lowest WVP values might be due to the high amount of –OH group in the phenolic compound of Rambutan peel, possibly interacting with the protein chain of fish gelatin, resulting in the lower content of free OH groups.

Besides, WVP values obtained for CSG0, which is the control films contain gelatin is, similar to the WVP values reported by Limpan et al. (2010), who obtained a value of 8×10^{-11} g m/Pa s m^2 for edible films from fish protein using glycerol as a plasticizer. It should be noted that WVP values of the CSG films are in the range of those reported for good protein films and more permeable to water vapor than that of synthetic polymer packagings such as LDPE and HDPE (WVP ≈ 10^{-13} g m/Pa s m^2) (Wihodo & Moraru, 2013). Therefore, although the concentration of CSE added does not affect the gelatin film's WVP values, the CSG films exhibit good films properties that are well permeable to water vapor. Decreased WVP of biopolymer-based films due to the incorporation of various extracts has also been observed by other researchers (Azlim et al., 2022; Liang et al., 2018; Sun et al., 2019).

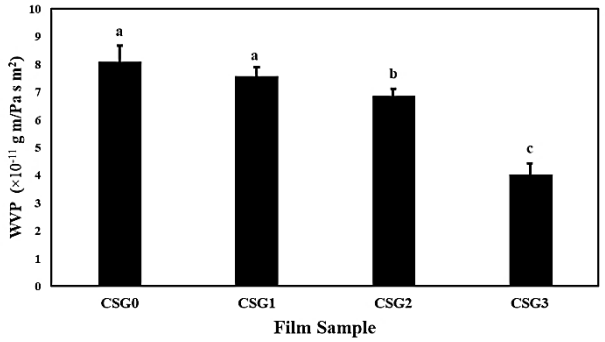

Fig. 1. Water vapor permeability of the gelatin/*Coleus scutellarioides* extract films. Data points with same letters indicate no significant different ($p > 0.05$) by Duncan's mean comparison test. CSG0, CSG1, CSG2, and CSG3 represent fish gelatin films with 0, 10, 20, and 30 mL of *Coleus scutellarioides* extract.

3.3. *Fourier transform infrared (FTIR) spectroscopy*

The intermolecular interactions between gelatin film and the concentration of CSE added were investigated using FTIR technique is presented in Fig. 2. The addition of CSE in the films, which is shown in Fig. 2 (b), (c) and (d) presented characteristic bands of polyphenols at 3745 cm^{-1} (O-H stretching), 1037 cm^{-1} (C-H deformation of aromatic rings) and 1633 cm^{-1} (C=C stretching of aromatic ring) (Yun et al., 2019). The peak situated around 1037 cm^{-1} in all spectra also might be related to the glycerol added as plasticizer (Bergo & Sobral, 2007). The bending vibration of N-H group and stretching vibration of C-N group at 1544 cm^{-1} (amide II) was observed in all the films due to the gelatin matrix. Gelatin film also showed the peak of amide I, representing the carbonyl group at 1633 cm^{-1} (Limpan et al., 2010). The intensity of amide I and amide II peaks increased with CSE addition. The spectra changes suggested the presence of protein-protein and protein–polyphenol interactions via hydrogen bond (Limpan et al., 2010). The band's intensity at 1037 $cm^{-1,}$ which is assigned as C-H deformation of aromatic rings (Silva-Pereira et al., 2015), increases by the addition of CSE. If there are noticeable band shifts, which can be found in FTIR spectra with the addition of each component, it means that the chemical interaction between components is present (Wu et al., 2009). Thus, this spectra analysis could identify chemical interactions between polymers that polyphenol of anthocyanins and protein-polymer from gelatin. Jiang et al. (2020) also reported similar results. These researchers found that new interactions occur between the components of CMC/starch films containing anthocyanin of purple sweet potato. Bao et al. (2022) also observed the interaction between the components of active films based on potato starch containing blueberry anthocyanins.

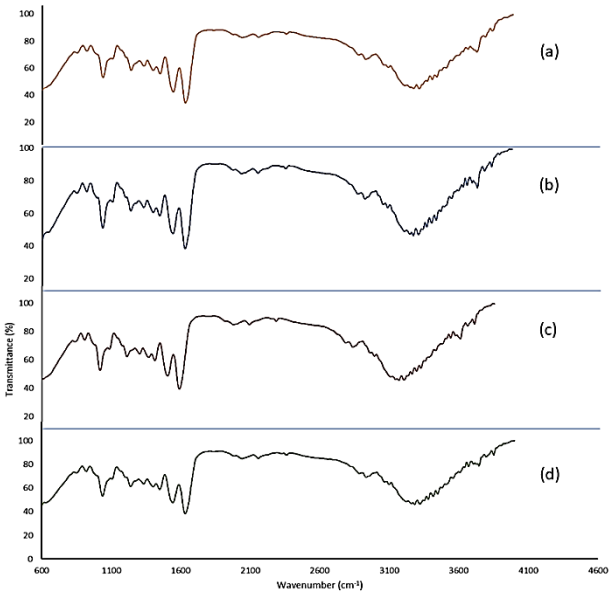

Fig. 2. FTIR spectra of (a) CSG0, (b) CSG1, (c) CSG2 and (d) CSG3. CSG0, CSG1, CSG2, and CSG3 represent fish gelatin films with 0, 10, 20, and 30 mL of *Coleus scutellarioides* extract.

3.4. *Scanning electron microscopy (SEM)*

SEM was used to investigate morphological changes of gelatin film associated with incorporating anthocyanins. Images of the cross-section of the films obtained by SEM are shown in Fig. 3. CSG0 is the control film, fish gelatin film without CSE added, while CSG1, CSG2, and CSG3 is the film that added CSE by 10 mL, 20 mL, and 30 mL, respectively. All the films showed fibrous structure, which may be associated with collagen fibrils in the gelatin film (Chambi & Grosso, 2006). All the films also show rough and uneven morphology attributed to lower interactions of the anthocyanin compounds with the protein and glycerol constituents (Prietto et al., 2017). They also reported that the surface roughness did not influence the color variation of the film as a function of the pH. Furthermore, cracks were observed in the CSG2 and CSG3, while no cracks were detected in CSG0 and CSG1. Similar to the study by Pourjavaher et al. (2017), they observed cracks and cavities in the films incorporated with anthocyanins. These might be due to the development of a heterogeneous film structure that leads to discontinuities in the polymer network after adding anthocyanins. Therefore, there are morphological changes of the fish gelatin film after incorporated with CSE such as cracks and cavities due to the presence of anthocyanins that result the discontinuities in the polymer network.

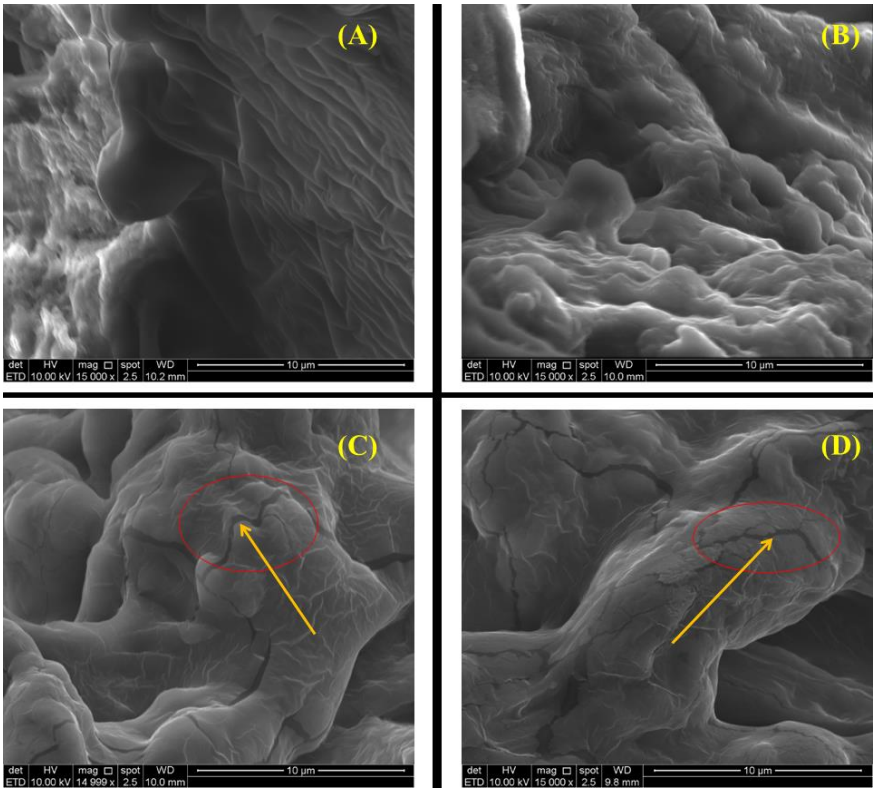

Fig. 3. SEM images of cross section of (A) CSG0, (B) CSG1, (C) CSG2 and (D) CSG3. CSG0, CSG1, CSG2, and CSG3 represent fish gelatin films with 0, 10, 20, and 30 mL of *Coleus scutellarioides* extract.

3.5. Total phenol content and antioxidant activity

DPPH radical is a highly stable free radical that contains central nitrogen. DPPH radical scavenging is one of the most widely used standard methods for investigating the antioxidant activity of food packaging films (Siripatrawan & Harte, 2010). Figs. 4 and 5 indicate the total phenol content (TPC) and antioxidant activity of active gelatin-based films containing different levels of CSE, respectively. As expected, the control gelatin film contained a very low amount of phenolic compounds (1.89 mg GAE/g of the film) and very low antioxidant activity (4.11%), and by adding CSE and increasing its level from 10 mL to 30 mL, the TPC and antioxidant activity of the films showed a significant increase ($p < 0.05$).

The TPC of CSG1, CSG2 and CSG3 films were 12.93, 19.25 and 28.73 mg GAE/g of film, respectively, and the antioxidant activity of these films was 49.24%, 70.92% and 83.24%, respectively. Moradi et al. (2012) also agreed with the present study results that there is a direct relationship between the concentration of incorporated active compounds and the free radical scavenging of the film. Improvement of the antioxidant activity of gelatin-based films by adding anthocyanin extracts was also observed by Ge et al. (2020), Wang et al. (2019) and Rawdkuen et al. (2020). In general, the researchers found that the antioxidant activity of packaging films depends on the release of active compounds, film's microstructure, and the interaction between the active compounds and the packaging polymer (Piñeros-Hernandez et al., 2017).

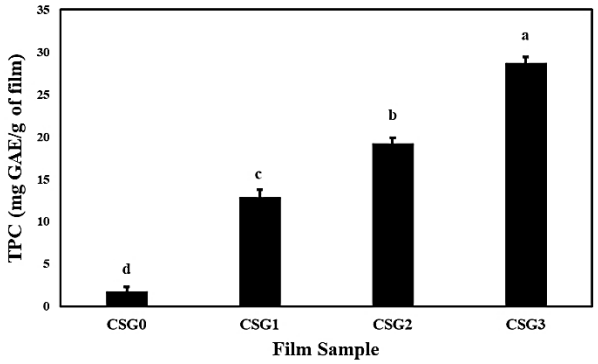

Fig. 4. Total phenol content of the gelatin/*Coleus scutellarioides* extract films. CSG0, CSG1, CSG2, and CSG3 represent fish gelatin films with 0, 10, 20, and 30 mL of *Coleus scutellarioides* extract.

4. Conclusion

The results of this study generally indicated a remarkable effect of CSE in reducing the sensitivity of gelatin films to moisture. Also, by adding the CSE to the films, the functional and antioxidant activity of the produced films improved significantly and a direct and positive relationship was observed between the content of phenolic compounds and the antioxidant activity of the films. In general, it can be concluded that CSE can be used as a rich source of antioxidants and functional compounds to prepare active food packaging films.

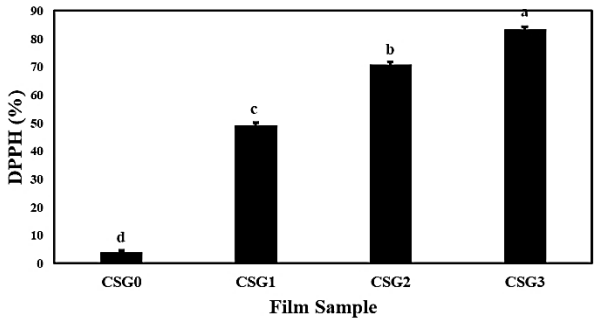

Fig. 5. Antioxidant activity of the gelatin/*Coleus scutellarioides* extract films. CSG0, CSG1, CSG2, and CSG3 represent fish gelatin films with 0, 10, 20, and 30 mL of *Coleus scutellarioides* extract.

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Conflict of interest

The authors declare that they have no known competing financial interests.

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