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FULL PAPER

Preparation and characterization of gelatin-based films with the incorporation of *Centella asiatica* (L.) urban extract

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Abstract

This study aimed to investigate the functional and antioxidant properties of chicken skin gelatin (CSG) films incorporated with *Centella asiatica* (L.) urban extract. Film formulations with different amounts of *C. asiatica* extract (0, 0.3 and 0.7 g) were prepared under mechanical stirring at a temperature of 45°C. Results revealed that CSG film with 0.7 g *C. asiatica* was optimal due to its high elongation at break (EAB) (249.62%) and low water vapor permeability (WVP) (1.09 x 10^{-9} kPa) rate. The FTIR assay presented a strong carboxyl group, while XRD revealed amorphous characteristics at a peak of 2Θ=20º. The film with 0.7 g *C. asiatica* extract also showed the highest antioxidant properties for DPPH (68.67%), total phenolic content (10.79 mg/mg gallic acid), and reducing power (14.4%). In conclusion, CSG film incorporated with *C. asiatica extract* has remarkable potential as an active film material, as it may have a positive influence on the environmental concern.

1. Introduction

Food packaging is an important discipline in the area of food technology, especially in terms of the preservation and protection of all types of foods, and specifically in those cases where oxidative and microbiological deterioration occurs (Tharanathan, 2003). Oxidative processes cause the degradation of meat proteins, pigments and lipids, which limits the shelf life of the food product (Liu *et al.,* 2010). Hence, active packaging may carry antioxidants to delay deleterious effects (Li *et al.,* 2014). The active films can be made from natural biopolymers, such as proteins, lipids and polysaccharides or the combination of these materials (Tharanathan, 2003). Among proteins, gelatin has been impressively used for the development of active packaging due to its relative abundance and good film-forming ability (Arfat *et al.,* 2014). Gelatins from tuna skin (Gómez-Esteca *et al.,* 2009), and bovine hide (Rivero *et al.,* 2010) have been shown to have good film forming properties. However, such gelatin films have poor light transparency, and inferior water vapor barrier and thermal properties. Therefore, antioxidative substances such as essentials oils, lipid, waxes and natural antioxidant from plant extract have been used to improve the functional properties of gelatin films (Wu *et al.,* 2013).

Currently, researchers are focusing on developing active food packaging with antioxidant agents from natural sources such as plant extracts (Li *et al.,* 2014). *C. asiatica*, commonly known as *pegaga* (Malaysia), pennywort (India) and *gotu kola* (Europe), has triterpenes as major biologically active ingredients which indicated strong antioxidant activity (Hashim, 2011). The use of chicken skin gelatin has proven to be a superior alternative in producing gelatin film due to its relative abundance and thus contribute to the environmental friendliness in the waste of poultry industry management. Chicken skin gelatin also exhibits excellent gel strength than that of bovine gelatin (Sarbon *et al.,* 2013; Nor *et al.,* 2016) and possessed good thermal properties as compared to mammalians gelatin (Sarbon *et al.,* 2013). However, to date, there is no published research on active film packaging from chicken skin gelatin incorporated with *C. asiatica* (L.) urban extract. Therefore, the objectives of this study were to investigate the antioxidant and functional properties of chicken skin gelatin films incorporated with *C. asiatica* extract.

2. Materials and methods

2.1 Materials

Chicken skins were purchased from local poultry processing industry and kept in ice during transport to the laboratory. The chicken skins were washed thoroughly in water and stored at -80ºC until further use.

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The whole plant of *C. asiatica* was purchased from a local market in Kuala Terengganu, Malaysia. All other chemicals used in this study were of analytical grade.

2.2 Methods

2.2.1 Sample preparation

Visible fat on the skin was mechanically removed and thoroughly rinsed in excessive water in order to remove the impurities. The skins were cut into 2-3 cm pieces and freeze-dried. The dried skins were ground before being defatted using the Soxhlet method (AOAC, 2006).

2.2.2 Chicken skin gelatin extraction

Chicken skin gelatin was prepared following the method of Sarbon *et al.* (2013) using acid-alkaline pretreatment. The defatted chicken skin was ground and soaked consecutively in 0.15% (w/w) sodium hydroxide, 0.15% (w/v) sulphuric acid and 0.7% (w/w) citric acid solution. Each solution was stirred and shaken slowly at room temperature for 30 mins before centrifuged at 3500 x *g* for 10 mins. The supernatant was removed, and each treatment was repeated three times to remove the pigments and non-collagenous proteins. The pellets were then thoroughly rinsed in distilled water to remove residual salts and placed in distilled water for overnight at control temperature (45ºC). The clear extract was filtered, concentrated by evaporation under vacuum, and freeze-dried.

2.2.3 C. asiatica (L.) urban extraction

A total of one kg of wet *C. asiatica* was freeze-dried before grinding to powder. The powder was extracted using boiling water in a Soxhlet apparatus (Siripatrawan and Harte, 2010). The resultant extract was freeze-dried and kept at temperature -20ºC prior to further use.

2.2.4 Development of gelatin films

Gelatin film was prepared using the casting technique as described by Suderman *et al.* (2016) with slight modifications. Briefly, 4.0 g of gelatin powder was mixed with different amounts of *C. asiatica* extract (0, 0.3 and 0.7%) in 100 mL of distilled water under mechanical stirring until it was completely dissolved. The film-forming solution was then added with 1.5 g glycerol as a plasticizer. All mixtures were stirred at 45° C for 20 mins in order to obtain a homogeneous gelatin film solution. Lastly, approximately 25 g of the filmogenic solution was poured into a clean Petri dish and dried at 45°C in an oven for 2 days. Dried films were then removed from the Petri dish and conditioned in desiccator prior to characterization.

2.3 Functionality properties

2.3.1 Determination of tensile strength (TS) and elongation at break (EAB)

Tensile strength (TS) and elongation at break (EAB) of the film were determined using texture analyzer (TA.TX Plus, Stable Micro System, USA) following Nur Hazirah *et al.* (2016). About 20 x 100 mm film strips were prepared using a cutting blade and placed onto grip pairs of AT/G probe which was attached to the texture analyzer with 5 kg load cell. The initial gap between the up and down parts of the grip was set to 20 mm. The film strips were stretched by the moving at headspace of 50 mm/min until broken. The TS (MPa) was calculated using the following equation:

$$\text{Tensile strength (MPa)} = \frac{F_{max}(N)}{A\ (m^2)}$$

Where F_{max} (N) is max load needed to pull the sample apart and A (m^2) is cross sectional area of film sample.

Meanwhile, the percentage elongation at break (EAB) was calculated as follows:

$$Elongation\ at\ break\ (\%) = \frac{l_{max} \times 100}{l_0}$$

Where l_{max} (mm) is the film elongation at the moment of rupture and l_0 (mm) is the initial grip length of sample.

2.3.2 Determination of puncture force

Puncture tests were performed in order to determine the breaking force and the breaking deformation of the films. The test was conducted using a method as described by Nur Hazirah *et al.* (2016). Films were placed in a cell with 5.6 cm in diameter and punched to the breaking point using the same texture analyzer, with a round-ended stainless-steel plunger 3 mm in diameter at a cross-head speed of 60 mm/min. The puncture force measurement was conducted in triplicate. Breaking force was expressed in terms of N and breaking deformation in percentage.

2.3.3 Determination of water vapor permeability (WVP)

Water vapor permeability (WVP) was measured using a modified ASTM method as described by Nor *et al.* (2016). The films were sealed onto a cup containing silica gel (0% RH) with silicone vacuum grease and a rubber band to hold the films in place. The cups with films were then weighted as initial weight. The cups were then placed in desiccators containing distilled water at 30°C and weighted at 1-hour intervals over 8 hrs of period. The determination of WVP was conducted in triplicate.

2.3.4 Determination of thermal property

Thermal properties of films were evaluated by differential scanning calorimetry (DSC, TA Q2000 Instrument, USA) according to the method of Nur Hazirah *et al.* (2016). Film samples (3 mg) were weighted in aluminium pans. The pans were sealed with TA sample encapsulation press. The samples were placed in the sample cells, while an empty pan was placed in the reference cell. Conditioned films were scanned between 0 to 100°C at a heating rate of 10°C / min. Melting temperature (T_m, °C) and enthalpy (ΔH, J/ g) were measured and data were determined in triplicate. Melting temperature (T_m) was calculated as the temperature where the endothermic peak occurs, and enthalpy (ΔH) was calculated as the area over the endothermic peak.

2.3.5 Determination of light barrier and transparency

Film samples from each formulation were cut into rectangular piece and placed directly in a UV-Vis spectrophotometer test cell (Pharo 300, USA). Then the light barrier properties of the film samples were measured at wavelengths between 200 nm and 800 nm according to the method by Bakry *et al.* (2017). Three replicates of each sample were tested, and the results expressed in terms of percentage transmittance:

$$T = - (\log T_{600}) / x$$

Where $AbsT_{600}$ is the value of absorbance at 600 nm and x is the film thickness (mm).

2.3.6 Determination of Fourier transform infrared spectroscopy (FTIR)

FTIR spectrum was used to measure the functional group that arose from the blending of film materials. Infrared spectra were recorded using a Thermo Nicolet 380 Spectrometer (Fisher Scientific Inc, USA) with a deuterated triglycerine sulphate (DTGS) detector as described by Suderman *et al.* (2016). The sample holder comprised a Multi-ounce horizontal attenuated total reflectance (HATR) plate of zinc selenite (ZnSe) crystal. The plate was cleansed with acetone and a background spectrum (without sample) was collected. Film samples were then affixed to the plate and spectra recorded. Resolutions varied between 4000 to 650 cm^{-1} over 32 scans. Each analysis was repeated three times.

2.3.7 Determination of film microstructure

To study the film microstructure, the cross-section and surface area of chicken gelatin films were viewed at 25,000 magnification employing scanning electron microscopy (SEM) (Hitachi S-4300SE, Hitachi Science System Ltd., Japan). The samples were prepared by treating the films in liquid nitrogen, mounting on aluminium stubs, and sputter coating with platinum (Bakry *et al.,* 2017).

2.4 Antioxidant properties

2.4.1 DPPH radical scavenging activity

The antioxidant activities of the films incorporated with *C. asiatica* were measured by the method stated by Li *et al.* (2014). Approximately 25 mg of each film sample was dissolved in 5 mL of distilled water by continuous stirring, then 0.1 mL of film extract solutions were added to 3.9 mL of the DPPH solution (0.1 mM methanol solution) followed by 60 mins incubation in the dark area at ambient temperature. The absorbance was measured at 517 nm against pure methanol. The percentage of DPPH radical-scavenging activity was calculated using the following equation:

$$DPPH\ scavenging\ activity\ (\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Where A is absorbance at 517 nm.

2.4.2 Reducing power

The reducing power was performed using a method described by Dashipour *et al.* (2015) with slight modification. About 1.0 mL of film samples were added to 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 10mg/mg potassium ferricyanide. The mixture was then incubated at 50°C for 20 mins. Then, the solution was centrifuged, and the 2.5 mL of supernatant was mixed with 2.5 mL deionized water and 0.5 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm after a 10 mins reaction. Higher absorbances indicate higher reducing power.

2.4.3 Total phenolic content

Total phenolic content of CSG films incorporated with *C. asiatica* extract was performed according to the method described by Dashipour *et al.* (2015). Approximately 25 mg of each film sample was dissolved in 5 mL of distilled water. The solution of 0.5 mL Folin-Ciocalteu reagent, 0.1 mL of the extract solution and 7 mL distilled water were then mixed and stored at ambient temperature for 8 mins. Next, 1.5 mL sodium carbonate (2%, w/v) and distilled water were added to obtain a final volume of 10 mL. The mixtures were stirred and shaken thoroughly before being kept at ambient temperature for 2 hrs prior to an absorbance reading at 765 nm against water using UV spectrophotometer. The following equation was used to express the results in terms of mg gallic acid equivalents (GAE mg/g) per gram of dried film:

$$T\ (mg/g) = C.V/M$$

Where T is the total content of phenolic compound (mg/g dried film, in GAE); C is the concentration of gallic acid obtained from the standard calibration curve (mg/g); V is the volume of film extract (mL); and M is the weight of dried film (g).

2.5 Statistical analysis

To characterize the functional and antioxidant properties of CSG film incorporated with *C. asiatica* (L.) urban extract, Minitab 14.0 software was used. Results were expressed as a mean (±SD) for each analysis. Comparative statistical analysis between mean and ANOVA was calculated with Minitab 14.0 to assess significant differences between treatments.

3. Results and discussion

3.1 Functional properties

3.1.1 Tensile strength (TS) and Elongation at break (EAB)

The tensile strengths (TS) of chicken skin gelatin (CSG) films containing *C. asiatica* extract are shown in Table 1. Results showed a significant difference ($p<0.05$) on TS of control and CSG films incorporated with 0.7 g *C. asiatica* extract, but there is no significant difference ($p>0.05$) on TS between control and CSG film with 0.3 g extract and between CSG film with 0.3 g and 0.7 g extract. It is clearly observed that the incorporation of 0.7 g *C. asiatica* extract into the CSG films showed a significant decrease of TS than that of control film. This may be due to the high polyphenolic compounds obtained in the films incorporated with the extract, in which the polyphenolic compound would increase the hydrophobicity of gelatin films, thus resulting in lower TS value. Besides that, the polyphenolic compounds that contained in *C. asiatica* extract could also form hydrogen and covalent bonds with amino and hydroxyl groups of polypeptide in gelatin, which would weaken the protein-protein interactions and stabilize the protein network, thus lowering the TS value (Bravin *et al.,* 2004).

In contrast, the value of elongation at break (EAB) of CSG films increased significantly as the amount of *C. asiatica* extract incorporated into the film increases. The EAB of CSG films containing *C. asiatica* extract is shown in Table 1. The results showed that there was a significant difference ($p<0.05$) with EAB of CSG films incorporated with *C. asiatica* (L.) urban extract between each sample. The improvement in EAB of the films incorporated with *C. asiatica* extract may be due to the interaction between polyphenolic compounds of *C.* extract and gelatin matrix. The formation of hydrogen bonds increased the flexibility and extensibility of the films. The results are in agreement with a study by Siripatrawan and Harte (2010) which found that the interaction between polyphenolic compounds grape seed extract and chitosan matrix increased the elongation at break of the films.

3.2 Puncture test

The puncture force of CSG films incorporated with *C. asiatica* extract was presented in Table 1. Results obtained showed that there was a significant difference ($p<0.05$) on the puncture force of CSG films incorporated with *C. asiatica* (L.) urban extract between each sample. The addition of the *C. asiatica* extract increases the puncture force of the films. This may be due to the alteration of the gelatin matrix as added with *C. asiatica* extract. Antioxidant extract might cause rearrangement of the protein network in a way which enhanced the matrix of the film (Tongnuanchan *et al.,* 2012). These results are in agreement with the study conducted by Tongnuanchan *et al.* (2012) who found that the phenolic compounds in essential oil might be able to cross-link gelatin chain, thereby increasing the film's rigidity, in which it will increase the puncture force. These phenolic compounds were able to react with more than one protein site and develop protein cross-links, thus increasing film rigidity and improving the mechanical properties of the films. However, this result was not in the same agreement with the study by Gómez-Guillen *et al.* (2007).

3.3 Water vapor permeability (WVP)

The primary function of food packaging is to prevent or decrease moisture transfer between the surrounding atmosphere and the food product, hence the water vapor permeability (WVP) of the good films should be as low as possible. The WVP of CSG films incorporated with *C. asiatica* extract were presented in Table 1. Results showed that there was a significant difference ($p<0.05$) on WVP of control and CSG films incorporated with 0.7 g *C. asiatica* extract, but no significant difference ($p>0.05$) on WVP between control and CSG film with

Table 1. Tensile strength (TS), elongation at break (EAB), puncture test (PT), water vapor permeability (WVP), and melting point (T_m) of chicken skin gelatin film incorporated with *C. asiatica* (L.) urban extract

Film formulation	TS (MPa)	EAB (%)	PT (N)	WVP (kPa)	T_m (°C)
Control	5.69 ± 0.16^{a}	113.87 ± 0.79^{c}	28.06 ± 0.09^{c}	$1.31 \times 10^{-9} \pm0.38^{a}$	50.99 ± 1.09^{b}
0.3 % extract	3.12 ± 0.06^{ab}	183.47 ± 0.96^{b}	31.44 ± 0.03^{b}	$1.27 \times 10^{-9} \pm0.95^{ab}$	54.04 ± 0.09^{ab}
0.7 % extract	2.48 ± 0.17^{b}	249.62 ± 0.56^{a}	36.95 ± 0.16^{a}	$1.09 \times 10^{-9} \pm0.71^{b}$	56.11 ± 0.44^{a}

Values as means ± SD for three determinations. ($^{a-c}$) indicate means with significant difference ($p<0.05$) within the column.

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0.3 g extract and between CSG film with 0.3 g and 0.7 g extract. The WVP of the films decreased as the amount of *C. asiatica* extract increases. There is a possibility that polyphenolic compounds may able to establish interactions with the gelatin matrix and forms hydrogen or covalent bonding with reactive groups of gelatin. The results obtained in this study may be due to the interaction of the polar groups of polypeptide in chicken skin gelatin with the polyphenols compound that contained *C. asiatica* extract which forms hydrogen and covalent bonds. These hydrogen and covalent bonds will restrain the availability of hydrogen groups to form hydrophilic bonding with water and decrease the affinity of gelatin films with water, thus lowering the WVP value (Curcio *et al.,* 2009).

3.4 Thermal property

The result obtained for the melting temperature (T_m) of CSG films incorporated with *C. asiatica* extract was presented in Table 1. Results obtained showed that there was a significant difference ($p<0.05$) on T_m of control and CSG films incorporated with 0.7 g *C. asiatica* extract, but no significant difference ($p>0.05$) on T_m between control and CSG film with 0.3 g extract and between CSG film with 0.3 g and 0.7 g extract. From the results, CSG film with 0.7 g *C. asiatica* extract possessed the highest melting point. It is observed that the melting point increased as the amount of *C. asiatica* extract incorporated into the film increases. This may be due to the formation of hydrogen and covalent bonds from the interaction between phenolic compounds of *C. asiatica* extract and gelatin. High amount of *C. asiatica* extract added to the films would promote the development of crosslinking between gelatin chains and polyphenolic compound, thus more energy needed to break the bonds. The results are in agreement with the study by Núñez-Flores *et al.* (2013), which found that the melting temperature of active tuna skin gelatin film increased as incorporated with 0.6% of lignin.

3.5 Light barrier and transparency

Food packaging film is required to protect food from the effects of light, especially UV radiation. Table 2 shows the results obtained for spectroscopic scanning of all film samples at visible wavelengths between 200 and 800 nm. The results showed that there was a significant difference ($p<0.05$) on the light barrier and transparency of CSG films incorporated with *C. asiatica* (L.) urban extract between each sample. Gelatin films incorporated with *C. asiatica* extract exhibited low light transmission than that of control film. The result showed that the film with 0.7 g *C. asiatica* extract had the lowest percentage of transmittance in UV light (1.44%) at 600 nm as compared to control film (1.74%). The incorporation of *C. asiatica* extract at a concentration of 0.7 g in gelatin film caused a significant increase in film opacity, in which it improved light barrier properties of the films. The decrease in light transmission and increase in film opacity may be due to the incorporation of polyphenols compounds and flavonoids contained in *C. asiatica* extract. In addition, the benzene ring contained in polyphenols compounds and flavonoids enhanced the absorption between 200 nm and 800 nm, resulting in the change of light transmission of the film (Li *et al.,* 2014). These results suggested that the gelatin film incorporated with *C. asiatica* extract can effectively restrain lipid oxidation in the food systems caused by UV light.

3.6 Fourier transform infrared spectroscopy (FTIR)

The infrared spectra of the control film and films incorporated with 0.3 g and 0.7 g *C. asiatica* extract are shown in Table 3. The results obtained showed that there was a significant difference ($p<0.05$) on infrared spectra of CSG films incorporated with *C. asiatica* (L.) urban extract. The peaks obtained between 3500 and 3000 cm^{-1} correspond to the stretching vibration of free hydroxyl and to asymmetric and symmetric stretching of the N-H bonds in the amino group. The intensity was lower in the control film as compared to those films incorporated with *C. asiatica* extract. The intensity of the peaks increased as the amount of extract incorporated increased. This showed that *C. asiatica* extract has a hydrophilic character, and its addition increased the amount of free water content in the films.

For film incorporated with *C. asiatica* extract, new peaks at 1700 cm^{-1} and 1650 cm^{-1} was observed. This peak around 1700 cm^{-1} refers to carbon-to-oxygen (C=O) stretching within the carboxylic group. A peak at around 1650 cm^{-1}, correspond to carbon-to-carbon (C-C)

Table 2. Film light transmission and transparency of chicken skin gelatin film incorporated with *C. asiatica* (L.) urban extract

Film formulation	Wavelength (nm)								
	200	280	350	400	500	600	700	800	T_{600}
Control	13.09 ± 0.02^{a}	14.89± 0.01^{a}	15.52±0. 02^{a}	16.48 ± 0.05^{a}	16.66± 0.02^{a}	17.26± 0.07^{a}	17.42± 0.03^{a}	18.01± 0.01^{a}	1.74± 0.01^{a}
0.3 % extract	10.32± 0.00^{b}	11.48± 0.05^{ab}	12.07± 0.00^{b}	12.79 ± 0.04^{b}	13.40± 0.03^{ab}	15.06± 0.01^{ab}	15.88± 0.01^{ab}	16.72± 0.04^{b}	1.51± 0.01^{b}
0.3 % extract	6.12± 0.04^{c}	6.87± 0.01^{b}	8.31± 0.04^{c}	9.48 ± 0.04^{c}	10.57± 0.02^{b}	11.09± 0.03^{b}	12.64± 0.06^{b}	14.22± 0.00^{c}	1.44± 0.01^{c}

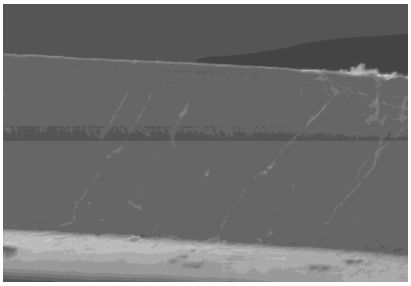
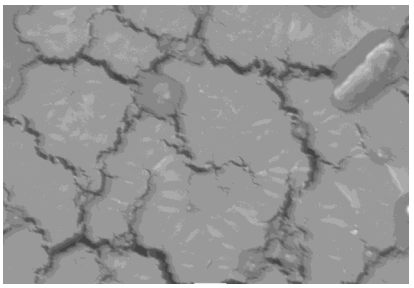
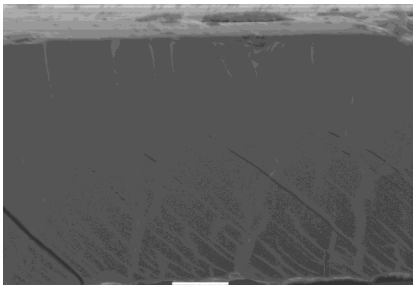
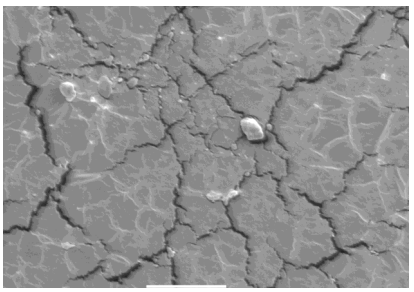
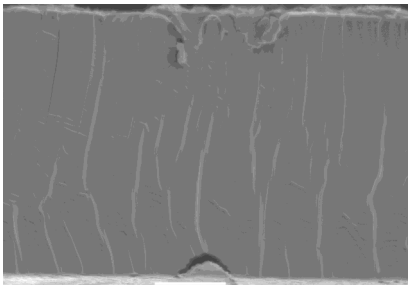
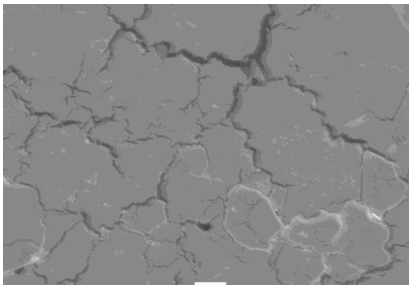
Values as means ± SD for three determinations. ($^{a–c}$) indicate means with significant difference ($p<0.05$) within the column.

Table 3. Infrared spectra of chicken skin gelatin film incorporated with *C. asiatica* (L.) urban extract

Film formulation	Amide A	Amide I	Amide II	Aliphatic Alcohol
	stretching vibration of N-H bands	C=O stretching, N-H bending vibration in amino group, C-N stretching vibration	bending vibration N-H group, stretching vibration of C-N group	hydroxyl groups (-OH), C-O stretching
	3275-3330 cm^{-1}	1648-1624 cm^{-1}	1510-1580 cm^{-1}	1250-1020 cm^{-1}
Control	3198.28±0.00^{b}	1635.64±0.00^{a}	1548.84±0.20^{a}	1039.63±0.40^{b}
0.3 % extract	3254.47±0.20^{b}	1637.56±0.40^{a}	1546.91±0.10^{ab}	1207.44±0.20^{ab}
0.7 % extract	3304.06±0.00^{a}	1645.11±0.20^{a}	1539.86±0.40^{b}	1216.39±0.30^{a}

Values as means ± SD for three determinations. ($^{a–b}$) indicate means with significant difference ($p<0.05$) within the column.

Table 4. Cross section and surface area of chicken skin gelatin film incorporated with *C. asiatica* (L.) urban extract

Film formulation	Cross section	Surface area
Control		
0.3% extract		
0.7% extract		

stretching within the aromatic ring, signify that the functional group of phenolic compounds was identified and appeared to be more apparent with increasing of *C. asiatica* extract concentration. The results show a particular arrangement in the films due to the interactions of hydroxyl and amino groups in chicken skin gelatin matrix with polyphenolic compounds of *C. asiatica* extract. These results were parallel with the study of active silver carp gelatin film added with green tea extract by Wu *et al.* (2013) which found new peak at the 1745 cm^{-1}.

3.7 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was conducted to study the microstructural changes in CSG film and to obtain the surface and cross-section topography of the films. The microstructure of CSG films incorporated with *C. asiatica* (L.) urban extract is presented in Table 4. SEM revealed that the cross-section of control CSG film possessed organized and compact microstructure. The cross-section became more compact as incorporated with *C. asiatica* extract, and the compactness continues to increase as the amount of *C. asiatica* extract added increased. On the other hand, it was observed that the control film showed homogeneous surface area with no bubbles and brittle areas. The micrograph of films depicted compact and smaller microstructure after the addition of *C. asiatica* extract. In addition, the film displayed smaller microstructure as more extracts were incorporated. The formation of this homogenous and smaller microstructure related to the increasing of *C. asiatica* extract dissolving in gelatin matrix as their amount increased to a higher concentration. The structural difference of films may be responsible for the improvement of WVP obtained in films.

3.8 Antioxidant properties

3.8.1 DPPH radical scavenging activity

Table 5 shows the radical scavenging activity in DPPH on CSG films incorporated with *C. asiatica* (L.) urban extract between each sample ($p<0.05$). Control CSG films showed some scavenging activity on DPPH. This may due to the scavenging activity that occurs between DPPH and gelatin molecules. This is because the radical scavenging mechanism of gelatin occurred when the free radical reacts with the residual free amino (NH_2) groups to form stable macromolecule radicals, and the NH_2 groups can form ammonium groups by absorbing a hydrogen ion from the solution (Siripatrawan and Harte, 2010). In films containing *C. asiatica* extract, the antioxidant activity increased as the amount of *C. asiatica* extract increased from 0.3 g to 0.7 g. From the results obtained, gelatin film with 0.7 g *C. asiatica* extract showed the highest scavenging activity against DPPH radicals. So, the higher antioxidant activities in CSG film with *C. asiatica* extract were due to high amounts of phenolic compound contained in *C. asiatica* extract. The results are in agreement with a study by Wu *et al.* (2013) who showed that the DPPH radical scavenging activity of silver carp skin gelatin film increased as the amount of green tea extract added to films increased.

3.8.2 Total phenolic content

The total phenolic content of CSG film incorporated with *C. asiatica* (L.) urban extract was presented in Table 5. The results showed the total phenolic content of CSG film increased with increasing concentration of *C. asiatica* extract ($p<0.05$). Phenolic content of gelatin films was the highest when incorporated with 0.7 g *C. asiatica* extract. The results obtained were parallel with the study by Hoque *et al.* (2011). In contrast, Li *et al.* (2014) obtained relatively low total phenolic content of bovine hide gelatin film as incorporated with oregano extract. The difference in the total phenolic content may be due to the difference in the phenolic compound contained in the extract used. High in phenolic content indicated that the film incorporated with high antioxidative compound. This is because phenolic compounds are the major contributors to the antioxidative activities of *C. asiatica*.

3.8.3 Reducing power

The reducing power of CSG film incorporated with *C. asiatica* (L.) urban extract was shown in Table 5. The results presented that, there was a significant difference ($p<0.05$) on reducing power between control and CSG films incorporated with 0.7 g extract and between CSG film with 0.3 g and 0.7 g extract, but there was no significant difference ($p>0.05$) on reducing power of control and CSG films incorporated with 0.3 g *C. asiatica* extract. No significant difference ($p>0.05$) in reducing power between control and CSG with 0.3 g *C. asiatica* extract may due to the amount of *C. asiatica* extract added to the film formulation was not sufficient to improve the reducing power of the films. However, the reducing power of gelatin films was the highest when incorporated with 0.7 g *C. asiatica extract*. The result is in agreement with Wu *et al.* (2013) who showed that the reducing power of silver carp skin gelatin films increased as the amount of green tea extract added to the films increases. The results obtained also indicated that the antioxidant activity of the CSG films incorporated with *C. asiatica* extract was significantly better than that of films without *C. asiatica* extract and continue to increase with the increasing *C. asiatica* extract concentration.

Table 5. DPPH, total phenolic content, and reducing power of chicken skin gelatin film incorporated with *C. asiatica* (L.) urban extract

Film formulation	DPPH (%)	Phenolic content (mg GAE/ g sample)	Reducing power (nm)
Control	48.34±0.34^{c}	9.52±0.11^{c}	49.50±1.02^{b}
0.3 % extract	55.42±1.11^{b}	10.18±0.05^{b}	52.32±0.80^{b}
0.7 % extract	68.67±1.20^{a}	17.79±0.13^{a}	65.44±0.91^{a}

Values as means ± SD for three determinations. ($^{a–c}$) indicate means with significant difference ($p<0.05$) within the column.

4. Conclusion

In conclusion, the urban extract of *C. asiatica* (L.) served as an important material which can be successfully employed as a natural active material to produce chicken skin gelatin films with good antioxidant properties with non-cytotoxic effect. *C. asiatica* showed relatively high antioxidant properties and offers adequate miscibility with gelatin. Therefore, this study showed that CSG film incorporated with *C. asiatica* is a potential material for developing active packaging film, as it boasts superior functional and antioxidant characteristics.

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