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Chitosan/Poly(vinyl alcohol)/Collagen Hydrogel Composites Containing Jackfruit Axis Extract for Wound Dressing Application

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Abstract

Jackfruit (Artocarpus heterophyllus Lam.) axis (JFA), a non-edible waste from jackfruit, has been reported for its phytochemical compositions along with antioxidant and antibacterial properties. The aim of this study was to fabricate and investigate the properties of wound dressing composites containing JFA extract. JFA was extracted using sonication in ethanol. The yield of extraction was about 4.93%. The films of chitosan (CS)/poly(vinyl alcohol) (PVA)/collagen (Coll) hydrogel composites containing JFA extract were prepared from the mixed solutions of 1% w/v CS, 1% w/v PVA, and Coll at various ratios including 5/4/1, 5/3/2, 4/5/1, and 4/4/2 by weight of solution. The JFA extract was added to the mixed solution at 0.25% w/w. A solvent casting was performed followed by crosslinking via glutaraldehyde vapor treatment. The obtained films were named JFA-CS/PVA/Coll 5/4/1, 5/3/2, 4/5/1, and 4/4/2. The actual JFA extract content was 19 ± 3.6% based on the weight of dry film. The antioxidant activity of JFA extract was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The half-maximal inhibitory concentration (IC50) of JFA extract was 0.250 mg/mL. The JFA extract only exhibited antibacterial activity against Staphylococcus aureus (S. aureus), but not for Escherichia coli (E. coli), as determined by an agar disc diffusion method. The release of JFA extract from the hydrogel composite films was studied by total immersion method in distilled water at 37°C during 0-8 h. The JFA-CS/PVA/Coll 4/4/2 showed higher amount of JFA extract released than those from the ratios of 4/5/1, 5/3/2, and 5/4/1, respectively. The degree of water swelling and weight loss of the films appeared in a similar trend to those of the release study. The higher content of CS and lower content of Coll led to the lower amount of water swelling, weight loss, and JFA extract released. Lastly, all types of JFA-CS/PVA/Coll films exhibited antioxidant activity of about 46-51% and antibacterial activity against S. aureus. However, the JFA-CS/PVA/Coll 5/4/1 showed the least antioxidant and antibacterial activities. Based on the overall results, the JFA-CS/PVA/Coll films revealed the potential for use in wound dressing applications.

Keywords: Jackfruit axis, Herbal extract, Chitosan, Poly(vinyl alcohol), Collagen, Wound dressing

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1. Introduction

Wound healing is a process where destroyed or damaged tissue is cured or regenerated and replaced by newly produced tissue. Wound healing materials have been developed and have been utilized in which an ideal wound dressing should provide moist environment to support the growth of living cells, absorb exudate from the wound, prevent infections, and even accelerate tissue regeneration. The achievement of wound dressing development depends on the selection of matrix materials and the loaded drugs or bioactive compounds that possess antibacterial, antioxidant, and anti-inflammatory properties. Hydrogels have been widely used as wound dressings according to their ability to be swollen but not dissolve in water, which allow them to absorb exudate from wounds and to maintain the moist environment as well. Poly(vinyl alcohol) (PVA) is one of the interesting hydrogels employed as a carrier for controlled-drug release in wound dressings [1-3]. Crosslinking of PVA is performed by using radiation [4] and by chemicals e.g. potassium persulphate [5], glyoxal [6, 7], and glutaraldehyde [6, 8] to enhance its physical stability and to control the degree of swelling and weight loss during wound healing period.

In terms of biological activity and environmentally friendly, biodegradable, biocompatible, biomaterials, non-allergic, and non-toxic materials are promising materials for use in biomedical applications. Chitosan (CS) is one of the most interesting biomaterials for pharmaceuticals, tissue engineering, and wound healing. CS is a linear polysaccharide consisting of randomly distributed $\beta(1 \rightarrow 4)$-linked D-glucosamine and N-acetyl-D-glucosamine. CS is also considered hydrogel due to its three-dimensional network structure and water absorption ability. Moreover, it was reported that CS contains antioxidant activity in which pristine CS films, CS nanoparticles, and those loaded with thyme oil [9] and clove essential oil [10] were tested and exhibited antioxidant activities. Additionally, CS has antibacterial activities against *Staphylo-*

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coccus aureus (S. aureus) [10, 11], *Escherichia coli (E. coli)* [10, 12], *Bacillus subtilis (B. subtilis)* [12], *Listeria Monocytogenes (L. Monocytogenes)* [10], and *Salmonella Typhi (S. Typhi)* [10].

In addition, various proteins or partially hydrolyzed proteins, for example, elastin, silk fibroin, gelatin, and collagen, have been utilized in wound dressing materials to improve biocompatibility, cell proliferation, and cell differentiation. Nanofiber mats of CS/Coll enhanced cell migration, re-epithelization, vascularization, and expression of protein differentiation [13]. The composite wound dressings of CS/Coll/alginate exhibited hemocompatibility, non-cytotoxicity, and a higher rate of wound healing in rats than in gauze or CS [14].

On top of that, development of wound dressings can be carried out by incorporation of herbal extracts. CS/Coll membranes containing red propolis extract showed antibacterial properties [15]. Films of PVA containing various types of flower extracts from lavender, peppermint, hemp, verbena, and sage plants demonstrated antibacterial properties towards *S. aureus* and *E. coli.* and were non-toxic to the rabbit fibroblasts [16].

Jackfruit *(Artocarpus heterophyllus Lam.)* tree belongs to the family of Moracea. It is widely grown in tropical countries, especially in Southeast Asia, including India, Bangladesh, Myanmar, Sri Lanka, Philippines, Pakistan, Malaysia, and Thailand [17]. Ripe fruits are sweet and always eaten fresh. Jackfruit is usually consumed as a dessert or as an ingredient in Asian recipes. It is abundant in nutrients, including carbohydrates, carboxylic acids, fibers, minerals, and vitamins. There are six main parts of jackfruit including, flesh or pods, core or axis, pulp or rag, seeds, seed shells, and rind. Jackfruit contains a broad spectrum of antibacterial, antioxidant, anti-diabetic, anti-inflammatory, and anti-helmintics properties [17, 18]. Not only for its flesh, jackfruit rag and jackfruit axis (JFA) were also revealed for their antibacterial [19] and antioxidant [20] properties, respectively. Cytoprotective effect on human liver hepatoma cells was also evident from using JFA extract [20]. JFA extract is composed of 53 compounds with many types of functional components, for example, glycosides, lipids, organic acids, amino acid derivatives, nucleic acids, thiols, terpenoids, esters, alkaloids, phenolics, phytosterol, saponins, and flavonoid as disclosed by Li et. Al. [20].

In the present work, JFA was extracted and loaded into the hydrogel composite films of CS/PVA/Coll. The antioxidant and antibacterial activity of JFA extract were determined. The potential for use of the JFA-CS/PVA/Coll films as a topical transdermal patch or wound dressings was investigated. The release characteristics of JFA extract from the films were studied by the total immersion method in distilled water at 37°C for 0-8 h. The degree of water swelling and weight loss of the films were evaluated. Lastly, the antioxidant and antibacterial activity of the JFA-CS/PVA/Coll hydrogel composite films were determined.

2. Experimental

2.1 Materials

Fresh jackfruit axis (JFA) was brought from a local market in Pathum Thani, Thailand. Poly(vinyl alcohol) (PVA; degree of hydrolysis: 86.0–89.0%, MW: 85,000–124,000 g/mol) was purchased from SD Fine Chemicals (India). Collagen (Coll; liquid, from lamb placenta) was purchased from Chemipan Corporation (Thailand). Glutaraldehyde (25% aqueous solution) was purchased from Acros Organics (USA). Ethanol, methanol, and glacial acetic acid were purchased from Carlo Erba (Italy). Chitosan (CS; MW: 100,000-300,000 g/mol) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich (USA).

2.2 Extraction of jackfruit axis

Fresh JFA was collected, cut into tiny pieces, and dried at 60°C for 8 h. Dried JFA was immersed in 99% ethanol at a solid:liquid ratio of 3 g : 45 mL. The mixture was sonicated for 30 min and further shaken at room temperature for 24 h. Solid residue was filtered out by vacuum filtration. JFA extract was collected as filtrate in which ethanol was subsequently evaporated by using a rotary evaporator. The obtained slurry was further dried in a vacuum oven at 75°C for 4 h. Lastly, dried JFA extract was obtained and kept in a desiccator. The percentage of yield of extraction was calculated according to an equation (1):

$$\text{Yield of extraction (\%)} = \frac{\text{mass of the dried JFA extract (g)}}{\text{mass of the initial dried JFA (g)}} \times 100 \quad (1)$$

2.3 Preparation of the JFA-loaded CS/PVA/Coll films

The solution of 1% w/v CS was prepared in 0.1 M acetic acid by continuously stirring the solution at room temperature for 3 h. Aqueous solution of 1% w/v PVA was prepared by stirring the solution at 80°C for 5 h until a homogeneous solution was obtained. Coll solution was used as obtained. The composite solutions were prepared by mixing 1% w/v CS, 1% w/v PVA, and Coll at different weight ratios of 5/4/1, 5/3/2, 4/5/1, and 4/4/2. JFA extract was later added to the composite solution. For 100 g of the composite solution, 0.25 g of JFA extract was added and mixed thoroughly. The JFA extract-loaded composite solutions were casted onto a plastic plate. The solvent evaporation was allowed to proceed at room temperature for 24 h in a hood.

The obtained hydrogel composite films were designated as JFA-CS/PVA/Coll 5/4/1, JFA-CS/PVA/Coll 5/3/2, JFA-CS/PVA/Coll 4/5/1, and JFA-CS/PVA/Coll 4/4/2, respectively. Lastly, crosslinking of PVA was performed using a glutaraldehyde vapor treatment. The JFA-CS/PVA/Coll hydrogel composite films were placed in a closed plastic box containing a cup of glutaraldehyde (25% in water) at 40°C under a saturated atmosphere of glutaraldehyde for 5 h of each side of film. The hydrogel composite films were later placed in a hood for 1 h to allow evaporation of the remaining glutaraldehyde in the films. Glutaraldehyde acts as a crosslinking agent for both PVA and CS. The crosslinking reactions are demonstrated in Figure 1. The hydroxyl groups of PVA and the amine groups of CS react with aldehydic groups of glutaraldehyde to form acetal bonds and imine bonds, respectively.

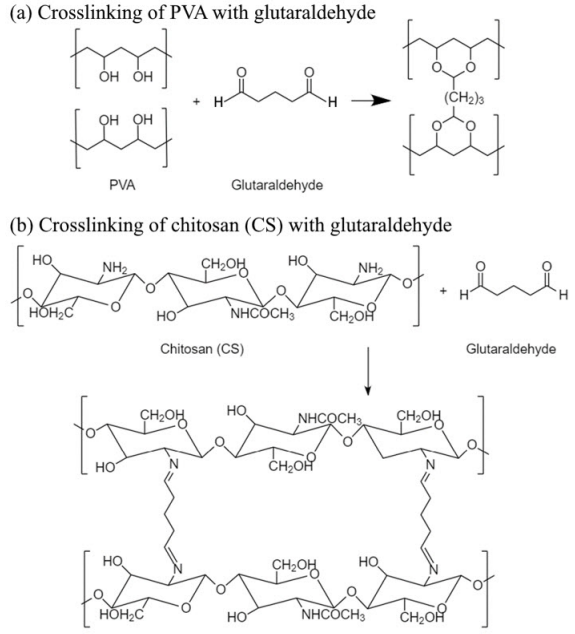

Figure 1: Crosslinking reactions of (a) PVA and (b) CS with glutaraldehyde.

2.4 *Water swelling and weight loss of the JFA-CS/PVA/Coll films*

The degree of water swelling and weight loss of the JFA-CS/PVA/Coll hydrogel composite films were determined after immersion in distilled water at 37°C for 4 h. Each sample was cut into a square shape of 2x2 cm2, dried in an oven at 40°C for 4 h, and immersed in 40 mL of distilled water. The initial dry weight of the sample was recorded as Mi. At 4 h of immersion, the weight of the wet sample was recorded as M. Afterwards, the wet sample was dried in an oven at 40°C for 4 h. The weight of the dried sample after immersion was recorded as Md. The percentages of water swelling and weight loss were calculated according to equations (2) and (3), respectively.

$$\text{Water swelling } (\%) = \frac{(M - M_i)}{M_i} \times 100 \qquad (2)$$

$$Weightloss(\%) = \left(\frac{M_i - M_d}{M_i}\right) \times 100 \qquad (3)$$

2.5 *Release of JFA extract from the JFA-CS/PVA/Coll films*

2.5.1 *Actual drug content*

Prior to investigating the characteristics of JFA extract release from the JFA-CS/PVA/Coll hydrogel composite films, the actual drug content (i.e., the actual amount of JFA extract in the films) was determined for use as base values in the release study. The film was cut into a square piece of 2x2 cm2 and was completely dissolved in distilled water by continuously stirring at 80°C for approximately 2 h. The obtained solution was diluted with distilled water and measured for its absorbance at a wavelength of 212 nm ($\lambda_{\max}$ of JFA extract) using a UV-vis spectrophotometer (Hanon I3). The amount of JFA extract was calculated against the predetermined standard curve plotted between the concentration of JFA extract and its absorbance at 212 nm.

2.5.2 *Release assay*

The release behaviors of JFA extract from the JFA-CS/PVA/Coll hydrogel composite films were studied by a total immersion method. The tested film was cut into a square shape of 2x2 cm2 and immersed in 40 mL of distilled water in a capped bottle at 37°C. The releasing medium was slowly stirred using a magnetic stirrer during the releasing time, ranging between 0 - 8 h. At each specified time point, 1.0 mL of the releasing medium was withdrawn and diluted with distilled water before measuring its absorbance at 212 nm. At each time of solution withdrawal, the same amount (1.0 mL) of distilled water was refilled into the bottle in order to keep a constant volume of releasing medium. The amount of JFA extract released were quantified from their absorbances against the predetermined standard curve of JFA extract in distilled water. The cumulative percentage of JFA extract released at each time point was calculated according to the equation (4):

$$\text{Cumulative JFA extract release}(\%) = \frac{C_t}{C_{total}} \times 100 \qquad (4)$$

where C_t is the cumulative weight of JFA extract released at time t and C_total is the weight of the JFA extract in the CS/PVA/Coll films. The experiments were carried out in triplicate.

2.6 Antioxidant activity

The antioxidant activity of JFA extract and the JFA-CS/PVA/Coll films were evaluated by the radical scavenging DPPH assay. For the antioxidant activity of JFA extract, the aqueous solutions of JFA extract were prepared in a range of concentrations of 0.156 – 10.00 mg/mL. Each 1.0 mL of JFA extract solution was mixed with 3.0 mL of 0.5 mM DPPH solution in methanol. The mixture was kept in darkness for 30 min and was measured for absorbance at 517 nm by the UV-vis spectrophotometer. The control solution was a pristine 0.5 mM DPPH solution which was prepared and stored in the same condition as those of the tested samples. The antioxidant activity was calculated according to the equation (5):

$$\text{Antioxidant activity (\%)} = \frac{(A_C - A_S)}{A_C} \times 100 \quad (5)$$

Where A_C is the absorbance of the control and A_S is the absorbance of the sample.

Later, the concentration of JFA extract at which 50% of DPPH free radicals are scavenged (IC50) was calculated.

For the antioxidant activity of the CS/PVA/Coll films and the JFA-CS/PVA/Coll films, the tested film was cut into a square shape of 2x2 cm2 and immersed in 40 mL of distilled water at 37°C for 8 h. After that, 1.0 mL of the releasing media was withdrawn and mixed with 3.0 mL of 0.5 mM DPPH solution in methanol. The mixture was further kept in the darkness for 30 min. The absorbance at 517 nm was determined. The antioxidant activity of these films was calculated according to the equation (5). The experiments were carried out in triplicate.

2.7 Antibacterial activity

The antibacterial activity of JFA extract, the CS/PVA/Coll films, and the JFA-CS/PVA/Coll films against Gram-positive Staphylococcus aureus (S. aureus: ATCC 6538) and Gram-negative *Escherichia coli* (*E. coli*: ATCC 8739) bacteria were evaluated by the agar disc diffusion method. The circular filter paper disc saturated with 0.6 mg/mL JFA extract and the various types of JFA-CS/PVA/Coll films with a diameter of 6 mm were placed on a plate containing bacteria in agar. The agar plate was incubated at 37°C for 24 h. The observed diameter of clear zone which included the diameter of disc was measured. The clear zone of inhibition was calculated from the subtraction of the diameter of disc (i.e., 6 mm) from the diameter of clear zone and later divided by 2. Deionized water was used as a negative control, while ethanol was used as a positive control. Specifically, vancomycin and gentamicin were used as the positive controls for*S. aureus* and *E. coli*., respectively.

3. Results and Discussions

3.1 Yield, antioxidant, and antibacterial activity of JFA extract

JFA was extracted by using 99% ethanol assisted with sonication and shaking for 24 h. The average percentage of yield was 4.93 ± 0.65%. The antioxidant activity of JFA extract based on DPPH free radical scavenging assay at the concentrations of 0.156, 0.312, 0.625, 1.250, 5.00, and 10.00 mg/mL were investigated. The antioxidant activities of JFA extract in a range of concentrations of 1.25 – 10 mg/mL were markedly high and were not much changed according to the change of concentration (see Figure 2), which was approximately 91 – 92%. For the lower range of concentrations, the antioxidant activity was decreased with decrease in JFA extract concentration. The half-maximal inhibitory concentration (IC50) of JFA extract (defined as the concentration at which 50% of DPPH free radicals are scavenged) was 0.250 mg/mL., which was determined from the data in a range of concentrations of 0.156 – 0.625 mg/mL.

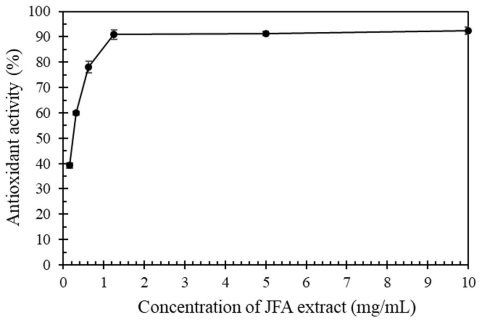

Figure 2: Antioxidant activity of JFA extract based on DPPH assay.

JFA extract was further investigated for antibacterial activity against Gram-positive S. aureus and Gram-negative E. coli bacteria by the agar disc diffusion method. The clear zone of inhibition are presented in Table 1. The JFA extract exhibited antibacterial activity against S. aureus only, but not for E. coli. Deionized water and ethanol were used as the negative and the positive control, respectively. Photographs of the bacteria cultured plates are shown in Figure 3.

From these results, the JFA extract possessed both the antioxidant and antibacterial activities and revealed the potential for use as an active ingredient in wound dressings. The hydrogel composite films of CS/PVA/Coll containing JFA extract were further fabricated. The study of the release behavior of JFA extract therefrom, water swelling, weight loss, antioxidant, and antibacterial properties of the films were performed.

3.2 Water swelling and weight loss of the JFA-CS/PVA/Coll films

The hydrogel composite films containing JFA extract were fabricated by solvent casting technique

Table 1. Antibacterial activities of JFA extract against S. aureus and E. coli as determined by the agar disc diffusion method.

samples	Clear zone of inhibition (mm)	
	S. aureus	***E. coli***
Negative control: deionized water	0.00 ± 0.00	0.00 ± 0.00
Positive control: ethanol	1.20 ± 0.15	0.50 ± 0.00
JFA extract	1.36 ± 0.42	0.00 ± 0.00

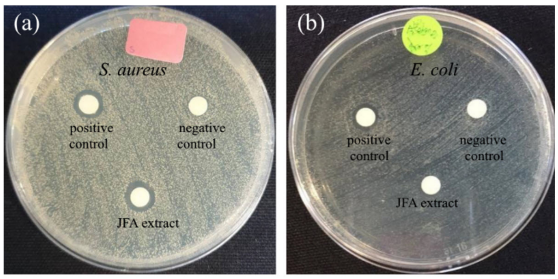

Figure 3: Photographs of the antibacterial activity testing by the agar disc diffusion method for the JFA extract against (a) *S. aureus* and (b) *E. coli.*

at various ratios of CS, PVA, and Coll. The obtained hydrogel composite films were designated as JFA-CS/PVA/Coll 5/4/1, JFA-CS/PVA/Coll 5/3/2, JFA-CS/PVA/Coll 4/5/1, and JFA-CS/PVA/Coll 4/4/2. These films were crosslinked by glutaraldehyde vapor treatment before use. The average thickness of the films was 135 ± 54 μm.

Degree of water swelling and weight loss are ones of the important parameters of the hydrogel, especially for use in wound dressing applications. The release behaviors can be explained in regards to the degree of water swelling in which the ability to hold water and to allow drugs to diffuse are related [21, 22]. Also, drug release according to the mass loss of the matrix is one of the release mechanisms [22].

Figure 4 shows the amount of water swelling and weight loss of the JFA-CS/PVA/Coll films at 4 h of immersion in distilled water. Similar trends of water swelling and weight loss were observed. These values ranked ascendingly as the JFA-CS/PVA/Coll films with the ratios of 5/4/1, 5/3/2, 4/5/1, and 4/4/2, respectively.

For the amount of water swelling, the values of the JFA-CS/PVA/Coll films with the ratios of 5/4/1, 5/3/2, 4/5/1, and 4/4/2, were 220.8 ± 8.2%, 296.5 ± 9.1%, 312.7 ± 12.8%, and 330.4 ± 13.3%, respectively. For the degree of weight loss, the values of the JFA-CS/PVA/Coll films with the ratios of 5/4/1, 5/3/2, 4/5/1, and 4/4/2, were 13.3 ± 1.2%, 15.2 ± 1.4%, 19.2 ± 2.6%, and 28.2 ± 3.1%, respectively.

Comparing the hydrogel composite films with different contents of CS, it seemed that the films with higher amount of CS (the ratios of 5/4/1 and 5/3/2) exhibited lower water swelling and weight loss than those of the films with lower amount of CS (the ratios of 4/5/1 and 4/4/2). The similar trends of observation were disclosed by other research that the crosslinked CS/PVA nanofibers had the lower water swelling and weight loss with the presence or increasing CS content [23, 24].

Comparing the hydrogel composite films with different contents of Coll, it seemed that the films with higher amount of Coll (the ratios of 5/3/2) exhibited higher water swelling and weight loss than those of the films with lower amount of Coll (the ratios of 5/4/1). The water swelling and weight loss of the hydrogel composite films with the ratio of 4/4/2 were also greater than those of the ratio of 4/5/1. Similar to the observations of Lan et al. [25] that the incorporation of Coll into porous PVA hydrogels for use in cartilage tissue engineering led to a higher degradation of hydrogels.

It is widely known that the amount of water swelling and weight loss notably correspond to the release behavior of molecules from the matrix [21]. The results of water swelling and weight loss are further discussed with the release characteristics in the next session.

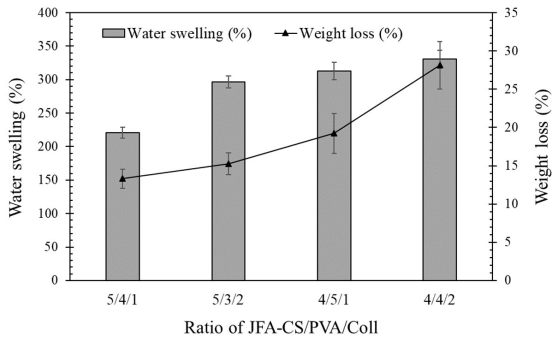

Figure 4: Water swelling and weight loss of the JFA-CS/PVA/Coll films at 4 h of immersion in distilled water.

3.3 Release of JFA extract from the JFA-CS/PVA/Coll films

Prior to studying the release characteristics of JFA extract from the JFA-CS/PVA/Coll hydrogel composite films, the standard curve of JFA extract was prepared. The plot between the concentration of JFA extract in distilled water and its absorbance at 212 nm (λ_{max} of JFA extract) was a straight line with the linear equation of $y = 0.002X + 0.0902$. The coefficient of determination (r^2) as to determine the best fit of the data was 0.929.

3.3.1 Actual JFA extract content in the JFA-CS/PVA/Coll films

The actual JFA extract contents in the JFA-CS/PVA/Coll films were determined for use as base values in the release study. The percentages of actual JFA extract contents were calculated from the actual weight of JFA extract presented in the film divided by the weight of the film. The average percentage of actual JFA extract content was 19 ± 3.6%.

3.3.2 Release characteristics of JFA from the JFA-CS/PVA/Coll films

The release of JFA extract from the hydrogel composite films was investigated by total immersion method in distilled water at 37°C during 0 – 8 h. Figure 5 shows the percentages of cumulative release amount of JFA extract from 4 types of films. The results were reported as the percentages of the cumulative weights of JFA extract released divided by the actual weight of JFA extract in the films. For all types of films, the burst release of JFA extract was observed at the initial time of release in a range of 0 – 20 min. Later, gradual release until reaching constant values appeared since approximately 180 min. The maximum amount of JFA released at 480 min (8 h) from the JFA-CS/PVA/Coll films with the ratios of 5/4/1, 5/3/2, 4/5/1, and 4/4/2 were about 24.1 ± 1.6%, 42.1 ± 3.1%, 44.4 ± 3.6%, and 47.1 ± 3.6%, respectively.

Interestingly, the results of the release study highly corresponded with the trend of the degree of water swelling and weight loss. Comparing the hydrogel composite films with different contents of CS, it was found that the films with higher amount of CS (the ratios of 5/4/1 and 5/3/2) exhibited lower amount of JFA extract released than those of the films with lower amount of CS (the ratios of 4/5/1 and 4/4/2). This was due to the lower water swelling ability of the JFA-CS/PVA/Coll film with the ratio of 5/4/1 and 5/3/2 films and therefore, the drug or substance molecules were more difficult to diffuse out from the matrix. Moreover, molecules can also be released from the mechanism of weight loss of matrix [22]. Since the JFA-CS/PVA/Coll 5/4/1 had the lowest weight loss among all types of films, it showed the lowest amount of JFA extract released.

Comparing the hydrogel composite films with different contents of Coll, it was noticed that the films with higher amount of Coll (the ratio of 5/3/2) exhibited much higher amount of JFA extract released than that of the films with lower amount of Coll (the ratio of 5/4/1). A similar trend was also observed for films with the ratios of 4/5/1 and 4/4/2, in which the film with higher amount of Coll (the ratio of 4/4/2) showed greater amount of JFA extract released than that of the film with lower amount of Coll (the ratio of 4/5/1). Repeatedly, this was due to the lower water swelling ability and lower weight loss of the film with the ratio of 5/4/1 compared to the ratio of 5/3/2 (also the ratio of 4/5/1 compared to the ratio of 4/4/2). Therefore, it was more inconvenient for the substance molecules to diffuse out from the matrix.

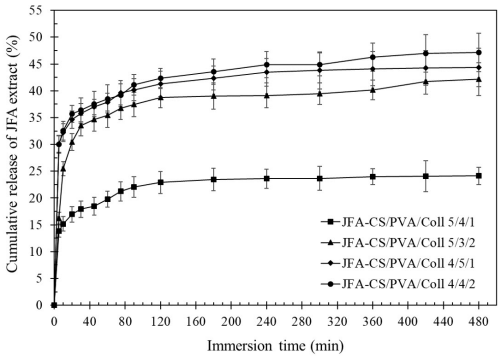

Figure 5: Cumulative release amounts of JFA extract from the JFA-CS/PVA/Coll films in distilled water at 37°C.

3.4 Antioxidant activity of the JFA-CS/PVA/Coll films

The antioxidant activity of the CS/PVA/Coll films (non-JFA extract loaded) and the JFA-CS/PVA/Coll films were examined by the DPPH assay. The scavenging of hydrogen radicals of DPPH can be evaluated by the decrease in absorbance at 517 nm (λ_{max} of DPPH) according to, the equation (5). The releasing media were collected at 4 h of immersion in distilled water and were quantified for the antioxidant activity.

For the CS/PVA/Coll films, the antioxidant activities of the films with the ratios of 5/4/1, 5/3/2, 4/5/1, and 4/4/2 were 38.68 ± 0.29%, 38.57 ± 0.35%, 38.12 ± 0.29%, and 38.84 ± 00.22%, respectively (see Figure 6). Even though there was no JFA extract presence, the CS/PVA/Coll films still exhibited antioxidant properties. The antioxidant ability could come from the presence of CS, which has been reported for its antioxidant activity [9, 10]. However, the difference of composition ratios had no effect on the antioxidant activity. For the JFA-CS/PVA/Coll films, the antioxidant activities of the films with the ratios of 5/4/1, 5/3/2, 4/5/1, and 4/4/2 were 46.40 ±0.31%, 50.79 ± 0.35%, 50.77 ± 0.32%, and 50.83 ± 0.35%, respectively (see Figure 6). Obviously, the films containing JFA extract (the JFA-CS/PVA/Coll films) exhibited greater antioxidant activities than those of the non-JFA extract-loaded films (the CS/PVA/Coll films). Notably, the presence of JFA extract helps improve the antioxidant activity of the films from about 38% to about 46 – 51%. However, the values of the films with the ratios of 5/3/2, 4/5/1, and 4/4/2 were not much different. Except for the films with the ratio of 5/4/1, it showed the lowest values among all types of films. This could be due to the lowest release amount of JFA extract from the film with the ratio of 5/4/1 and, therefore, there was the least antioxidant activity.

Table 2. Antibacterial activities of CS/PVA/Coll and JFA- CS/PVA/Coll films against S. aureus and E. coli as determined by the agar disc diffusion method.

samples	Clear zone of inhibition (mm)	
	S. aureus	***E. coli***
Vancomycin (positive control for S. aureus)	3.24 ± 0.24	N/A
Gentamicin (positive control for E. coli)	N/A	3.70 ± 0.05
CS/PVA/Coll 5/4/1	0.06 ± 0.01	0.00 ± 0.00
JFA- CS/PVA/Coll 5/4/1	3.30 ± 0.70	0.00 ± 0.00
JFA- CS/PVA/Coll 5/3/2	4.14 ± 0.18	0.00 ± 0.00
JFA- CS/PVA/Coll 4/5/1	4.02 ± 0.59	0.00 ± 0.00
JFA- CS/PVA/Coll 4/4/2	4.14 ± 0.84	0.00 ± 0.00

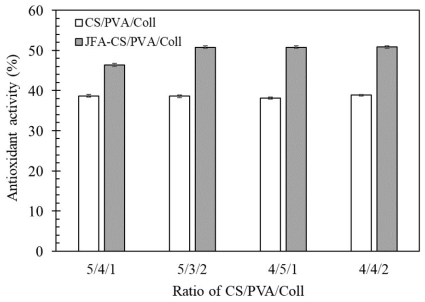

Figure 6: Antioxidant activities of the CS/PVA/Coll films and the JFA-CS/PVA/Coll films at 4 h of immersion.

3.5 Antibacterial activity of the JFA-CS/PVA/Coll films

Antibacterial activity of the CS/PVA/Coll films (only for the ratio 5/4/1) and the JFA-CS/PVA/Coll films against the Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria was evaluated by the agar disc diffusion method. Table 2 shows the clear zones of inhibition that were measured from the photographs of the cultured plates (see Figure 7). The clear zones of inhibition of the positive control for*S. aureus* (vancomycin) and for *E. coli* (gentamicin) were also reported. For the CS/PVA/Coll 5/4/1 films, it had no antibacterial activity against *E. coli* as the inhibition zone was not observed, but it slightly inhibited the growth of *S. aureus*. The antibacterial activity against S. aureus could come from the presence of CS, which has been reported for its antibacterial properties [10, 11].

For all types of the JFA-CS/PVA/Coll films, they had no antibacterial activity against E. coli. However, they expressed excellent antibacterial activity against S. aureus., as observed from the large inhibition zones, which were even greater than that of the positive control (vancomycin). It should be noted that the antibacterial activity of the JFA-CS/PVA/Coll 5/4/1 film was the lowest among other JFA-CS/PVA/Coll films. This result corresponded well with the release study in which the JFA-CS/PVA/Coll 5/4/1 film had the lowest release amount of JFA extract and therefore led to the lowest antibacterial activity.

Based on the overall results, all types of the JFA-CS/PVA/Coll films exhibited excellent antioxidant and antibacterial activity against S. aureus. Even though the JFA-CS/PVA/Coll 5/4/1 expressed the least ability among these films. It was revealed that all types of the JFA-CS/PVA/Coll hydrogel composite films can be promising materials for use as topical transdermal patches or wound dressings.

4. Conclusions

In the present contribution, jackfruit axis (JFA) was extracted and loaded into the hydrogel composite films of CS/PVA/Coll. The films were prepared at various ratios of CS/PVA/Coll, including 5/4/1, 5/3/2, 4/5/1, and 4/4/2 by weight. The actual JFA extract content was 19 ± 3.6% based on the weight of dry film. Antioxidant activity of JFA extract as evaluated by DPPH assay showed that the half-maximal inhibitory concentration (IC_{50}) of JFA extract was 0.250 mg/mL. Antibacterial activity as determined by an agar disc diffusion method revealed that JFA extract inhibited growth of *S. aureus*, but not *E. coli*. The release of JFA extract from the hydrogel composite films (JFA-CS/PVA/Coll 5/4/1, 5/3/2, 4/5/1, and 4/4/2) was studied by the total immersion method in distilled water at 37°C during 0-8 h. The JFA-CS/PVA/Coll 4/4/2 showed higher amount of JFA extract released than those from the ratios of 4/5/1, 5/3/2, and 5/4/1, respectively. The amount of JFA extract release was in accordance with the trends of degree of water swelling and weight loss in which the higher content of CS and lower content of Coll led to the lower amount of water swelling, weight loss, and JFA extract release. Lastly, all types of JFA-CS/PVA/Coll films exhibited antioxidant activity in a range of 46-51% and antibacterial activity against S. aureus. To be noted, the JFA-CS/PVA/Coll 5/4/1 showed the least antioxidant and antibacterial activities among all types of films, which corresponded with the lowest amounts of JFA released. Based on the overall results, the JFA-CS/PVA/Coll films at all ratios which exhibited excellent antioxidant and antibacterial properties revealed the potential for use as topical transdermal patches or use in wound healing applications.

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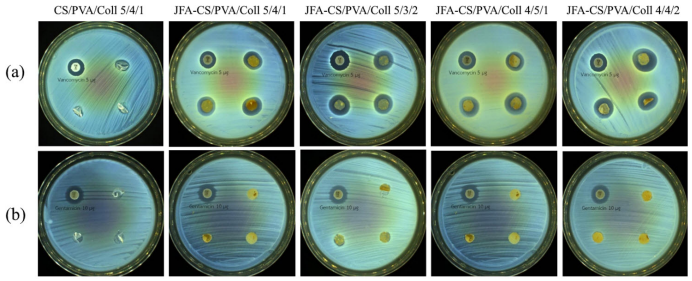

Figure 7: Photographs of the antibacterial activity testing by the agar disc diffusion method for the CS/PVA/Coll and the JFA- CS/PVA/Coll films against (a) *S. aureus* and (b) *E. coli.*

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