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# Bitter Leaf Crude Extracts-Loaded Alginate Films as Potential Wound Dressings

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ABSTRACT

The bitter leaf (BL), a tropical herbal plant which possess antioxidant activity and several pharmacological properties, was extracted using water, ethanol, and ethyl acetate as solvents. The yield of BL crude extract from water (BL/W) was the highest, followed by those from ethanol (BL/E), and ethyl acetate (BL/EA), respectively. The BL/W and the BL/E exhibited the antioxidant activity as determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and slightly antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* as determined by agar disc diffusion method. The alginate films containing 20% w/w of either the BL/W or the BL/E were fabricated from the solvent-casting technique. The release characteristics of the BL extracts therefrom were investigated by the total immersion method and the transdermal diffusion through a pigskin method in either the acetate buffer (pH 5.5) or the phosphate buffer (pH 7.4) solutions during 0-48 h, at the temperatures of 32° or 37°C, respectively. For total immersion method, the maximum released amounts of the BL/W were higher than those of the BL/E. Inversely, the maximum released amounts of the BL/E were higher than those of the BL/W in the case of the transdermal diffusion method. For both the total immersion and the transdermal diffusion methods, the released amounts of both BL/W and BL/E in the phosphate buffer were greater than those in the acetate buffer solutions. Both types of alginate films containing either BL/W or BL/E exhibited the antioxidant and antibacterial activities which showed the potential for use as topical transdermal and wound dressing materials.

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## INTRODUCTION

*Vernonia amygdalina*, known as bitter leaf (BL), is largely found in Asia and Africa. It is used as traditional food and medicine for the treatment of many diseases (1). It is a shrub in the Asteraceae family that grow 2-5 m high. It had shown to possess several pharmacological properties such as anti-inflammatory (2), antioxidant (3), anti-cancer (4), anti-malaria (5), anti-fungal (6), anti-microbial (7), anti-diabetes (3), and anti-allergic (8). BL contains carbohydrates, proteins, fats, amino acids, fibers, minerals, and vitamins (9, 10). Many phytocompounds have been extracted from BL which include flavonoids, phenolics, terpenes, sesquiterpenes, edotides, alkaloids, and xanthones (11, 12). The antioxidant activities of herbal extracts were found to be related with the levels of flavonoid and phenolic compounds (13). The outstanding properties of BL in medicinal uses had drawn attentions to be used as a bioactive ingredient in wound healing (14, 15).

The achievement of wound dressings depends on the selection of the matrix materials and the loaded bioactive compounds. Biodegradable, biocompatible, non-allergic, and non-toxic materials are suitable for use as wound dressings (16). Hydrogels have been widely utilized as wound covering materials due to their ability to keep moist environment and absorb exudate from wounds. The crosslinking of some functional groups in polymer molecules allows hydrogel formation that can be swollen but not soluble in water. Alginate is one of the natural hydrogels that had been employed in biomedical applications, including tissue engineering (17) and carriers for a controlled-drug release in wound healing (18-20). Sodium alginate is a derivative of alginic acid, which is a linear copolymer of (1,4)-linked β-D-mannuronic (M) and its C-5 epimer α-L-guluronic (G) acids (21). It is a natural polymer which can be extracted from a cell wall of brown algae. For use as topical transdermal patch and wound dressings, alginate had shown many satisfied properties including biocompatibility, biodegradability, anti-bacterial, providing moist environment, accelerating re-epithelialization, non-immunogenicity, and controlled release properties (22-25).

In the present contribution, the crude extracts of BL obtained from extraction by different types of solvents were loaded into alginate solutions and were fabricated into films. The antioxidant and antibacterial activities of BL extracts were evaluated. The release behaviors of BL extracts from the BL extracts-loaded alginate films were investigated by the total immersion and the transdermal diffusion through a pig skin with either the acetate buffer (pH 5.5) or the phosphate buffer (pH 7.4) solutions as to simulate the human skin and the physiological condition of wounds, respectively. Lastly, the antioxidant and antibacterial activities of the BL extracts-loaded alginate films were determined to evaluate the potential for use in wound dressing applications.

## MATERIALS AND METHODS

*Materials*

Bitter leaf (BL) was collected from Pathumthani province, Thailand. Sodium alginate was purchased from Acros Organics (USA). Ethanol, methanol, glycerol, ethyl acetate, and calcium chloride were purchased from Carlo Erba Reagents (Italy). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Aldrich (USA). The chemicals for the acetate buffer (pH 5.5) preparation including glacial acetic acid, and sodium acetate and for the

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phosphate buffer (pH 7.4) preparation including anhydrous disodium hydrogen orthophosphate ($Na_2HPO_4$) and sodium dihydrogen orthophosphate monohydrate ($NaH_2PO_4.H_2O$) were purchased from Carlo Erba Reagents (Italy). All chemicals were of analytical grade and used without further purification.

*Extraction of BL*

BL were collected, washed with water, dried at room temperature for 30 min, and were kept in refrigerator. BL was crushed into fine solid with mortar and mixed with each solvent including water, ethanol, and ethyl acetate at a solid:liquid ratio of 10 g : 20 ml. The mixture was sonicated at 40 kHz for 24 h at room temperature. The liquid extract of BL was collected after removing the solid residue by vacuum filtration. The extract was further freeze-dried and kept in a desiccator. The yield of extraction was calculated from an equation (1).

$$\text{Yield } (\%) = \frac{\text{weight of the dry extract (g)}}{\text{weight of the BL leaf}} \times 100 \qquad (1)$$

*Antioxidant activity of BL extracts*

The DPPH assay was conducted to evaluate the antioxidant activity of the BL extracts. The solutions of BL extract from various solvents were prepared at 1, 2, 3, and 5 mg/mL for this study. The DPPH solution was prepared at 0.5 mM in methanol. Then, 3 mL of DPPH solution was mixed with 1 mL of the tested solution and was kept in dark for 30 min. The control sample was the pristine DPPH solution which was kept in the same condition. The absorbance of the obtained solution was measured by the I3 Hanon UV-vis spectrophotometer at a wavelength of 517 nm. The antioxidant activity was calculated from an equation (2):

$$\text{Antioxidant activity } (\%) = \left(\frac{A_c - A_s}{A_c}\right) \times 100 \qquad (2)$$

where, $A_c$ and $A_s$ are the absorbance at 517 nm of the control and the tested sample, respectively.

*Antibacterial activity of BL extracts*

Antibacterial activity of BL extracts against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) was studied by the agar disc diffusion method. The circular filtered papers (diameters of 6 mm) saturated with the BL extracts, distilled water (a negative control: NC), and ethanol (a positive control: PC) were placed in agar plate and incubated at 37°C for 18 h. The length of inhibition zone was measured from the edge of the sample to the end of clear zone.

*Preparation of BL extract-loaded alginate films*

The weighed amount of sodium alginate powder was dissolved in distilled water at a concentration of 2% w/v and was stirred until the homogeneous solution was obtained. The BL extract was added into the alginate solution at a concentration of 20% w/w based on the weight of alginate powder. Glycerol, as a plasticizer, was further added into the solution at 3% w/v. The solvent-casting technique was used to fabricate the BL extract-loaded alginate (designated as alginate/BL) films. 20 g of the alginate/BL solution was poured into the 10 mm-diameter petri-dish. The dish was dried at 45°C for 21 h. The obtained alginate/BL film was immersed in 15 mL of the 2%w/v calcium chloride solution for 5 min to crosslink the alginate. Lastly, the film was rinsed with distilled water and was dried at room temperature for 24 h.

*Preparation of acetate and phosphate buffers*

In the release study, the acetate buffer (pH 5.5) was used as the releasing medium in order to simulate the pH condition of human

skin for the proposed application as topical transdermal patches. In addition, the phosphate buffer (pH 7.4) was used as another releasing medium to simulate the physiological condition for the proposed application as wound dressing. For preparation of 1 L of the acetate buffer solution, 15 g of sodium acetate was dissolved in about 500 mL of distilled water and followed by 1.5 mL of glacial acetic acid. Lastly, the final volume of the solution was adjusted to 1 L using distilled water. Few drops of sodium hydroxide solution or hydrochloric acid could be added to adjust the pH to 5.5. For preparation of 1 L of phosphate buffer solution, 20.2 g of $Na_2HPO_4.7H_2O$ and 3.4 g of $NaH_2PO_4.H_2O$ were dissolved in distilled water. The final volume was adjusted to 1 L. The pH of the obtained phosphate buffer solution was 7.4.

*Release of BL extracts*

The release behaviors of BL extracts from the alginate/BL films were investigated by two methods i.e., total immersion and transdermal diffusion through a pig skin. For both methods, the release medium was either acetate (pH 5.5) or phosphate (pH 7.4) buffer solutions. The temperatures of the release experiments in the acetate and the phosphate buffer solutions were either at the human skin temperature of 32°C or the physiological temperature of 37°C, respectively.

Prior to study the release of BL extracts therefrom, the actual drug content (i.e., the actual amounts of BL extracts) was determined for use as base values in the release study. The film was cut into a square piece of 2x2 $cm^2$ and was dissolved by continuously stirred in 20 mL of distilled water at 80°C. The actual amount of BL extracts was quantified from its absorbance at 324 nm using the UV-vis spectrophotometer.

For the release by total immersion method, the film was cut into a square piece of 2x2 $cm^2$ and was immersed into a capped bottle containing 40 mL of either acetate or phosphate buffer solutions. The solution was slowly stirred during the period of release study which was in a range of 0-48 h.

For the transdermal diffusion through a pig skin method, the abdomen pig skin was used. The pig skin was treated by removing epidermal hair, subcutaneous fat, and underlying tissues in which the final thickness was about 1-1.2 mm. The modified Franz diffusion cell with the expose diameter to the tested film of 13 mm was full-filled with the buffer solution. The tested film was placed on top of the pig skin which, in turn, was placed on the modified Franz diffusion cell. At a specified time point of release, either 1.0 mL of the releasing medium in case of the total immersion method or 0.3 mL in case of the transdermal diffusion method was withdrawn, diluted with the buffer solution, and measured for the absorbance by the UV-vis spectrophotometer at 324 nm. The amounts of released BL extract were calculated against the pre-determined standard curve of BL extract in each type of medium. After each time of withdrawal of the releasing medium, the same amount of fresh releasing medium was added into the bottle to keep the constant volume. The experiments were carried out in triplicate. The percentages of cumulative release amounts of BL extract were calculated from the comparative amounts of BL extract released at a given time point and the actual drug (BL extract) content.

*Water swelling of alginate/BL films*

The degree of water swelling of the alginate/BL films was determined after submersion in either the acetate buffer (pH 5.5)

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or the phosphate buffer (pH 7.4) at the temperature of 32°C and 37°C, respectively, for 48 h according to an equation (3):

$$\text{Water swelling } (\%) = \left(\frac{M-M_i}{M_i}\right) \times 100 \quad (3)$$

where, $M_i$ and $M$ are the initial weight of sample and the weight of sample after submersion in a buffer solution for 48 h, respectively.

*Antioxidant activity of alginate/BL films*

The antioxidant activity of the alginate/BL films was evaluated by the DPPH assay. The tested solution was collected from the released media from the transdermal diffusion release study at 48 h. The procedure of DPPH assay mentioned in the earlier section was performed. Also, an equation (2) was used to calculate the antioxidant activity.

*Antibacterial activity of alginate/BL films*

The alginate/BL films were cut into circular discs with diameter of 6 mm. Antibacterial activity of the tested films against *S. aureus* and *E. coli* was studied by the agar disc diffusion method as described in the earlier section. The length of inhibition zone was measured.

## RESULTS AND DISCUSSION

*Yield and antioxidant activity of BL extracts*

BL were extracted by using 3 types of solvents with different polarity. The obtained BL extracts from water, ethanol, and ethyl acetate were hereafter designated as BL/W, BL/E, and BL/EA, respectively. The percentages of yield of BL extracts are presented in Figure 1. The BL/W was obtained in the highest yield (2.99 ± 0.16%), followed by BL/E (1.46 ±0.05%), and BL/EA (0.45 ±0.08%), respectively. According to a very low yield of BL extract obtained from ethyl acetate, only BL/W and BL/E were used in the further study. The BL/W and the BL/E were further investigated for their antioxidant activities based on the DPPH assay at the concentrations of 1, 2, 3, and 5 mg/mL. For both types of extracts, the higher concentration of extracts contributed to the higher antioxidant activity (see Figure 2.). Obviously, the BL/W exhibited greater antioxidant activity than the BL/E at the same concentration. The antioxidant activities of the BL/W were in a range of about 49 -89% whereas those of the BL/E were in a range of about 0-51%. Interestingly, the antioxidant activity of the BL/W was as high as that of gallic acid released from the electrospun cellulose acetate at a comparable amount of antioxidant substances (26). The antioxidant activity of approximately 80.0% at 3.2 mg/mL gallic acid released was reported (26). However, the BL/E exhibited less antioxidant activity than the BL/W. A similar trend was reported by Atangwho, *et.al*., (3) that the water extract of BL exhibited the higher antioxidant activity as measured by DPPH, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assays than the alcohol (i.e., methanol) extract.

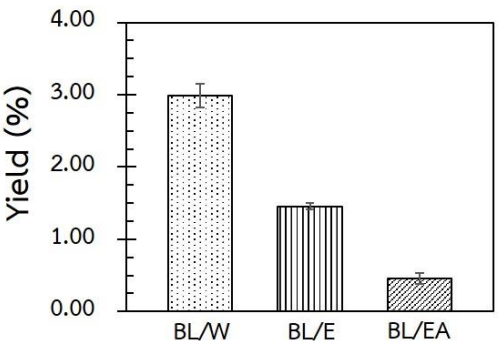


**Figure** 1 Yield of BL extracts in various types of solvents.

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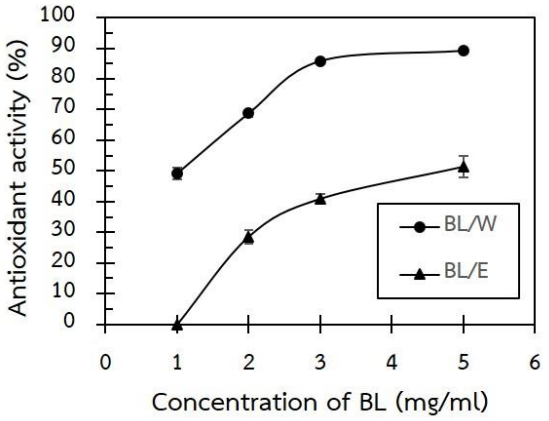


**Figure 2** Antioxidant activity of BL extracts from water and ethanol.

*Antibacterial activity of BL extracts*

Antibacterial activity of BL extracts against *S. aureus* and *E. coli* was determined by the agar disc diffusion method. The lengths of inhibition zone are shown in Table 1. Both BL/W and BL/E exhibited slightly antibacterial activity against both types of bacteria which can be observed from the small values of inhibition zone compared with that of a positive control. Photographs of the bacteria cultured plates were presented in Figure 3. However, the photograph of the BL/E is not available.

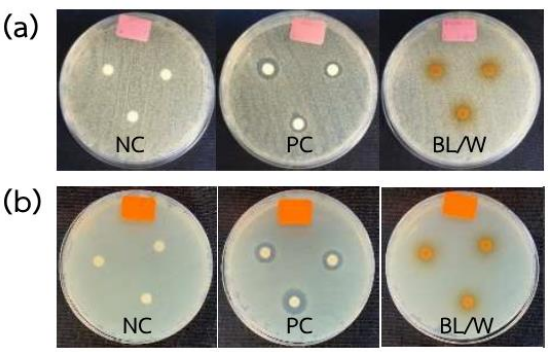


**Figure 3** Antibacterial activity of a negative control (NC), a positive control (PC), and BL/W extracts against (a) *S. aureus* and (b) *E. coli* as determined by agar disc diffusion method.

Both of the BL extracts (BL/W and BL/E) were further loaded into the alginate solution at a concentration of 20% w/w based on the weight of alginate powder in which the solution was subsequently fabricated into films. The average thickness of the alginate/BL films was 134 ± 59 μm. Furthermore, the release of the BL extracts from the alginate/BL films were investigated.

**Table 1** Lengths of the inhibition zone for a negative control, a positive control, and BL extracts against *S. aureus* and *E. coli.*

| Samples | Lengths of inhibition zone (mm) | |
|---|---|---|
| | *S. aureus* | *E. coli* |
| Negative control | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Positive control | 2.33 ± 0.53 | 2.33 ± 0.58 |
| BL/W | 1.24 ± 0.51 | 1.26 ± 0.45 |
| BL/E | 1.21 ± 0.45 | 1.25 ± 0.44 |

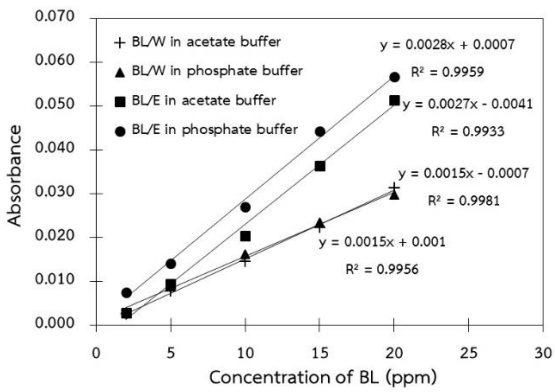


**Figure 4** Standard curves of BL/W and BL/E in acetate and phosphate buffer solutions.

*Release of BL extracts*

Prior to study the release of the BL extracts from the alginate/BL films, it is necessary to prepare the standard curves of each BL extract in both release media (i.e., the acetate and the phosphate buffer solutions). Figure 4.

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shows the standard curves of the BL/W and the BL/E in each buffer solution along with the equations corresponded to each curve.

In the further section, the amounts of the BL extracts released could be quantified according to the measured absorbance at the $\lambda_{max}$ of the BL extracts (324 nm) against the equation of the standard curve. The best fits of the data were validated from the coefficient of determination ($R^2$) which were above 0.99 for all curves.

Moreover, the actual amounts of the BL extracts were determined for use as base values in the release study. The percentages of actual amounts of BL/W and BL/E in the films which were calculated from the actual amounts divided by the loaded amounts of BL extracts were 95.1% ± 3.4% and 94.6% ± 4.1%, respectively.

*Release of BL extracts from total immersion method*

The release of the BL/W and the BL/E from the alginate/BL films was investigated by total immersion method during 0-48 h. Figures 5(a) and 5(b) show the percentages of cumulative release of BL/W and BL/E in the acetate buffer (pH 5.5) at 32°C and in the phosphate buffer (pH 7.4) at 37°C, respectively. The results were reported as the percentages of the cumulative weights of BL extracts released divided by the actual BL extracts content in the sample. For both media and both types of BL extracts (i.e., BL/W and BL/E), the burst release was observed at the initial 200 min of release. Later, the gradual release until reaching the plateau amounts was noticed. The maximum amounts of the BL/W and the BL/E released in the acetate buffer solution at 2,880 min (48 h) were about 50% and 42%, respectively. The greater released amounts of the BL/W than the BL/E were evidenced. Moreover, the similar trend of release was observed in the phosphate buffer solution where the maximum released amount at 2,880 min of the BL/W (about 67%) was higher than that of the BL/E (about 58%).

There are a number of factors affected the rate and the amount of a substance released into media, for example, the degree of weight loss or the dissolution of matrix (27), the degree of swelling of matrix (28), and the polarity of substance (29). The comparable solubility of the substance released and the medium allows the greater rate and amount of substance released (29). The reason of the noticeable greater amounts of the BL/W released than the BL/E in both media could be from the fact that the chemical compositions in the BL/W were more polar than those in the BL/E. Therefore, the molecules in BL/W can diffuse and dissolve into either acetate or phosphate buffer solution which were aqueous solutions better than the molecules in BL/E.

Further observations to be discussed is that, for any type of the BL extract, the released amounts in the phosphate buffer solution were higher than those in the acetate buffer solution. The temperature of the phosphate buffer solution was controlled at 37°C which is the physiological temperature of wound, whilst the temperature of the acetate buffer solution was controlled at 32°C which is the temperature of human skin. The higher temperature of the experiment in the phosphate buffer than that in the acetate buffer solution could be the main reason that attributed to the greater released amounts of the BL extracts. The molecules would have higher kinetic energy and therefore can diffuse out easily at the higher temperature.

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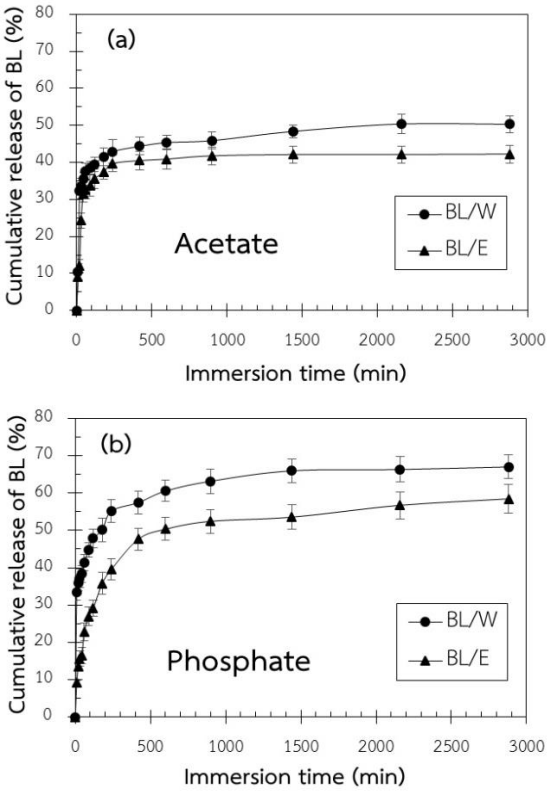


**Figure 5** Cumulative release amounts of BL/W and BL/E from alginate/BL films in (a) acetate (pH 5.5) and (b) phosphate (pH 7.4) buffer solutions from total immersion method.

*Release of BL extracts from transdermal diffusion through a pig skin method*

According to the attempt to investigate the release behavior of the BL extracts in the most similar conditions of the applications in wound dressings or topical transdermal patch, the study of release by transdermal diffusion through a pig skin method was performed. Figures 6(a) and 6(b) present the percentages of cumulative release of the BL/W and the BL/E in the acetate buffer (pH 5.5) at 32°C and in the phosphate buffer (pH 7.4) at 37°C, respectively. The maximum amounts of the BL/W and the BL/E released in the acetate buffer solution at 2,880 min (48 h) were about 7% and 28%, respectively. While, the maximum amounts of the BL/W and the BL/E released in the phosphate buffer solution at 2,880 min were about 39% and 45%, respectively.

Interestingly, the released amounts of the BL/E were significantly higher than those of the BL/W in both buffer solutions. Opposite to the results in the total immersion method, the chemical compositions in the BL/E which were less polar than those in the BL/W could easily diffuse and penetrate into the non-polar membrane of pig skin. The lipid bilayers in epidermis layer of mammal skins act as a non-polar membrane (30) that allows a non-polar substance to penetrate or diffuse conveniently (31, 32).

In addition, for any type of the BL extract, the released amounts in the phosphate buffer solution were higher than those in the acetate buffer solution. The higher temperature used for the phosphate buffer experiments could contribute to the higher amounts of BL extracts released as mentioned earlier.

*Water swelling of alginate/BL films*

The degree of water swelling of the carriers for the drug-controlled release applications is one of the important properties to explain the behavior of release (28). The degree of water swelling of the alginate/BL films was determined after submersion in either the acetate buffer (pH 5.5) or the phosphate buffer (pH 7.4) for 48 h at the temperature of 32°C or 37°C, respectively. The degree of water swelling of the pristine alginate, the alginate/BL/W, and the alginate/BL/E films in the acetate buffer solution were 1339 ± 84%, 1345 ± 56%, and 1329 ± 135%, respectively. The alginate films with different types of BL extracts had the comparable degrees of water swelling. Additionally, the degree of water swelling of the pristine alginate, the alginate/BL/W, and the

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alginate/BL/E films in the phosphate buffer solution were 513 ± 59%, 581 ± 66%, and 455 ± 74%, respectively.

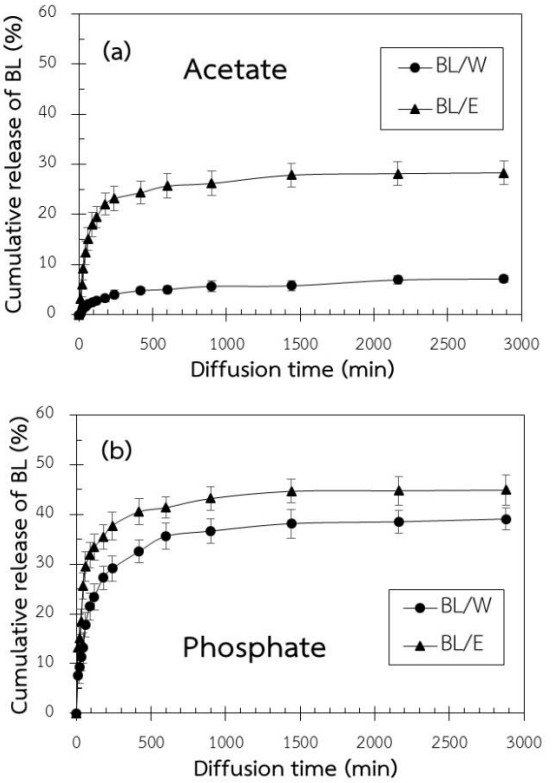


**Figure 6** Cumulative release amounts of BL/W and BL/E from alginate/BL films in (a) acetate (pH 5.5) and (b) phosphate (pH 7.4) buffer solutions from transdermal diffusion through a pig skin method.

According to the higher amounts of BL extracts released in the phosphate buffer than the acetate buffer solution that was mentioned in the earlier section, the higher degree of water swelling of the alginate/BL films in the phosphate buffer solution was expected. However, the contrast results were obtained. During the experiment of determination of water swelling, the high mass loss of the films was observed in the case of the phosphate buffer solution. This mass loss affected to the lower degree of water swelling which was evaluated from the mass of the films after submersion in media. The mass loss of the alginate films when they were submersed in the phosphate buffer solution could be due to the presence of phosphate ion that can remove calcium from the crosslinked alginate molecules and produce calcium orthophosphate. Therefore, the structure of the crosslinked alginate might be partly destroyed and the films could be eroded and lost some mass into media.

*Antioxidant of alginate/BL films*

The antioxidant activity of the alginate/BL films was evaluated by the DPPH assay. The DPPH radical can donate hydrogen radical and become a non-radical form. The decrease in amounts of DPPH radical induced by the antioxidant species was measured by the reduction in its absorbance at 517 nm according to an equation (2). The releasing media collected from the transdermal diffusion through a pig skin method for 48 h were determined for the antioxidant activity. The antioxidant activities of the alginate/BL/W and the alginate/BL/E films in the acetate buffer solution were 23 ± 3% and 34 ± 4%, respectively (see Figure 7). While, these values in the phosphate buffer solution were 40 ± 5% and 41 ± 6%, respectively.

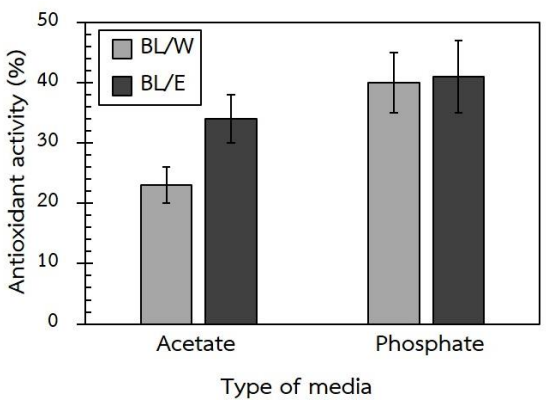


**Figure 7** Antioxidant activity of alginate/BL films after immersion in buffer solutions for 48 h.

These observations were corresponded well with the amounts of BL extracts released which were discussed in the earlier section. Comparing 2 types of releasing media, the higher amounts of BL extracts released (either BL/W or BL/E), in the phosphate buffer solution contributed to the higher antioxidant activity than those in the acetate buffer solution. In addition, comparing between 2 types of BL extracts, the higher amounts of BL/E released in either acetate or phosphate buffer solutions contributed to their higher antioxidant activity than those of the BL/W.

**Table 2** Lengths of the inhibition zone for a negative control (NC), a positive control (PC), and alginate/BL films against *S. aureus* and *E. coli*.

| Samples | Lengths of inhibition zone (mm) | |
|---|---|---|
| | *S. aureus* | *E. coli* |
| Negative control | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Positive control | 2.05 ± 0.05 | 2.03 ± 0.05 |
| Pristine alginate film | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Alginate/BL/W | 0.56 ± 0.09 | 0.51 ± 0.11 |
| Alginate/BL/E | 0.54 ± 0.08 | 0.50 ± 0.09 |

*Antibacterial activity of alginate/BL films*

For the proposed application as wound dressings, the antibacterial activity is one of the important properties. The antibacterial activities of the alginate/BL films against *S. aureus* and *E. coli* were evaluated by the agar disc diffusion method. The length of inhibition zone are shown in Table 2. These values of a negative control (distilled water) and a positive control (ethanol) were also reported. Obviously, the pristine alginate film without BL extracts had no antibacterial activity as the inhibition zone was not observed. The alginate/BL/W and alginate/BL/E films exhibited slightly antibacterial activity against both types of bacteria which can be observed from the small values of inhibition zone.

Interestingly, both the alginate/BL/W and alginate/BL/E films possess their free radical scavenging ability and antibacterial activity. Even though, their antibacterial activities were not outstanding, some other antibacterial substances, for example, silver nanoparticles might be recommended to be incorporated into the films in the future work. Based on the overall results, the alginate/BL/W and the alginate/BL/E films exhibited the potential for use as carriers for topical transdermal delivery and wound healing applications. Even though the BL/E was obtained in the lower yield than the BL/W, the BL/E exhibited the greater released amounts and therefore greater antioxidant activity. While, the BL/W which was gained in the higher yield than the BL/E, exhibited lower amounts of release and therefore lower antioxidant activity. Possibly, the BL/W would be suggested to be loaded at the higher amounts than 20% w/w in the films which was currently used in this study to improve their antioxidant activity.

## CONCLUSION

In the present study, the bitter leaf (BL), a tropical herbal plant which possess antioxidant activity and several pharmacological properties, was extracted by using different solvents. The BL extracts from water (BL/W) and from ethanol (BL/E) showed the antioxidant and slightly antibacterial activities. The alginate films containing 20% w/w of either the BL/W or the BL/E were fabricated. The release characteristics of the BL extracts therefrom were investigated in either the acetate buffer (pH 5.5) or the phosphate buffer (pH 7.4) solutions at 32° or

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37°C, respectively. For total immersion method, the release amounts of the BL/W were higher than those of the BL/E in a given type of medium. The higher polarity of the molecules in the BL/W could contribute to the higher ability to diffuse and dissolve in the aqueous media. In contrast, for the transdermal diffusion through a pig skin method, the release amounts of the BL/E were greater than those of the BL/W. In a similar manner, the less polarity of the molecules in the BL/E could contribute to the greater ability to penetrate into a non-polar membrane of a pig skin. The proposed application of the alginate/BL films as wound dressings was investigated by evaluating their antioxidant and antibacterial properties. Both types of alginate films exhibited the antioxidant and slightly antibacterial activities which showed the potential for use as carriers for topical transdermal delivery and wound healing applications.

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