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Development and Antioxidant Activity Analysis of Bio-Cellulose Containing Indian Gooseberry Extract

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ABSTRACT--- Bio-cellulose containing Indian gooseberry extract was preliminary developed for facial mask products in this research. Two Indian gooseberry fruits viz. wild and Kaset varieties were extracted with ethyl acetate. The total phenolic content and % radical scavenging activity of the extract were analyzed and it was observed that wild Indian gooseberry had higher total phenolic content with higher antioxidant activity as compared with Kaset Indian gooseberry. In addition, the two Indian gooseberries exhibited higher antioxidant activity than ascorbic acid. Wild Indian gooseberry extract was, therefore, selected for incorporating into bio-cellulose by using absorption process. Release of the absorbed extract from bio-cellulose samples was also investigated in acetate buffer pH 5.5 to simulate the release of the extract under human skin pH condition. It was found that the extract of wild Indian gooseberry could absorb well into bio-cellulose and its release was about 3.5-4.5% with high antioxidant activity. This suggests the potential development of bio-cellulose containing Indian gooseberry for facial mask products.

Keywords - Indian Gooseberry, bio-cellulose, total phenolic, antioxidant activity

I. INTRODUCTION

Bio-cellulose is a natural material obtained from fermentation process of coconut water using Acetobacter bacteria. This bacterial bio-cellulose is finer and purer than plant cellulose (Okiyama et al., 1992; Li et al., 2015). At present, bio-cellulose has been used widely in various fields e.g. medicals, cosmetics and food. Bio-celllose is used to produce artificial skin for temporary wound covering in medical field. For cosmetics, contact lens and facial masks can be produced from bio-cellulose (Keshk., 2014). Anti-wrinkle property of bio-cellulose masks containing vitamin E has been reported by Reveny et al. (2017).

Indian gooseberry (*Phyllanthus emblica*) is a herb belonging to *Euphorbiaceae*, found in Thailand. Indian gooseberry is also found in sub-tropical and tropical countries like India, China, Indonesia and Malaysia. Indian gooseberry fruits have been used in medicinal application (Liu et al., 2008) as they contain many active ingredients e.g. gallic acid, tannins, flavonoids, phenolics and vitamin C. Vitamin C contained in Indian gooseberry fruits, is twice of the amount found in orange or lemon.

Active ingredients consisting in Indian gooseberry exhibit antioxidant, antibacterial and antimicrobial activity (Iqbal et al., 2017; Singh et al., 2015). It was reported that Indian gooseberry extracts could help prevent UVB- photo-aging on human skin fibroblasts and had a potential to be used as anti-aging cosmetic product (Adil et al., 2010). Therefore, utilizing Indian gooseberry extract for cosmetic product development is very encouraging.

II. PROBLEM STATEMENT

Facial masks are popular as ready-to-use and easy-apply skincare products. Many of these products are based on synthetic materials enriched with skin pampering agents like antioxidants, moisturing agents, etc. added to improve facial skin conditions. As most of facial masks have short service life, they pose a disposal problem after uses for those from synthetic-based ones. Therefore, utilizing biomaterials like bio-cellulose as facial masks would be promising for development of these products and also incorporation of natural potential antioxidant from plants i.e. Indian gooseberry would be encouraging for entirely eco-friendly and user-safe facial mask products.

III. AIM OF THE RESEARCH

Development of bio-cellulose containing Indian gooseberry was established in the current research, aiming for gaining an insight into the application of this bio-cellulose as a facial mask for skin anti-aging. Incorporation of Indian gooseberry extract into bio-cellulose was studied and the release of the extract from bio-cellulose was conducted in buffer pH 5.5 solution. The antioxidant activity of the extract released from bio-cellulose was analyzed.

IV. METHOD OF THE RESEACH

1. Extraction of Indian gooseberry fruits

In this study, Indian gooseberry fruits used were those from 2 varieties viz. wild and Kaset gooseberries, supplied from Khon Kaen province, Thailand.

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Wild Indian gooseberry is naturally grown native variety while Kaset Indian gooseberry is of the larger-fruited, cultivated varieties. Deseeded fruits were dried at 40°C for 3 days and later pulverized into a fine powder. Extraction was conducted using method modified from Liu et al. (2008). Indian gooseberry fruit powder (40g) was extracted with 250ml ethyl acetate in the soxhlet extraction unit for 12 hours. The extracted solution was then evaporated the solvent out with rotary evaporator. The extract was then dried in hot-air oven at 70°C for 3 days.

The extracts of wild and Kaset Indian gooseberry fruits were analyzed for their total phenolic content by using method modified from Maisuthisakul et al. (2007) and Sabir et al. (2015). The extract solutions were prepared by dissolving 0.01g extracts in 100 ml ethanol. Two ml of 10% Folin-Ciocalteu reagent was mixed with 0.4 ml extract solutions and after that, 1.6 ml of 7.5% sodium carbonate was added. The solution was allowed to react in the dark at room temperature. After 30 minutes, the absorbance values were measured at 765 nm with a Specord UV/vis spectrophotometer. The experiment was done in three replicated measurements. Gallic acid (EMD Millipore Corporation.) was used as a standard to produce calibration curve for total phenolic content calculation. The total phenolic contents were expressed in mg of gallic acid equivalents (GAE)/g of the extracts.

Antioxidant activities of two Indian gooseberry extracts were examined with DPPH radical scavenging assay method modified from Liu et al. (2008) and Singh et al. (2015). Indian gooseberry extracts (0.025g) were dissolved in 50 ml ethanol. Ten ml of the extract solutions were mixed with 10 ml of DPPH solution (0.08 mM in ethanol) and let to react in the dark at room temperature for 20 minutes. Then, the absorbances were measured at 517 nm with a Specord UV/vis spectrophotometer. Ascorbic acid was used as a standard to extablish calibration curves. The absorbances were converted into percentage DPPH radical scavenging activity using the following equation 1.

$$\%\text{DPPH radical scavenging activity} = \frac{(Abs_c - Abs_s)x100}{Abs_c} \quad (1)$$

where Abs_c is absorbance of control and Abs_s is absorbance of mixed solution of DPPH and the extract.

2. Preparation and properties of bio-cellulose containing Indian gooseberry extract

Bio-cellulose sheets being soaked in pH 3 water was provided by Fruitia Food Processing Co., Ltd. Bio-cellulose was cut into 25 mm x 35 mm x 3mm size and then boiled in 0.5% NaOH solution for 15 minutes and later rinsed with running water for another 10 minutes. The washed bio-cellulose was kept by soaking in distilled water for 24 hours. Absorption of Indian gooseberry extracts into bio-cellulose samples was conducted by immersing biocellulose (4g) into 1%w/v extract solution in ethanol. The container was then covered with aluminium foil and left standing at room temperature for 1, 2 and 3 hours. After each immersion time periods, bio-cellulose sample was taken to weigh and the absorbance of the solutions were measured with a Specord UV/vis spectrophotometer. The percentage Indian gooseberry extract absorption on biocellulose was calculated according to equation 2.

$$\%\text{ Extract absorption} = \frac{(Abs_o - Abs_t)x100}{Abs_o} \quad (2)$$

Where Abs_o is absorbance of initial extract solution and Abs_t is absorbance of the extract solution after each designate times.

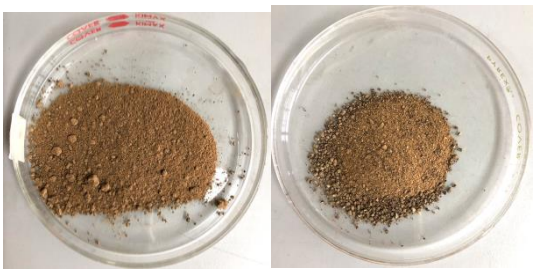
Release of Indian gooseberry extract from biocellulose samples was investigated in acetate buffer pH 5.5 medium in order to simulate skin pH condition. Bio-cellulose samples that already absorbed Indian gooseberry extract for 1, 2 and 3 hours were soaked in 50 ml of acetate buffer pH 5.5 at 37°C for 30, 60 and 120 minutes. Release of Indiam gooseberry extract was monitored by measuring the absorbance of soaking solutions with a Specord UV/vis spectrophotometer and the % extract release from bio-cellulose was calculated against the initial extract absorbed into bio-cellulose. Along with this study, the antioxidant activity of the solution containing release extract was analyzed by DPPH radical scavenging assay.

V. ANALYSIS AND DISCUSSION

Indian gooseberry fruits from 2 varieties viz. wild and Kaset, are illustrated in Figure 1. Wild Indian gooseberry bears smaller sized fruits as compared that of Kaset cultivated variety.

a) b)

Figure 1. Indian gooseberry fruits; a) wild gooseberry fruits; b) Kaset gooseberry fruits



a) b)

Figure 2. Powder of dried wild (a) and Kaset (b) Indian gooseberry fruites

Total phenolic content and % radical scavenging activity of wild Indian gooseberry fruits were found higher than that Kaset gooseberry ones. Interestingly, percentage radical scanvenging activity of both Indian gooseberry fruits were higher than that of ascorbic acid, indicating their high antioxidant properties.

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This antioxidant result agrees with the works of Liu et al. (2008) and Iamsaard (2014). Wild Indian gooseberry exhibiting as high as 87% radical scavenging activity was, therefore, chosen for bio-cellulose incorporation study in the next section.

Table 1. Total phenolic content and % radical scavenging activity of Indian gooseberry fruits & Results

Sample	Total phenolics (mg GAE/g extract)	% Radical scavenging activity
Wild	330.67 ± 1.18	87.05 ± 0.003
Kaset	316.5 ± 5.16	83.70 ± 0.007
Ascorbic acid	-	77.33 ± 0.004

Absorption of Indian gooseberry extract into bio-cellulose sample was studied in order to prepare the bio-cellulose containing Indian gooseberry extract aiming for facial mask application. Bio-cellulose samples themselves (4g) contained large amount of water accounting for 99% being absorbed in their structure. By immersing in Indian gooseberry extract solution, weight of bio-cellulose samples increased with increasing immersion time (Table 2.). In the meantime, percentage extract absorption onto bio-cellulose, determined by absorbance measurement of the extract solutions, increased significantly within the first 2 hours and then reached a plateau when longer immersion time was employed (3 hours). From this result, it points out that absorption into bio-cellulose of wild Indian gooseberry extract in ethanol is optimal after 2 hours immersion and about 20% absorption could be achieved.

Table 2. Absorption of wild Indian gooseberry extract into bio-cellulose

Immersion time (hr.)	% Weight increment	% extract absorption	Sample appearance
0	0	0	
1	3.88 ± 0.692	8.54 ± 1.279	
2	8.16 ± 1.055	20.02 ± 2.651	
3	11.52 ± 0.916	21.84 ± 4.669	

Release ability of wild Indian gooseberry extract from bio-cellulose sample is a crucial property for preliminary determining the application performance of this material for developing into facial mask products. In pH 5.5 buffer medium, being the same as human skin condition, bio-cellulose samples containing wild Indian gooseberry extract could release approximately 3.5-4.5% of the extract after 30 minute exposure to pH 5.5 solutions (Table 3). For those underwent 2 hour absorption, they did release the extract to about the same extent as those bio-cellulose absorbed for 1 and 3 hours, although they exhibited differing degree of absorbed extracts as seen from Table 2.

Table 3. % Release of wild Indian gooseberry extract from bio-cellulose

Absorption time (hr)	% Extract release after immersing for		
	30 min	1 hr	2 hrs
1	3.49 ± 0.277	2.14 ± 0.047	3.29 ± 0.134
2	3.62 ± 0.295	2.72 ± 0.306	3.31 ± 0.168
3	4.57 ± 0.304	2.5 ± 0.245	4.42 ± 0.159

Table 4. % Radical scavenging activity of wild Indian gooseberry extract from bio-cellulose

Absorption time (hr)	% Radical Scavenging activity after immersing for		
	30 min	1 hr	2 hr.
1	82.39 ± 0.011	76.16 ± 0.008	79.14 ± 0.034
2	81.95 ± 0.004	79.11 ± 0.004	80.82 ± 0.016
3	83.28 ± 0.018	77.42 ± 0.008	80.57 ± 0.007

All bio-cellulose sample displayed the same trend in releasing wild Indian gooseberry extract and the % release declinded after 1 hour immersion in acetate buffer solution, however, it seemed to rise up to the same extent as 30 minute ones again after 3 hours. The result infers a high affinity of Indian gooseberry extract to bio-cellulose material.

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About 3-5% of the absorbed extract was released within the most applicable time (30 minutes) regarding to usage as facial mask products that are normally applied on skin for 30 minutes. The antioxidant activity of the release extract into actate buffer solution was determined and it was found correspond to the degree of extract release as seen in Table 4. Although a relative small release of Indian gooseberry extract was observed in the pH buffer solution, it still exhibited high degree of antioxidant activity. The extract released had about 80% radical scavenging capacity. This suggests a strong antioxidant activity of wild Indian gooseberry extract and its potential for use as anti-aging agent for facial mask products.

VI. CONCLUSION

A study on extraction of two Indian gooseberry gives the information that fruits of wild Indian gooseberry has higher phenolic content and better antioxidant activity than Kaset Indian gooseberry. Therefore, it was chosen for a further study in incorporating into bio-cellulose material. Wild Indian gooseberry extract could absorbed from ethanol into bio-cellulose to about 20% within 2-3 hours of immersion. While the percentage release of this extract from bio-cellulose showed that about 3.5-4.5% of the extract desorbed into acetate buffer pH 5.5, being used to mimic pH condition of human skin. This release amount of the extract could still show a strong antioxidant activity, approximately 80% radical scavenging activity being observed. The results inform utilizing potential of wild Indian gooseberry extract for developing bio-cellulose facial masks with antioxidant activity.

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