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Formulation and evaluation of facial mask from gelatinous pulp of *Dillenia* fruit

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Abstract

Formulation and evaluation of facial mask from gelatinous pulp of *Dillenia* fruit

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Gelatinous pulps from *Dillenia indica* Linn. fruit are rich in pectin and nutrients. Mainly, gelling property and the presence of phenolic antioxidants are expected to apply for cosmeceutical applications. The gelatinous pulp was used as gel base for formulation natural facial mask. Properties of gelatinous pulp including pectin quantity, degree of esterification (DE), viscosity, pH, and antioxidant activity were determined. Various concentrations of sucrose and calcium chloride were varies by 0.5, 1, 2, and 4 times from pulp weight for receiving an appropriate gel base. Chosen gel base was then used to formulate facial mask with effective ingredients of licorice extract, honey, and concentration parabens. Stability and skin irritate testing of the product were evaluated. The gelatinous pulp composed 1.1% pectin and 57.9%DE, behaved as pseudo plastic fluid, and exhibited pH about 3.5. Increasing proportional of sucrose and calcium was increased viscosity but reduced pH of gel base. Formulated facial mask was 93.9 cP viscosity, exhibited pH at 4.6, and presented antioxidant activity at IC_{50} = 9.47 mg/ml. It could stable during storage time under switching temperature, as well as there was no skin irritation found in all volunteers. Consequently, skin friendly of gelatinous pulp could be modified and used for skin care and other cosmeceutical applications.

Keywords: *Dillenia indica* L., pectin, facial mask

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Introduction

Dillenia indica Linn. (commonly called Saan or Mataad; Family – Dilleniaceae) grows in the moist and evergreen forests of Thailand.[1,2] The hard fruit are 4-5 inch in diameter, consisting of five tightly fitted imbricate sepals enclosing numerous seeds embedded in a gelatinous pulp. Its succulent gelatinous pulp is rich in nutrients, active compounds, and especially pectin. The fruit is sour in taste and used as flavoring agent in curries as well as gelling agent in jam and jelly preparations. Several parts of *D.indica* such as leaf, bark, and fruit are widely used as traditional medicine. The extracts of the fruit exert an antioxidant activity *in vitro*[3] and the gelatinous fruit pulp could be used as mucoadhesive agent obtained in gel formulation.[4]

Pectin is a complex polysaccharide consisting of galacturonic acid, galacturonic acid methyl ester and sugar units.[5] It is abundantly found in the primary cell walls of terrestrial plants, the middle lamella of fruit, and vegetable tissues. Pectins form skeletal tissues of plants and combine with proteins and other polysaccharides. Due to the molecular size and its complex structure, pectin possesses a key property of gelling.[5] The properties of pectin gel are mainly depended on degree of esterification (DE) or degree of methylesterification (DM) as well as the various factors including temperature, pectin type, pH, sugar, calcium, and other solutes.[5] High methoxyl pectin with DM ≥ 50% can form gels in the presence of sugar, while low methoxyl pectin with DM < 50% can form gels in the presence of divalent cations. The applications of pectin are widely used in food and beverage industries such as jams, jellies, fruit juices and milk as its properties of gelling agent, stabilizer, thickener, emulsifier, and source of dietary fiber.[6] Moreover, pectin possesses high pH buffering capacity, which could help quickly and stabilize skin pH, resulting in maintain the function of permeability barrier and antimicrobial defense.[7] The pectin gels also provide moisture absorption of skin, form excellent films, and being skin friendly.[7]

This study intended to apply gelatinous pulp of *D.indica* fruit as gel base for facial mask formulation. The influence factors of sucrose and Ca^{2+} were studied there effect on gelatinous pulp gelling properties including viscosity, pH, and other appearances. An appropriate gel base was chosen and used for formulation the product and then evaluated by viscosity, pH, antioxidant activity. All formulations were compared with gelatinous pulp gel base and commercial pectin gel.

Materials and Methods

1. Materials

Pectin was brought from Danisco (Bangkok, Thailand). Ascorbic acid was purchased from Riedel-de Haen (Seelze, Germany). DPPH (2,2-diphenyl-1-picrylhydrazly) was from Fluka chemica (Buch, Switzerland). Sodium chloride, sodium hydroxide, and calcium chloride were brought from Ajax (NSW, Australia). All reagents used in this study include methanol (RCI Labscan, Bangkok, Thailand), ethanol and hydrochloric acid (Merck, Darmstadt, Germany).

The fruits of *Dillenia indica* Linn. were ripened from Phisanulok province in December 2011.

2. Preparation of the gelatinous pulp from *Dillenia indica* Linn.

The gelatinous pulp was separated from *D. indica* fruits and removed the numerous seeds, resulting in clear light-orange gelatinous pulps. The clear gelatinous pulps were then pasteurized by heating at 65˚C for 30 min and transferred to 0˚C immediately.

3. Composition characteristics of gelatinous pulp from *Dillenia indica* Linn.

3.1 Determination of pectin content

Pectin in gelatinous pulp from *D. indica* fruits was extracted by the method modified from Dedduang (2010)[8]. In brief, the gelatinous pulp was cleaned by boiling in ethanol at 75˚C for 10 min, filtered, and kept precipitation part. The precipitate was then extracted by diluted HCl, heated at 100˚C for 1 hr, and filtered. Thereafter, the filtrate was mixed with ethanol at 40:45 ratios, and stirred until pectin was precipitated. The precipitate was dried in hot air oven until weight stable. Yield of pectin from gelatinous pulp was calculated.

3.2 Determination of degree of esterification (DE)

The degree of esterification could be measured by quantify the methoxyl content. The determination was performed by saponification of the pectin and titration of the liberated carboxyl group.[8] Firstly, gelatinous pulp was reacted with NaCl and titrated with 0.1 N NaOH for calculating an equivalent weight. Then, the equivalent weight of the sample, containing 0.5 g, was mixed with 0.25 N NaOH, and allows standing for 15 min at room temperature (26.7 ± 2˚C). Finally, the solution was neutralized by 0.25 N HCl, and titrated with 0.1 N NaOH. The methoxyl content or %DE was calculated by the following solution.

$$\%DE = \frac{NaOH\ volume\ 2}{NaOH\ volume\ 1 - NaOH\ volume\ 2} \times 100$$

When NaOH volume 1 is volume of 0.1 N NaOH for calculating an equivalent weight and NaOH volume 2 is volume of 0.1 N NaOH for the methoxyl content calculated.

4. Effect of sucrose and Ca^{2+} on gel properties

The influence factors of sucrose and Ca^{2+} were investigated by adding various concentrations of sucrose and $CaCl_2$. Each weight of them was 0.5, 1, 2, and 4 times from a weight of gelatinous pulp. The mixture of sucrose and $CaCl_2$ with different ratio between 0 to 8 g, were also investigated. Physical properties of each gel were evaluated.

5. Formulation of facial mask using gelatinous pulp from *Dillenia indica* Linn.

An appropriate gel base selected from above study was used to formulate facial mask. Licorice extract, honey, and concentration parabens were mixed into the gel base and heated at 60°C with gentle agitate until homogenize gel was obtained. The properties of the formulation including viscosity, pH, and antioxidant activity were evaluated. Equivalent content of commercial pectin was used to compare. Gel base without ingredients was also used as control.

All products were testing the stability by heating-cooling cycle method. The stability was performed by using temperature between 4° and 45°C, running 12 hr/round for 7 rounds.

6. Analysis of gelatinous pulp and formulations

6.1 Physicals analysis of gelatinous pulp and formulation

Base gelatinous pulps and formulated gelatinous pulps were evaluated the pH values and viscosity at ambient temperature (27 ± 2°C). Base gels and formulated gels made from pectin which equivalent the pectin content in the gelatinous pulp were used to compared.

6.2 Antioxidant Activity

The antioxidative potential of gelatinous pulp, the formulated gelatinous pulp was assayed by DPPH method described elsewhere.[9] Vitamins C was used as positive control. Each sample was done at 5 concentrations, adjusted with methanol and added finally with DPPH solution. After 30 min, the reacted mixtures were measured by UV-spectrophotometer at 517 nm. The absorbance was plotted against concentration and % inhibition was calculated by the following formula. The effective concentration showing 50% inhibition (EC_{50}) was reported.

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

7. Skin irritation testing in Human Volunteer

Thirty healthy volunteers (20-45 years) were participated in this study. The participants were briefed on the study procedures, and each was given written informed consent. Each facial mask and control about 0.5 g was applied once on a surface area of 1 $inch^2$ on lateral arms. After 20 min, the samples were washed and evaluated by Draize technique.

8. Statistical analysis

All values are the arithmetic mean ± SD. Statistical significance was assessed using the unpaired Student's *t*-test or by the one-way ANOVA with a level of significance set at $p < 0.05$.

Results and Discussion

1. Physicochemical characteristics of gelatinous pulp from *Dillenia indica* Linn.

1.1 Determination of pectin content and degree of esterification (DE) in gelatinous pulp

Quantity of pectin consisting in *D.indica* pulp was found in 1.08% of fresh gelatinous pulp weight. The content of methoxyl group represented by %DE, was around 57.9% that was classified as high methoxyl pectin.

The pectin content in the gelatinous pulp was quite low, that might be depended on type and part of plant materials.[5] Pectin content from Krueo Ma Noy leaves (*Cissampelos pareia* L.) was reported around 7% on fresh matters[10], various kinds of guava (*Psidium guajava* L.) was shown around 8-19% [8], and apple pomace contains 10-15% of dry matters.[6] Moreover, types and conditions (e.g. temperature, time, and pH) of extraction method were also affected to the quantity of pectin.[10] The number of DE could be affected by the same influent factors of pectin content.

1.2 Viscosity and pH value

Viscosity of gelatinous pulp was plotted versus spindle flow rate as shown in Figure 1. Increasing the proportion of speed reduced viscosity of the gelatinous pulp. As this result, gelatinous pulp revealed the non-Newtonian or pseudo plastic behavior, which is the same as the behavior found in moderate concentration of pectin solutions.[6] It was in contrast in the commercial pectin solution. Maintained viscosity around 3.14 ± 0.13 cP of the commercial pectin solution behaves as the Newtonian fluid. That might be the reason of pectin structure in gelatinous pulp. Long chain polymers with linkages of pectin were formed network structure and this skeleton could bind cells and other inside molecules together, resulting in higher gel strength than another one. Thus, at equivalent content of both pectins, they act different viscous behavior. The results confirm the viscosity of pectin solution is related not only to molecular weight and DE but also the factors of gel strength.[6]

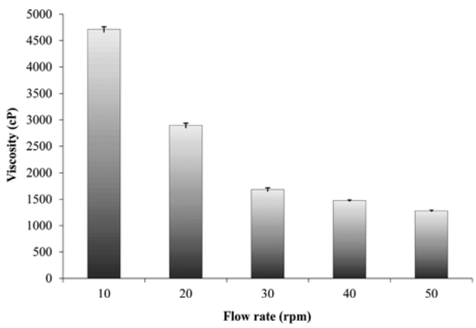

Figure 1 Viscosity of gelatinous pulp at various spindle flow rate, measured at 27 ± 2°C (n = 3).

The pH of gelatinous pulp and commercial pectin were 3.53 ± 0.02 and 3.18 ± 0.02, respectively. Those were in the normal range of high methoxyl pectin, 2.0 – 3.5.[8]

2. The effects of sucrose and Ca^{2+} on gel properties

Figure 2 was presented the effect of sucrose and Ca^{2+} on viscosity of pectin gels from gelatinous pulp and commercial. The gelatinous pulp gels were higher viscous than commercial pectin gels. Increasing the proportional of Ca^{2+} with and without sucrose increased gel viscosity, especially the significant highest was found in the present of 8 g sucrose ($p < 0.05$). The viscosity of each gel was also increased as the sucrose concentration manner. Mixture of high concentration of sucrose (8 g) and Ca^{2+} (4 or 8 g) provided the highest condensed gels. That was confirmed the effects of sucrose and Ca^{2+} on pectin gel forming via cross-linking to form three dimension crystalline networks. Sucrose could stick together in smooth regions with ester groups to form gel network.[11] There have been reported about the large concentrations of sucrose is mainly effect to gelling properties of high DE pectin.[6,12,13] While in the presence of Ca^{2+}, crystalline network could be formed by calcium cross-linking between free carboxyl groups of galacturonic acid chains.[11]

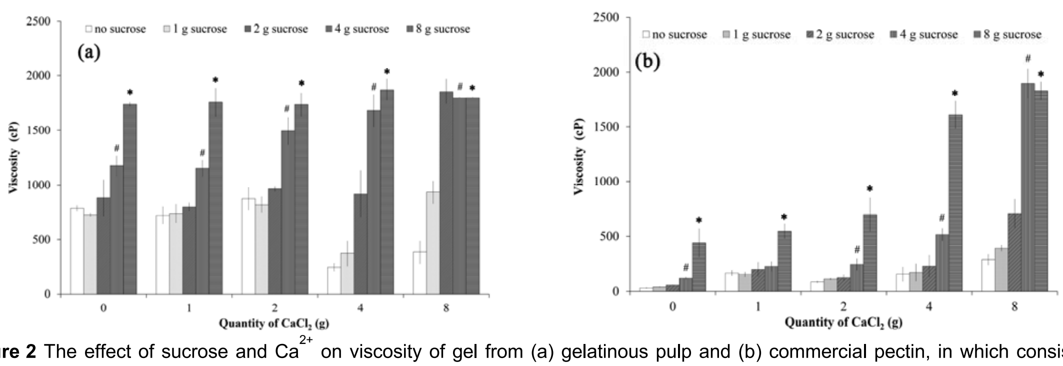

Figure 2 The effect of sucrose and Ca^{2+} on viscosity of gel from (a) gelatinous pulp and (b) commercial pectin, in which consist of the system without sucrose (gray column), with sucrose at 1 g (dotted column), 2 g (stripe column), 4 g (line column), 8 g (steak column); measured at 27 ± 2°C (n = 3).

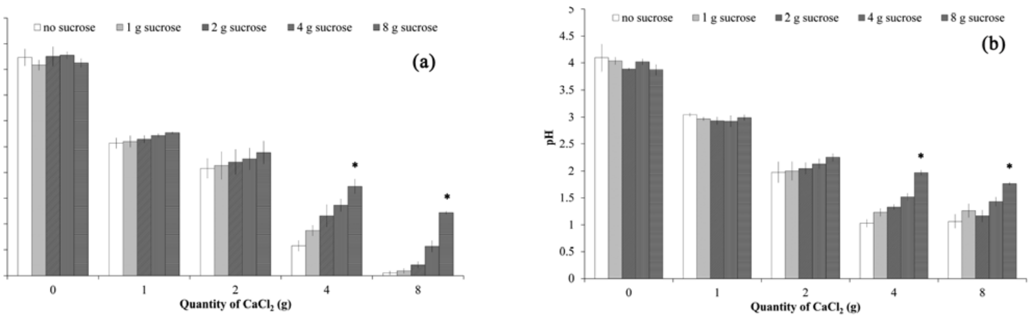

Figure 3 The effect of sucrose and Ca^{2+} on viscosity of gel from (a) gelatinous pulp and (b) commercial pectin, in which consist of the system without sucrose (gray column), with sucrose at 1 g (dotted column), 2 g (stripe column), 4 g (line column), 8 g (steak column) ; measured at 27 ± 2°C (n = 3).

The pH of gels from gelatinous pulp and commercial pectin, affected by sucrose and Ca^{2+}, were shown in Figure 3. Both type of gels revealed the same trend, which increasing of Ca^{2+} decreased pH of gels to strong acid region. Ca^{2+} might substituted proton in pectin structure and provided proton into gel, resulting in pH approach to strong acid. There was slightly effected from sucrose, found in the presence of low Ca^{2+} content (2 and 4 g) and without. At high concentrate of Ca^{2+}, high sucrose molecules might compete to cross-link and obstruct calcium atoms, reducing proton release.

3. Characteristics of facial mask from *Dillenia indica* Linn. gelatinous pulp

Modified gel base from 1 g sucrose was chosen for facial mask formulation. Facial mask formulation was performed by selected gel base, licorice extract, honey, and concentration parabens. The product possessed brown-green color, clear gel, and licorice extract smell. Product from gelatinous pulp was shown higher viscous than commercial pectin over 12 times (data not shown). The pH of both products was 4.60 ± 0.02 and 5.38 ± 0.02 for sample and control. That pH is in the same range of human skin pH varied from 4.1-5.8.

4. Antioxidant activity

The gelatinous pulp and modified gel base were able to scavenge free radicals of DPPH, while commercial pectin and its modified gel base were not (Table 1). This could be explained that the gelatinous pulp from *D. indica* fruits consist the effective compounds which possess hydrogen donating capabilities to act as antioxidant. There were reported the antioxidative activity of extracted *D. indica* fruits[3] and active compounds contained in this fruit were flavonoids, steroids and triterpenoids such as lupeol, betulin aldehyde, betulinic acid, and cycloartenone.[2] The potential of facial mask products were influenced by the contained licorice extract and honey. Gelatinous pulp and its products provided good results for using as skin care, although the activity was lower than vitamin C.

Table 1 Antioxidant potentials of gel from *D.indica* gelatinous pulp and commercial pectin

Materials	IC_{50} (mg/ml)
Gelatinous pulp	21.40 ± 0.31
Modified gel base	52.55 ± 1.62
Facial mask product	9.47 ± 0.99
Commercial pectin	0
Modified gel base	0
Facial mask product	10.38 ± 0.71
Licorice extract	35.40×10^{-3} ± 0.43
Vitamin C	2.78×10^{-3} ± 0.29

5. Stability and skin irritation testing

Figure 4 was shown the results of stability testing. Viscosity of samples from gelatinous pulp presented significantly changed from initiation time ($p<0.05$), while a little bit change was found in commercial pectin samples. That might be reason of the storage time and temperature in which caused by some part of pectin breakdown occurs. Changing of structure and molecular size were key factors, affected to gel strength and viscosity. Commercial pectin samples revealed slightly pH reduction and some precipitates were found. Texture, color, and pH of gelatinous pulp samples were also still maintained.

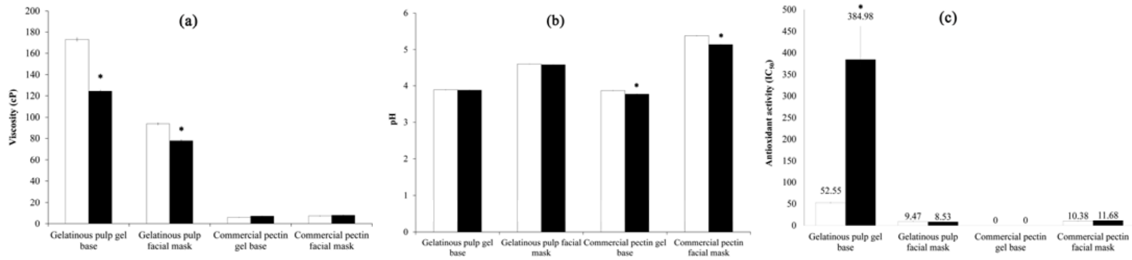

Figure 4 Properties of gel base and facial mask made from gelatinous pulp and commercial pectin including (a) viscosity, (b) pH, and (c) antioxidant activity at initiation time (blank column) and the end of storage time (gray column) (n = 3).

6. Skin irritation testing

There was no skin irritations found in all volunteers. That confirmed the safety of pectin and all ingredients used in facial mask formulation.

Conclusion

Gelatinous pulp from *Dillenia indica* Linn. fruit provided interesting properties of viscosity, pH, texture, and especially antioxidant activity for using as source of pectin or gel base. Gel base from this material could be adjusted for widely applications by using sugar or divalent ion. Moderate viscous, mild pH as physiological skin pH, considerable antioxidative activities, and safety product of facial mask from gelatinous pulp would be alternative way to apply natural pectin for health and cosmeceutical applications.

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