

# Cosmeceutical Effects of Galactomannan Fraction from *Arenga pinnata* Fruits *In vitro*

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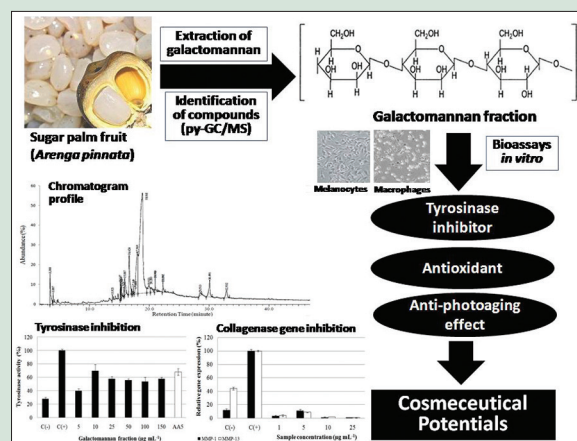
## ABSTRACT

**Background:** Cosmeceuticals refer to natural cosmetics with medical-like benefits due to their bioactive contents. Sugar palm fruit (*Arenga pinnata*) extract has been claimed for its anti-aging effect *in vitro*. However, its active compounds for cosmeceuticals is still unclear. **Objective:** This study was aimed to extract galactomannan from *A. pinnata* fruits and test its efficacy for tyrosinase inhibition, antioxidant, and anti-photoaging activities *in vitro*. **Materials and Methods:** Galactomannan from *A. pinnata* fruits was extracted by freeze drying and identified for its chemical compounds by using pyrolysis gas chromatography-mass spectrometry (py-GC/MS). Galactomannan was tested for its tyrosinase inhibition in both cell-based (melanocytes) and enzymatic assays, antioxidant activity using ferrous ion chelating assay (FCA) assay, and anti-photoaging activity for inhibiting the gene expression of matrix metalloproteinase-1 (MMP-1) and MMP-13 in macrophages using quantitative real-time polymerase chain reaction (qRT-PCR) analysis. **Results:** Identification of galactomannan fraction from *A. pinnata* fruits by py-GC/MS mainly consisted of oxonium ion and glucosides. For cellular assay, galactomannan at 5 µg/mL inhibited >50% of tyrosinase activity in melanocytes induced by phorbol myristate acetate. At the enzymatic level, galactomannan at similar concentration showed less tyrosinase activity inhibition (~20%). FCA results showed that galactomannan at 10 µg/mL exerted >50% of antioxidant activity. The qRT-PCR data indicated that galactomannan at 5 µg/mL inhibited >50% of MMP-1 and MMP-13 gene expressions in ultraviolet B-treated macrophages. **Conclusion:** Galactomannan fraction from *A. pinnata* fruits has efficacy for enlightening effect, antioxidant, and anti-photoaging activity in the dose-independent pattern, indicating its cosmeceutical effects for skin healthcare.

**Key words:** *Arenga pinnata*, cosmeceutical agent, galactomannan fraction, sugar palm fruit

## SUMMARY

- *A. pinnata* fruit containing galactomannan has cosmeceutical potentials through enlightening effect, antioxidant, and anti-photoaging activity *in vitro*.
- Galactomannan fraction has inhibitory effect on tyrosinase activity in both cellular melanocytes and enzymatic systems.
- Galactomannan fraction has strong protection against UVB-irradiation effect by inhibiting collagenase genes (MMP-1 and MMP-13) in macrophages.



**Abbreviations Used:** Py-GC/MS: Pyrolysis-Gas Chromatography-Mass Spectrometry; FCA: Ferrous chelating activity; MMP: Matrix metalloproteinase; qRT-PCR: Quantitative Real-Time Polymerase Chain Reaction; PMA: Phorbol myristate acetate; UV: Ultraviolet; RPMI: Roswell Park Memorial Institute; DMEM: Dulbecco's modified eagle media; FBS: Fetal bovine serum; PBS: Phosphate buffered saline; MTT: 3-(4,5-diethylthiazol-2-yl)-2,5-dipheniltetrazolium bromide; L-DOPA: L-3,4-dihydroxyphenylalanine; EDTA: Ethylenediaminetetraacetic acid; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; DPPH: 1,1-diphenyl-2-picryl-hydrazyl; SPF: Sun protection factor

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## INTRODUCTION

Cosmeceuticals are first popularized by Kligman and defined as cosmetic products containing biologically active compounds that have medical-like benefits.<sup>[1]</sup> Cosmeceuticals are described as the combination of cosmetics and drugs. Cosmetics refer to products that use for cleansing, beautifying the skin, promoting attractiveness, and altering appearance, while drugs are associated with products for preventing, mitigating, and treating diseases by affecting the structure and body function.<sup>[2,3]</sup> Cosmeceuticals are used topically, but its ingredients influence skin biological function; it delivers nutrients to maintain the skin health. Nowadays, cosmeceuticals are claimed to improve skin tone, while reducing wrinkle (aging). Aging is a normal process in life by the passage of time. Aside from our whole body

metabolism, extrinsic factors, such as photoaging by ultraviolet (UV) irradiation from the sun, may cause aging faster.<sup>[4]</sup> A stressful life condition also becomes one of the most important factors in this very high technology era. Thus, people now concern about their skin

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appearance and want to prevent aging or other degenerative skin conditions.

In early 1980's, Kligman introduced the term of cosmeceuticals through the use of retinoid acid as an active compound in developing a formulation to improve UV damaged and skin wrinkle.<sup>[1]</sup> Some cosmeceuticals are natural, while others are synthetic, but all contain active ingredients.

Several reports showed that cosmeceutical peptides and plant cosmeceuticals have been the focus of investigation in the development of a formulation for skin healthcare. Some plants extracts and bioactive compounds such as green tea, ferulic acid, and grape seed have been proven to improve structure and function of the skin due to their antioxidant and sun protection effects to skin.<sup>[5]</sup> Concerning this issue, natural polysaccharides have been known to protect skin from damaging factors. In the previous study, glucomannan isolated from *Candida utilis* exerted a potential photoprotective effect against the damaging effects of UVB exposure.<sup>[6]</sup> Sugar palm fruit (*Arenga pinnata*) is rich in polysaccharide contents, particularly galactomannan gum that has beneficial health effects, such as anticancer and high antioxidant activities.<sup>[7]</sup> However, its cosmeceutical effect for skin healthcare is still unclear. This study was aimed to extract galactomannan fraction from *A. pinnata* fruits and test its efficacies as tyrosinase inhibitor, antioxidant, and anti-photoaging agent *in vitro*.

## MATERIALS AND METHODS

### Galactomannan extraction

Young (half-ripe) sugar palm fruits (*A. pinnata*) were purchased from the traditional market at Jakarta (Indonesia). Before extraction, the fruit endosperms were rinsed with clean water. Galactomannan as polysaccharide fraction was extracted by freeze drying method.<sup>[8]</sup> After suspending the endosperms for 3 days, they were homogenized using a blender and then centrifuged. An equal amount of 95% ethanol was added to the clear supernatant, and the white precipitate was formed. The white precipitate was separated by decantation and freeze dried as galactomannan fraction.

### Compound identification by pyrolysis gas chromatography-mass spectrometry

The composition of the galactomannan fraction was identified by pyrolysis gas chromatography-mass spectrometry (py-GC/MS) using QP2010.<sup>[9]</sup> The sample was injected to the capillary column with 60 m × 0.25 mm ID film thickness. Pyrolysis temperature was set to 280°C. Helium was used as the carrier gas.

### Cytotoxicity assay of galactomannan fraction in phorbol myristate acetate-treated melanocytes

The cytotoxicity assay was done using the 3-(4,5-diethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay.<sup>[10]</sup> The B16F10 melanocytes (ATCC CRL-6475) represented skin cell model for testing sample toxicity. Cells were cultured in Roswell Park Memorial Institute media supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin A, and 100 U/mL streptomycin. Cell culture was incubated at 37°C for 24 h in humidified atmosphere of 5% CO<sub>2</sub>/95% air. For MTT assay, cells were grown until the confluence was reached 50–60% in a 96-well plate. Cells were washed with phosphate-buffered saline (PBS) and added with media. Cell media were induced with 10 ng/μL of phorbol myristate acetate (PMA) and treated with galactomannan fraction from *A. pinnata* fruits at various concentrations (5–150 μg/mL). Cell media were incubated at 37°C in humidified atmosphere of 5% CO<sub>2</sub>/95% air for 48 h. After incubation, media were removed, and cells were washed with PBS. A 20 μL of MTT

reagent was added to the cells, followed by incubation for 3 h at 37°C. Ascorbic acid (5–100 μg/mL) as a reference cosmeceutical agent was prepared in a similar procedure. The absorbance of the cell color changes was measured at 590 nm using microplate reader.

### Tyrosinase assay for galactomannan fraction

Tyrosinase assay was done to determine whether galactomannan fraction from *A. pinnata* fruits inhibited tyrosinase activity for blocking hyperpigmentation. Tyrosinase activity was conducted in two systems, cellular and enzymatic level. Cellular assay for tyrosinase activity was done in B16F10 melanocytes (ATCC CRL-6475). Cells produced melanin that was controlled by tyrosinase. Cells were stimulated with PMA (10 ng/mL) to induce hyperpigmentation in cells and treated with galactomannan fraction at various concentrations (5–150 μg/mL). Cells were incubated at 37°C in humidified atmosphere of 5% CO<sub>2</sub>/95% air for 48 h. After washing with PBS, then cells were lysed with trypsin.<sup>[11,12]</sup> Cell lysate was centrifuged, and cell extract was obtained. A 100 μL of cell extract was mixed with fresh L-3,4-dihydroxyphenylalanine (L-DOPA) and incubated at 37°C for 30 min. Ascorbic acid (5 μg/mL) as a reference cosmeceutical agent was prepared in a similar procedure. Absorbance was measured at 490 nm using microplate reader.

Enzymatic assay was done by adding galactomannan fraction (5–150 μg/mL), tyrosinase (333 unit/mL in 50 mM PBS pH 6.5), and fresh L-DOPA 12 mM into a 96-well plate.<sup>[13]</sup> After incubation in 37°C for 30 min, the absorbance was measured using microplate reader at 510 nm. Ascorbic acid (5 μg/mL), quercetin (25 μg/mL), and arbutin (10 μg/mL) were used as reference cosmeceutical agents.

### Antioxidant assay for galactomannan fraction

Antioxidant assay was conducted to determine if galactomannan fraction from *A. pinnata* exerted antioxidant activity using ferrous ion-chelating assay (FCA) for reduction of free radicals and protection against photodamage in skin.<sup>[14]</sup> Ferrous ions as the most effective prooxidants interact with hydrogen peroxide and form reactive hydroxyl radicals. A ferrozine forms stable magenta-colored complexes with ferrous ion. In the presence of other chelating agents from galactomannan antioxidant, the complex formation is disrupted, and the color of the complex decreases. One milliliter of galactomannan fraction at 5–500 μg/mL was mixed in 3.7 mL ddH<sub>2</sub>O and 0.1 mL ferrous chloride. After 3 min incubation, a 0.2 mL of ferrozine was added, then incubated at room temperature for 10 min. Absorbance was measured at 562 nm against blank. Control (ethanol) without sample was prepared in a similar procedure. Ethylenediaminetetraacetic acid (EDTA) at similar concentrations was as a reference standard. The chelating activity was calculated using this equation: Chelating activity (%) = (Abs control – Abs sample)/Abs control × 100%.

### Anti-photoaging assay of galactomannan fraction

Anti-photoaging activity assay was done in the RAW 264.7 macrophages (ATCC TIB-71) *in vitro*.<sup>[15]</sup> Cells were used to represent skin aging model where the cells were exposed with UVB to induce photoaging condition, and galactomannan fraction from *A. pinnata* were tested to see its efficacy for recovering wrinkle condition. Cells were cultured in Dulbecco's modified eagle media supplemented with 10% FBS, 100 U/mL penicillin A, and 100 U/mL streptomycin. Cell culture was incubated at 37°C for 24 h in humidified atmosphere of 5% CO<sub>2</sub>/95% air. Cells were grown to 50–60% confluence. Cells were washed and added with PBS, followed by irradiation with UVB at 30 mJ/cm<sup>2</sup> for 30 s. PBS was then removed, and cells were incubated with serum-free media with galactomannan fraction at various concentrations (1–25 μg/mL)

for 24 h. The media was separated, and cells were lysed using trypsin. The cellular lysate was further used for mRNA extraction.

### Quantitative real-time polymerase chain reaction

The quantitative real-time polymerase chain reaction (qRT-PCR) was done to quantify the effect of galactomannan fraction at various concentrations (1–25 µg/mL) on the expression of collagenase genes (matrix metalloproteinase-1 [MMP-1] and MMP-13) in UVB-treated macrophages.<sup>[16]</sup> Total RNA from the cellular lysate was extracted using TRIzol reagent (Life Technologies, California, US) according to the manufacturer's protocol and quantified spectrophotometrically at 260/280 nm using NanoDrop. cDNA was synthesized using iScript One-Step RT-PCR Kit with SYBR Green and KAPA SYBR FAST qPCR Kits for analysis by qRT-PCR. Glyceraldehyde 3-phosphate dehydrogenase was used as housekeeping gene control. Primer sequences for qRT-PCR were listed in Table 1. The gene expression of MMP-1 and MMP-13 were calculated and expressed as ratios.

### Statistical analysis

Duplicate experiments were performed throughout this study. All data are presented as mean ± standard deviation. The significant difference between control and treated groups were statistically analyzed by the *t*-test ( $P < 0.05$ ).

## RESULTS

### Galactomannan fraction from *Arenga pinnata* and its chemical compounds identification

One gram of dried powder of galactomannan fraction was extracted from 600 g of *A. pinnata* young fruits (with yield extraction of 0.167%). Galactomannan fraction had characteristics, including white opaque color, transparent coating, plastic-like texture, water soluble, and odorless. Chromatogram profile showed that galactomannan fraction from *A. pinnata* consisted of 20 different chemical compounds that were grouped into several classes, including oxonium ions (56.17%), pyrans (14.04%),

light oxygenates (6.39%), steroids (5.61%), furans (3.24%), anhydrosugars (0.55%), and others (11.2%) [Figure 1 and Table 2]. There were two major compounds found in galactomannan fraction, i.e. 1,2,3,4-cyclopentanetetrol and methyl  $\alpha$ -D-glucopyranoside.

### Cytotoxicity of galactomannan fraction in phorbol myristate acetate-treated melanocytes

MTT results demonstrated that PMA as an inducer for hyperpigmentation did not significantly affect the cell viability of B16F10 melanocytes [Figure 2]. Meanwhile, galactomannan fractions from *A. pinnata* showed various responses on melanocytes growth. At lowest concentration, galactomannan fraction showed less cytotoxicity effect (<20%), while at higher concentration (>5 µg/mL), it reduced cell viability up to ~40%. Ascorbic acid at various doses (5–100 µg/mL) had no effect on cell viability.

### Tyrosinase activity of galactomannan fraction

For cellular assay, B16F10 melanocytes were stimulated with PMA for hyperpigmentation condition due to the increased activity of tyrosinase. Our results showed that galactomannan fraction at 5 µg/mL exerted highest inhibition (~60%) on tyrosinase activity in PMA-treated melanocytes [Figure 3a]. In contrast, ascorbic acid standard at a similar concentration only reduced < 30% of tyrosinase activity. For enzymatic assay, galactomannan fraction dose-independently showed less inhibition (~20–30%) on tyrosinase inhibitory effect [Figure 3b]. At 10 µg/mL, its inhibitory effect was quite similar with arbutin (10 µg/mL) and quercetin (25 µg/mL). Both results indicated that galactomannan fraction exerted tyrosinase inhibition in the dose-independent manner.

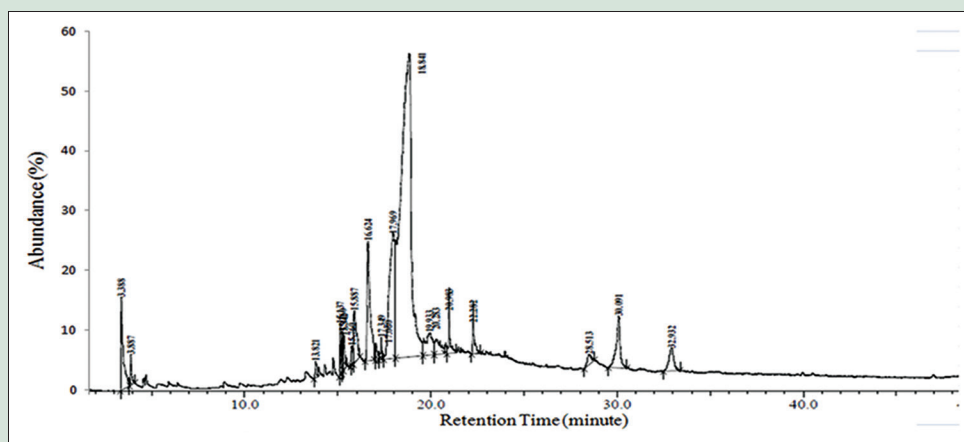
### Antioxidant activity of galactomannan fraction

To test the ability of galactomannan fraction from *A. pinnata* as an antioxidant for skin protection, FCA was conducted. Our results demonstrated that galactomannan fraction at 10 µg/mL showed higher antioxidant activity (~50%) compared to that of ascorbic acid

**Table 1:** Oligonucleotide primer sequences for quantitative real-time polymerase chain reaction

| Gene                | Forward primer         | Reverse primer         |
|---------------------|------------------------|------------------------|
| GAPDH <sup>a</sup>  | CTGGAGAAACCTGCCAAGTATG | CCCTGTTGCTGTAGCCATATTC |
| MMP-1 <sup>b</sup>  | CTCTGACATTCACCAAGGTCTC | AACATCACCAGTGAAGGTGTAG |
| MMP-13 <sup>c</sup> | GGCGACTTCTACCCATTGA    | ATCTCCTGGACCATAGAGAGAC |

<sup>a</sup>Glyceraldehyde 3-phosphate dehydrogenase; <sup>b</sup>Matrix metalloproteinase-1; <sup>c</sup>Matrix metalloproteinase-13

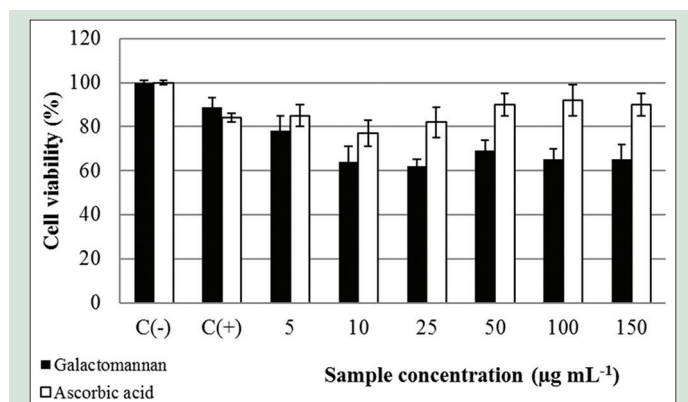


**Figure 1:** Chromatogram of galactomannan fraction derived from *Arenga pinnata* fruits by pyrolysis-gas chromatography mass spectrometry

**Table 2:** Chemical compounds of galactomannan fraction derived from *Arenga pinnata* fruits

| Peak | Retention time | Compounds   | Concentrations (%) | Group         |
|------|----------------|---|--------------------|---------------|
| 1    | 3.388          | Dimethylamine   | 3.48               | Amines        |
| 2    | 3.887          | 1-cyanohexan-5-one  | 0.60               | Alkanes       |
| 3    | 13.821         | Hydroxy dimethyl furanone   | 0.58               | Furans        |
| 4    | 15.137         | Trans-5,6-epoxydecane   | 0.84               | Alkanes       |
| 5    | 15.260         | 3,5-dihydroxy-2-methyl-4H-pyran (hydroxymaltol)                                 | 0.88               | Pyrans        |
| 6    | 15.342         | 3-methyl-1,2-cyclopentanediol   | 0.71               | Alcohols      |
| 7    | 15.760         | 1,4-anhydro-d-mannitol sugars   | 0.55               | Anhydro       |
| 8    | 15.887         | HMF   | 2.66               | Furans        |
| 9    | 16.624         | Heptyl acetate oxygenates   | 6.39               | Light         |
| 10   | 17.060         | 2-butene, 1-butoxy-3-methyl-  | 0.46               | Ethers        |
| 11   | 17.349         | n-pentadecane   | 0.40               | Alkanes       |
| 12   | 17.969         | Methyl alpha-D-glucopyranoside  | 13.16              | Pyrans        |
| 13   | 18.841         | 1,2,3,4-cyclopentanetetrol  | 56.17              | Oxonium ion   |
| 14   | 19.933         | 2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-1,4,4.alpha.,5,8,8a.beta., s | 2.79               | Aldehydes     |
| 15   | 20.283         | Roridin E   | 1.56               | Baccharanoids |
| 16   | 20.983         | Palmitic acid   | 1.29               | Fatty acids   |
| 17   | 22.282         | Oleic acid  | 1.13               | Fatty acids   |
| 18   | 28.513         | Cis-octadec-9-enal  | 0.73               | Fatty acids   |
| 19   | 30.091         | 3-hydroxy-20-oxo-pregnane   | 3.60               | Steroids      |
| 20   | 32.932         | Ergosta-4,6,22-trien-3-one  | 2.01               | Steroids      |

HMF: Hydroxymethyl furfural

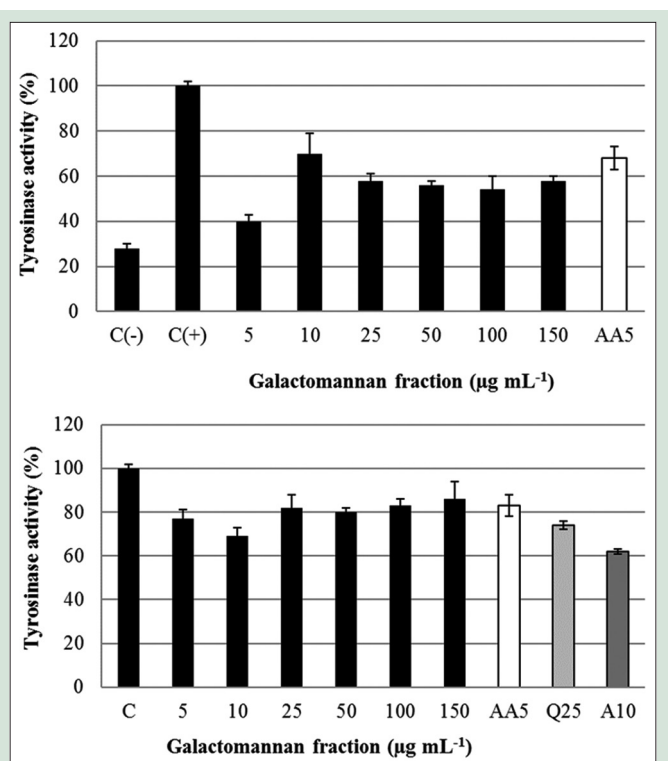


**Figure 2:** Cytotoxicity of galactomannan fraction in phorbol myristate acetate-treated melanocytes. Cells were induced with phorbol myristate acetate (10 ng/mL) and treated with galactomannan fraction (5–150 µg/mL) and ascorbic acid standard (5–150 µg/mL). Cells without phorbol myristate acetate and sample were negative control (C–) and cells treated with phorbol myristate acetate only were positive control (C+). Data were shown as mean ± standard deviation from duplicate experiments.  $P < 0.05$  against phorbol myristate acetate-treated cells

standard [Figure 4]. Galactomannan fraction did not show dose effect on its antioxidant ability, while ascorbic acid exerted an antioxidant effect in the dose-dependent manner.

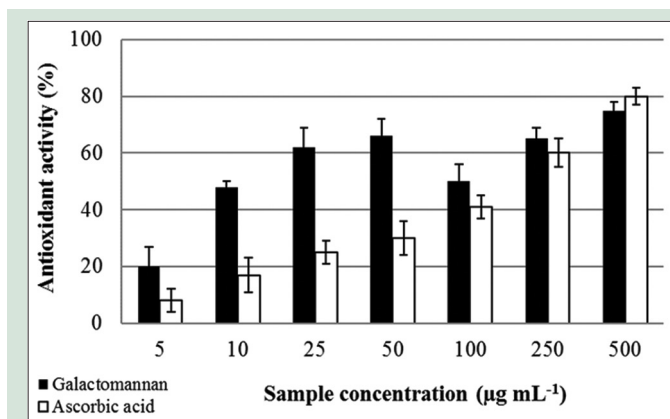
### Gene expression of gelatinases (matrix metalloproteinase-1 and matrix metalloproteinase-13) by galactomannan fraction in ultraviolet B-treated macrophages

The qRT-PCR results showed that UVB irradiation (30 mJ/cm<sup>2</sup>) strongly induced the gene expression of gelatinases (MMP-1 and MMP-13) in UVB-treated macrophages. Galactomannan fraction at various concentrations (1–25 µg/mL) significantly blocked >90% of both MMP-1 and MMP-13 gene expressions in cell system in the dose-independent manner [Figure 5].



**Figure 3:** Tyrosinase activity of galactomannan fraction using cellular (a) and enzymatic (b) assays. Cellular assay used melanocytes and cells were induced with phorbol myristate acetate (10 ng/mL) and treated with galactomannan fraction (5–150 µg/mL). Ascorbic acid (5 µg/mL; AA) was used as a reference agent. Cells without phorbol myristate acetate and sample were negative control (C–) and cells treated with phorbol myristate acetate only were positive control (C+). Enzymatic assay was conducted by using L-3,4-dihydroxyphenylalanine substrate, tyrosinase, and galactomannan fraction at various concentrations (5–150 µg/mL). Ascorbic acid (5 µg/mL; AA), quercetin (25 µg/mL; Q), and arbutin (10 µg/mL; A) were used as reference agents. Control (C) was L-3,4-dihydroxyphenylalanine and tyrosinase only. Data were shown as mean ± standard deviation from duplicate experiments.  $P < 0.05$  against control





**Figure 4:** Antioxidant activity of galactomannan fraction (5–500 µg/mL) by FCA. Ethylenediaminetetraacetic acid at similar concentrations was used as the reference agent. Data were shown as mean ± standard deviation from duplicate experiments

## DISCUSSION

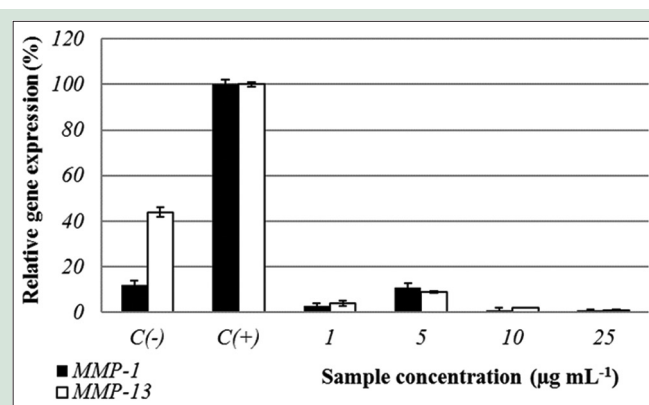
In this study, we isolated polysaccharide fraction, i.e. galactomannan, derived from *A. pinnata* fruits and explored further for its bioefficacy as a promising cosmeceutical agent through mechanisms of tyrosinase inhibition, antioxidant, and anti-photoaging *in vitro*. Our data showed that galactomannan fraction obtained from young *A. pinnata* endosperms was similar with the previous study.<sup>[17]</sup> The galactomannan content varies in a very low amount. It is noted that the content depends on different pretreatments and the endosperms maturity stage.

Mature *A. pinnata* endosperms are associated with higher galactomannan contents, appearance, and moisture properties.<sup>[18,19]</sup> This also indicates in the appearance of the extract; the higher opacity value has higher galactomannan concentration. It is correlated to the phenol compound concentration because the opacity is caused by interaction between polysaccharide and phenol compound.<sup>[18]</sup> Galactomannan forms gel at temperature 30–70°C which depends on its structure and its solubility behavior. This characteristic is very useful to make products that are sensitive to high temperature.

The most abundant compound found in galactomannan fraction from *A. pinnata* is the 1,2,3,4-cyclopentanetrol [Figure 1], an ion related to a carbonic acid. It is used to stabilize reactive compounds and decrease the reaction. It can be generated by splitting reactions which are subject to certain selective controls.<sup>[20]</sup> The second most abundant compound, i.e. methyl- $\alpha$ -D-glucopyranoside, is known as a glucoside (monosaccharide) as the result of glucose catalyzed by methanol. Glucoside is a glycoside with glucose as its sugar constituent. It is an emulsifier applied for personal care products and cosmetics; act to reduce tacky feel and synergistic humectant performance.

Other sugar compounds found were sugar derivatives and intermediate compounds from mannose (1,4-anhydro-D-mannitol) or cellulose (5-hydroxymethylfurfural). The previous study also showed that the 1,6-anhydro- $\beta$ -D-glucopyranose, known as levoglucosan (used as preservatives), was found as the most abundant compound in *A. pinnata* extract.<sup>[17]</sup> Levoglucosan is an important intermediate in cellulose pyrolysis that is released from hemicellulose combustion. In this study, levoglucosan broke down to form pyrans, furans, light oxygenates, and anhydrosugars as identified by py-GC/MS. The second most abundant compound was 1,2,3,4-cyclopentanetrol (7.50%), which is the most abundant compound in this study.

Cell cytotoxicity refers to the ability of compounds to destroy living cells. In drug development, this assay is used to determine the safety dose of



**Figure 5:** Effects of galactomannan fraction on the gene expression of matrix metalloproteinase-1 and matrix metalloproteinase-13 in ultraviolet B-treated macrophages by quantitative real-time polymerase chain reaction. Cells were induced with ultraviolet B (30 mJ/cm<sup>2</sup>) and treated with galactomannan fraction (1–25 µg/mL) for 24 h. Cells without ultraviolet B and sample were negative control (C–) and cells treated with ultraviolet B only were positive control (C+). Data were shown as mean ± standard deviation from duplicate experiments.  $P < 0.05$  against ultraviolet B-treated cells

compounds toward cell growth. MTT results showed that galactomannan fraction from *A. pinnata* at various doses had weak cytotoxicity against cell viability of PMA-treated melanocytes [Figure 2]. According to López-García *et al.*, cytotoxicity is considered as nontoxicity at >80% cell viability; 80–60% is weak; 60–40% is moderate and <40% is strong cytotoxicity.<sup>[21]</sup> PMA inducer only caused less toxicity effect against cell growth. PMA was used to enhance hyperpigmentation by increasing tyrosinase-related protein-1 gene expression encoded tyrosinase in melanocytes.<sup>[22]</sup>

Tyrosinase activity was conducted using both cellular and enzymatic assays to determine enlightening effect of galactomannan fraction from *A. pinnata*. Our results demonstrated that galactomannan fraction was found to be more effective to inhibit tyrosinase activity in PMA-treated melanocytes than that of enzymatic system [Figure 3]. It is indicated that galactomannan fraction is more responsive to block melanogenesis in PMA-treated melanocytes. Its efficacy at low dose (5 µg/mL) exerted higher inhibition on tyrosinase activity compared to that of ascorbic acid standard [Figure 3a]. Meanwhile, enzymatic assay is considered as a fast screening tool for tyrosinase inhibitor candidates. Interestingly, both galactomannan fraction and ascorbic acid standard had a similar inhibitory effect on tyrosinase activity in the conversion of L-DOPA to dopaquinone [Figure 3b].

Ascorbic acid is a potent water-soluble natural skin care ingredient that actively works in reducing melanin formation, neutralizing free radicals, and increasing collagen synthesis in skin. Ascorbic acid is a well-known antioxidant and tyrosinase inhibitor.<sup>[23]</sup> Other standards including quercetin and arbutin isolated from plants are also recognized as skin protectors against damage caused by free radicals and skin whitening agents through inhibiting tyrosinase activity that is responsible for melanin formation.<sup>[24,25]</sup> Tyrosinase controls synthesis rate of melanin that governs skin and hair color and also has harmful UV rays protection. However, overproduction and accumulation of melanin due to UV radiation exposure could lead to various skin disorders.<sup>[11]</sup>

*A. pinnata* is known as a palm native to tropical Asia, from Eastern India east to Malaysia, Indonesia, and the Philippines in the East. Various water and alcoholic extracts of *A. pinnata* different parts (leaves, stems, bark, roots, fruit, flowers or flower buds, seeds, and sap) have been reported for their biological and beneficial results when applied to skin. *A.*

*pinnata* extracts were shown to possess antioxidant and *in vitro* MMP-1 inhibitory properties, but they were ineffective toward lipoxygenase and tyrosinase inhibition.<sup>[26]</sup> Thus, *A. pinnata* extracts could be developed as promising ingredients for topical skin care products.

Antioxidant activity of galactomannan fraction at various doses was tested using FCA assay with principle of metal-chelating activity. Galactomannan fraction at low dose (10 µg/mL) demonstrated higher antioxidant activity (>50%) than EDTA standard [Figure 4]. This data are in linear with antioxidant activity of galactomannan isolated from seed extract of *Gleditsia triacanthos*.<sup>[19]</sup> Interestingly, our previous study demonstrated that the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) results for antioxidant activity of galactomannan fraction from *A. pinnata* at similar dose exerted less antioxidant activity (<40%).<sup>[17]</sup> It is noted that galactomannan may act as an antioxidant by chelating metal ions and neutralizing free radicals, indicating that FCA assay is more sensitive than DPPH assay.

In FCA assay, ferrous ion is one of the most effective pro-oxidants among all metal ions which can catalyze hydrogen peroxide oxidation in the body and generate hydroxyl radical. One of the antioxidant mechanisms is by chelating the metal ion to prevent oxidation.<sup>[27]</sup> Ferrous ion can form Fe<sup>2+</sup>-ferrozine complex with magenta color in the result. If there is ferrous chelating agent, Fe<sup>2+</sup> will be chelated before the complex formation. The complex is disrupted, followed by decreasing of the magenta color. Measurement of the color reduction, therefore, estimates the chelating activity.<sup>[28]</sup>

Our qRT-PCR results demonstrated that galactomannan fraction had strong protection against UVB-irradiation effect, indicating by low expression of MMP-1 and MMP-13 genes in UVB-treated macrophages [Figure 5]. This data are in linear with the previous study by Florence *et al.* that showed the efficacy of *A. pinnata* extracts on inhibition of MMP-1 expression at gene and protein levels.<sup>[26]</sup> *A. pinnata* extracts significantly increased collagen synthesis in skin cells through blocking the expression of MMP-1 gene and protein *in vitro*.

MMP-1 and MMP-13 are specific collagenases with unique ability to denature various collagen substrates, including Type I, II, and III. MMP-1 and MMP-13 are expressed mostly in various cancer, inflammation, primary tumor, and premature aging cells after irradiation with infrared and UV.<sup>[29]</sup> It is also known that human keratinocytes and squamous epithelial cells specifically expressed collagenases, including MMP-1, MMP-9, and MMP-13.<sup>[30]</sup> A recent report from Apraj and Pandita showed that the peel of *Citrus reticulata* Blanco exerted skin anti-aging potentials via antioxidant, anti-collagenase, and anti-elastase effects.<sup>[31]</sup> Other study also revealed that the use of sunscreen products containing various herbs, such as aloe vera, jojoba, cucumber, wheat germ, and olive, with sun protection factor range 10–40 was enough potential to protect skin from harmful UVA and UVB exposures based on photo-stability and psychometric evaluation.<sup>[32]</sup>

Several studies on edible palm products, i.e. edible fruits (endosperms), edible hearts, sap, and edible starch, and their characteristics and applications have been published by most researchers from Asian regions. Torio *et al.* showed that galactomannan extracted from *Arenga saccharifera* endosperms at different maturity stages (young, mid-mature, and mature) exerted different characteristics in terms of solvent solubility, specific gravity, water-holding capacity, viscosity, mannose: Galactose ratio, total sugar content, and soluble protein concentration.<sup>[18]</sup> A recent study from Navaro stated that galactomannan gum from *A. pinnata* endosperms has been purified in terms of high yield. Gum appears as hard translucent white solid and is soluble in various aqueous solutions, resistant to low pH, and unstable at extreme temperature.<sup>[33]</sup> However, both studies were only focused on isolation and characterization of

galactomannan from sugar palm fruits, but not explored on bioefficacy of galactomannan for food and cosmeceuticals applications. Our results demonstrated the effect of galactomannan fraction from *A. pinnata* fruits for cosmeceuticals study.

It is widely known that the major prominent products from palm tree are palm sap, fruits, and fibres. Several studies demonstrated the utilization of palm starch as a promising biodegradable agent for food industry. A study by Ishak *et al.* has been focused on the use of edible starch from *A. pinnata* for biofibers, biopolymers, and biocomposites.<sup>[34]</sup> The combination of starch-based biodegradable composites with date palms and flax fibers significantly enhanced the characteristics and provided a competitive eco-friendly biocomposites for various applications.<sup>[35]</sup>

Sugar palm starch from *A. pinnata* has been also developed as biodegradable films for food packaging.<sup>[36]</sup> The new thermoplastic starch derived from *A. pinnata* starch was successfully developed in the presence of biodegradable glycerol as a plasticizer.<sup>[37]</sup> The previous study from Adawiyah *et al.* reported that both edible starches from *A. pinnata* and *Metroxylon sagu* exerted similar characteristics in terms of amylase contents and peak gelatinization temperature, but not in the breaking properties and texture profile.<sup>[38]</sup> Other application is the use of palm sap from *A. pinnata* for the production of palm sugar. Ho *et al.* demonstrated that thermal processing affected the physicochemical composition of palm sugar from *A. pinnata* sap, including its sugar color, soluble solids (Brix), amino acid contents, sucrose, and pH value.<sup>[39]</sup>

## CONCLUSION

Galactomannan fraction from *A. pinnata* exerted cosmeceutical potentials for enlightening effect by inhibiting tyrosinase activity, antioxidant activity by chelating Fe<sup>2+</sup> ion, and anti-photoaging activity by inhibiting MMP-1 and MMP-13 gene expression. Further study is needed to formulate cosmeceutical products containing *A. pinnata* galactomannan and test its efficacy using *in vivo* animal model for natural skin care product development.

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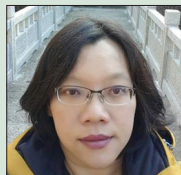
## Conflicts of interest

There are no conflicts of interest.

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