Development of Lightening Cream from Mangosteen Pericarp Extract with Olivoil Emulsifier

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Abstract. The objective of this study is to determine the antioxidant and tyrosinase inhibition activities of the mangosteen (Garcinia mangostana L.) extracts and finally to develop a lightening cream. The results showed that the methanol (MeOH) and ethyl acetate (EtOAc) extracts exhibited antioxidant activities at IC₅₀ = 0.04 mg/ml and IC₅₀ = 0.05 mg/ml respectively. The G. mangostana pericarp extract also inhibited the tyrosinase enzyme but only in EtOAc extract which showed 61.11% of inhibition at 0.05 mg/ml. Based on laboratory findings, the lightening cream comprising the G. mangostana EtOAc extract was formulated into oil-in-water (O/W) cream and the physicochemical properties such as organoleptic properties, stability, viscosity and pH were conducted.

Keywords: Garcinia mangostana, antioxidant, tyrosinase inhibition, lightening cream, mangosteen pericarp extract.

1. Introduction

“Manggis” in Malaysia, Philippines and Indonesia, “Mang khút” in Thailand or Garcinia mangostana Linn. in scientific name is often referred to as the “Queen of Fruit” in Southeast Asia. G. mangostana is a tropical fruit distributed from Malaysia, India, Myanmar, Philippines, Singapore, Sri Lanka, Thailand and Vietnam. The pericarp of the fruit is dark red with 6-10 mm in thickness. The edible portion of fruit is whitish color, soft texture and juicy with a slightly acidic and sweet flavour with a pleasant aroma [1-3]. For centuries, G. mangostana fruit has been used as a medicinal agent by Southeast Asians in the relief of diarrhea [2,4-7], skin infections and wounds [8,9], tuberculosis [10,11] as well as acne [12-13].

G. mangostana fruits have been reported to contain the major active substances which are secondary plant metabolites, xanthones [3,14-17]. Xanthones are phenolic compounds which have been isolated from pericarp, whole fruit, bark, and leaves of G. mangostana fruits. Several studies have shown that xanthones obtained from G. mangostana fruits have possess biological activities [11] included antityrosinase [18], antioxidant [3], antibacterial [18-19], anti-inflammatory [20-21], anti-malarial [22], and anti-atherosclerotic [21].

There are fifty xanthones that have been isolated from G. mangostana pericarp. α-, β-, and γ-mangostins, gartanin and 8-deoxygartanin are the most studied xanthones. The major xanthone in pericarp of G. mangostana fruit is α-mangostin that is proven to be antityrosinase [18] and a strong antioxidant capability. According to W. Pothitirat et al., (2009) [33], the pericarp of G. mangostana at mature stage contains highest amount of α-mangostin.

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The xanthones that have been isolated from *G. mangostana* pericarp has potentially to be an alternative skin lightening agent to treat hyperpigmentation disorders due to its ability to act as tyrosinase inhibitor, where it can inhibit enzyme tyrosinase activity in melanogenesis pathway. According to Briganti *et al.*, (2003) [23], most of the inhibitors are phenol or catechol derivatives, structurally similar to tyrosine or DOPA. The binding at the catalytic site of enzyme tyrosinase blocks the formation of pigment by the deep cells on the skins and reduces the melanogenesis activity [24-25]. The major xanthone in pericarp of *G. mangostana* fruit, α-mangostin is shown to be a strong antioxidant capability.

Antioxidants play an important role to protect the human body against damage by reactive oxygen species released excessively from cells or generated in tissue or blood [26]. The antioxidant properties of phenolics are mainly due to their redox properties. They act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [27-28].

An essential element in constructing female attractiveness in Asian cultures is by having a healthy and fair skin. According to Crawford (1985) [64], having a healthy skin is a matter of self-control, self-discipline, self-denial, and will power. Skin lightness does not only affect women’s beauty perceptions but it also affects marital and job prospects, social status, earning potential [65-67], and self-esteem. Asian countries have long histories of exploiting white skin as a main criterion of personal beauty. In Korea, flawless skin like white jade and an absence of freckles and scars have been desired since the first dynasty in Korean history (the Gojoseon Era, 2333-108 B.C.E) [39]. At a theoretical level, whiteness is a source of symbolic cultural capital [68-69] that is linked with upper class image, luxury, prestige, and success in Asian cultures [70].

In 2004, nearly 40% of women surveyed in Malaysia, China, the Philippines and the Republic of Korea have reported using skin lightening products (UNEP 2008). Skin lightening products does not commercialized for cosmetic purposes only but for clinical treatment to treat hyperpigmentory disorders such as freckles, melasma, pregnancy marks and age spots [71] as well as even out skin tone. Today, many developing countries have evolved into cosmetic industry in developed lightening products. There are a lot of lightening products visible in market that claim to be the effective, but this is not without adverse effects as many harmful chemicals are used in them. Skin lightening compounds, such as mercury and hydroquinone are often used to treat hyperpigmentation disorders.

In Asian markets, skin lightening products dramatically growth and have recorded over the past two decades and become top-selling product categories in the Asian beauty industry in line with technological advances and marketing forces [39]. According to Jennifer *et al.*, (2012) [40], the natural skin lightening from plant extracts are more effective, more safe, non-toxic and cost effective when compared with chemical skin lightening agents such as mercury and hydroquinone which can cause adverse effects. Mercury may cause adverse effects since it can be absorbed through intact skin [41-42], toxic renal [43-45], neurologic [46-47], and dermal [47-50]. Chronic adverse events related to exposure to hydroquinone include ochronosis, nail discoloration, conjunctival melanosis and corneal degeneration [51-52]. Ochronosis is the most common chronic complication related to long-term use of hydroquinone [53-59]. For many years, hydroquinone also has been known highly cytotoxic to melanocytes and potentially mutagenic to mammalian cells [60-61].

2. Materials and Methods

2.1. Plant materials and crude extraction method

*G. mangostana* fruits were purchased from a local market in Johor Bahru, Malaysia. Crude extraction of *G. mangostana* pericarp was performed according to the methods of Arasali and Kadimi, (2009) [34] and Sarin *et al.*, (2009) [18] with some modifications. The fruits were rinsed with distilled water to remove impurities such as dust before the pericarp was separated from the fruits manually. The *G. mangostana* pericarp were chopped into small pieces and dried at 40°C. The dried *G. mangostana* pericarp was ground into powder using blending machine. The powdered of *G. mangostana* was thoroughly extracted with two different organic solvents, methanol (MeOH) and ethyl acetate (EtOAc). Extractions were carried out for 24h for MeOH and EtOAc extracts. The filtrates were pooled and concentrated by a rotary evaporator at 40°C to
give crude extract. The crude extract were kept in air-tight amber bottles and stored at room temperature until they were analyzed.

2.2. 2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity assay

Scavenging of free radicals is the basis of common antioxidant assays. DPPH is a dark-colored crystalline powder composed of stable free radical molecules. Antioxidants react with DPPH, which are stable free radicals, and convert it to 1,1-diphenyl-2-(2,4,6-trinitrophenyl) hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant compounds [63]. In this assay, the antioxidant activity of the extracts was measured accordingly to the method of Wang (2003) [35] with some modifications. 0.4 mg of DPPH was dissolved in MeOH at a volume of 10 ml and left in the dark at room temperature. 100 µl of methanolic DPPH solution was added to 100 µl MeOH and EtOAc of G. mangostana pericarp extracts solution. The absorption was monitored at 30 min after incubation. Absorbance values were corrected for radical decay using blank solutions. The absorbance was measured at 515 nm using microplate reader and the IC\textsubscript{50} value of the mangosteen extracts were determined and compared with positive control, ascorbic acid (0.5 mg/ml). The scavenging activity was calculated using the following equation:

\[
\text{Scavenging activity (\%)} = \left( \frac{A_{515 \text{ of control}} - A_{515 \text{ of sample}}}{A_{515 \text{ of control}}} \right) \times 100
\]

The inhibition concentration (IC\textsubscript{50}) is the amount of antioxidant necessary to decrease the initial DPPH by 50%. The lower the IC\textsubscript{50} is, the higher the antioxidant activity

2.3. Tyrosinase inhibition assay

Tyrosinase is an enzyme that catalyzes the hydroxylation of tyrosine and the subsequent oxidation of DOPA to dopaquinone. When tyrosine aqueous solution was incubated with tyrosinase, its color turned red-brown due to formation of dopachrome [62]. Tyrosinase inhibition activity was determined by the tyrosinase inhibition assay accordingly to the method of Sritularak (2002) [36] with some modifications. The 96-well plate was prepared by applying 80 µl of phosphate buffer pH 6.8, 40 µl of tyrosinase (100 u/ml), 40 µl of sample, and 40 µl of 2.5 mM L-DOPA. After incubation for 30 minutes, the activity was determined by measuring the absorbance at 515 nm with reference 655 nm using the microplate reader. Kojic acid (0.5 mg/ml) was used as positive control. Three independent experiments were performed and each experiment was run triplicate. The percentage of tyrosinase inhibition was calculated as follows:

\[
\text{Inhibition (\%)} = \left( \frac{A_{515 \text{ of control}} - A_{515 \text{ of sample}}}{A_{515 \text{ of control}}} \right) \times 100
\]

2.4. Preparation of lightening cream from G. mangostana EtOAc extract

Table 1. List of instrument and processes used in formulation preparations

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electronic weight balance</td>
<td>Excipients weighing</td>
</tr>
<tr>
<td>Stirrer</td>
<td>For uniform mixing</td>
</tr>
<tr>
<td>Homogenizer</td>
<td>For uniform mixing/dispersion</td>
</tr>
<tr>
<td>Thermometer</td>
<td>Adjustment of temperature</td>
</tr>
<tr>
<td>pH meter</td>
<td>Adjustment of pH</td>
</tr>
</tbody>
</table>

Table 2. Phases for formulation.

<table>
<thead>
<tr>
<th>Phase A</th>
<th>Phase B</th>
<th>Phase C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>Glyceryl monostearate</td>
<td>Vitamin E</td>
</tr>
<tr>
<td>Carbopol 2020</td>
<td>Refined coconut oil</td>
<td>Propylene glycol</td>
</tr>
<tr>
<td>Glycerine</td>
<td>Olive oil emulsifier</td>
<td>Microkill cos</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>Mangoosteen EtOAc extract</td>
<td></td>
</tr>
</tbody>
</table>

The instruments shown in Table 1 were used to prepare lightening cream from G. mangostana EtOAc extract. In this study, oil-in-water (O/W) cream was prepared contemporaneously phases A and B (see Table 2) accordingly to the method Kotta et al., (2011) [37] with some modifications. Distilled water and phase B were heated up to (70-75)°C in glass beaker. The carbopol 2020 then was added into the distilled water and stirred well. The carbopol 2020 was continuing stir until well dispersed followed by addition of glycerine. Homogenizer was used to mix well. When the indicated temperature of the two precedent phases has been
reached, the heat was stopped. The item in phase B was pour slowly into phase A beaker and completely homogenize. The product was left to cool until the temperature drop to 40°C. The item of phase C shown in Table 2 was then mixed one at a time.

2.5. Evaluation of physiochemical properties of lightening cream from *G. mangostana* EtOAc extract

All tests were performed on the cream from *G. mangostana* EtOAc extract formulation. The results were analyzed and summarized in Table 4.

- **Organoleptic properties:** The color of the creams was evaluated against dark background and the odor of the creams was determined as well. The average of three reading was recorded.
- **Stability:** The stability studies of formulated cream were carried out by placed in the centrifuge tubes, vigorously shaken for half an hour at 25000 rpm and observed whether there was a separate section or not.
- **Viscosity:** The viscosity of formulated cream was determined by viscometer using spindle number 64 and determinations were carried out in triplicate and the average of three reading was recorded.
- **pH:** The pH of the creams were measured by pH meter to a depth 0.5 cm in a beaker. The determinations were carried out in triplicate and the average of three reading was recorded.

3. Results and Discussion

3.1. Antioxidant activity

![Antioxidant activity of G. mangostana extracts](image)

The DPPH assay using ascorbic acid as a standard was analyzed to evaluate the antioxidant activity of the *G. mangostana* extracts. The data showed that the MeOH and EtOAc extracts exhibited the antioxidant activities at IC\textsubscript{50} = 0.04 mg/ml and IC\textsubscript{50} = 0.05 mg/ml respectively (see Fig. 1). The positive control, ascorbic acid showed IC\textsubscript{50} = 0.0015 mg/ml. The inhibition concentration (IC\textsubscript{50}) is the amount of antioxidant necessary to decrease the initial DPPH by 50%. The lower the IC\textsubscript{50} is, the higher the antioxidant activity. Therefore, the MeOH extract of the *G. mangostana* showed DPPH scavenging activity [29], and declared to have a very strong antioxidant since the IC\textsubscript{50} value < 0.05 mg/ml [30]. According to Sherwin (1978) [32], the antioxidants donate hydrogen from the phenolic hydroxyl group and neutralize the free radical chain of oxidation forming a stable end product, which does not initiate or propagate further oxidation.

According to Sarin et al., (2009) [18], *G. mangostana* fruits also possessed tyrosinase inhibition activities, which are responsible to inhibit activity of tyrosinase in melanogenesis pathway.

3.2. Tyrosinase inhibition assay

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration (mg/ml)</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kojic acid</td>
<td>0.50</td>
<td>93.31</td>
</tr>
<tr>
<td>EtOAc</td>
<td>0.05</td>
<td>61.11</td>
</tr>
<tr>
<td>MeOH</td>
<td>0.20</td>
<td>7.46</td>
</tr>
</tbody>
</table>
Tyrosinase inhibition assay was performed on the *G. mangostana* extracts to determine the inhibitory effect on tyrosinase activity. In this assay, a well-known tyrosinase inhibitor, kojic acid was used as a standard. The data indicated the *G. mangostana* extract inhibited the tyrosinase enzyme in EtOAc extract, 61.11% at 0.05 mg/ml (see Table 3). According to Briganti *et al.*, (2003) [23], most of the inhibitors are phenol or catechol derivatives, structurally similar to tyrosine or DOPA. Since *G. mangostana* EtOAc extract showed the percentage of tyrosinase inhibition activity, it has potentially to mimics the amino acid tyrosine and binding at the catalytic site of enzyme tyrosinase blocks the formation of pigment by the deep cells on the skins and reduces the melanogenesis activity [24-25].

Based on the data obtained, *G. mangostana* EtOAc extract were used in skin lightening agent [18] due to its potentially to inhibit activity of enzyme tyrosinase, a key components of the skin’s pigmentary system to produce melanin [31].

### 3.3. Physiochemical properties

#### Table 4: The physiochemical properties of lightening cream

<table>
<thead>
<tr>
<th>Formula</th>
<th>Organoleptic properties</th>
<th>Stability 25000 rpm, ½ h</th>
<th>Viscosity 30 rpm (cP)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Cream, white emulsion, lime odor</td>
<td>No separation</td>
<td>7291 ± 251</td>
<td>5.66 ± 0.08</td>
</tr>
<tr>
<td>B</td>
<td>Cream, white emulsion, lime odor</td>
<td>No separation</td>
<td>4359 ± 20</td>
<td>5.6 ± 0.12</td>
</tr>
<tr>
<td>C</td>
<td>Cream, white emulsion, lime odor</td>
<td>No separation</td>
<td>3339 ± 20</td>
<td>5.60 ± 0.07</td>
</tr>
<tr>
<td>D</td>
<td>Cream, white emulsion, lime odor</td>
<td>No separation</td>
<td>1560 ± 20</td>
<td>5.54 ± 0.08</td>
</tr>
</tbody>
</table>

The physicochemical properties of lightening cream from *G. mangostana* EtOAc extract were summarized in Table 4. It was determined that there are no changes in the organoleptic properties of *G. mangostana* EtOAc extract at four different formulas. The increasing EtOAc extract concentrations do not affect color and odor parameters of the lightening cream.

The stability of the lightening cream were evaluated by placed in the centrifuge tubes and vigorously shaken for half an hour at 25000 rpm. The formulas were observed there was no separate section between oil and water phases. Viscosity value of formulas was determined using viscometer at 30 rpm (spindle no: 64; run time: 30 s; temperature: 27.9°C). Increasing extract concentrations proportionate to viscosity values. According to Ashish *et al.*, (2013) [38], the higher the viscosity of cream indicates the cream is easily spreadable by small amounts of shear. Finally, pH values of the lightening cream were determined using pH meter. Increasing extract concentrations caused increasing pH values, probably due to the acidity of the extract. The pH of the cream found to be in range of 5.6 to 6.8 is good for skin pH [38].

### 4. Conclusion

In this study, MeOH extract showed better antioxidant activity at IC<sub>50</sub> = 0.04 mg/ml compared to EtOAc extract at IC<sub>50</sub> = 0.05 mg/ml. However, in the tyrosinase inhibition assay EtOAc extract inhibit the tyrosinase enzyme which showed 61.11% of inhibition at 0.05 mg/ml. Based on laboratory findings, the lightening cream comprising *G. mangostana* EtOAc extract was formulated into oil-in-water (O/W) cream consists of emollient, Olivoil emulsifier. The prepared cream formulations were subjected to study the physicochemical properties on the lightening cream. From the result it is clearly evident that the physicochemical properties included organoleptic properties, stability, viscosity and pH were found to be satisfactory. These studies suggest that Formula A is the best result because it is more stable and safe, as well as easily spreadable by small amounts of shear.

### 5. Acknowledgements

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