Determination of Vitamin A and Mineral Levels (Zinc and Selenium) in Asam Kandis Fruit Peel Extract (*Garcinia Cowa* Roxb)

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Abstract. Traditional medicine has historically employed the West Sumatran herb *Garcinia cowa* Roxb, often known as asam kandis. Flavonoids are one of many phytochemicals found in the rind of asam kandis. Since the rind of G. cowa has been shown to have antioxidant action, it is frequently utilized as a medicinal plant. Mineral and vitamin A content of *Garcinia cowa* Roxb is unknown as of this writing. Active ingredients including vitamin A, selenium, and zinc can shield the skin from oxidative damage and are crucial for both the prevention and treatment of aging skin. In this study, the amount of vitamin A, zinc, and selenium in the rind extract of G. cowa will be examined. The preparation of G. cowa rind samples used the maceration extraction method and 96% ethanol. UV-Vis Spectrophotometry was used to analyze the amounts of vitamin A, at a wavelength of 286 nm. Meanwhile, an X-Ray Fluorescence Spectrometer was used to analyze the amounts of zinc and selenium (XRF). According to the findings, there was 1.16% of vitamin A and 0.212% and 0.002% of zinc and selenium, respectively, in the rind of asam kandis.

Keywords: Garcina Cowa Roxb · Flavonoid · Vitamin C · HC

1 *I*ntroduction

Indonesia is blessed with innumerable and diverse kind of plants which can be considered as a potential to be used as a source of active ingredients for natural medicines and cosmetics [1]. Cosmetic products containing active ingredients (cosmeceuticals) are currently flourishing in the industry due to their benefits in improving physical appearance, health and beauty of the skin, as well as preventing skin disorders [2]. Nowadays, the development and innovation of cosmeceuticals derived from natural active ingredients spark great interest because of their wide availability and fewer side effects compared to those of synthetics [3]. Numerous plants containing secondary metabolites, vitamins, or minerals are formulated as natural antioxidants in skin care products. Plant extracts with vitamins and antioxidant activity stimulate the development of phyto cosmetics field in

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providing cosmetics that can maintain health and prevent premature skin aging [4, 5]. Active ingredients derived from plant extracts are also regarded as safe and has fulfilled the Food and Drug Administration (FDA) criteria for topical use [5].

Garcinia cowa Roxb. (Guttiferae, Cluciaceae) is also well known as asam kandis in West Sumatra and Cha Muang in Thailand. It has been utilized as a cooking spice by the people of West Sumatra. The fruit, twigs, and stems of asam kandis are considered as the greatest sources of secondary metabolites, each of which provides flavonoids, phloroglucinols, xanthones, etc. [6, 7]. The rind of the G. cowa fruit has been shown to possess antioxidant properties, making it a popular therapeutic plant. The rind of the G. cowa fruit is a source of flavonoids and hydroxycitric acid (HCA). [7, 8]. Plant metabolites such as flavonoids and organic acid hold a potential to be utilized in cosmetics [4]. Based on their chemical structure, HCA is included under the hydroxy acid group (AHAs) [9]. AHAs are compounds used extensively in skin care cosmetics and have an effect in repairing photo aging skin [10, 11]. Meanwhile, flavonoids are poly phenolic compounds containing antioxidants and hold the benefit in protecting the body and skin against free radicals [12]. The use of flavonoids derived from plants as active ingredients in cosmeceuticals has a role as antioxidant, absorbing ultraviolet radiation, and anti-inflammatory activity [13, 14]. Due to its various biological activities, G. cowa has the potential to be investigated as a new bioactive component in skin care cosmetics due to its diverse biological activity. Studies on the minerals and vitamin A content of Garcinia cowa Roxb have not been widely conducted, nevertheless. Antioxidants including vitamin A, zinc, and selenium have been widely used in cosmetics and are essential for preventing and treating aging skin [15, 16]. This study aims to analyze the content of vitamin A, zinc, and selenium contained in *G. cowa* fruit rind extract which can potentially be used as a source of bioactive substances in skin care cosmetics. The results of this study can be used as the basis for developing *G. cowa* potentials as a bioactive ingredient in phytopharmaca cosmetics.

2 Research Method

2.1 Tools and Materials

First, This research used a measuring flask, a UV-Vis Spectrophotometer (Analytic Jena's Specord 210 plus), a rotary evaporator, a set of maceration equipment, an X-Ray Fluorescence (XRF) Spectrometer (PANalytical Epsilon 3), measuring pipette, analytical balance (precisa), a container maceration (dark bottle), magnetic stirrer, separating funnel, Buchner funnel, aluminum foil, glassware, oven, analytical balance, 100 mesh test sieve, mortar, stampler, porcelain cup, porcelain crucible, beaker, and other glass wares.

The materials used are technical grade maceration solvent (food grade), asam kandis fruit rind, ethanol PA (Merck), ethanol (Merck), Aquadest (Merck), acetone (Merck), petroleum ether (Merck), Na2SO4 anhydrous (Merck), and Vitamin A (retinol).

2.2 Research Stages

1) *Sampling:* The *asam kandis* (*G. cowa*) used in this study was taken in Tampunik Kanagarian Kambang, Pesisir Selatan Regency. Its identity has been confirmed in

Andalas University Herbarium, Faculty of Mathematics and Natural Sciences with collection number 239/K-ID/ANDA/V/2021.

2) *The Production of Ethanol Extract of Asam Kandis fruit Peel:* A total of 2.5 kg of *G. cowa* samples were cleaned and the wet skin was separated from the flesh. The extract was made through maceration by using 96% of ethanol. Around 5 L of 96% ethanol were added to the G.cowa's wet skin in the macerator, and it was let to soak for 24 h. With the same kind of sample and solvent used in each iteration, the macerate was separated and the procedure was done twice.After that, separate the macerate and evaporate it with a rotary evaporator until a thick extract is obtained.
3) *Determining vitamin A levels in G. cowa Fruit Peel Extract* [17]:

   a) *Preparation of vitamin A Standard Solution:* First, a 1,000 ppm standard solution of vitamin A was made by weighing 0.1 g of vitamin A retinol and dissolving it in ethanol. Then, standard vitamin A solutions at concentrations of 200, 300, 400, and 500 ppm were created.
   b) *Determining Maximum Wavelength:* One concentration of the standard solution was taken, and its absorption was measured in the wavelength range of 185–1100 nm. The wavelength showing the high absorption value is the maximum wavelength.
   c) *Developing vitamin A Standard Curve:* A standard curve was made by connecting the concentration of the standard solution with the absorption results obtained from the measurements using an ultraviolet-visible (UV-VIs) spectrophotometer at the maximum wavelength.
   c) *Determining vitamin A Level from sample:* Distilled water, acetone, and petroleum ether were used to partition 1 g of asam kandis, which was then centrifuged at 4 °C for 5 min at 2,000 rpm. The sample was then divided once more using petroleum ether, acetone, and distilled water. Anhydrous $Na2SO_4$ was added to the filtrate after centrifugation in order to separate the filtrate's aqueous phase

4) *Determining Mineral (ZINC and Selenium) Levels in G. cowa Fruit Peel Extract* [18]: The asam kandis fruit peel extract was then measured using an XRF Spectrometer. It was placed in an XRF holder, measured at 8 and 12 KV with an intensity of 0.32–0.34 mA. The results of the analysis were a print-out data containing composition and mineral content of asam kandis extract. The results of XRF analysis revealed the percentage (%) of the elements contained in the asam kandis extract. The percentage of the results was obtained from a relative calculation of the total peak area which appeared in the absorption pattern, meaning that a certain peak area of a certain element is compared to the total peak area.

3 Result and Discussion

3.1 Sample Identification and Extraction

Maceration is a simple method of extraction carried out by soaking the simplicia powder in a solvent. The solvent will penetrate the cell wall and enter the cell cavity containing

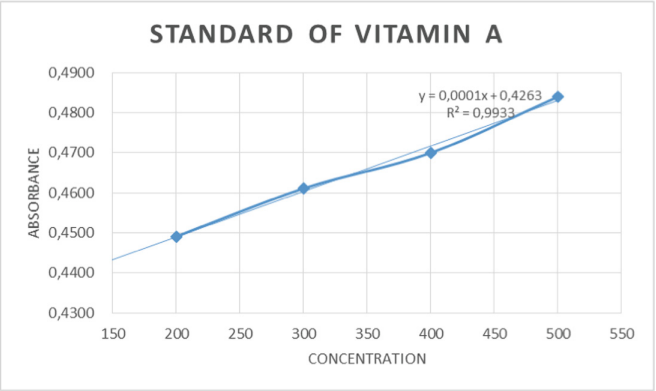

Fig. 1. Standar curve of vitamin A

Table 1. Vitamin A level in *G. cowa* fruitpeel extract

Extract	VIT A level in the sample (%)
Fruit rind	1,16%

the active substance. The active substance will dissolve and due to the difference in concentration between the active substance inside the cell and outside the cell, the concentrated solution is pushed out. This process is repeated so that there is a concentration balance between the solution outside and inside the cell. Maceration is utilized for simplicia extraction containing active substances which dissolve easily in the solvent, does not contain liquid that easily expands in the solvent, does not contain benzoite, stirak and others. The solvent utilized can be water, ethanol, ethanol-water, or other solvents [19].

3.2 The Total Content of Vitamin A in Garcinia cowa Fruit Peel Extract

The vitamin A level contained in *G. cowa* was analyzed using an ultraviolet-visible spectrophotometer (UV-Vis) at a wavelength of 286 nm. The standard solution used was vitamin A (retinol) 1. The results of the absorbance value of the standard solution are connected with the concentration of vitamin A, thus the standard curve of vitamin A was obtained (Fig. 1).

Based on the calibration curve, the results of the regression equation for quercetin absorbance are Y = 0.0073x–0.0057 has a close-to-1 correlation coefficient of r = 0.9998, indicating that the regression equation is linear.

Table 1 shows that the total vitamin A content in *G. cowa* fruit peel extract was 1,16 g/100 g extract.

Utilizing spectrophotometry, the amount of vitamin A found in the fruit rind of asam kandis was determined to be 1.16%. The UV-Vis Spectrophotometry method in vitamin

A examination was an analysis of interaction between near ultraviolet radiation (190–380 nm) and visible light (380–780 nm). Vitamin A is a fat-soluble vitamin which is needed for the body metabolism to maintain body and skin health [15, 19]. It can reduce Reactive Oxygen Species (ROS) in the skin and prevent skin aging [15, 20]. Many derivatives of natural vitamin A come from plants such as β-carotene and retinoids. Vitamin A can play a part in inhibiting cell division if there is excessive proliferation and can activate it when the process is too slow. Topical vitamin A can increase the epidermal thickness by stimulating the epidermal keratinocyte proliferation. Vitamin A regulates the process of keratinization in the skin which normally occurs within 21–28 days [15, 16].

The biologically active form of vitamin A is able to promote remodeling of reticulin fibers and the synthesis of new capillary tissue in the dermis by stimulating endothelial cell proliferation and activating fibroblasts. This process influences the condition of the dermis connective tissue, increasing its firmness, hydration, and elasticity [15]. Vitamin A increases collagen deposition and collagen synthesis in the skin suffering from photo aging. In clinical studies, vitamin A (retinol) given topically could help to reduce wrinkles in photo aging [15, 16]. Thus, vitamin A is able to solve skin aging by repairing the epidermis and dermis of the skin by affecting the three main types of skin cells: epidermal keratinocytes, dermal endothelial cells, and fibroblasts [15, 16].

3.3 The Total Content of Zinc Level in Garcinia cowa Fruit Peel Extract

The level of mineral level (zinc and sekenium) in *G. cowa* was measured using XRF (X-Ray Fluorescence) spectrometer. The final result containing the type and level of zinc in *G. cowa* is presented in Table 2. The measurement using XRF spectrometer was carried out to determine the number of moles of each element in asam kandis extract. Then, the number of moles was converted into mass percent by multiplying them with the relative atomic mass of each appearing element. The result of the mass percentage of each element was multiplied by the weight of asam kandis extract after it underwent destruction process or the mineral sample of asam kandis extract, so that the weight of each element was discovered. The weight of each element was the weight of each mineral per 100 g of *G. cowa* extract. X-Ray fluorescence (XRF) spectrometer can be put to use for quantitative analysis of micronutrients or minerals such as Zinc (Zn), Selenium, Calcium (Ca) and others (296). The use of an XRF spectrometer in determining mineral level hold several advantages such as it does not require a lot of samples, faster, does not require standards and high accuracy, able to measure various mineral levels in biological materials, and the results can be directly obtained [21]. Minerals are considered as essential micronutrients, but the body is unable to synthesize them. Therefore, they must be obtained through nutrition. Skin health problems such as structure and function disorder of skin can occur due to the lack of micronutrients. Several minerals such as zinc, selenium, copper, and silicon have a role and contribution to initiate skin aging [15].

Table 2 shows the average zinc content in *G. cowa* extract of 0,212 g/100 g.

Zinc is a mineral found in the epidermis and dermis layers of the skin. The epidermis of the skin contains higher level of zinc in the epidermis, this is because zinc is needed

Table 2. Zinc level in I *G.cowa* fruit peel extract

Extract	Zinc level in the sample (%)
Fruit peel	0,212%

Table 3. Selenium level in *G. cowa* peel extract

G.cowa Extract	SE level in the sample (%)
Fruit peel	0.002%

in the process of proliferation and proliferation and differentiation of epidermal keratinocytes [15, 20].) Zinc contains antioxidant activity to protect body cells against the negative effects of free radicals. It is also involved in the enzymatic antioxidant activity such as cooper zinc superoxide dismutase (Cu/Zn-SOD) which acts to neutralize superoxide radicals [15, 22]. Table 2 displays the outcomes of measuring the total zinc content of G. cowa extract.

Zinc is needed to maintain normal skin function and can be used topically to treat various skin problems such as melasma, aging, and so forth [23]. Zinc plays a role in preventing skin aging through biosynthesis and regeneration of elastin fibers [24]. A study on the topical application of zinc to facial skin suffering from photo aging showed that after 8 weeks there was a regeneration of elastic fibers in the papillary dermis which led to the disappearance of wrinkles. Zn also possess effective properties against melasma through its role as an anti-inflammatory, anti-oxidant, sunscreen, and healing agent [24]. Zinc is beneficial as a skin mechanical barrier because it effectively blocks UV rays at a wavelength of 340–400 nm. Zinc can also increase resistance to oxidative stress, prevent hyperpigmentation, and influence collagen metabolism [22, 25, 26]. Furthermore, Zinc is widely used as a broad-spectrum physical sunscreen. Its advantages lie in its low cost and outstanding safety profile. It has been used alone and in combination with other physical sunscreen (titanium oxide) or other chemical ingredients. Zinc may also act as an anti-inflammatory by reducing the level of cytokines that lead to inflammation and biomarkers of oxidative stress, such as C-reactive protein [14]. As an anti-inflammatory, Zn is used to treat various skin diseases such as rosacea, seborrheic dermatitis, eczema, etc. [26]. The role of zinc as an antioxidant, photo protection, anti-inflammatory, and elastic regenerative can be utilized for the development of effective antiaging therapies. Lemon flesh contains vitamin C compounds with vitamin C content of 0.66 mg/g sample [31]. The vitamin C content of *G. cowa* peel extract is quite high when compared to the vitamin C content of various kinds of oranges and lemons.

3.4 The Total Content of Sselenium in Garcinia cowa Fruit Peel Extract

Table 3 shows the average Selenium content of *G. cowa* rind of 0,002 g/100 g.

The results of selenium examination using XRF spectrophotometer are obtained in Table 3. Aging is regarded as an inevitable natural process which is constantly accompanied by aging-related diseases. Excessive accumulation of ROS (Reactive Oxygen Species) which triggers oxidative stress is an early causative factor of aging. [27]. Se possess beneficial effects on skin health and can be applied topically to the skin [28]. As an antioxidant in the form of selenoprotein, selenium can reduce the production of ROS and inflammation. Se suppresses ROS reduction through oxidation-reduction regulation thereby preventing aging [27]. Se is present in the cell of the skin, both in the epidermis and dermis layer, and is a cofactor or part of the thioredoxin reductase and glutathione peroxidase antioxidant enzymes. As a cofactor of thioredoxin reductase in cell membranes of keratinocytes, Se provides protection against free radicals generated by UV ray [28, 29]. Several studies have shown that Se application can protect keratinocytes, melanocytes, and fibroblasts from the damage and cytotoxicity of UV ray [27].

Selenium is able to reduce Matrix metalloproteinase (MMP) which can trigger the aging process of fibroblasts and elastic fibers in the skin. Thus, the application of Se to the skin can enhance collagen synthesis and promote the proliferation of skin fibroblasts as an anti-aging and anti-wrinkle. The selenium-containing antioxidant which is applied to the skin exhibit improvement in moisture, wrinkles, and smoothness [27]. Se contained in plants accumulates in the form of organic compounds, especially selenomethionine and selenocysteine. Selenium is most easily absorbed in the form of organic compounds due to the presence of vitamins A, D, and E [27]. Therefore, Selenium is often used as an ingredient in anti-aging skin care products because of its role as an antioxidant, anti-inflammatory, and co-factor in enzymatic antioxidants formation (glutathione peroxidase) [27].

4 *c*onclusion

Based on the results of the study, it can be concluded that *Garcinia cowa* roxb extract contains Vitamin A 1.16%, Zinc 0.212% and Selenium 0.002%. The level of vitamins A, zinc dan selenium can add to the scientific knowledge and can be further developed into cosmeseuticals products.

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