

Phenolic Content, Antioxidant Activity, and Tyrosinase Inhibitor Potential for both Skin and Physical Health: Test of Extract, Skin Content and Content of Garcinia Cowa Fruit

Contenido fenólico, actividad antioxidante y potencial inhibidor de la tirosinasa para la salud física y de la piel: prueba de extracto, contenido cutáneo y contenido de la fruta Garcinia Cowa

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Abstract. Skin aging leads to reduced structural integrity and reduced skin function. Medicinal plant extracts that have antioxidant properties and are able to inhibit tyrosinase have become a trend in various uses for anti-aging. One of the commonly used plants in traditional medicine that grows in many Asian countries is *garcinia cowa*. Currently, no one has studied the fruit from two different aspects, namely for the skin and to help in physical improvement. The purpose of this study is to test the phenolic content, antioxidants, and tyrosinase inhibition activity of the extract from the skin and pulp of *garcinia cowa* as well as its effectiveness on the physical ability and function of the skin. The stages carried out start from the collection of *garcinia cowa* fruits, the determination of total phenolic levels, the determination of antioxidant activity, and the determination of tyrosinase inhibition activity. The total phenolic content was determined by the folin-ciocalteu method. The phenolic content of *garcinia cowa* fruit peel extract is 17,789 mg of gallic acid equivalent extract (GAE)/g, while the meat extract is 12,078 mg GAE/g extract. Furthermore, the flesh of *garcinia cowa* showed weak antioxidant activity with an IC50 value of 490.2 µg/mL, but the fruit peel extract showed moderate antioxidant activity at 243.3 µg/mL. The skin of *garcinia cowa* fruit shows tyrosinase inhibition activity in vitro, which is more significant than the pulp. *Garcinia cowa* peel and pulp extracts both contain phenolic compounds and show the antioxidant activity, but it was found that the fruit peel extract has a higher content. This means that with its antioxidant content, it can ward off free radicals, prevent cell damage, and reduce the risk of chronic diseases. The combination of phenolic content, antioxidant activity, and tyrosinase inhibitor potential provides significant benefits for skin health and overall physical health.

Keywords: Medicinal plant, extract, antioxidant, physical activity

Resumen. El envejecimiento de la piel conduce a una reducción de la integridad estructural y la disminución de la función de la piel. Los extractos de plantas medicinales que tienen propiedades antioxidantes y son capaces de inhibir la tirosinasa se han convertido en una tendencia en diversos usos para el anti-envejecimiento. Una de las plantas más utilizadas en la medicina tradicional que crece en muchos países asiáticos es la *garcinia cowa*. Actualmente, nadie ha estudiado la fruta desde dos aspectos diferentes, a saber, para la piel y para ayudar en la mejora física. El propósito de este estudio es probar el contenido fenólico, los antioxidantes y la actividad de inhibición de la tirosinasa del extracto de la piel y la pulpa de *Garcinia cowa*, así como su eficacia sobre la capacidad física y la función de la piel. Las etapas llevadas a cabo partieron de la recolección del fruto de *Garcinia Cowa*, la determinación de los niveles fenólicos totales, la determinación de la actividad antioxidante y la determinación de la actividad inhibidora de la tirosinasa. El contenido fenólico total se determina por el método folin-ciocalteu. El contenido fenólico del extracto de cáscara de fruta de *Garcinia cowa* es de 17.789 mg de extracto equivalente de ácido gálico (GAE)/g, mientras que el extracto de carne es de 12.078 mg de extracto GAE/g. Además, la pulpa de *Garcinia Cowa* mostró una actividad antioxidante débil con un valor IC50 de 490,2 µg/mL, pero el extracto de cáscara de fruta mostró una actividad antioxidante moderada a 243,3 µg/mL. La piel del fruto de *Garcinia Cowa* muestra actividad de inhibición de la tirosinasa in vitro, que es más significativa que la pulpa. Los extractos de cáscara y pulpa de *Garcinia Cowa* contienen compuestos fenólicos y muestran actividad antioxidante, pero se descubrió que el extracto de cáscara de fruta tiene un mayor contenido. Esto significa que, con su contenido de antioxidantes, puede protegerse de los radicales libres, prevenir el daño celular y reducir el riesgo de enfermedades crónicas. La combinación de contenido fenólico, actividad antioxidante y potencial inhibidor de la tirosinasa proporciona beneficios significativos para la salud de la piel y la salud física en general.

Palabras Clave: Planta medicinal, extracto, antioxidante, actividad física

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Introduction

A progressive decline in the physiological function of organs, including the skin, is a sign of a biological process known as aging. The skin experiences various problems because the structural integrity and function of the skin decreases as the skin ages (Rinnerthaler et al., 2015). Skin aging also causes clinical signs such as lentigo (age spots), xerosis (dry skin), rough wrinkles on the skin, and color imbalance (Mohiuddin, 2019). Reactive oxygen species (ROS) are the main factors that affect skin aging. ROS is created by exposure to ultraviolet rays, which can accelerate skin aging (Kammeyer & Luiten, 2015). This is a natural process that occurs with age and many outdoor activities that result

in physical changes in the skin such as wrinkles, skin thinning, and loss of elasticity.

In recent years, the use of anti-aging ingredients such as cosmetics has become very popular among people around the world. Almost all the use of anti-aging drugs and cosmetics uses chemicals that are a big threat to skin damage in the future (Shanbhag et al., 2019). Not only does it threaten skin damage, but it will have an impact on other body organs (Sorg et al., 2017). However, currently there are also many companies that are trying to maximize natural ingredients in the manufacture of cosmetics to prevent anti-aging (Ahmed et al., 2020). Some of the natural ingredients used are plants that contain antioxidants and anti-tyrosinase activity (tyrosinase blocker). On the other hand, one form of

activity carried out to overcome skin aging is to do physical activity which plays a very important role. With physical activity, it will be able to increase blood circulation which will be able to help deliver oxygen and nutrients to skin cells (Zhang et al., 2024). This will help keep the skin healthy and speed up the cell regeneration process. Physical activity can also stimulate the production of collagen, an important protein that can keep the skin tight and elastic (Kastella et al., 2024). Because of age, collagen production will decrease, so special handlers are needed to help slow down the process.

One type of natural plant that is currently widely used in overcoming skin aging is the medicinal plant *garcinia cowa* or also known as Kandise Acid which is widely found in Southeast Asia and South Asia. The plant is believed to have a lot of high antioxidant content which has many benefits. In traditional medicine, the leaves and fruits of *garcinia cowa* are used to improve blood circulation, expectations, and digestion (Phukhatmuen et al., 2020; Minerva et al., 2023). Phytochemicals of *garcinia cowa* include flavonoids, phloroglucinol, and xanthenes (Chouni & Paul, 2023). The skin of *garcinia cowa* fruit also contains vitamin C and hydroxy citric acid (HCA), which belong to the hydroxy acid group (Angami et al., 2021).

A number of studies have shown the biological activity of the fruit of *Garcinia Cowa*, including its anti-inflammatory and antioxidant properties, which suggests its possible use as an active component in medicines (Wanna et al., 2023). Other studies have found that *garcinia cowa* ethanol extract shows anticancer activity (Jhofi et al., 2021). Based on the results of other researches, it shows that the extract from *garcinia cowa* fruit contains compounds such as flavonoids and xanthenes that have antioxidant and anti-inflammatory properties that can help fight free radicals and reduce inflammation which has the potential to reduce the risk of chronic diseases (Espirito Santo et al., 2020).

However, until now there have been no studies that can show the specific content of these types of plants, both the content of the fruit and the content of the fruit skin. By knowing the clear content, it can be analyzed in depth the benefits that can be obtained from the plant, especially the impact it has on the skin and physical health. Therefore, it is very important to conduct research related to tyrosinase inhibition activity against *garcinia cowa* extract because it has never been done at all in detail so it is considered very important for skin health and physical health. The potential use of *garcinia cowa* fruit as an active ingredient in cosmetics and anti-aging health has not been widely reported. While some investigation of the biological characteristics of plant extracts, such as their anti-tyrosinase and antioxidant abilities, is needed to determine their potential as a source of new active ingredients for cosmetics.

Materials and Methods

Sample Collection

Garcinia cowa fruit taken and used as a test material comes from Southeast Asia. Fresh fruit samples from

garcinia cowa with a total weight of 2.5 kg were collected and cleaned. After the removal of the pulp, the skin is removed using the maceration procedure, which requires two 24-hour cycles of 96% ethanol as a solvent. After collecting the fibers, they are dried at 40°C in a rotary evaporator to produce a viscous extract, which is then weighed. Next, both extracts are stored for additional testing at 4-8°C.

Procedures Determination of Total Phenolic Content

A spectrophotometer was used to measure the total phenolic content of *garcinia cowa* extract using the Folin-C method. 200 mg of *garcinia cowa* extract is added to 25 ml of methanol. 500 µl of Folin-Ciocalteu reagent is inserted into 100 µl of sample in a yellow vial, which is then incubated at room temperature for 8 min and added with 400 µl of 1M NaOH to each sample.

Determination of Antioxidant Activity

The determination of antioxidant activity using 2,2-azino-bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) is based on a method developed by Nature and Hameed (Hameed et al., 2017) with some adjustments.

Measurement of Tyrosinase Inhibitory Activity

Tyrosinase inhibitory action was assessed in vitro using kojic acid as a positive control and L-DOPA as a substrate. The test method proposed by Klomsakul and Momtaz is applied with slight modifications.

Results

Phenolic Content of *Garcinia Cowa* Extract

The total phenolic content is calculated by ultraviolet spectrophotometry. As standard, gallic acid is used. As can be seen in Figure 1, the standard curve for gallic acid shows the relationship between the standard concentration and the absorbance value.

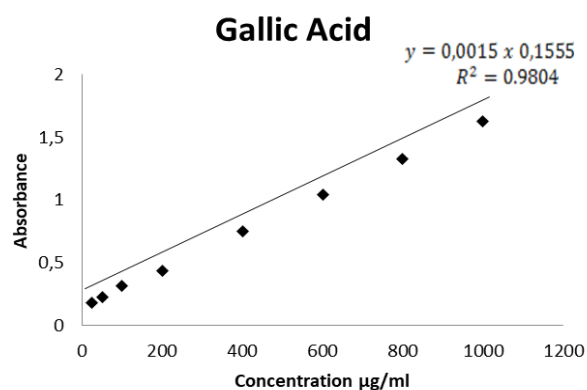


Figure 1. Standard curve of gallic acid. The absorbance value of the various standard concentrations is expressed as the average \pm standard deviation ($n = 3$)

The total phenolic content (TPC) in *G. Cowa* extract is recognized as the gallic acid equivalent (GAE), a common reference for measuring the number of phenolic compounds in an ingredient. The TPC value for *garcinia cowa*

fruit peel extract was 17,789 mg of GAE/g extract, and for *garcinia cowa* fruit pulp extract, the TPC value was 12,078 GAE/g extract. The findings of the study show that the skin of *garcinia cowa* fruit has a higher total phenolic content than the pulp.

Antioxidant Activity of *Garcinia Cowa*

The results showed the percentage of variables inhibiting antioxidant activity in the peel and pulp of *garcinia cowa* extract. There is an increase in antioxidant activity as the concentration increases, as shown in Figure 2. The skin of *garcinia cowa* fruit shows higher antioxidant activity at all concentrations than meat. Fruit peels showed 86.3% inhibition at 500 µg/mL, but flesh showed 84.1% inhibition. The best inhibition (82.64%) was obtained at the highest extract concentration of 10 mg/mL.

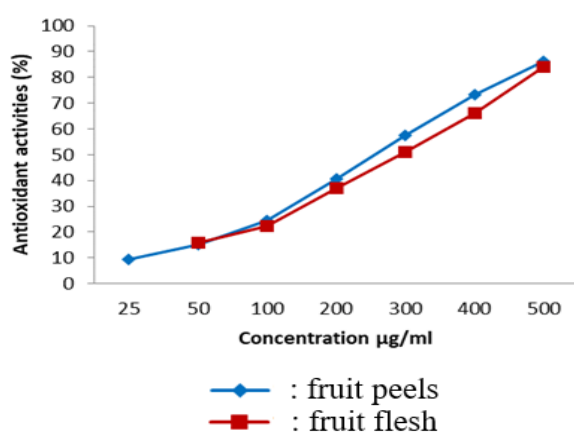


Figure 2. Antioxidant properties of the extract from the pulp of *garcinia cowa* and its peel at varying concentrations (average \pm SD, n = 3)

Antioxidants sourced from plant extracts or secondary metabolites have been used extensively in medicine so far to guard against disorders associated with free radicals and oxidative stress. The antioxidant activity and phenolic content of the extract were found to be positively correlated. The stronger the antioxidant activity, the greater the concentration of phenolic substances. Likewise, current investigations show that *garcinia cowa* fruit peel extract, which has higher levels of phenolics than the pulp, also shows higher antioxidant activity. The increase in phenolic content will also increase free radical scavenging activity.

Garcinia Cowa Tyrosinase Activity

In vitro tyrosinase activity of *garcinia cowa* was measured using an enzyme-linked immunosorbent assay. The skin of *garcinia cowa* fruit shows higher tyrosinase inhibition activity at all concentrations than *garcinia cowa* flesh. The highest inhibitory activity was found in kojic acid at all concentrations. Tyrosinase was inhibited in 21.4% of fruit skin, 18.3% of fruit pulp, and 65.6% of fruit at a kojic acid concentration of 250 µg/mL. The highest IC₅₀ of tyrosinase inhibition was found in meat extracts, followed by kojic acid and fruit peels. Fruit peels and meat extracts showed the highest levels of inhibitory action, with a concentration

of 250 µg/mL. Tyrosinase inhibition of fruit peel and meat extracts at doses of 31.25 µg/mL and 15.625 µg/mL did not differ significantly. Skin and meat have the lowest tyrosinase inhibitor activity in vitro, while kojic acid has the largest.

Discussion

Tests conducted on *garcinia cowa* fruits in general have shown very impressive results. Several important ingredients for health are obtained, both for skin health and for physical health. In the flesh and skin of the fruit shows tyrosinase inhibition activity which means that it has high antioxidants. However, the additional information was obtained from the test results that the antioxidant content of fruit peels turned out to be higher than fruit pulp.

A number of previous studies have also shown a link between phenolic chemicals and antioxidant action. Ascorbic acid and fruit peel have the lowest content and the highest IC₅₀ value, respectively, while meat extracts have the greatest value. This suggests that *garcinia cowa* bark extract has a higher free radical reduction power than the pulp extract but less than ascorbic acid. An important enzyme involved in the formation of melanin is tyrosinase (Logesh et al., 2023; Anugrah et al., 2024). This enzyme oxidizes L-DOPA to dopaquinone (quinone molecule) and hydroxylation of L-tyrosine (monophenol) to L-DOPA (diphenol) (Tesoro et al., 2023). In addition, under certain pH conditions, dopaquinone turns into dopa and dopachrome, which then instantly turn into melanin. Tyrosinase enzymes are often used to assess the capacity of a substance to prevent melanogenesis (Hassan et al., 2023).

The more prominent inhibition of the skin of the *Garcinia Cowa* fruit can be caused by its phenolic content, which is also higher than the pulp. A previous study reported that this phenolic compound is mainly responsible for inhibiting tyrosinase activity (Zuo et al., 2018). Compounds containing more phenolic hydroxyl groups have more anti-tyrosinase and antioxidant activity and have been used to treat skin depigmentation (Panzella & Napolitano, 2019; de la Cruz Sánchez et al., 2008). Furthermore, phenolic compounds were found to have anti-tyrosinase and antioxidant properties by Srisupap and Chaicharoenpong. According to (Abate et al., 2022) albalplant's anti-tyrosinase and antioxidant properties have led to its use in cosmetic applications. As a result, the antioxidant and anti-tyrosinase activity of *garcinia cowa* fruit peel in vitro can find application as a new cosmetic ingredient.

So far, antioxidants derived from plant extracts or secondary metabolites have been used extensively in medicine to protect against disorders caused by free radicals and oxidative stress (Engwa, 2018). In general, plants that have antioxidant content have been widely consumed by the public, including sportsmen, which are useful for maintaining skin health and physical health in general (Ayaz et al., 2024; Sellami et al., 2018). Herbal plants that contain phenolic active substances have antioxidant potential and can be used as anti-aging (Chaiyana et al., 2021; López et al., 2022).

Certain extracts that have antioxidant activity can be developed as antiaging agents to increase the longevity of human skin cells. Some studies suggest that antioxidant supplementation can protect muscle cells from oxidative damage, reduce muscle fatigue, and speed recovery (Canals-Garzón et al., 2022).

The content of phenolic compounds and flavonoids in *garcinia cowa* can help reduce oxidative damage to the body's cells (Biswas, 2017). *Garcinia cowa* extract may have anti-inflammatory effects, which are beneficial in reducing chronic inflammation (Dewi et al., 2024). Some of the bioactive components in these plants are reported to have antiproliferative activity against cancer cells, although human research is still very limited. *Garcinia cowa* has also been tested for its potential in reducing cholesterol and lipid levels in the blood (John et al., 2019).

On the other hand, antioxidants and physical activity have a complex relationship, where physical activity can affect the status of antioxidants in the body, and conversely, antioxidants can affect the body's ability to recover from exercise (Nieman & Wentz, 2019; Petracci et al., 2020). During physical activity, especially high-intensity exercise, the production of free radicals in the body increases (Alimuddin et al., 2024). This happens due to increased oxygen consumption and energy metabolism (Bouzid et al., 2015). Excess free radicals can cause oxidative damage to muscle cells, lipids, proteins, and DNA, which contributes to muscle fatigue, inflammation, and injury (Mustafa, 2024; Liza et al., 2024). Antioxidants help neutralize free radicals, reduce oxidative damage, and repair damaged cells (Chaudhary et al., 2023; Unsal et al., 2021). By reducing oxidative damage, antioxidants can speed up muscle recovery and improve muscle adaptation to exercise, such as increased strength and endurance.

During intense physical activity, the body produces free radicals that can cause oxidative damage to muscle cells and tissues (El Assar et al., 2022; Cardona et al., 2022). Phenolic compounds act as powerful antioxidants, helping to neutralize free radicals and reduce oxidative damage. This can speed up muscle recovery and reduce fatigue. Intense exercise can lead to inflammation of muscles and tissues. Phenolic compounds have anti-inflammatory properties that can help reduce inflammation and muscle soreness after exercise (Tanabe et al., 2021). It can also help prevent injuries and speed up the recovery process. Therefore, with the results of research that has been carried out with the analysis of *garcinia cowa* fruit extract, it can be recommended as one of the herbal plants that can be used for skin health and physical health in general. *Garcinia cowa* offers great opportunities as a nutraceutical and therapeutic ingredient in dermatology, impact of its antioxidant, anti-inflammatory, antibacterial, and wound-healing properties (Abate et al., 2022). *Garcinia cowa* extract-based products can help in addressing various skin problems and their relationship with physical activity. More in-depth and focused research is needed to fully understand its potential as well as establish safe and effective dosage guidelines.

Conclusion

Garcinia cowa skin and pulp extract contains phenolic compounds and has antioxidant and tyrosinase inhibitor activities that allow it to be utilized for anti-aging such as cosmetic mixtures as well as health supplements for physical activity. In addition, another result obtained is that fruit peel extract has a higher content of phenolic compounds than that of fruit pulp, so in terms of benefits for anti-aging such as cosmetics, it is better to recommend *garcinia cowa* fruit peel as one of the natural ingredients. However, for the benefits of physical health, it is more recommended to use the pulp. With the results of research that has been carried out regarding the content of the *garcinia cowa* plant, it can be recommended as one of the herbal plants that can be used for skin health and physical health in general.

Conflict of Interest

No conflict of interest with any person, company, or institution

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