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Determination of Vitamin C, Vitamin A and Flavonoid Levels in Garcinia cowa Roxb Fruit Flesh Extract

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***Abstract*—Garcinia cowa Roxb is a plant used as traditional medicine containing antioxidant activity. Plant extracts with antioxidant activity are often used as bioactive ingredients in anti-aging cosmetics. Studies on bioactive compounds and the use of this plant in cosmeceuticals have yet to be widely reported. This study aims to determine and analyze the levels of flavonoids, vitamins C, and vitamin A in G. cowa fruit flesh extract. These three compounds are bioactive ingredients that are beneficial in reducing the symptoms of photo-aging on the skin. The G. cowa used in this study was obtained from Tampunik Kambang, Pesisir Selatan Regency, and was extracted by maceration method using 96% ethanol. The testing of vitamin C levels in the extract of G. cowa fruit flesh was carried out through High-Performance Liquid Chromatography (HPLC) method using a UV detector with a wavelength of 264 nm. Quantitative HPLC analysis was carried out based on the resulting peak area data from the chromatogram. Vitamin A and flavonoid levels were measured using ultraviolet-visible spectrophotometry (UV-VIS) at maximum wavelengths of 286 and 429 nm. The results show that the levels of vitamin C and vitamin A in G. cowa was 151.4 mg/100g extract and 1.23 g 100g extract, while the flavonoid level was 69.5 mg/100g extract. The level of vitamins C, vitamin A, and flavonoids in G. cowa can contribute to natural bioactive substances and can be developed further into anti-aging cosmeceutical products.**

***Keywords*—G. cowa; vitamin C; vitamin A; flavonoid.**

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I. INTRODUCTION

Photo-aging is one of the problems in skin aging that many people experience and is currently becoming a concern. Photo-aging is an extrinsic type of skin aging that occurs due to exposure to the sun's ultraviolet rays [1]. Photo-aging leads to several complaints such as dull skin, pigment changes, wrinkles on facial skin, and so forth [1]. In Southeast Asians, the dominant clinical symptom of photo-aging is pigment changes. These include lentigines, melasma, and hyperpigmented spots [2]. Based on research conducted by Rachmantyo and Indramaya [2], in 3 years at the medical cosmetic section of dr. Soetomo Hospital Surabaya, 455 people, experienced new photo-aging problems, with 77,4 % the most common complaints of changes in skin color. Dewiastuti and Hasanah [3] also found that photo-aging problems occur earlier in female students aged 18-21 years, which should only occur at 28-30 years.

One of the efforts that can be carried out to prevent and reduce photo-aging is the use of cosmeceuticals. Cosmeceuticals are current cosmetic advancements, referring to cosmetics containing bioactive ingredients to improve skin function and health and cure minor skin disorders [4], [5], [6]. The development of cosmeceuticals with active ingredients derived from the plant extract turns into a current trend because natural ingredients are believed to be of higher quality and are safer than the others [6], [7], [8]. Increasing interest in natural anti-aging bioactive ingredients; therefore, research aimed to figure out natural anti-aging active ingredients within the country needs to be carried out [9].

A plant that can potentially be used as a source of natural anti-aging cosmeceutical bioactive ingredients in West Sumatra is GarciniacowaRoxb, commonly known as G.cowa. G. cowa is rich in bioactive phytochemical sources, including flavonoids, phloroglucinols, and xanthones [10], [11]. Flavonoids found in plants can act as antioxidants and tyrosinase inhibitors to reduce hyperpigmentation in photo-aging skin [12], [13]. Research by Hang et al. [14] states that G. cowa fruit has a pharmacological effect as an antioxidant and anti-inflammatory, which is very suitable as a source of natural ingredients of bioactive substances to be used in

medicine and pharmacy. Studies related to the use of G. Cowa in cosmeceuticals and its bioactive compounds (Vitamin C, A, and flavonoid) have yet to be widely reported. These three compounds are bioactive ingredients with benefits in reducing the symptoms of photo-aging on the skin, and are the most commonly selected for cosmeceutical formulation inclusion [12], [15]. This study aims to analyze the levels of vitamins C, vitamin A, and flavonoids in the flesh of G. cowa. This research can contribute to the scientific knowledge that g.cowa has the potential as natural bioactive substances as well as develop the use G.cowa in anti-aging cosmeceuticals products

II. Material and Method

A. Material

The material used was Fresh G.cowa fruit from Tampunik Kambang, Pesisir Selatan Regency, technical grade maceration solvent, quercetin (fitopure), ethanol pa (Merck), 10% $AlCl_3$, sodium acetate, distilled water, ascorbic acid (Merck), 0.1% acetic acid (Merck), Aquabidest, Methanol (Merck), Aquadest (Merck), Acetone (Merck), Petroleum ether (Merck), anhydrous Na_2SO_4 (Merck) and Vitamin A retinol.

B. Tools

The tools used were a rotary evaporator, HPLC (Shimadzu), a set of maceration tools, UV-Vis Spectrophotometer (Shimadzu Pharmaspec 1700), measuring flask, UV-Vis spectrophotometer (Alytikjena brand type Specord 210 plus), measuring pipette, analytical balance (precise), burette, 0.24 µm nylon filter, maceration container (dark bottle), Autoclave 3, Magnetic stirrer, Magnetic stirrer separate funnel, Separatory funnel, Buchner funnel, aluminum foil paper, and other glassware.

C. Method

1) Making Ethanol Extract from Fresh G.cowa Flesh: 2.5 kg of G.cowa fruit is separated between the wet bark and the flesh. 96% ethanol was used to make the extracts which were carried out by the maceration method. The G.cowa wet flesh was then put in a macerator. Then 5 liters of 96% ethanol were added and soaked for 24 hours. Separate the macerate with the same solvent; this process is repeated twice. Then use a rotary evaporator to evaporate to get a thick extract. After the extract was obtained, organoleptic parameters were observed for early introduction and identity of the extract as a basis for testing the extract during storage. Then, the thick extract was tested to determine the pH.

2) Determining Vitamin C Levels [16], [17]*:* Vitamin C in the flesh of G. cowa fruit was measured by the HPLC method using a UV detector with a wavelength of 264 nm, a flow rate of approximately 1 ml/minute, and an injection loop: 10 µl. 0.1% acetic acid and methanol were used as a mobile phase with a ratio of 94:6 (v/v) ratio. 0.1% acetic acid was prepared by pipetting 1 ml of 100% acetic acid solution and then the volume was increased to 1000 ml with pro-injection aquabidest in a volumetric flask and then filtering.

The Vitamin C comparison solution was prepared by dissolving 10 g of Vitamin C in 0.1% acetic acid in aquabidest to obtain the first 10 ml of the solution. Then 1 ml of the first solution was added in 0.1% acetic acid in aquabidest to obtain 10 ml of solution 2. From the second solution, various serial concentrations were made; those were 4, 6, 8, 10, 12, and 14 ppm. Filter solution with a 0.24 µm nylon filter and then injected into the HPLC tool, and the area was obtained. Then the linear regression equation and correlation value were determined.

The sample preparation test was carried out by weighing 10 mg of each G.cowa fruit flesh extract, dissolved in 0.1% acetic acid in aquabidest to 10 ml to obtain a solution of 1,000 ppm. Perform three repetitions and filter the solution with 0.24 m nylon before being injected into the HPLC apparatus.

3) Determining Vitamin A Levels [18]*:* Standard solution of vitamin A was prepared by weighing 0.1-grams of vitamin A retinol and dissolved in ethanol to a concentration of 1,000 ppm. Then a standard solution of vitamin A was made with a concentration series of 200, 300, 400, and 500 ppm, and the absorbance was measured. Connect the concentration of the standard solution to the absorbance value measured by an ultraviolet-visible (UV-VIS) spectrophotometer to obtain a standard curve.

Next, the determination of vitamin A levels from the sample of G.cowa fruit flesh extract was carried out where 1 gram of G.cowa was partitioned using distilled water, acetone, and petroleum ether centrifuged at 4°C at the speed of 2,000 rpm for 5 minutes. The sample was then partitioned again using distilled water, acetone, and petroleum ether. The filtrate was centrifuged, and anhydrous Na_2SO_4 was added to separate the aqueous phase contained in the filtrate. The filtrate was then measured for absorbance with a wavelength of 286 nm.

4) Determining Total Flavonoid Levels [19]*:* The total flavonoid levels in G. cowa flesh were determined using the Indonesian Herbal Pharmacopoeia Method 2 (2011) by using UV-Visible Spectrophotometry. Preparation of quercetin Standard Solution was made by weighing 0.01 g of quercetin, and it was dissolved with ethanol p.a to a volume of 10 ml, and 1000 ppm of the prime solution was obtained. After that, the prime solutions were produced with various concentrations, including 6.25 ppm, 12.5 ppm, 25 ppm, 50 ppm, 100 ppm, and 200 ppm. Then 0.10 ml of 10% $AlCl_3$, 0.10 ml of 1M sodium acetate, and 2.80 ml of sterile aqua were added. The mixture was mixed until it became homogeneous, and it was set aside at room temperature for 30 minutes. Then measure using an ultraviolet-visible spectrophotometer (UV-VIS) with the maximum wavelength to get the absorbance value. Connect the absorbance value with the concentration of the standard solution so that the standard quercetin curve was obtained

The total flavonoid levels were determined by doing the following procedures. A sample of G.cowa flesh extract (27 mg) was dissolved in 10 ml of ethanol p.a so that the concentration of a sample solution of G.cowa fruit extract (2700 ppm) was obtained. Separately 0.5 ml of the test solution and the control solution were pipetted, and each solution was added with 1.5 ml of ethanol p.a, 0.1 ml of 10% $AlCl_3$ (aq), 0.1 ml of 1M sodium acetate, and 2.8 ml of distilled water. The mixture was mixed until homogeneous, and at room temperature, it was set aside for 30 minutes. Then

use an ultraviolet-visible spectrophotometer (UV-VIS) to measure the absorption value.

III. Result And Discussion

The Garciniacowa fruit used as samples in this study were derived from Tampunik Kambang, Pesisir Selatan Regency. The results of sample identification which was conducted at the Andalas Herbarium, Mathematics and Natural Sciences Faculty, Andalas University, indicate that the samples utilized are GarciniacowaRoxb ex Chiosy species from the Clusiaceae family. 450 mg of fresh G. Cowa flesh as a sample was extracted using 96% ethanol by maceration method, resulting in 46 mg of a thick extract with 9.28% immersion. The organoleptic parameters of G.cowa obtained a thick extract with a distinctive aroma, slightly greenish-brown color, and sour taste. The pH value of the extract was 2.10, indicating that the sample was very acidic.

A. Vitamin C Level of GarciniaCowa Fruit Flesh Extract

The results of examining vitamin C comparison solution at various concentrations using HPLC gained the area (AUC) and retention time on the chromatogram can be seen in table 1. The standard curve of vitamin C is a graph presenting the relationship between standard concentrations and the area (AUC), as shown in Figure 1.

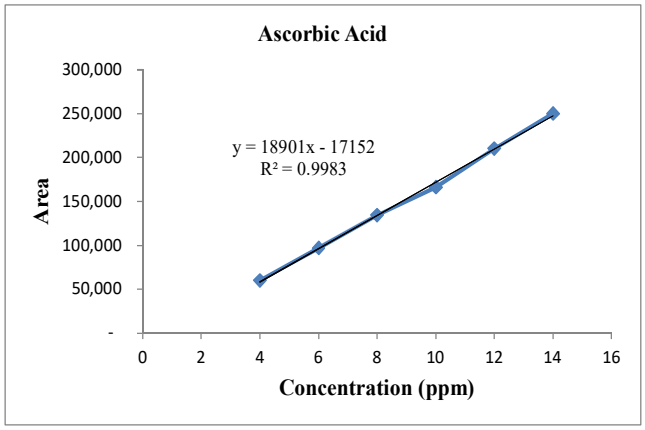

Fig. 1 Vitamin C Standard Curve

Based on the standard curve in figure 1, the regression equation is

$$y = 18901x - 17152 \quad (1)$$

y is the area, x is the standard concentration, and the value of $R^2$is 0.9983, which is close to 1, which means the regression equation is linear and shows that area and concentration are suitable.

The standard curve equation gained was used to determine the vitamin C level in the sample. The sample AUC value is transferred to the standard curve regression equation as y value so that the vitamin C level of the sample is obtained.

Measurement of vitamin C levels in the flesh of G. cowa was carried out with three repetitions, and the results are presented a show in table 2. The examination in this study was performed using the high-performance liquid chromatography (HPLC) method because it is a more precise and susceptible method to prescribe the vitamin C level in fruit and pharmaceutical samples.

TABLE I
AUC VALUE VITAMIN C COMPARISON

Concentration (ppm)	Retention Time (min)	AUC
4	3.074	59,997
6	3.078	96,716
8	3.051	134,426
10	3.056	166,148
12	3.054	210,382
14	3.058	250,064

Besides that, the HPLC method is relatively quick, and it does not necessitate many samples and chemicals compared to the spectrophotometer method. Also, the measurement of vitamin C is more precise than iodometric titration [20], [21]. HPLC quantitative analysis was conducted based on peak area data. The ascorbic acid peak area for each standard solution series concentration was attained from the chromatogram results. The standard curve is utilized to observe the correspondence between the detector response gained and the analyte concentration. The suitability is in the form of a linearity parameter measured from the correlation coefficient value (r) 0.998.

Vitamin C is advantageous in reducing the symptoms of photo-aging on the skin and is often used as an active ingredient in anti-aging cosmeceuticals [15], [22]. The antioxidant activity of vitamin C can minimize the effects of exposure to UV rays of the sun by abating the symptoms of erythema and DNA damage caused by UV rays of the sun and preventing premature aging of the skin [15], [22]. Vitamin C contributes to skin fibroblasts that play a role in collagen synthesis and sustain the balance of collagen and elastin in the dermis layer. Vitamin C acts as a co-factor for collagen (proline and lysine) hydroxylase in the skin. The application of vitamin C preserves the skin's barrier integrity by preventing water loss from the skin [22]. Vitamin C has a role in stratum corneum formation and epithelial differentiation in general [22]. Vitamin C can lighten the skin by reducing melanin through the tyrosinase enzyme activity and oxidized dopaquinone in the synthetic melanin pathway [22], [23]. Vitamin E is one of the active substances that has benefits in preventing aging because of its photoprotective mechanism, tightening, hydrating, and increasing elasticity. Vitamin C and vitamin E work synergistically, where Vitamin C can regenerate the oxidized form of vitamin E. The combination of vitamin C and vitamin E applied topically increases its photoprotective effect and reduce melanin in age spots or melasma [12].

Natural sources of vitamin C are extensively accessible in fresh fruits and vegetables such as citrus fruits, tomatoes, papayas, green and red peppers, strawberries, melons, etc. In a study by Najwa and Azrina [20] using the HPLC method, it was found that the vitamin C level in oranges was 43.61 mg/100g, lemons 31.33 mg/100g, grapefruit 26.40 mg/100g, and limes 22.36 mg. /100g. Oranges are the best source of vitamin C

TABLE II
ANALYSIS OF VITAMIN C LEVELS IN G. COWA FRUIT FLESH EXTRACT

Sample	No. of Repetition	Concentration (ppm)	Retention Time (minutes)	Area under Curve (AUC)	Vitamin C Level in The Sample (ppm)	Vitamin C Level in The Sample (mg/100g sample)	Average Level (mg/100g sample)
G.cowa Fruit Flesh Extract	I	1,020	3.070	10.732	.1,04	144.6	151.4
	II	1,030	3.082	11.534	1.51	147.3	
	III	1,030	3.083	14.460	1.29	162.3	

In the related study, the vitamin C level in papaya fruit ranged from 59.9 ± 3.4 mg/100 g to 112.4 ± 12.6 mg/100g[24]. The measurement results of vitamin C in G.cowa are higher than other vitamin C sources such as oranges and papayas. The presence of vitamin C in G. cowa supports the statement by Hang T et al. [14] that G. cowa contains 57.75-64.75 mg/100 g of vitamin C and is very suitable as a source of natural ingredients to provide bioactive substances

B. Vitamin A Level of GarciniaCowaFruit Flesh Extract

The results of investigating standard solutions of vitamin A at various concentrations using UV VIS spectrophotometry, the absorbance values are attained in table 3. The standard curve of vitamin A is obtained by connecting the relationship between vitamin A concentrations and absorbance values in Figure 2.

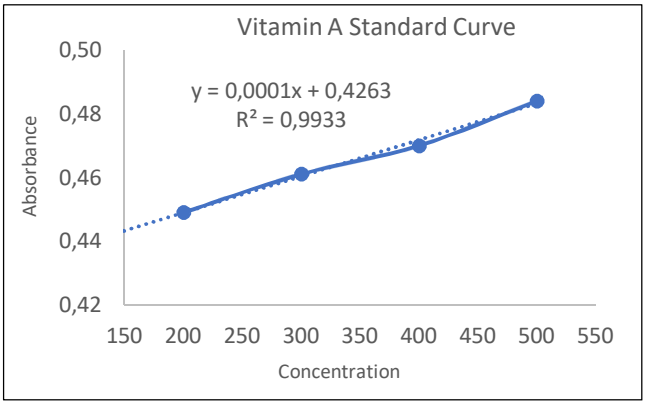

Fig. 2 Standard curve of vitamin A

with the following regression equation:

$$y = 0.0001x + 0.4263 \quad (2)$$

Y is the absorbance, X is the concentration of vitamin A. From the regression equation, it is obtained that R^2 is 0.9933 so that it is interpreted regression equation is linear. The standard curve of vitamin A was used to determine the content of vitamin A in G cowa extract.

TABLE III
ABSORBANCE VALUE VITAMIN A STANDARD SOLUTION

No	Standard Concentration	Absorbance
1	200 ppm	0,4491
2	300 ppm	0,4611
3	400 ppm	0,4700
4	500 ppm	0,4840

G. cowa fruit flesh extract test results using a visible ultraviolet (UV-VIS) spectrophotometer at a wavelength of 286 nm gained a vitamin A content of 1.23g/100g extract. Vitamin A is fat-soluble and is found in the epidermis layer of the skin, dominated by retinol esters [12]. Vitamin A (Retinol), at this time, is the substance most often used as an anti-aging compound. Vitamin A derivatives influence the metabolism and synthesis of collagen and elastic fibers. The antioxidant activity of Vitamin A can protect cells from free radicals such as peroxyl radicals and singlet oxygen [12], [20], [25]. Fatonah et al. [26] determined that the vitamin A level in carrots and mustard greens from Bumiaji village using the spectrophotometer method is 345.7mg/100g and 52mg/100g. In addition, research by Widyamoko et al. [18] determined that the vitamin A level in bengkoang is 17.9 mg/100g extract. The level of vitamin A in G. cowa is relatively high compared to some plants, which are the primary sources of vitamin A.

Vitamin A applied to the skin helps regenerate skin by playing an active role in the exchange stratum corneum and is also involved in the proliferation epidermis layer. Vitamin A also regulates epithelial keratinization in the skin [12], [27]. The barrier in the epidermis can be strengthened by vitamin A to prevent the occurrence of transepidermal water loss (TEWL) on the skin surface [28], [29], [30]. The study showed that facial skin contours enhanced after 12 weeks of treatment using vitamin A (retinol 13]. The topical use of vitamin A affects melanocyte function and melanin distribution so that it can inhibit the transfer of melanin to skin epidermal cells. Inhibition of the melanogenesis process by vitamin A can also diminish melanin's amount in the skin [12]. Vitamin A can also be used to lessen skin inflammation because it controls mast cell activity.Th2-mediated exacerbation of skin inflammation and activation of mast cells may occur due to vitamin A deficiency [27]. UV rays oxidize cell components so that a lipid peroxide reaction is formed; at this time, the oxidation process of vitamin E occurs. Vitamin A has the ability to block lipid peroxidation reactions and increase levels of alpha-tocopherol (vitamin E). Vitamin E (Alpha-tocopherol) also shows a synergistic effect with vitamins A (retinol) and C (ascorbic acid) in the product combination, providing a photoprotective effect and antioxidant action so that it has the potential to prevent photo-aging [12], [28]**.** Vitamin A in G. cowa can potentially be developed in cosmeceuticals because, according to Draelos [15], vitamin A is measured an active compound in cosmeceuticals which is extensively utilized in anti-aging formulas.

C. Flavonoid Level in GarciniaCowaFruit Flesh Extract

The total flavonoid content of the extract was calculated as Quercetin Equivalent (QE). Quercetin is the most important flavonoid group as an antioxidant compound. Quercetin is a

marker compound of flavonoids. Quercetin standard curve is a graph of the relationship between standard concentration and absorbance value and from which it is obtained a standard curve linear regression equation shown in Figure 3. The linear regression equation on the Quercetin standard curve is used to determine the total flavonoid level of the sample, and the results of testing the total flavonoid level of G. cowa extract can be seen in table 4.

In this study, a quantitative test of flavonoid levels was carried out using UV VIS spectrophotometry with a wavelength of 429 nm. The standard solution used is quercetin. Quercetin standard curve is obtained by relating the absorbance value of Quercetin standard solution measurement of various concentrations to the standard concentration. Quercetin standard curve was used to analyze the total flavonoid level of the fruit flesh extract of G. cowa. The total flavonoid level of the extract calculated as quercetin can be seen in table 4. Flavonoids can provide health benefits related to their chemical structure and bioavailability [12]. The antioxidant activity of flavonoids works by inhibiting several free radical reactions (hydroxyl, superoxide, nitric oxide), thereby preventing the formation of Reactive Oxygen Species (ROS) and suppressing pro-inflammatory cytokines [31], [32]. Flavonoids can also protect photo-aging symptoms by photoprotection of skin cells against UV radiation and reduce sunburn and prevent UV-induced skin damage [12], [33]. The combination of Tocopheryl acetate (vit E) with Bioflavonoids can provide a photoprotective effect to prevent sunburn [12], [28]. Flavonoids have anti-inflammatory activity through various mechanisms, inhibiting the release of histamine inflammatory mediators and reducing inflammation and erythema on the skin surface. Flavonoids can affect human skin color and influence the process of melanogenesis, including tyrosinase, thereby inhibiting melanin synthesis [12].

The accumulation of old cells can cause many pathological changes in the skin resulting in skin aging. Changes in tissue structure and function occur because aging cells produce an aging-associated secretory phenotype (SASP). Production (SASP) and causes of diseases related to cell aging are due to the presence of NF-kb as a transcription factor. Several flavonoid compounds derived from flavonoid plants can block this SASP by interfering with NF-B activation and related pathways [34].

Previous research conducted by Ritthiwigrom et al. [10] (qualitative test) mentions that G. cowa contains positive flavonoids as well as antioxidant activity. Determination of total flavonoid levels of G. cowa in this study is regarded as necessary, considering that plants containing flavonoids are often used in various nutraceuticals and cosmetics due to their antioxidant and anti-inflammatory activities that can slow down skin aging [35]. The total flavonoid level of G. cowa was 69.50 mg/100g. Tea, apples, and grapes are rich sources of flavonoids proven to offer health benefits. Cempaka et al [36] also determined the flavonoid level as Quercetin in fresh manalagi apples at 40.65mg/100g and Fuji apples at 27.28mg/100g. Meanwhile, Fajar et al [37] determined the flavonoid level of quercetin in green tea at 252.3±1.71mg QE/g-extract. The levels of flavonoids are in the moderate category compared to that of flavonoids found in green tea and apples. Garcinia cowa Roxb is widely used as traditional medicine. The fruit and leaves have been used to treat digestive disorders and to improve blood flow. C.cowa is a medicinal plant that has been recognized to have various activities such as anticancer, anti-inflammatory, antibacterial, and antioxidant.[10], [11], [14].

The level of flavonoids in G. cowa can be potentially developed further in pharmaceutical products. The presence of vitamin C, vit A and flavonoids in G.cowa fruit flesh extract has the potential to be used as a new bioactive ingredient in anti-aging cosmeceuticals. This is because vitamins A, C, and flavonoids are bioactive ingredients that are often used in anti-aging cosmetics and reduce the symptoms of photo-aging on the skin [12], [15].

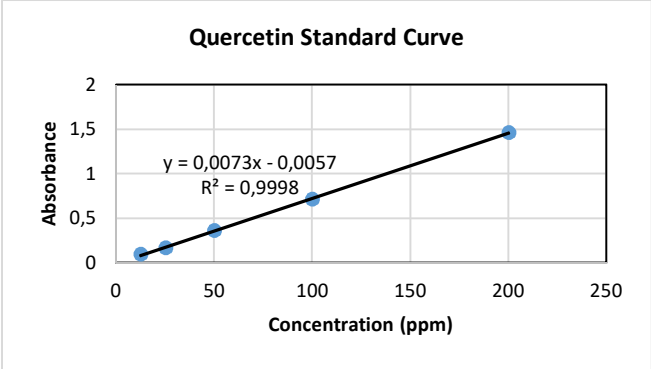

Fig. 3 Quercetin Standard Curve

TABLE IV
TEST RESULT OF TOTAL FLAVONOID EXTRACT

Extract	Average Absorbance	Total flavonoid concentration (ppm)	Total flavonoid (mg QE/100g extract)
G. Cowa Fruit Flesh	0.008	1.876	69.50

IV. CONCLUSION

Garcinia cowa Roxb contains vitamin C and vitamin A in the levels of 151.4 mg/100g and 1.23 g/100g, while the flavonoid level is 69.5 mg/100g. G. cowa has the potential to be used as a new bioactive ingredient in anti-aging cosmeceuticals.

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