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# Antioxidant Activity Of Combination Of Garcinia Manggostana Pericarp And Hibiscus Sabdariffa Calyxes

## E. Hanani<sup>1</sup>, B. Elya<sup>1</sup>\*, B. Katrin<sup>1</sup>

## <sup>1</sup>Faculty of Pharmacy, University of Indonesia, Depok 16424, West Java, Indonesia

## \*Corres.author: elya64@yahoo.com Phone no.:6281314161497

**Abstract:** *Garcinia mangostana* (GM), belongs to Garcinia family, it is an indigenous plant from Indonesia that has been known for its fruit, and the fruit pericarp just becomes as waste. Roselle calyxes (RC) from the *Hibiscus sabdariffa* Gaertn. were frequently used in folk medicine in the middle-east and some Asian countries for the promotion of good health. The purpose of this study was focused on utilization of mangostana pericarp (MP) with RC to make herbal tea formula in the tea bag. We prepare three formulas with different concentration of MP and RC. The antioxidant activity of these formulas, were measured by DPPH method. Hedonic test is also performed in this study to measure public acceptance for taste, aroma and color of the best formula. The result showed that the combination of MP and RC in formula with the composition 1.5 g of MP and 1.0 g of RC possessed the highest antioxidant activity (IC<sub>50</sub> = 148.1 µg/ml). This formula also contains 60 mg stevia extract which is preferable for the panelist in the hedonic test.

Keywords: Antioxidant activity, Garcinia mangostana, herbal tea, Hibiscus sabdariffa.

### **Introduction**

*Garcinia mangostana* is a tree which attains a height of 15 meters, cultivated as a fruit tree, in tropical countries. The methanol extract of GM have antioxidant and cytoprotective activities also on inflammation caused by *Propionibacterium acne*<sup>1,2</sup>.

Spiral incisions through the bark from the ground upward permit the resinous emulsion in the cortex and phloem to exude. The leaves are shiney and dark green above, dull pale green beneath, or yellow-green on both surfaces. Flowers with yellow-green outer sepals, about 2 cm long, staminodes shorter than ovary. The edible fruit mangosteen has dark-purple pericarp, depressed-globose, with sessile, large stigma, 1 - 3 seeds and has a sweet taste. Xanthon compound are the major component in the GM that have antioxidant activity<sup>3</sup>.

The calyxes from the *Hibiscus sabdariffa* Gaertn are used in folk medicine in some Asian countries for treatment of many ailments, to reduce weight, to decrease blood viscosity, as diuretic, diaphoretic, cholagogue, and hypotensive<sup>4,5,6</sup>. The plant is an erect annual herb which belongs to the Malvaceae family with reddish cylindrical stem, nearly glabrous. The leaves are simple, having petiole, with blade 3-5 lobes. Flowers are solitary, axially, nearly sessile, 5-7 cm diameter, calyx is thick, red and fleshy, cup-like, petals 5, yellow, twice as long as calyx. The aqueous extract from RC has inhibition activity to DPPH up to more than 90%<sup>7</sup>.

MP and RC can be used to support community health as a healthy beverage. This opportunity is obvious because (MP) it is usually considered as an un-useful waste. Both plants grow abundantly in Indonesia but are still yet to be used optimally. MP is known having an antioxidant activity while RC is commonly used as a component in beverage. Combining the two of this compound in tea form is simple, cheap, and easy to produce in relatively small environment. Finally developing the formula for beverage from the MP and RC combination allows an opportunity to support the community to create a small/home industry. It is not only a health promotion matter, but also economic development of the community.

#### Materials and Methods

#### Plant collection and authentication

Rosella calyxes were collected in February 2012, from Indonesian Medicinal and Aromatic Research Institute. Botanical identification was done by Center for Plant Conservation Bogor Botanical Garden, Indonesian Institute of Sciences, Bogor, and voucher specimen was deposited at the herbarium of Pharmacognosy laboratory, Faculty of Pharmacy, University of Indonesia. MC was collected from the mangosteen fruit which was purchased from fruit market in Jakarta.

#### **Determination of total ash**<sup>8</sup>

Accurately weight about 2.5 g of ground air-dried material, and spread the material in an even layer. Heat in the oven until indicating the carbon was absence. Cool and allow it to return until it's at a constant weight.

#### **Determination of acid soluble ash**<sup>8</sup>

To the crucible containing the total ash add 25 ml of HCl, and boil for 5 minutes. Filter and wash the residue with hot water until the filtrate is neutral. The filter paper transfers to the crucible, dry on a hot-plate, until to constant weight.

#### Water and ethanol-soluble extractive

Water and ethanol-soluble extractive were carried out by water-chloroform, ethanol maceration of samples for 6 hours, and allow to stands for 18 hours. Filtered, 20 ml of filtrate evaporates to dryness.

The characteristic of the RC and MP were presented at Table 1.

#### Determination of total flavonoids (for RC) (8)

Accurately weight 3 g, add 0,5% HTM (hexamethylene tetramine solution), acetone 20 ml, and 5 ml 25 % HCl , hydrolyzed for 30 minutes.

Filter and the residue was extracted with 30 ml acetone, all filtrate were mixed. Filtrate was extracted with ethylacetate 3 times, each 15 ml, then added AlCl3 solution, measured the absorbance at 425 nm in a spectrophotometer, and quercetin used as a standard.

#### **Determination of anthocyanin** (for RC)

The aqueous RC extract add with potassium chloride buffer (pH = 1) and sodium acetic buffer (pH = 4.5), and the absorbance was recorded at wave length max = 518 nm.

#### **Determination of total phenolic content**(for MP)<sup>9</sup>.

The total phenolic content was determined by Folin-Ciocalteu method, and gallic acid was used as a standard reference. The aqueous extract of MP was added with Folin-Ciocalteu reagens, allowed to stand for 10 minutes, added with 2.0 ml sodium carbonate solution (1,5% w/v). The mixture was incubated for 20 minutes at 40oC, than the absorbance measured at 755 nm using spectrophotometer uv-vis.

#### **TLC Chromatogram**

TLC profile of the RC and MP were carried out using silica gel as stationary phase and chloroformmethanol (9:1) as mobile phase for RC, whereas hexane – ethyl acetate (15:8) for MP. Aluminum trichloride solution (5% in methanol)) was used as a sprayed reagent (Figure1).

#### Antioxidant activity<sup>10</sup>

In this study, three formulas were prepared, formula A contain RC: MP = 1: 1.5, B = 1.5: 1.0 and C 2.0: 0.5. The antioxidant activity of these formulas was determined by DPPH method, and used ascorbic acid as a standard reference. In the experiment the three formulas of MP and CR were dissolved in an aliquot of methanol, added to 0.2 ml DPPH solution (in methanol, 0.2 mM). After 30 minutes incubated at room temperature, the absorbance was measured at 518 nm. The percentage of scavenging activity was calculated using the following equation:

$$I(\%) = (Abs. control -- Abs. sample) \times 100\%$$

#### Abs control

 $IC_{50}$  value was calculated by the comparison of sample concentration with percentage value.

#### **Hedonic Test**

The formula that showed the most promising antioxidant activity was developed in three formulas (A1, A2 and A3) with the addition of sweetening agent (stevia extract). The test was conducted by spreading questioners to 30 panelists in order to see any differences in favored color, aroma, and taste. The panelists would fill the form with the mark scaling 1-5, from excellent to very poor.

The RC contains total flavonoids 0.24 - 0.26 % calculated as quercetin, and anthocyanin 4.13 - 4.19

%. Phenolic total content in the MP was 0.032 - 0.040 % calculated as gallic acid.

The formula A showed the strongest antiradical activity with  $IC_{50}$  value 148.1 µg/ ml, B 226.7 and C 279.99 µg/ml respectively.

In the TLC chromatogram of MP were shown 4 spots (ethanol extract), 6 spots (dichloromethane extract) and 7 spots (*n*-hexane extract), after  $AlCl_3$  sprayed. The RC TLC chromatogram showed only 2 spots appear.

#### **Results**

Table 1. The characteristic of the rosella calyx and mangosteen pericarp				
Sample	Total ash	Acid - insoluble	Water- extractive	Ethanol –
	(%)	ash (%)	(%)	extractive (%)
MP	2.09 - 2.14	1.09 – 1.23	15.47 – 16.98	20.64 - 22.42
RC	5.55 - 5.61	1.81 – 2.33	36.95 - 37.45	25.45 - 25.60



а



b

Figure 1. TLC chromatogram of MP (a), RC (b) under UV 366 nm, after sprayed using AlCl<sub>3</sub> solution, A= quercetin as standard, B = ethanol extract, C = dichloromethane extract, D = n-hexane extract.

#### **Discussion**

Characterization for plant material were done to measure the standard plant material, and those have been used in this study to make herbal tea product. Some character as total ash, acid-insoluble ash, water soluble extractive, ethanol soluble extractive and TLC chromatogram have been done. It was determined that it usually applies plant materials is used for food or drugs.

We prepare three formulas with different concentration of MP and RC. These differences are based on the different empirical use from MP and RC. The antioxidant activity of these formulas, were measured by DPPH method. DPPH method is useful in the measure of antioxidant activity as it gives a quick estimation and this method is sensitive. To evaluate the antioxidant activity from the two aqueous extracts, the extracts were reacted with a 1,1-diphenyl-2-picrylhydrazyl stabile radical (DPPH) in methanol solution. DPPH reduction as indicated by monitoring the absorbance change at a characteristic wave length during reaction. In radical form DPPH absorbs at 518 nm, but upon reduction of antioxidant (AH) absorbance disappears. DPPH•  $+ AH \rightarrow DPPH-H + A \bullet$ 

Antioxidant activity was evaluated by the decrease of the absorbance value at 518 nm, and the inhibition percentage value was calculated using the formula.

Formula A showed the strongest antiradical activity with  $IC_{50}$  value 148.1 µg/ml, compared to B and C (226.7; and 279.99 µg/ml). The  $IC_{50}$  value of ascorbic acid in comparison, were 4.79 µg/ml.

Formula A1, A2, and A3 were prepared with different stevia extract concentration, 20, 40 and 60 mg, it was based on the comparison of sucrose which is commonly used. Stevia extract is natural sweetener that has calories low, but sweeter then sugar up to 200 times. It is a useful sweetener agent for a low calorie diet. Hedonic test is also performed in this study to measured public acceptance for taste, aroma and color of the best formula among the three tea herbal. The hedonist data are analyzed by SPPS program. The highest concentration of stevia extract

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(60 mg) was the highest acceptance formula that was indicated by the highest hedonic value.

#### **Conclusion**

The characteristic from the RC, including the total ash, acid-insoluble ash, water-extractive, ethanol extractive and total flavonoid and anthocyanin content were 5.55 - 5.61%, 1.81 - 2.33%, 36.95 - 37.45%, 25.45 - 25.60%, 0.24 - 0.26% (calculated as quercetin) and 4.13 - 4.19% respectively. Whereas for MP it was 2.09 - 2.14%, 1.09 - 1.23%, 15.47 - 16.98%, 20.64 - 22.42% respectively and total phenolic compound was 0.032 - 0.040% (calculated as gallic acid).

The MP TLC chromatogram gave 4 spots (ethanol extract), 6 spots (dichloromethane extract) and 7 spots (hexane extract), after AlCl<sub>3</sub> sprayed. Whereas RC TLC chromatogram showed that only 2 spots appear.

Formula A (contained RC- MP = 1: 1.5 g) have the highest antioxidant activity. Herbal tea that contains RC - MP (1: 1.5 g) and stevia extract (as sweetener) 60 mg was the best formula that was in acceptance by the volunteers in hedonic test.

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