

Aktivitas Antioksidan dan Senyawa Bioaktif yang Terkandung dalam Minuman Fungsional dari Ekstrak Kulit Manggis dan Rosela.
Antioxidant Activity and Bioactive Compound in a Functional Instant Drink Made from Mangosteen Peel and Rosella Extract

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ABSTRACT

The research aims to identify the effect of mangosteen peel and rosella formulation through antioxidant activity and bioactive compound contained in functional instant drink and to determine its highest formulation. The method utilized was complete random method that consists of 6 (six) stages. The variables observed were total of phenol, vitamin C, tannin and antioxidant. The data were analyzed using SPSS 17.0 version, and further analyzed with Duncan test. The research showed that the formulation was significantly effect on levels of phenol, tannin, ascorbic acid (Vitamin C), as well as on the capacity of antioxidant. The higher level of phenol and tannin cause the higher antioxidant capacity in functional instant drinks. The functional instant drink with the highest antioxidant capacity was made of mangosteen peel, rosella flower and water formulation, with a ratio of 4:1:50.

Key words: *Bioactive compound, antioxidant activity, functional instant drink, mangosteen peel, rosella flower.*

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INTRODUCTION

Mangosteen peels are now found to be developed as medicinal treatment, because it contains of compounds with pharmacology activity such as antioxidant, anti inflammatory, heart disease treatment, anti bacterial, anti fungal, also for therapy of HIV drug. The primary compound of mangosteen peel is found as it contains of pharmacology activities which is xanthone compounds. Xanthone compound is identified with 1,3,6- trihydroxy-7-metoxy-2,8-bis(3-methyl-2-butenhyl)- 9H-xanten-9-on and 1,3,6,7- tetrahydroxy- 2,8-bis(3-methyl-2-butenhyl)- 9Hxanten- 9-on. Both xanthenes are so called alpha mangosteen and gamma mangosteen (Jinsart, 1992). Jung *et al* (2006) successfully identified the content of xanthone from soluble extracts within dichloromethane, which are 2 xanthenes prenyl oxygenated and other 12 xanthenes. 2 xanthenes prenyl oxygenated consist of 8-hidroksiquadraxanthone G, and mangostingon [7-methoxy-2-(3-methyl-2- butenyl) – 8 - (3-methyl-2-okso-3-butenyl) -1,3,6-trihydroxyxanthone.

Another identified xanthenes are quadraxanthone G, 8- deoxygartanin, garsimangoson B, garsinon D, garsinon E, gartanin, 1-isomangostin, alfamangostin, gamma-mangostin, mangostinon, smeathxanthon A, and tovofillin A. Other research found that mangosteen peel has antihistamine (Nakatani *et al.*, 2002a), anti inflammatory (Nakatani *et al.*, 2002b and Nakatani *et al.* 2004), antioxidant

(Weecharangsan *et al.*, 2006 and Jung *et al.*, 2006), anti cancer (Ho *et al.* (2002), Matsumoto *et al.* (2003) and Moongkarndi *et al.* (2004)), anti-microorganism (Suksamrarn *et al.* (2003), Sakagami *et al.* (2005) and Mahabusarakam *et al.* (2006). Jujun *et al.* (2006) stated that acute and sub chronic toxicity testing had demonstrated that mangosteen peel was edible.

Rosella is an annual resin herbal plant. It grows on the stem side and is classified as *planta uniflora* which means one flower grows in one stem. It has 8 to 11 fury petals with 1 cm length. Its base is attached and colored red. The calyx is mostly pointed as the flower and processed as drink or food (Maryani and Kristiana, 2008). On its physiochemical characteristics, rosella has a high content of vitamin C with a low glucose, contains of 2 predominant organic acids; succinate and oxalic. It also contains of ascorbic acid, higher compared to orange and mango. The calyx contains of vitamin A and other 18 amino acids that body needs. One of them is arginine that keeping cells young. Beside that, it also contains of protein, calcium, and other beneficial essences. The 18 amino acids mentioned are *arginine, cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, valine, aspartic acid, glutamic acid alanine, glycine, proline* dan *serine* (Okasha *et al.*, 2008).

Vitamin A and C can maintain the body immune system and also as an anti aging and fight cataract. The high calcium contained in rosella also helps to support bones and teeth. Combined

vitamin A, C and Calcium benefit the eye health, skin and bone and the fibre can cure the digestion (Arellano *et al.*, 2004). Flavonoid, which is contained in calyx, has function to prevent cancer, caused by free radical, such as gastric (stomach) cancer and leukemia. Another benefit is that flavonoid has a protective effect toward cardiovascular diseases including hypertension (Kusmardiyana *et al.*, 2007). Flavonoid compound can prevent the microorganism growth, since it is able to form a complex compound with protein through bonds of hydrogen. Polyphenol or phenolic compounds form an antibacterial activity to denaturate cell protein and harm plasm membrane (Arellano *et al.*, 2004).

Usually, natural of functional food and drink have a strange flavor. Like bitter taste on mangosteen peel or sour taste in rosella flower. These two different taste combinations, bitter and sour, are expected to synergized to boost its function and to overcome the unusual taste they have. Based on the above background elaborated, the purpose of this research is to identify the beneficial effect of mangosteen peel and rosella to their anti oxidant and bioactive compound contained in a functional instant drinks and to determine which functional instant drinks have highest antioxidant activity and bioactive compound.

METHODS

Material and Chemical

Materials used in this research were mangosteen peel and rosella flower were

collected from Ubud regency, methanol, hexane, etil acetate, aquadestilata, maltodextrin (MD), tween 80, whatmann filter paper, kits of total phenol, vitamin C, total tannin, pH, and antioxidant capacity (DPPH radical scavenging).

Equipments

The equipments used in this study are spectrophotometer, analytical balance, separation, flask, rotary vacuum evaporator, incubator, oven drier, vacuum filter, magnetic stirrer, GC-MS, and glass ware.

Preparation of Samples

The method in this research using a simple Randomized experimental research with one factor, which is formulation of functional instant drink. The formulation factor comprises of 6 (six) stages and repeated 3 (three) times that resulting 18 units of experiments.

R5M0

rosella : water = 1 : 10

R4M1

mangosteen peel : rosella : water
= 1 : 4 : 50

R3M2

mangosteen peel : rosella : water
= 2 : 3 : 50

R2M3

mangosteen peel : rosella : water
= 3 : 2 : 50

R1M4

mangosteen peel : rosella : water
= 4 : 1 : 50

R0M5

mangosteen peel : water = 1 : 10

The research was started by having the mangosteen peel and rosella dried in

an oven, until the 12% of water content. After that the formula of mangosteen peel, rosella, and water was made. The 3(three) substances were blended with a speed of 1500 rpm for 3 (three) minutes, and kept or drown for 1(one) hour. The mixing then filtered using filter paper whatman no.1. Filtrate was added 0.75% Tween 80, stir and add with 20% maltodextrine.. The mixing was stirred for 30 minutes. Then it was put into petridish and dried in an oven dryer at 60°C until the water content was about 5%. After drying, the extract was refined and sieved to gain 60 mesh of instant powder. Data analysis is conducted using SPSS 17.0 version and once there is a significant influence, then continued with Duncan experiment.

The parameters

The parameters were observed as followed:

Phenol Total

The analysis was using a reactor of *folin-cioccalteu phenol*. A sample with level of contrentation 1000 ppm was taken in 3 ml, reactor of *folin-cioccalteu phenol* 1,5 ml was added. The test tube was gently shaken, added with 7.5 ml of sodium carbonate 20%. The mixture is widely opened, then after 20 minutes, the solution is measured using spectrophotometer with a 750 nm wave length. After the result of absorbance is taken, then in order to get the sample concentration, the sample of absorbance is attached using standard curve. The standard curve was using (+)- gallic acid. Once the absorbance figure is taken, the total of phenol is determined with a

regression linear from standard solution curve (Liu *et al.*, 2002).

Antioxidant activity (DPPH Scavenging)

DPPH solution is made (1,1-diphenyl-2-picrylhydrazyl) $7,5765 \times 10^{-5}$ mol/L in ethanol. 1 ml is taken and added with 3 ml distilled water, counted the absorbance with 516 nm wave length until reaching 0.8 of absorbance. Sample then dissolved in water with concentration of 1000 ppm, taken 1ml then put into a test tube, added with 3 ml of DPPH solution ($7,5765 \times 10^{-5}$ mol/L in ethanol). Next is to vortex the test tube containing mixture and kept it opened for 20 minutes. The mixture then counted using spectrophotometer with length wave of 516 nm. The capacity of scavenging free radical DPPH is measured using standard solution curve with gallic acid concentrate volume of 0, 50, 100, 150, 200, 250, and 300 ppm. After having the result of standard absorbance solution, then proceeded by making the regression linier curve of standard solution (Okawa *et al.*, 2001).

Content of Vitamin C (Ascorbic Acid)

Analysis method of vitamin C is examined following the procedure of Apriyantono *et al.*, 1989. The content measurement of vitamin C is as follows: Place a 500 mg vitamin C tablet on paper and weigh the two together. Dissolve the tablet in about 150 mL of distilled water using a 250 mL conical flask, and then filtered. Add to the flask 5 mL of 1.0 M HCl, 10 mL of 0.6 M KI solution, and drops of freshly starch indicator

solution. Carefully add KIO₃ solution from buret until the solution takes on a permanent bluish purple color. After that, place 500 mg sample and analyzed as above. Vitamin C :

500mg vitamin C/vol. Titrant need for vitamin C tablet= a mg vitamin C/vol titrant needed for sample.

RESULT AND DISCUSSION

Phenol Total

Formulation of mangosteen peel and rosella formulation showed high significantly effect to the phenol total of instant drink powder. The lowest content of phenol total in rosella formulation and water was a formula with a ratio of 1:10 was 14.98 mg GAE/L, while the highest phenol total found is on formulation of rosella, mangosteen peel and water is a ratio of 1:4:50 was 26.11 mg GAE/L. The average of phenol total on the treatment can be seen on Figure 1.

The mangosteen peel contains of xanthone, which is included in phenol group. The identified xanthone comprises of quadraxanthone G, 8- deoxygartanin, garsimangoson B, garsinon D, garsinon E, gartanin, 1-isomangostin, alfamangostin, gamma-mangostin, mangostinon, smeathxanthon A, and tovofillin A (Jung *et al* 2006), while rosella has phenol compound of flavonoid, which is anthocyanin, a red to purple colored pigments. This research points that phenol contained in mangosteen peel is higher compared to rosella.

Content of Tannin

The lowest tannin content was found on the formulation of tannin and water, with a ratio of 1:10, while the highest is on the ratio of rosella, mangosteen peel and water 1:4:50. Formulation between mangosteen peel extract and rosella showed the significantly effect of tannin content . The average of tannin content was shown in Figure 2

Tannin is polyphenol compound which has a characteristic of astringent bitter taste. Tannin functions as an antioxidant as well as antimicrobial agents which is able to precipitate protein. There are 2 (two) classes of tannin *hydrolysable tannin* and *condensed tannin*. *Hydrolysable tannin* is a tannin can be hydrolyzed with acid, alkaline or enzyme into simple compounds such as sugar and tannic acid (gallic and ellagic acid). *Condensed tannin* or proanthocyanidins are oligomeric flavonoids, which are oligomers of catechin and epicatechin, such as procyanidin B-2 (Hagerman 2002).

Content of Vitamin C

The lowest content of vitamin C was 0.277 mg/100 g (mangosteen peel and water, with a ratio of 1:10). The highest is the ratio of rosella and water formulation 1:10 which is 133.39mg/100g. Rosella has a higher content of vitamin C, compared to orange and mango. Besides that, it also contains of citric, succinate and oxalic acid (Okasha *et al.*, 2008). During the research, vitamin C contained in rosella is higher than mangosteen peel. The

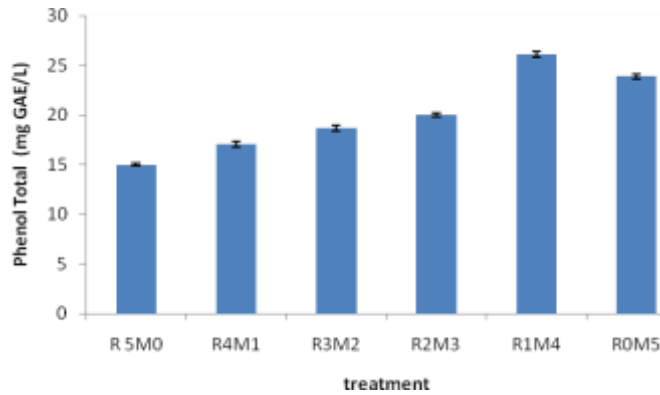


Figure 1. Content of Phenol Total in Mangosteen Peel and Rosella Powder in Average

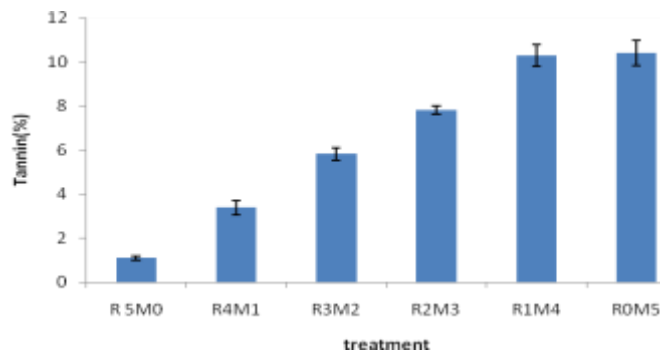


Figure 2. Content of Tannin in Mangosteen Peel and Rosella Formulation in Average

average of vitamin C contents can be seen on Figure 3.

Human does not have the ability to synthesize vitamin C, because does not have glunolactone oxydase enzyme which can synthesize ascorbic acid from glucose. Therefore, human needs to intake vitamin C by consuming food or drinks. Vitamin C has a characteristic of water soluble, easily absorbed in body as well as come out. When the intake is too much, then body will take them out by itself (Carr and Frei, 2002 *in* Cadenas and Packer, 2002). The sources of vitamin C can be found in fruits,

vegetables and rosella that contain L-ascorbic acid. It is also very sensible on temperature and oxygen. The chemical structure of vitamin C is drawn on picture 2.2 Padayatty *et al.*, 2002 *in* Cadenas and Packer, 2002).

The function of vitamin C is to help absorption of iron in the body, prevent the nitrosamine (cancer triggering elements), improving immune system, strengthening teeth and gums, capillaries, prevent fat oxidation and help to heal wound (Carr and Frei, 2002 *in* Cadenas and Packer, 2002 ; Kumalaningsih, 2006).

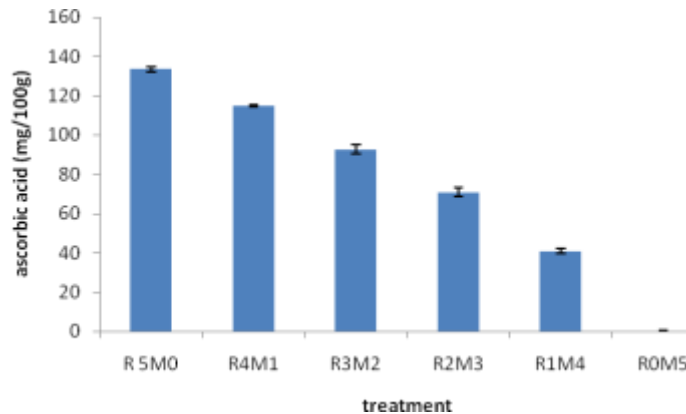


Figure. 3. The Content of Vitamin C in Mangosteen Peel and Rosella Formulation in Average

Antioxidant Activity

Formulation of mangosteen peel and rosella formulation showed significantly effect to the antioxidant activity of instant drink powder. The lowest capacity is seen on the formulation of rosella and water with a ratio of 1:10 which is 19,29%, while the highest capacity goes to formulation of rosella, mangosteen peel and water with a ratio of 1:4:50 which is 27,15%.

The antioxidant activity in functional drink product made of mangosteen peel and rosella is taken from the bioactive compounds, such as phenol and ascorbic acid as the main ingredients of the drinks. The compounds are synergized one to another that result high antioxidant activity in the product. Product with formulation of rosella, mangosteen peel and water with ratio of 1:4:50 with a total phenol 26.11 mg GAE/L, tannin 10.30% and vitamin C 40.74 mg/100g. It is in line with a research by Wrasati (2011) who found that the highest capacity of antioxidant in *Plumeria cendana* powders that rich of

vitamin C, total phenol, and tannin concentrate. The average antioxidant activity of each treatment was shown in Figure 4.

CONCLUSION

Formulation of mangosteen peel and rosella formulation showed significantly effect on the phenol total, tannin, ascorbic acid (vitamin C) and activity of antioxidant in functional instant drinks. The higher content of phenol total and tannin, the higher activity of the antioxidant in the drinks. The functional instant drinks with a highest antioxidant was a formulation of mangosteen peel, rosella and water with ratio 4:1:50.

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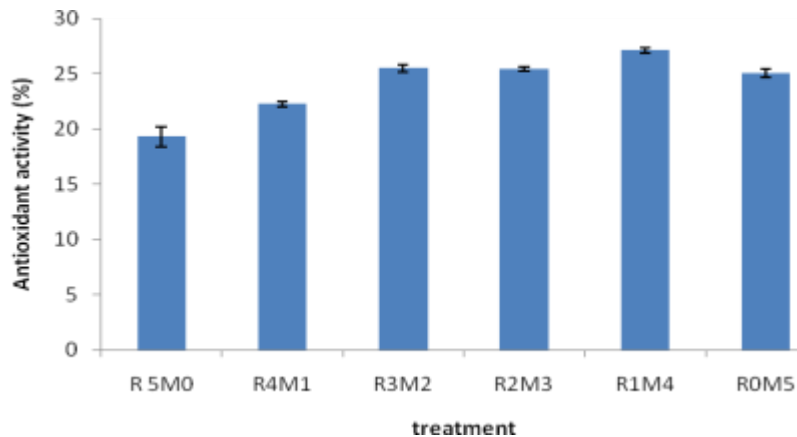


Figure. 4. The Content of Vitamin C in Mangosteen Peel and Rosella Formulation in Average

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