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PRODUCTION AND POTENCY OF LOCAL RAMBUTAN AT EAST JAVA AS A CANDIDATE PHYTOPHARMACA

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ABSTRACT

Rambutan is a tropical fruit that grow well in Indonesia and the peel is considered as waste. Many researchers' showed that rambutan peel contains polyphenol that could be expected to avoid obesity. The objective of this study was to explore the increasing production of local rambutan and to identify the promising phytochemical compounds on its peel as phytopharmaca candidate against obesity. Survey was conducted on the production of rambutan, potential plantation area, and marketing. Sample of rambutan peel collected from the sub-district Kanigoro, Blitar. Phytochemical compounds were analyzed using TLC, HPLC and FT-IR. Bioassay analysis used obesity rat models. The survey result showed a mean of rambutan production increased 2.6% in 2007-2012. Average production of rambutan 70-120 kg/tree. Vegetative multiplication usually done to maintenance of rambutan quality. The main compound of Rambutan peel extract (RPE) is flavonoids, tannins, ellagic acid and the major functional group of CH_3, aliphatic CH_3, and C=O. These compounds have a potential activity against obesity. RPE 30mg/kgBW dose was significantly inhibit the weight gain of obese rats and reducing the adipocyte size ($p<0.05$).

Key words: potency, production, local rambutan, blitar, obesity

INTRODUCTION

Rambutan (*Nephelium lappaceum,* L; Sapindaceae) is a lowland plant and can be found in Indonesia, Philippines, and Latin American countries. This plant grows well at 30-500 m asl and requires a humid climate with annual rainfall about 1500-2500 mm. The rambutan flowering time needs approximately 3 months in dry season (Tindall, 1994).

Rambutan tree grow up to 15–25m, with simpodial branching type. The stem is woody, round, and white. The leafs are single, scattered, oval-shaped, flat edge, tip and base are tapered, pinnate, and green with cylindrical stalk. The leaf size is about 10-20 cm length and 5-10 cm width. Flowers are arranged in bunches at the ends of branches, fragrant, small sized, and light green. Male flowers and female flowers grow apart in a single tree. Fruits are buni with 4-5 cm long and oval-shaped with thick integument. The peel is green and becomes yellow or red when ripe. Seeds are elliptical with woody-seed coat and wrapped in transparent white coat which is edible and contain lots of water with sour to sweet in taste. Rambutan usually flowers at the end of the dry season and when the fruit forms at the wet season, around November to February (Samsuraida *et al.,* 2009).

Indonesia has 22 varieties of rambutan derived from pure variety and grafting from two different varieties. The distinguished characteristics are in the fruit (transparent white-coat, water content, shape, skin color, and hair length). Several varieties are often cultivated due to its high economic value (Tindall, 1994).

Rambutan contains carbohydrates, proteins, calcium, vitamin C, iron, phosphorus, macro and micro minerals, and fats. The fruit peel contains flavonoids, tannins and saponins. The seed

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contains fat and polyphenols while the leaves contain tannins and saponins. The bark contains tannins, saponins, flavonoids, pectic substances and iron (Okonogi *et al.,* 2007). Rambutan production in Indonesia reached 811,993 tons per year and the waste from peel reached 487,195 ton (Statistic Indonesia and Diretorate Generale of Horticulture, 2012). One gram of rambutan fruit can be consumed only 0.4 g while the remaining of 0.6g is peel and seed. The rambutan peel known waste material and have not been used. Rambutan peel contain polyphenol have a strong antioxidant activity (Okonogi *et al.*, 2007; Thitilertdecha *et al.*, 2010).The main polyphenol compounds are tannins and flavonoids (Ling, *et.al*, 2010) which were identified as geraniin, coraligin, and ellagic acid (Thitilertdecha *et al.*, 2010). According to this information, we suggest that the function of some compounds of rambutan peel extract (RPE) be able to control body weight and adipocyte size.

MATERIALS AND METHODS

Survey Potency of Local Rambutan

The location was purposively determined in the district of Kanigoro, Blitar regency. The survey was about potential area, measuring the number of plants and its production. The fruit production data was taken from the Central Bureau of Statistics District and used to predict the amount of waste that will be produced and that has not been utilized.

Phytochemical Compound Analysis of Local Rambutan Peel

Rambutan peel was washed and cut into tiny pieces to prepare for the drying step. The pieces were dried for 14 days on straw paper and later grinded. The powder was sieved using 24 mess. An amount of 100 g of rambutan peel powder was macerated with 500 ml of 70% ethanol for 3 days and the results of subsequent maceration was evaporated using rotary evaporator until the compound becomes semi-solid liquid. 5 g semi-solid liquid was dissolved in 100 ml distilled water and centrifugated at 10000 rpm for 5 minutes. The supernatant was filtered with Millipore 0.45 µl and labeled as 50 mg/ml concentration. Phytochemical compounds identification was performed nanodrop UV-vis. Flavonoids and tannins identification were performed by TLC, while the identification of a compound that is suspected to be ellagic acid was performed by HPLC and the functional groups identification was performed by FT-IR.

Bioassay analysis

Bioassay analysis was performed on male obese rat (*Rattus norvegicus*) which was obtained from the D'Wistar Laboratory, Bandung-Indonesia. Obese rats were fed with a high calorie diet. Criteria of obese rats based on the Lee Index criteria (Campos, *et al.*, 2008). The rats were divided into six treatment groups, non-treatment (NT), ellagic acid (EA 15 µg/kg BW), placebo (water and corn oil), and three different dose of rambutan peel extracts (RPE: 15 mg/kg BW, 30 mg/kg BW, and 60 mg/kg BW). During the treatment, rats were kept in standard rat cages at Bioscience Laboratory and fed with phokphan 551 as a source of high calorie source of diet without physical exercise. The RPE treatment was given via oral administration every two days for three months. Rats body weight gain, food intake, and amount of feces were measured once every week. After 3 months, the rats were dislocated without anesthesia and dissected on the abdominal to take all of the caudal visceral fat. Then, 0.5 g of visceral fat, were fixed on 4% PFA and embedded with liquid paraffin. Paraffin blocks were sliced for providing microanatomy slides stained using haematoxylin-eosin to measure the adipocyte size. This study was approved by the ethical review committee of Brawijaya University Research Ethics Committee as a member of the National Research Ethics Committee of Republic of Indonesia.

RESULT AND DISCUSSION

Distribution and Yield Potencies of Local Rambutan

Rambutan is one of the leading commodities in East Java province. The production centers in East Java are in Blitar, Tulung Agung, Kediri, Malang, Jember and Lumajang (Statistic Indonesia and Diretorate Generale of Horticulture, 2012).

Rambutan fruit production in the Blitar district, has increased from 2007 to 2012 (Fig.1). This is advantageous because rambutan is one of tropical fruit that demanded in international markets.Rate of production rambutan was 2.6% for five years. Largest production increased at 2010 was 6.6%. The result of survey production area field of rambutan indicate the need for effort to

improve and optimize the production area. There is show approximately 35% productive area of rambutan planted with another plant, such jackfruit and calliandra. Potential area to increase the rambutan production spreaded at 22 sub-district at Blitar. East Java province is the second largest rambutan producer after West Java province. International market demand towards tropical fruit is potential opportunities for East Java province to optimize area for rambutan planting. An accordance with the directorate of horticulture policy (2013) conduct a strategies refocusing of the activities to increase production, productivity and quality of horticulture product. Main refocusing to development of primary commodities based on the potential location, availability of facilities and infrastructure, open access to local and international market. These is policies in support of local fruit production on Blitar district to improving the fruit of rambutan. The propagation of rambutan plant, usually by vegetative 93.7% and 6.7% by generative. The advantage of vegetative propagation is expedite fruit production and quality maintenance of rambutan. Rambutan production sale was wholesale 65%, selling directly in the market 28% and 7% consume themself. Sales price on wholesale system ranges from 100-200 thousand/tree, while direct sales price range 3,200-4,000/kg fruit. The farmer prefer wholesale because the practicality.

The areas for planting rambutan are about from 1601 ha, and almost all citizens have this plant on their gardens. The number of rambutan trees in 2010 was about 385,437 trees with average production of 70-120 kg/tree. The income from rambutan may reach to 17 million rupiah per hectare by the number of 100 trees which is sold to middlemen at 170 thousand for every tree.

Some efforts to increase the number of rambutan fruit production has been done by the department of agriculture by focusing on the empowerment of farmer and horticulturist groups, in addition for enhancing partnerships with the private sector. Over all, the national production also increase over 5.28% (Statistic Indonesia and Diretorate Generale of Horticulture 2012). The increasing market demand for rambutan will also increase its waste because rambutan peel is still considered as waste and has never been used yet. Peel of rambutan has a potential as an afford able herbal medicine due to its phytochemical compounds (Ling *et al*., 2010; Wulandari and Lestari, 2012).

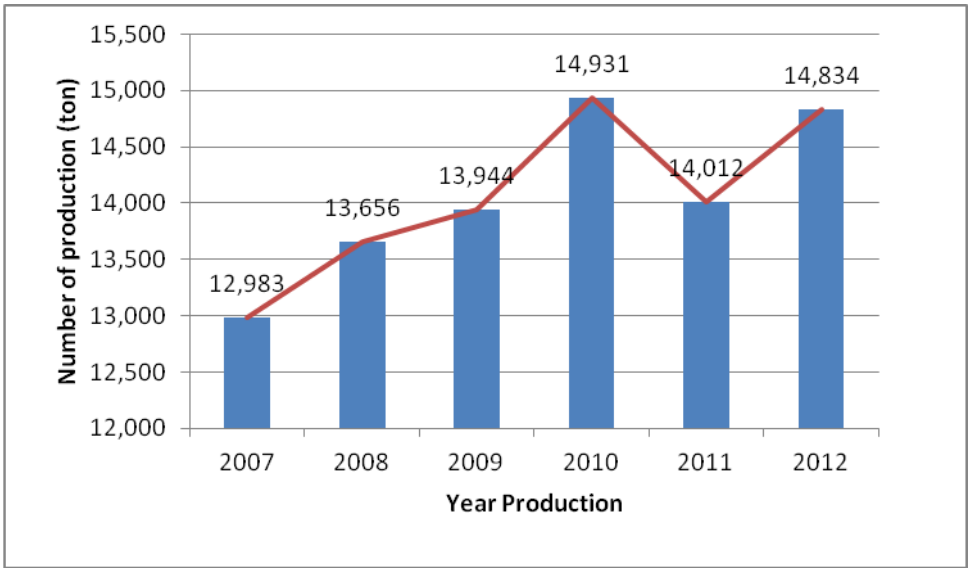
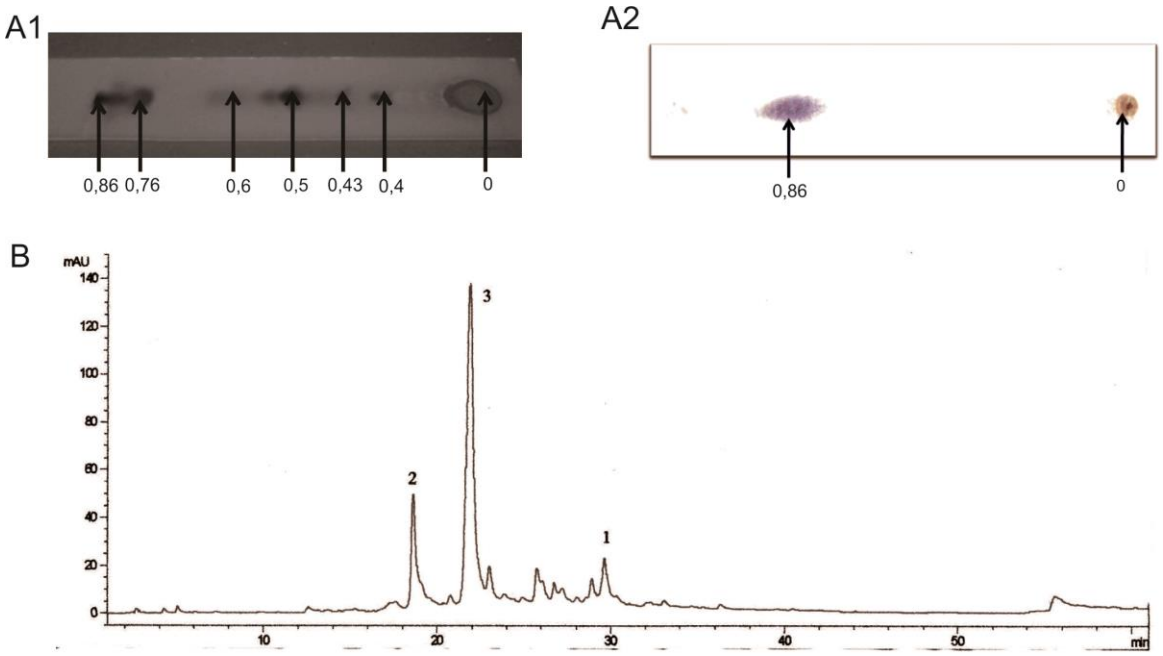


Figure 1. Rambutan fruit production in the Blitar district. A. Rambutan production table in 2007-2012. B. Graphic of increased productionrambutan for five years.



C

Area spectra	Bunch of interpretation
3348,19 and 2943,17	CH3
2360,71	C-H alifatis
1708,81; 1614,31 and 1521,73	C=O
1344,29	CH3

Figure 2. Identification compound contained in RPE. A1 Identification flavonoids compounds by TLC, A2 Identification tannins compounds by TLC, B HPLC chromatograms, peak 1 is ellagic acid, peak 2 is coraligiin and peak 3 is geraniin, C FT-IR interpretation RPE fuctional bunch

Phytochemical Compounds of Local Rambutan Peel Extract

The collection of rambutan fruit as much as 8 kg generated 2.4 kg wet weight rambutan peel. Furthermore, the peel became 600 g dried rambutan and yielded 368 g of peel powder with 1.56% rendement of peel powder extract. The tests of TLC, HPLC, and FT-IR (Fig. 2) showed that contains some compounds such as flavonoids, tannins, ellagic acid and the major functional group of CH_3, aliphatic CH_3, and C=O. These compounds has a role in suppressing the adipogenesis process through inhibition of triglyceride absorption and Fatty Acid synthetase inhibition with IC_{50} values ranging from 5.59 to 204.40 µM (Zhao *et al*., 2011).

Fractionation with ethanol, n-butanol and water, generally resulting hydrophobic fractions that consists of terpenoids, steroids, lignans and flavonoids. Another fractions found are tannins, saponins, glycoside, and hydrophilic fraction containing sugars, amino acids and peptides. The proportion of RPE consists of 37.08% hydrophobic fraction, 8% n-butanol fraction and 54.92 % water fraction.

The peel of rambutan contain a lot of phenols. The main compounds are ellagic acid, coraligin and geraniin (53.5 mg ellagic acid, 71.9 mg coraligin, and 568.0 mg geraniin obtained from methanolic extracts of 1g of rambutan peel) (Thitilertdecha *et al*., 2010). Ellagic acid is a plant secondary metabolites that are often found

in cell vacuoles. These compounds are known to have antioxidant properties, anti proliferative, chemo preventive, and anti-atherogenic (Atkinson *et al*., 2006).

Local Rambutan Potency in Controlling Body Weight Gain

Rambutan peel extract (RPE) treatment caused a reduction in weight gain compared to non treatment. The student t-test showed that there was significantly different between calorie intake and weight gain on normal and obese rats. There was correlated between treatment and body weight gain. Treatment with RPE reduced body weight gain significantly at all concentrations. At 30mg/kg BW the gain was reduced by about 25%, similar to 15 µg/kg BW of EA. 15 mg/kg BW of RPE had a smaller effect and, surprisingly, 60 mg/kg BW of RPE also had a smaller effect compared to non treated animals and to the placebo control ($p<0,05$). Obese rats consumed significantly less food but considering the higher calorie content, this difference was reversed in that the obese rats consumed 30% more calories. Food consumption was not significantly affected by any of the treatments. Obese rats produced approximately 60% less feces which may be partially explained by the lower consumption in grams, but this cannot be entirely accounted for the increased body weight gain.

The results of the ANOVA showed that there is significant different between the amount of feed consumed and the amount of feces produced on normal and obese rats. The results of the regression analysis showed that there is no correlation between the amount of feed consumed and the amount of feces produced to the body weight gain. There was a tendency, the less calories intake which is consumed make decreased body weight gain. As expected, the microanatomy slides of visceral fats showed that the adipocytes in obese rats are significantly larger. After RPE treatment, adipocytes seemed to be smaller in obese rats.

Many researchers recently reported that bioactive compound of herbal therapy is one alternative of anti-obesity in animal's model. Firdausi & Indra (2012) reported that black tea treatment could decrease body weight in rats. (Bajerska *et al*., 2011) used an obese rats model demonstrated that tea catechins (another kind of tannin) caused a significant increase in lipid catabolism in the liver. Zheng *et al*., (2004) indicated that catechins and caffeine were synergistic in anti-obesity activities. The findings described by Ito *et al*., (2008), confirm that catechins (much more than caffeine) at clinically appropriate doses, affect lipid metabolism in non-obese and obese subjects.

The putative active phytochemical contents of RPE, ellagic acid and flavonoids, may play an important role in affecting body weight gain. In addition, RPE also contain tannins, a large number of phenolic groups which provide interaction sites with carbonyl groups of peptides. Though tannins mainly exert their effects on proteins, they also have effects on carbohydrates, particularly hemicellulose, cellulose, starch and pectin's. They may cause are substrate deprivation or enzyme inhibition.

RPE may prevent the onset of obesity by efficiently reducing body weight gain before an extensive therapy is required or to prevent regain of body weight after a weight loss therapy. RPE could be a promising food ingredient to suppress weight gain and its long-term consumption could contribute to the maintenance of optimal weight.

There was no appreciable changes in calorie intake depending on the treatment. However, the treatment seemed to affect the weight gain of rats, especially with 30 mg/kg BW of RPE, whereas 15 mg/kg BW and 60 mg/kg BW had a smaller effect. The lowest weight gain was observed with 15 µg/kg BW of pure EA as has been previously described (Ho *et al*., 2005). The reduced weight gain observed with ellagic acid and RPE may be caused by the suppression of lipid absorption, reduction in biosynthesis of fatty acid, or enhancement of fatty acid oxidation. Clearly, the intake of calories was not changed with EA or RPE treatment.

The accumulation of Igf-1 and Igf-1R expression in the treated and untreated animals which is this protein and receptor regulated the adipogenesis pathway (data not shown). It appears that RPE function is upstream regulation of the Igf-1 and Igf-1R expression. We observed a pronounced reduction of the proteins in the EA and RPE treated animals. Consequently, the size of adipocytes was also reduced after treatment.

Recently some research reported that EA was considered the main active ingredient for

reduction of body weight gain (Ho *et al*., 2005; Min *et al.*, 2012). The comparison of RPE and pure EA treatment revealed that there was apparently a combination effect of EA and other compounds in RPE since the calculated dosage of EA was much higher than described as effective in the literature (Zhao *et al*., 2011)

CONCLUSSION AND SUGGESTION

The results indicated production of rambutan at 2007-2012 increased by 2,6%. Average of rambutan production was 70-120 kg/tree. The farmer more fond a sale of rambutan of wholesale system. Propagation of rambutan plant has done by vegetative system. Productive area has not been used optimally so that need to be improve the utilization.

Rambutan peel contains flavonoids, tannins, ellagic acid and the major functional group of CH_3, aliphatic CH_3, and C=O compounds. The RPE can inhibit the increase of body weight gain and also reduce the size of adipocyte in obese rats model. This is a new finding that reveal the utilization of RPE as a basic for further research about anti-obesity therapy. Further experiments are required to elucidate the mechanism of action of RPE and to understand if the activity is caused by EA alone or rather by a combination of bioactive substances in the extract.

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