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ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT OF STAR FRUIT LEAVES (*Averrhoa carambola* L), A RAW MATERIAL FOR BALINESE TRADITIONAL FOOD (*LAWAR*)

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Abstract: ***Oxidative stress induced by free radicals has been well known to cause degenerative related diseases, such as cancer, coronary heart attack, and premature aging. These free radicals are form of reactive compounds and known to have unpaired electron on the outermost of their electron orbit, and therefore in such condition, they become very reactive to other compounds in human body and resulting in induction to various diseases to arise. To counter this bad side effect of free radicals, compounds function as antioxidants are needed by our body to neutralize such free radicals, so that damage caused by these free radicals can be reduced. Antioxidants in some levels can be produced naturally by our body, but often in insufficient quantity, and therefore there is a need to regularly consume antioxidant containing food. This background triggered the authors to extract antioxidant compounds from leaves of star fruit plants with a view to develop antioxidant containing products for future use to fight free radicals in human body, so that cases of degenerative-related diseases can be reduced. The antioxidant activity of methanol extract of star fruit leaves (Averrhoa carambola L) was conducted at the laboratory of physiology, Udayana University. These leaves have been used as one of raw materials in a Balinese traditional food (Lawar). The leaves were normally harvested from two types of star fruit plants (plants with sweet fruits and plants with sour fruits or belimbing besi). Leaves from the later type of the plant are preferable for raw material of lawar, because they are more elastic than that the other type. The method of DPPH was applied in the determination of the capacity of antioxidant activity. The results showed that the Activity Antioxidant Index (AAI)of leaves of sweet star fruit plant and sour star fruit plants were 0.17 and 0.63, respectively. This indicated that the leaf methanol extract of sweet star fruit plant had weak antioxidant activity (the value of AAI <0.5), while the leaf methanol extract of sour star fruit plant had moderate antioxidant activity (the value of AAI >0.5 – 1). The IC_{50} of leaf extract of sweet star fruit plant and sour star fruit plants were 158.3 and 69.2 respectively. This indicated that leaf methanol extract of sour star fruit plants had high active capacity to decrease DPPH radicals, while sweet star fruit plant had moderate capacity. It was also found that the flavonoid and vitamin C contents of sour star fruit leaf extract were higher than that of sweet star fruit leaf extract.***
Keywords: Star fruit leaves, Antioxidant Activity Index, DPPH

INTRODUCTION

Free radicals are molecules that contain one or more unpaired electrons in their outermost electron orbit, and therefore they become very reactive and unstable. To achieve their stability, these free radicals will react with other atoms or molecules around them to stabilize their electrons (pairing with electrons from other atoms or molecules). Such reactions will continuously take place in human body and causing chain reactions that damage cell structure, and if they are not stopped, they will result in various degenerative related diseases (references …).

Some diseases, such as cancer, arthritis, coronary heart attack, diabetes mellitus, and retardation of brain function can be caused by free radicals. Excessive numbers of free radicals can attack any compounds, such as lipid and protein and may result in many types of degenerative-related diseases. Therefore, formation of free radicals in our body must be reduced as much as possible by applying antioxidants. Antioxidant compounds play important role to protect our body from bad side effects of free radicals. These compounds can prevent the formation of free radical molecules, reduce radical molecules, cure oxidative defects, as well as preventing mutation. Antioxidants are electron donor compounds that play

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important role to inhibit oxygen-facilitated oxidation processes. Antioxidant compounds can prevent bad effects caused by free radical compounds and therefore they play important role to protect our body from various diseases (Percival, 1998). Many types of foods can be sources of natural antioxidants. These include spices, tea, chocolate, and vegetables. Antioxidant sources are dominated by plants which contain Flavonoid found in all parts of the plant body. Synthetic antioxidants can have high effectiveness, but they are relatively unsafe for our health, and therefore their use in many countries has been tightly supervised (Pujimulyani, 2003). Therefore, investigation of chemically active compounds of plants is urgently needed so that their use as pharmaceutical can be decided.

A type of plant abundantly found in Indonesia and its use in medicine rarely found is sweet star fruit plant (*Averrhoa carambola*). Until recently, the fruits of this plant have been widely used by people in Indonesia as a medicine of diabetes mellitus. The leaves of this plant however have not been used in this aspect. In Bali, the leaves of this plant have been use as a raw material in the making traditional food called '*lawar*'. The leaves of two types star fruit plants (sour and sweet star fruit plants) are normally used for this purpose, but leaves of sour star fruit plant are preferable as they are more elastic. The use of such leaves in the *lawar* making is believed to have good effect on human health. In this research, the pharmacologic potency of the star fruit leaves was partly elucidated with the main objective to find novel source of natural antioxidant to replace the use of synthetic antioxidant.

MATERIALS AND METHODS

Extraction

In this research, leaves of the two types of star fruit plants were used. The collected leaves were washed with flowing water, chopped to reduce the leaf size, air dried, and powdered using a warring blender. The leaf powder was then macerated in 70% methanol for 72 hours with regular mixing, filtered, evaporated in a vacuum rotary evaporator at 40 °C until a concentrated extract was obtained.

Measurement of Antioxidant Activity by applying DPPH method

The method of DPPH was applied to measure the antioxidant activity. An amount of 25 mg crude extract was diluted in methanol to obtain stock solution of crude extract with a concentration of 1000 ppm. This stock solution was then further diluted to obtain various concentrations of working crude extract solutions (4 ppm, 8 ppm, 12 ppm and 16 ppm). These were then added with 5 ml DPPH 0.5 mM solution. The blank solution was prepared by diluting 5 ml 0.5 mM DPPH solution in methanol with a final volume of 25 ml. The absorbance of DPPH of each prepared solutions above was measured using a spectrophotometer (at the wavelength of 515 nm) at regular 5 minute intervals for 30 minutes. The antioxidant activity was measured on the basis of reduction of DPPH absorbance as a result of sample addition. The percentage of inhibition (values of absorbance of DPPH solution before and after crude extract addition) was calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{(A_{control} - A_{sample}) \times 100\%}{A_{control}}$$

Note:
$A_{control}$= Absorbance of blank solution
A_{sample}= Absorbance of sample

The results of the calculation were then plotted which shows a relationship between extract concentration (X axis) and % inhibition (Y axis) in order formulate linear regression. The IC_{50} was then determined on the basis of linear equation derived from the linear regression formula of Y = aX + b (Zuhra *et al*., 2008).

Test of Vitamin C content

This was conducted by applying iodometric titration. To the samples, a starch solution was added and titrated with iodine solution until the blue color appeared.

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Measurement of Flavonoid content

Total flavonoid content was measured using reagent $AlCl_3$ as applied by Chang *et al*. (2002). $AlCl_3$will react with the *keto* group located at C4 and hydroxyl group (OH) at C3 or C5 of flavon or flavonol of the sample to form stable complex compounds.

RESULTS AND DISCUSSION

Antioxidant activity measured as IC_{50} is a concentration needed to produce reduction of DPPH activity by 50%. It implies that the lower the value of $IC_{50}$the stronger the antioxidant activity of a sample. It was found in this research that the IC_{50}values of sour and sweet leaves were 69.20 and 159.68, respectively. According to Molyneux (2004), samples with a value of IC_{50} less than 200 ppm can be claimed to have antioxidant activity. If their IC_{50}value falls between 200 and1000 ppm, the samples are less active, but still have potential as antioxidant. Specifically, a compound can be considered as a very strong antioxidant, if its IC_{50}value is less than 50 ppm, strong if its IC_{50}value falls between 50 and100 ppm, moderate if the value falls between 100 and 150 ppm, and weak if the value falls between 151 and 200 ppm. The results of our study showed that both methanol extracts of star fruit leaves had IC_{50}values of less than 200 ppm. This means that methanol is a good solution for use to extract antioxidant compounds from leaves of both type of star fruit plants. Methanol is a relatively polar solution which is appropriate to be used in this purpose. Based on the above categories, the crude leaf extract of sour star fruit plant had strong antioxidant activity with IC_{50}value of between 50 and 100 ppm, while the leaves of sweet star fruit plant had weak antioxidant activity with IC_{50}value of between151 and 200 ppm (Figure 1).

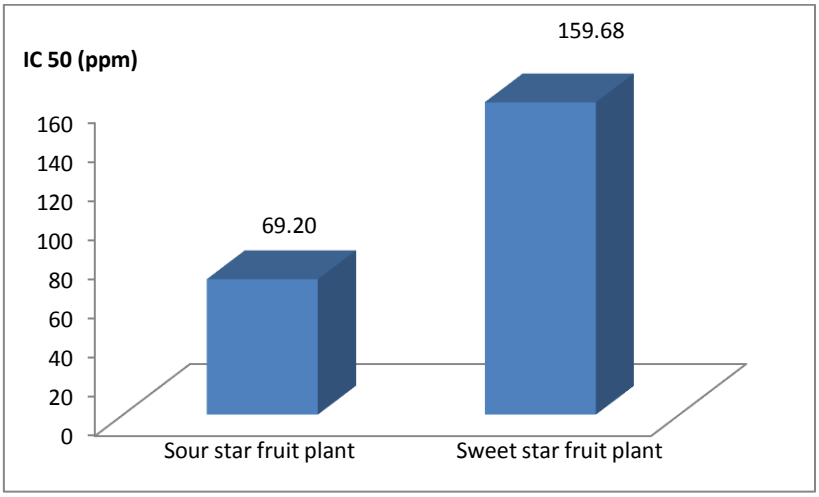

Figure 1: IC_{50}values of methanol extract of leaves of sour and sweet star fruit plants.

Based on the antioxidant activity test, the leaf extracts of sour and sweet star fruit plants had antioxidant activity index (AAI) of 0.6 and 0.2, respectively. These indicated that the methanol extract of sweet star fruit leaves can be considered to have weak antioxidant activity as the value of AAI of this plant is less than 0.5 (AAI < 0.5), while those of sour star fruit leaves had moderate as its AAI value fell between 0.5 and 1 (AAI > 0.5 – 1) (Figure 2).

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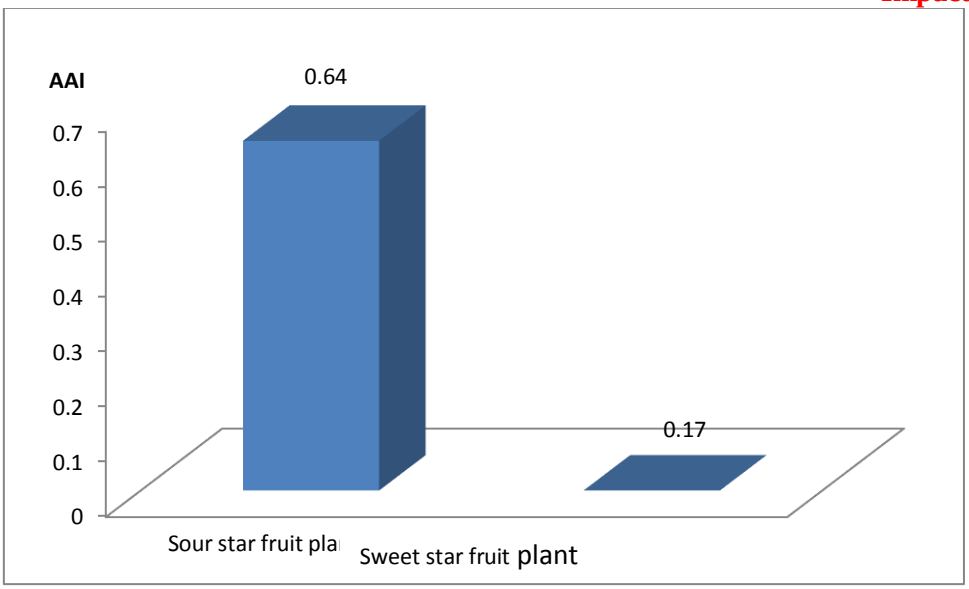

Figure 2: Antioxidant Activity Index (AAI) of sour star fruit leaf extract and sweet star fruit leaf extract (*Averrhoa carambola* L).

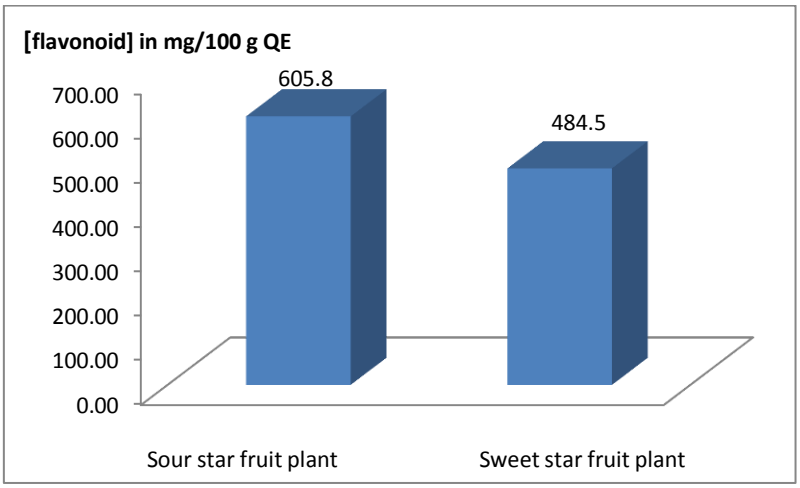

Figure 3: Flavonoid content of methanol leaf extract of sour and sweet star fruit plants.

Analysis of flavonoid content is a measurement of total flavonoid contained in samples which was conducted by using a spectrophotometric UV-Vis using aluminum chloride ($AlCl_3$) with *kuersetin*as standard. *Kuersetin*is a type of flavonoid commonly used as standard in the determination of flavonoid concentration. Biologically, this compound has a very high antioxidant activity (Sugrani, 2009).

In the determination of flavonoid concentration, each extract was reacted with $NaNO_2$and $AlCl_3$, so that yellow color appeared. In this reaction, $AlCl_3$forms stable acidic complex with ketone group or hydroxyl group of flavon and flavonol and turn the solution to yellow. The results of our study showed that the flavonoid content of the leaf extracts of sour and sweet star fruit plants were 605.8 mg quercetin per 100 mg sample and 484.5 mg quercetin per 100 mg sample, respectively (Figure 3). Flavonoid in the groups of flavon, flavonol, and isoflavon contained in the leaf extract of sour and sweet star fruit plants contribute to neutralize free radicals, and therefore can be considered to have antioxidant activity. Derivate of flavon has biological activity as well as pharmacologic effect, such as anti-inflammation, anticancer, and antioxidant. Isoflavon on the other side is known to have potential as anti-aging or preventing degenerative diseases.

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According to Inggrid and Santosa (2014) flavonoid can also play a role as anti-inflammation and antioxidant so that this compound can prevent oxidative related in cardiovascular related diseases and neurodegenerative. Therefore flavonoid has important contribution in human health. For health maintenance, it is recommended to consume daily some flavonoid containing food as this compound functions as anti-mutagenic agent and anti-carcinogenic agent. Besides that flavonoid also has antioxidant, anti-allergic, anti-inflammation activities, as well as preventing oxidation of LDL (Low Density Lipoprotein).

Vitamin C concentration of the leaf extracts of sour and sweet star fruit plants is shown in Figure 4. Vitamin C is dissolved in water and play important role in the prevention of various diseases. Vitamin C is within a group of antioxidant vitamins with capability to neutralize various types of free radicals. The results of this study showed that leaf extract of sour star fruit plant contained relatively higher vitamin C than that of sweet star fruit plant (Figure 4).

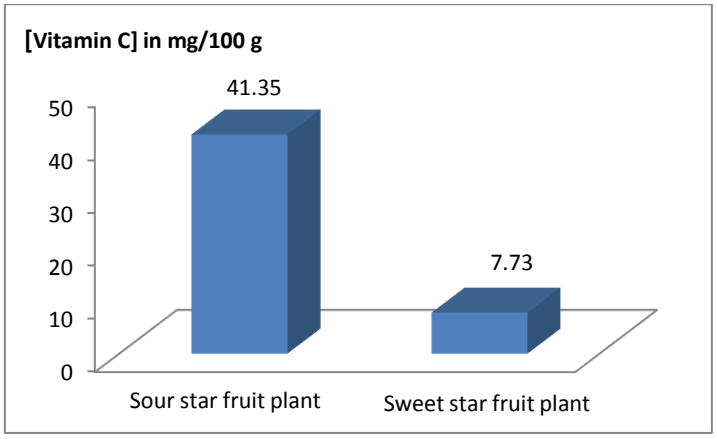

Figure 4: Vitamin C concentration of methanol leaf extracts of sour and sweet star fruit plants (*Averrhoa carambola* L).

CONCLUSION

Leaf extract of sour star fruit plant had higher antioxidant activity than that of sweet star fruit plant with antioxidant activities measured as IC_{50} of between 50 and 100 (strong) and between 151 and 200 (weak), respectively. Flavonoid and vitamin C contents of leaf extract of sour star fruit plant were higher than that of sweet star fruit plant. Antioxidant Activity Index (AAI) of sour star fruit plant was higher than that of the sweet star fruit plant, and this indicated that methanol extract of the former plant had higher antioxidant than that of the later one, and therefore can be considered as an excellent natural source of antioxidant

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