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Analysis of Phenolic and Tannin Contents in the Methanol Extract of Sweet and Sour Star Fruit Plants (*Averrhoa carambola* L) Leaves Commonly Used as Raw Materials of *Lawar* (A Balinese Traditional Food)

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Abstract. *Lawar*, a special Balinese food made of leaves of star fruit plant, is commonly served in any Balinese traditional events, such as temple festival or wedding parties. The leaves of sweet and sour star fruit plant are used to make this traditional food. In this research, phenolic and tannin content of leaves of these types of plant were analyzed with a view to investigate their potential as antioxidant source. Phenolic compound has been well known to have antioxidant activity by neutralizing free radicals and stabilizing singlet oxygen. This compound has capacity to reduce free radicals by chelating metal ions or inhibiting enzymatic system, such as cyclo-oxygenase, mono-oxygenase or xanthine oxidase that catalyze formation of free radicals. [4] reported that plants with high content of phenolic compounds tend to have high nutritious and antioxidant activities Tannin is a compound belongs to polyphenolic group. Such compound is abundantly found in any plants. This compound has capacity to bind proteins, alkaloid, and gelatin. In human body, this compound play a very complex role, such as chelating metal ions, precipitating proteins, and functioning as biological antioxidant. The results of this study showed that the phenol content of sour and sweet star fruit plants were 2830.99/100 mg GAE and 1959.77/100 mg GAE, respectively, while the tannin content of these two plants were 18810.43 mg/100 gr TAE and 11035.75 mg/100 gr TAE, respectively, indicating that the leaves of sour star frit plant had better antioxidant activity than the sweet one.

Keyword*:* Star Fruit, phenol, tannin

I. INTRODUCTION

Degenerative related diseases, such as cancer, coronary heart attack, and premature aging can be due to free radicals-induced oxidative stress. Free radicals are atoms with one or more unpaired electrons, so that they become very reactive and tend to continuously react and form new free radicals. Free radicals continuously produced during normal metabolic process have been claimed to cause damage of cell functions and resulting in degenerative related diseases.

The role of antioxidant is very important to reduce bad side effects of free radicals that induce degenerative diseases, such as diabetes mellitus (DM), hypertension, coronary heart attack, atherosclerosis, and premature aging. Reaction of free radicals can generally be inhibited by either synthetic of natural antioxidants. This is related to the capacity of antioxidant to function as inhibitor in oxidative reactions induced by free radicals, and therefore reduce the chance of having degenerative related diseases [9].

Many of raw materials of foods, such as spices, tea, chocolate, seeds of plants, vegetables, enzyme, and other proteins are sources of natural antioxidant compounds. Natural antioxidant sources are dominated by plants that contain flavonoid and phenolic compounds spreading in all parts of the plants. Some research reported that all parts (seeds, fruits, leaves, and bark of its roots) of mengkudu plant has capacity as anti-dislipidemia [10], antioxidant [2], curing agent of wound in diabetes [8], hepato-protector [7], inhibiting agent of *Angiotensin Converting Enzyme* (ACE) [11], analgesic agent [1], hipoglychemic , antiinflammation, and chemo-preventive cancer [5]. This activity is suspected to be due to flavonoid and phenolic content of *Morinda citrifolia* L fruits [12].

Flavonoid is the biggest group of phenolic compound. Being an antioxidant, this compound has capacity to stabilize free radicals by adding electron deficit of free radicals and resulting in inhibition of chain reaction due to formation of new free radicals. Flavonoid is a secondary metabolite produced by all green plant, except algae. Flavonoid can be found in all parts of plants, such as roots,

leaves, barks of plant stems, stamens, fruits, and seed of plants, and flowers [13].

Tannin is a high molecular weight of phenolic compound containing hydroxyl group or carboxyl-like group and has capability to form effective complex compound with proteins and other macromolecules under certain conditions. Tannin has potential as antiseptics, astringent, antioxidants, anti-mites, anti-fungi, and metal chelating agent.

Leaves of sour and sweet fruit star plants are used as raw materials *lawar* making in Bali and often served in any Balinese traditional events, such as wedding ceremony. *Lawar* made of these leaves is a popular food among Balinese society. However scientific explanation on the active contents within these leaves is very rare. Therefore our research investigated phenolic and tannin compounds in leaves of these two types of star fruit plants. The main objective of this research was to investigate the potential of these materials as antioxidant.

II. RESEARCH METHODS

Extraction

The leaves of the plants were collected from Tabanan regency-Bali. These materials were air dried, powdered with a warring blender, and macerated with methanol solution for 48 hours, and evaporated with a rotary evaporator at 50 ^{o}C, until concentrated crude extract was obtained.

Measurement of total phenol

Phenolic content was determined by using *Folin-Ciocalteu* solution [6]. The principle of this method is to oxidize hydroxyl group of the phenolic compounds. Samples of the leaves extract were reacted with this solution and its absorbance was then measured at the wavelength of 725 nm. Total phenol of samples was determined on the basis of a standard curve produced from various concentrations of Gallic acid solutions.

Determination of total tannin

Total tannin was determined by applying small modification of method specified by [3]. Some 0.5 g The leaf powder was extracted with 10 mL methanol for 20 hours, filtered, its residue was boiled in 100 mL of distilled water for 2 hours, cooled down at ambient temperature, and filtered. The filtrate was added with distilled water and its final volume was adjusted to 100 mL.

Diluted filtrate amounted at 0.1 mL was then added with the same volume of reagent Folin Ciocalteu, vortexed, added with 2 mL Na2CO3 and vortexed again, incubated at ambient temperature for 30 minutes, before being measured for absorbance at the wavelength of 760 nm. The reading values were then plotted on a standard curve of tannic acid previously prepared. The tannin contents are presented in mg tannic acid/100 g samples.

III. RESULTS AND DISCUSSION

Test of total phenolic activity is used as the basic of antioxidant activity test, because this compound is known to play role in preventing oxidation to occur. Total phenol is an estimation of phenolic compound in plant samples. Total content of phenolic compounds measured with Folin-Ciocalteau was on the basis of a redox reaction, and its total content was measured by comparing this value with those specified in a standard curve established with Gallic acid. Gallic acid was used in the establishment of standard curve, because this compound is very effective to form complex with reagent Folin-Ciocalteu, [4.] The data obtained from this research showed that leaves extract of sour star fruit and sweet plants contained total phenol of 2830.993 mg Gallic acid /100 g extract and 1959.767 mg Gallic acid /100 g extract, respectively, indicating that sour star fruit plant has higher potential as antioxidant than the sweet one (Figure 1).

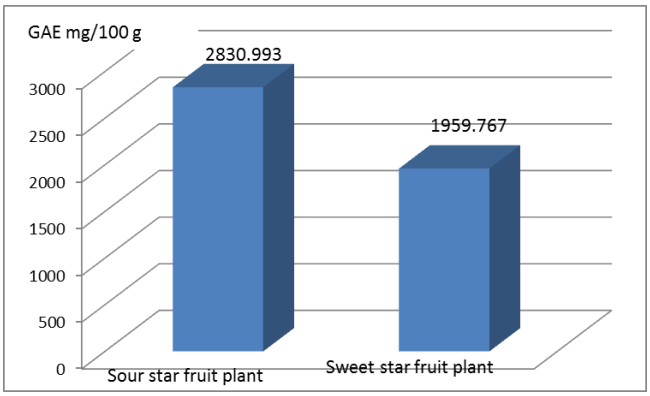

Figure 1: Phenol total content of methanol leave extracts of 100g of sweet and sour star fruit plants

Polar solvent was found to better extract phenolic compound from plant samples which resulted in higher yield. Polarity level of solvents used in the extraction will determine the level the structure and types of phenolic compounds extracted from samples of plants. Phenolic compounds with higher content of hydroxyl groups will result in higher content of total phenol. When compared with the results of [6] and [4] who reported total phenol of green kiwi fruit (88,5-155,5 mg Gallic acid per 100 gram sample, extracted with a mix of acetone a hexane with a ratio of (2:3) and total phenol of the same fruit (40 mg/100g, extracted with water), respectively, our results were higher than those two reports.

Results of tannin content

Generally, tannin content is defined as poly phenolic compound with high molecular weight (MW of higher than 1000 Dalton). These compounds can form complex with protein. The results of our study showed that methanol leave extracts of sour and sweet star fruit plants contained total tannin of 18810.433 mg TAE / 100 g sample and

11035.747 mg TAE / 100 g sample, respectively. Tannin is an excellent antioxidant compounds in plants and their activity depends of their quantity. As they are composed of poly phenol compounds, they have activity to neutralize free radicals. As shown in Figure 2, leave extract of sour star fruit plant contains higher total tannin than that of sweet star fruit plant, indicating that the former has higher antioxidant than the later one.

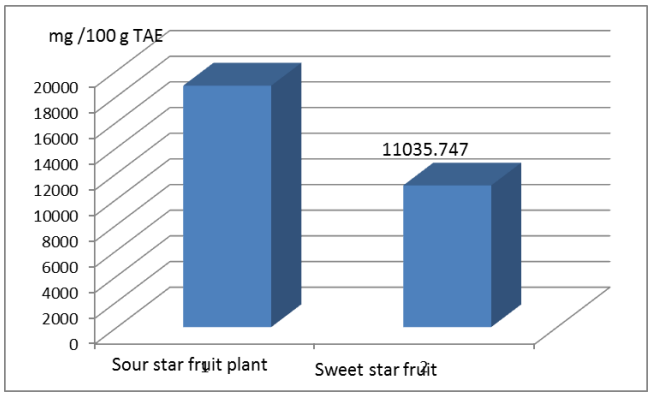

Figure 2: Total tannin in 100g of methanol leave extracts of sour and sweet star fruit plants

IV. CONCLUSION

Total phenol and tannin of sour star fruit plant was found to be higher than that of sweet one, 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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