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Abdi Wibowo Prakoso
Department of Pharmacology, Faculty of Pharmacy, Pancasila University Jl. Srengseng Sawah, Jagakarsa, South Jakarta, Indonesia

Correspondence
Syamsudin Abdillah
Department of Pharmacology, Faculty of Pharmacy, Pancasila University Jl. Srengseng Sawah, Jagakarsa, South Jakarta, Indonesia

Antioxidant and anti-AGEs (Advanced glycation end-product) activities of ethanolic extract of *Ficus carica* Linn from Indonesia

Syamsudin Abdillah and Abdi Wibowo Prakoso

Abstract
Background and Objective: The tin leaf contains flavonoid and phenol compounds, which are known to act as antioxidants. The objective of this study was to investigate the antioxidant activity and inhibition of Advanced Glycation End-Products (AGEs) from 96% tin ethanol extract (*Ficus carica* L.) based on the inadequacy of DPPH and AGEs compound levels formed.
Materials and Methods: Different concentrations of tin leaf extract were tested with a radical compound of DPPH, with Vitamin C as a positive control. The inhibitory activity of AGEs compound formation was studied by reacting high concentrated sugar solution with protein in the form of Bovine Serum Albumin. With quercetin used as a positive control for barrier testing on the formation of AGEs. The reaction of tin leaf extract was determined by measuring the total content of the phenol compound using Folin-Ciocalteu reagent.
Results: The results showed that 96% of ethanol extract had a potent antioxidant activity, buthad little ability to inhibit AGEs. With IC_{50} values of 55.38 µg/ml as an antioxidant and 750.29 µg/ml inhibiting AGEs, total phenol content of tin leaf extract was 0.71%.
Conclusion: Based on this information, it can be concluded that the 96% ethanol extract of tin leaf has a strong and an antioxidant activity. However it is in inhibiting AGEs compounds.

Keywords: Antioxidant, fig leaves (*Ficus carica* Linn.), Advanced Glycation End-products, AGEs, total phenolic compounds

Introduction
Oxidative stress is defined as an imbalance between the body's free radical production and its ability to neutralize their harmful effects through production of antioxidants. The human body will always produce free radicals (ROS). Therefore, in order mitigate the impact, antioxidant compounds are produced to defend the body against ROS [1, 2]. Antioxidants are molecules that block or prevent free radicals from stealing other atoms' electrons. They work both outside and inside the cells [3]. The human body has two defenses against free radicals. These are detoxification enzymes (enzymatic antioxidants) and molecular antioxidants (non-enzymatic antioxidants [4]. Pathophysiological conditions are aggravated by the formation of freeradicals. This leads to severe complications in some diseases, and causes the harmful compounds, such as final product of glycation process, commonly called Advanced Glycation End-Products (AGEs) to build up [5].
The AGEs compound is a complex and irreversible product of glycemic reaction. This compound causes biomacromolecular damage for diabetics by altering the molecular configuration of lipid proteins, carbohydrates, and other substances. Accumulation of AGEs in the tissues plays an important role in pathologies such as Alzheimer's disease, and can cause atherosclerosis or renal failure diabetic persons. Because of this, there is a potential therapeutic benefit in researching compounds that lower intracellular and extracellular levels of AGEs. Natural antioxidants (AO) with true anti-AGEs capability is one of the most promising canditates for this cause [5, 6].
The tin leaf (*Ficus carica* Linn.) is one member of the Moraceae plant family which grows well in Indonesia. One study, by Trifunschi and Ardelean (2012) found that antioxidant activity in Romanian *F. carica* Linn leaf extracts, have high antioxidant activity [7]. *F. carica* Linn leaves are widely known as a rich source of phenolics, with high antioxidant capacity, giving the extrats potential to overcome many diseases [8]. However research on the effects of Indonesian tin leaf on AGEs activity has never been done before. This study aims to determine the antioxidant activity and anti-AGEs potential of Indonesian *F. carica* Linn's ethanol extract.

Materials and Methods

Plant material

Fresh tin leaves were collected from Syanira Garden in Bogor, West Java, in January 2018. The washed plant leaves were cut into small pieces and dried in 60 °C oven. Then, the dry simplicias were mashed and sifted with 4/18 sieves, resulting in tin leaf powder. One-hundred grams of ten leaf powder was extracted with 400 mL of ethanol for a period 24 hours. The solution was then filtered, and dried using a 30-35 °C evaporative vacuum. The resulting ethanol extract of *F. carica* Linn had a dark brown color with a weight of 13.5 grams. (13.5% w/w) and kept in an air-tight containers stored in a refrigerator for use.

The antioxidant activity test using DPPH method

10 mg of *F. carica* Linn leaf extract was dissolved in 10.0 mL methanol pro-analysis (1000 µg/ml). The test solution was portioned in100, 200, 300, 400 and 500 µL into a 5.0 mL measurable flask in order to obtain the sample concentrations of 20, 40, 60, 80 and 100 µg/mL, respectively. 0.4 mM. DPPH solution and 10.0ml methanol pro analysis was added into each of the test solution and the comparator (positive control). The DPPH without inhibition (blank) test solution, and the comparative solution were both immediately incubated for 30 min at 37 °C, then measured at 517 nm wavelength.

Inhibition percentage was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Blank absorption} - \text{sample absorption}}{\text{Blank absorption}} \times 100\%$$

The value of IC_{50} (Inhibitor Concentration) or the concentration of antioxidants that can inhibit 50% free radicals. The value of IC_{50} is obtained by using a linear regressive equation, with the concentration represented by *x* and inhibition percent by *y* [9].

The inhibition test of Advanced Gycation End-Products (AGEs)

Analysis of anti-AGES activity was conducted *in vitro* using a modified Lee *et al*., (2006) method. ±50 mg of tin leaf extract were added into 10.0 mL (5000 µg/mL) measurable flask. Then, the solution was dissolved with a phosphate buffer, pH 7.4, to the line (the main liquid). This was piped in 5 µL, 10 µL, 50 µL, 100 µL, 500 µL and 1000 µL to obtain concentrations of 5 µl/mL, 10 µl/mL, 50 µl/mL, 100 µl/mL, 500 µl/mL and 1000 µl/mL. Then, a 5.0 mL test tube was prepared, for mixing 1.0 mL of tin leaf extract solution, 2.0 mL of BSA solution, 10 mg/ml, and 2.0 fructose glucose solution 0.2 M. The mixture was homogenized by closing it with aluminum foil. The solution was incubated at 37 ºC for 7 days. Absorption was measured at a maximum wavelength of 278.6 nm, using a UV-Vis spectrophotometer [10].

The determination of total phenol.

The *F. carica* Linn leaf extract was weighed, approximately 125 mg, and then was poured into a 25 mL measuring flask. The solution was dissolved with 2 mL of 96% ethanol and diluted with distilled water until it reach the mark. It could be filtered by filter paper if it is necessary. A total of 1.0 mL of solution, 80 µL of *Folin Ciocalteu* reactant was let stand for 8 min, 600 µL of 7.5% Sodium Carbonate, and water were added to a 25 mL flask. This micture was shaken and sterilized for a stable period, then absorption was measured at maximum wavelength. Total phenol content was calculated by using the equation: Au = a + b. Cu regression line which was previously obtained from the standard curve of galat acid [7, 10].

Results

The *F. carica* Linn (local name tin) leaf has a shape reminiscent of a hand with long fingers. The plant grows as a singular unit, and has a light green appearance with a length of around 12 to 25 cm high and a width of around 10 to 18 cm. The leaves have a fuzzy texture, and the plant bares fruit year round.

Fig 1. *F. carica* Linn

The strength of the antioxidant activity 96% ethanol tin leaf extract was examined using the DPPH method, to see the extent to which the extract can prevent free radical production and AGEs by calculating IC_{50}. This number was then compared to quersetin as positive control.

Table 1: The result of antioxidant activity test by DPPH method and the test of inhibition activity of the AGEs compound formation from *F. carica* Linn leaf ethanol extract seen from the IC_{50} value

Sample	IC_{50} (mg/ml) ± SD	Antioxidant capacity	IC_{50} (mg/ml) ± SD	Anti-AGEs capacity
Ethanolic extract of *F. carica* Linn leaves	58.68 ± 0.234	Strong	523.56 ± 9.87	Moderate
Quercetin	0.098 ± 0.001	Strong	361.69±12.34	Strong

From the DPPH antioxidant activity test, and quersetin as positive control above, the value of IC_{50} obtained amount to 0.098 which means the ethanolic extract of *F. carica* Linn leaves has very strong antioxidant activity. The smaller the

value of IC_{50}, the stronger the ability of a compound to prevent free radicals. The IC_{50} value of quercetin is used as a comparison to determine how strong 96% ethanol extract of tin leaf (*Ficus carica* Linn.) is in counteracting free radicals.

Table 2: The data result of total phenol of 96% ethanol extract of *Ficus carica* Linn leaf

Sample	Repetition	Weight (mg)	Absorption	Value (%)	Average (%)
96% ethanol extract of *F. carica* Linn leaf	I	125.1	0.5553	0.72	0.71
	II	126.4	0.5527	0.71	
	III	125.5	0.5426	0.69	

From Table 2., it can be seen that the total phenol content in 96% ethanol extract of leaf tin is 0.71%. It generates the antioxidant power in *Ficus carica* Linn leaf extract. The total phenolic content of each plant may also differ based on where it grows. This could be determined by using Folin Ciocalteau. These reagents are used because phenolic compounds react with folin to form a measurable color solution. The principle of the Folin Ciocalteau method is that the formation of blue compounds can be measured at a 760 nm of wavelength. This reactant oxidizes phenolics (alkali salts) or phenolic-hydroxy groups, reducing the heteropolyac acid (phosphomolibdat-phosphotungstat) contained in the Folin Ciocalteau reagent into a tungsten-molybdenum complex. The phenolic compound reacts with the Folin Ciocalteau reagent only in an alkaline atmosphere, causing proton dissociation in the phenolic compound, creating phenolic ions. In order to facilitate the basic conditions, Na2CO3 7.5% is used. The hydroxyl group of phenolic compounds which reacts with Folin-Ciocalteau reagents forms a blue molybdenum-tungsten complex that can be detected by a spectrophotometer. The greater the concentration of phenolic compounds, the more phenolic ions reduce the heteropoly acid (phosphomolibdat-phosphotungstat) into a molybdenum-tungsten complex that makes the blue color more concentrated [7, 12].

Discussion

In this study, the antioxidant activity of 96% ethanol extract of tin leaf's IC_{50} value of 58,68µg/mL. This shows that tin ethanol extract is capable of inhibiting free radical production. There was also research conducted by Agustina E. (2017) that mentioned tin leaf extract with water methanol solvent had a very strong antioxidant activity level, with IC_{50} value 13.61 µg/mL[12]. Natural components such as phenolic compounds work non-enzymatically. Several researchers report the capability of total phenolic compounds when it comes to protecting DNA from free radical attack. These compounds work by involving the chelating processes of metal ions such as copper and iron. Phenolic compounds such as flavonoids form the complexes with copper or iron, preventing the activation of ROS. It is also able to resist the free radical attacks as *OH, H2O2 dan O2*. Phenolic compounds contained in the tin leaf are able to cut the chain reaction of free radicals or scavenger free radicals, rendering them inert. Phenolic compounds also have an ability to donate hydrogen atoms and form a relatively radical compound that is more stable [12]. Phenolic compounds play an important role as antioxidants by forming cleates with metal ions compounds such as quercetin will bind to copper ions and form chelates. But quercetin was known to be carcinogenic in high concentrations, and may damage the DNA sequence.

Phenolic compounds have antioxidant characteristics and can absorb the free radical as DPPH by providing a hydrogen atom or by electron donation. Through a donation of hydrogen atoms and electrons, phenolic compounds are capable of capturing free radicals and converting them into non-radical compounds or more stable radical compounds [13]. The flavonoid in phenolic compounds is also a potential antioxidant. The inhibit the process of fat oxidation in the body. This ability is related to the mechanism of action, by forming chelates with metal ions such as copper and iron. These metal ions are useful for catalyzing O_2^- into radical compounds H_2O_2 [14].

The average value of IC_{50} obtained in the AGEs inhibitory activity test on *F. carica* Linn leaf extract was 523.56 ± 9.87µg/ml. This shows that 96% ethanol extract in tin leaf has medium strength as an inhibitor of Advanced Glycation End-Products, while quercetin, the positive control, has an IC_{50} value of 391.69 ± 80.70 µg/ml, indicating that quercetin is strong in the inhibition AGEs formation. Antioxidant compounds are one of the mechanisms in the AGEs inhibition process. These compounds are s thought to work by inhibiting the process of amadori product formation from the schiff base and preventing auto-oxidation. These two processes produce precursors AGEs formation,such as methylglioxal and another unstable sugars. Antioxidant compounds also prevent the process of carbonylation of glucose, thus further inhibiting the formation of AGEs [15].

Advanced Glycation End-Products (AGEs) are a heterogeneous molecule group formed during the Maillard Reaction. This non-enzymatic reaction involves reducing sugars such as glucose or ribose and the primary amino group of proteins (lysine, arginine). It is formed by the reaction between high-concentration sugar and protein molecules [15, 16].

The accumulation of AGEs in the body can aggravate pathophysiological conditions. The principle of the inhibitory mechanism of the AGEs compound is stopping the reaction of the dicarbonyl compounds and impeding th formation of the AGEs precursors. Mechanisms of the AGEs compounds inhibition can be accomplished in several ways:1. Inhibition of the formation of ROS compounds in the glycation process; 2. inhibition of the Schiff base pathway and the production of amadori products, inhibiting formation of dicarbonyl compounds; 3. detoxifying precursors of AGEs, such as methylgioxal by glycosylase; 4. inhibition of the formation of AGEs precursors, which result from amadorous products; and 5. influencing AGEs to bind with their receptors. Based on its AGEs inhibition strength, the anti-glastic compounds can be classified as either strong (having an IC_{50} below 500 µg/ml) pr weak (having an IC_{50} of 500-1000 µg/ml). The latter, it is said to have no activity as AGEs inhibitors when IC_{50} has more than 1000 µg/ml [16, 17].

The compound with good antioxidant activity could potentially inhibit the formation of AGEs. The association between antioxidants and the inhibition of AGEs is surmised from free radical pathways' assistance in the formation of AGEs. This, including ROS activation, could cause AGEs precursors such as methylglioxal, 1-deoxyglucosone, and glyoxal. The inhibition of the AGEs precursor affects the AGEs compound produced. The precursor passes through a dicarbonyl line, that changes the precursor compound into an end-product in the form of AGEs [18, 19]. The increased

oxidative stress leads to changes in various protein and lipid compounds forming a reactive radical compound that could damage the cells [20].

Conclusion

In this study, the 96% ethanol extract of tin leaf was also found to be active in inhibiting the formation of AGEs compounds as seen from IC_{50} values of less than 1000 µg/ml. The 96% ethanol extract of tin leaf has an IC_{50} value of 750, 29 µg/ml, so *F. carica* Linn leaf extract is classified as moderate in the inhibition of the AGEs compound.

Meanwhile IC_{50} value of extract of *F. carica* Linn leaf on the antioxidant test was 55,38 µg/ml, considered strong in inhibiting free radicals. This might happen because the inhibitory process of AGEs is partial with antioxidant activity. Although there is a connection between them, these antioxidant pathways only have a minor role in inhibiting AGEs.

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