Antioxidant Activity of *Amaranthus spinosus* L. (Spiny Pigweed) and *Annona squamosa* L. (Custard Apples)

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ABSTRACT

Aim : The aim of this research was to evaluate antioxidant activity of spiny pigweed and custard apples leaves by using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity with ascorbic acid as standard drug.

Methods : Extraction of leaves was perfomed by maceration method using ethanol 70% as a solvent. Identification of secondary metabolites was executed by phytochemicals screening.

Results : Based on the experiments, the extract of custard apples showed higher activity than spiny pigweed extract with their IC_{50} values were 48.63 µg/mL and 272.09 µg/mL, respectively. Furthermore, the results of phytochemicals screening showed that the extracts contained alkaloid, polyphenolic, tannin, flavonoid, and steroid.

Conclusion : The custard apples extract had strongly active and the spiny pigweed extract had weak antioxidant activity. Based on qualitative assay, the flavonoid compound had antioxidant activity.

Key words: Antioxidant, DPPH, custard apples, spiny pigweed.

INTRODUCTION

Ayurvedic or commonly known as traditional medicine is the oldest available method of treatment in India. The main aim of *ayurvedic* is the preservation of health for normal persons and treatment of sick individuals using only natural methods and sources [1].

A compounds from some of the herbs used in the treatment of *Ayurveda* such as *Amaranthus spinosus* (Spiny pigweed) and *Annona squamosa* (Custard apples) are antioxidant agents. In *Ayurveda* treatment, *Amaranthus spinous* leaf is used to treat rheumatic pain, ulcers and joint inflammation [2]. *Amaranthus spinosus* has been shown to have excellent free radical retention system through superoxide dismutase analysis, catalase, ascorbic peroxidase, glutathione reductase and phenolic peroxidase enzyme activity [3].

Annona squamosa leaves are used to treat ulcers and wounds traditionally. Beside that, *Annona squamosa* leaf water extract had antioxidant activity as evidenced by increased scavenging enzyme and superoxide dismutase (SOD) activity [1,4].

Free radicals are molecules that unstable and highly reactive because they contain unpaired electrons in their outermost orbital. To achieve the stability of atoms or molecules, free radicals will react with the surrounding molecules to obtain an electron pair. This reaction will take place continuously in the body and if not stopped it will cause various diseases such as cancer, arteriosclerosis, heart disease, cataracts, premature aging, and other degenerative diseases caused by tissue damage due to oxidation. Thus, an antioxidant is required to capture free radicals that can not induce these diseases [5].

Antioxidants are compounds which could prevent dangerous agents that generated from the oxidation reaction. These compounds may serve to inhibit the possibility of degenerative diseases [6]. Free radicals can be prevented with antioxidant compounds [7].

One of the most commonly used antioxidant activity assays is through free radical scavenging using *2,2-diphenyl-1-picrylhydrazyl* (DPPH) radicals. The activity is known from a measurement of uptake by UV-Vis spectrophotometry [8].

The DPPH is a free radical compound that is stable in its storage, stored in dry form and in good storage conditions. This method is quite simple and easy to work [9]. The strength or absence of antioxidants

can be measured by IC_{50} value. The IC_{50} is a value that indicating the concentration of extract (µg/mL) or ppm which is capable of inhibiting 50% oxidation [8].

MATERIAL AND METHODS

Plants Material

The fresh leaves of *A. spinosus* (Spiny pigweed) and *A. squamosa* (Custard apples) were collected from Manoko-Lembang, West Java, Indonesia.

Extraction

The dried plant material was powdered by using a grinder. It was extracted using maceration method with ethanol 70% as a solvent. The maceration method has been used for 3 x 24 hours with solvent replacement every 24 hours. Then, the solvent was evaporated under reduced pressure using rotary evaporator, and a greenish-black colored sticky residue were obtained. The amounts of dried plant and precentage of yield are presented in Table 1.

Phytochemical Screening

The crude ethanolic extracts of *A. spinosus* and *A. squamosa* leaves were tested for the presence of alkaloid, phenolic, tannin, flavonoid, monoterpenoid, sesquiterpenoid, steroid, triterpenoid, quinone and saponin [10] with some modification.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of the plant's extracts and preparation of DPPH solution were adopted from with making some modifications [11]. The diluted working solutions of the test extracts and DPPH were prepared in ethanol. Each extract (various concentrations) was mixed with DPPH solution at a concentration of 50 µg/mL (1:1) to initiate the reaction. After 30 minutes of incubation, the absorbance was read at λ518 nm wavelength using spectrophotometer Ultraviolet-visible. Ethanol p.a. (1.5 mL) and DPPH solution (50 µg/ml) (1:1) were used as a blank. Based on the reduction of DPPH absorbance, the antioxidant activity of each extract was determined by calculating a percentage of radical scavenging activity, as follow :

$$Radical\ scavenging\ activity\ (\%) = \frac{Ao - As}{Ao} x100$$

Where Ao was absorbance of DPPH in ethanol and As was absorbance of DPPH in ethanol plus sample solution (1 : 1). IC_{50} was obtained by plotting the correlation of extract concentration and radical scavenging activity (%). Ascorbic acid was used as the reference. The analysis was done in triplicate for each extracts and standard.

Thin Layer Chromatography (TLC)

The qualitative evaluation by using TLC was performed on the extract that has higher antioxidant activity. The compound that has antioxidant activity will provide a yellow spot with a dark purple background in TLC after sprayed by DPPH (0.2%) and $AlCl_3$ was used to identified flavonoid.

Table 1. Result of Extraction

Extract	Weight of crude extract (g)	Weight of dried plants (g)	Rendement (%)
A. spinosus	160,49	612,00	26,22
A. squamosa	342,07	990,00	34,55

RESULT AND DISCUSSION

The phytochemical screening of crude ethanolic extracts of *A. spinosus* and *A. squamosa* revealed the presence of some secondary metabolites such as alkaloid, polyphenolic, tannin, flavonoid, and steroid as shown in Table 2.

Quinone was detected in the *A. Squamosa* but not in the *A. Spinosus* leaves extract. The phytochemical compounds detected are known to have medicinal importance. For example, alkaloids have been reported as powerful poison and many alkaloids derived from medicinal plants show biological activities like anti-inflammatory, cytotoxicity, antispasmodic and pharmacological effects. Similarly, steroids derived from plants are known to have cardiotonic effect and also possess antibacterial and insecticidal properties They are very often used in medicines due to their well-known biological activities. Tannins, according to research, are known to have antibacterial antitumor and antiviral activities. They work by precipitating microbial protein thus making nutritional protein unavailable for them [12]. Especially, phenolic and flavonoid have been reported as antioxidant agents [13,14]. The results of antioxidant activity with DPPH radical scavenging assay are presented in Table 3.

Table 2. Results of phytochemical screening of ethanolic extract plants

Metabolites	*A. spinosus*	*A. squamosa*
Alkaloid	+	+
Phenolic	+	+
Tannin	+	+
Flavonoid	+	+
Monoterpenoid & Sesqui-terpenoid	-	-
Steroid	+	+
Triterpenoid	-	-
Quinone	-	+
Saponin	+	-

+ = presence , - = absence

Table 3. Results of antioxidant activity of plant extract

Sample	Concentration (μg/mL)	Inhibition (%)	Linear Regression	IC_{50} (μg/mL)
A. squamosa	10	9,99	y = 1.0643x - 1.753 R^2 = 0.9913	48,63
	20	18,71		
	30	30,31		
	40	38,62		
	50	53,25		
A. spinosus	100	17,28	y = 0.2067x - 6.242 R^2 = 0.9771	272,09
	150	23,04		
	200	32,37		
	250	44,59		
	300	58,17		
Ascorbic acid	2	3,22	y = 6.515x - 9.528 R^2 = 0.9963	9,14
	4	15,64		
	6	31,77		
	8	41,98		
	10	55,20		

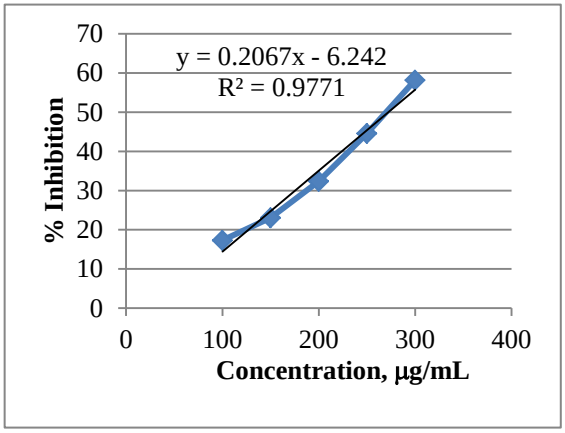
Water, methanol and ethanol are solvents commonly used in extraction processes. Since the antioxidant compounds found in plants have different polarities, the antioxidant activity of the extract and the yield depends on the selected solvent [15]. Based on the results obtained in Table 3, it can be seen that a percent of inhibition was directly proportional to concentration. The higher concentration gave a higher antioxidant activity as well.

The absorbance was inversely proportional to concentration. The linear regression curve of sample is attached in Figures 1. The result showed that *A. squamosa* ethanolic extract had higher antioxidant activity compared to *A. spinosus* ethanolic extract, with IC_{50} value are 48,63 and 272,09 μg/mL, respectively. The

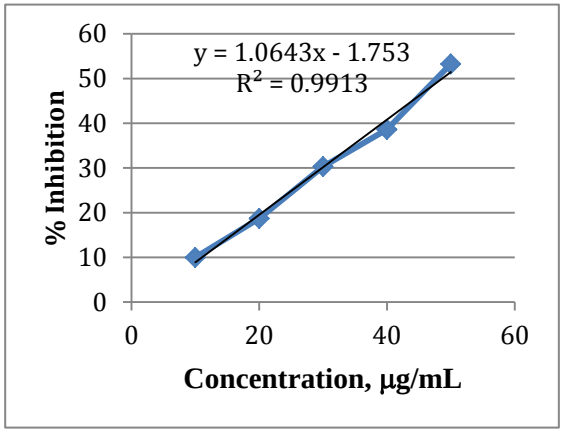
antioxidant activity with an IC_{50} value of <10 µg/mL could be considered as a powerful antioxidant, IC_{50} value of 10-50 µg/mL could be considered as a strongly active, > 50-100 µg/mL moderately active, 100-250 µg/mL weakly active and 250 µg/mL is inactive [16].

The qualitative evaluation by using TLC is performed on the extract that had higher antioxidant activity, and it was *A. squamosa* ethanolic extract. The thin layer chromatography profile was observed using a Silica GF_{254} plate with mobile phase buthanol : acetic acid : water (4 : 1 : 5). The results of spot from TLC, can be seen in Table 4.

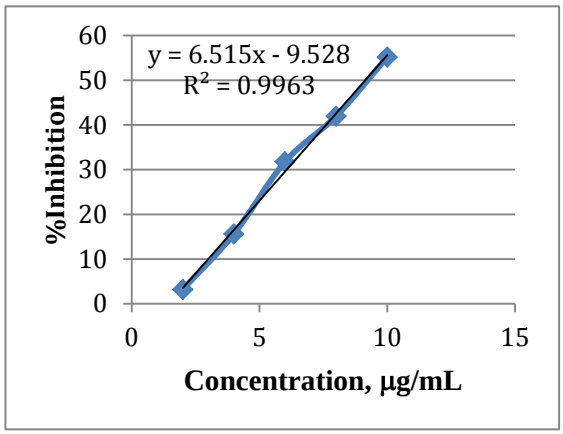
Based the results obtained, it was suspected that spots 5 and 6 are flavonoid because after sprayed by $AlCl_3$, fluorescent greenish-yellow spot can be seen under UV λ366 nm. Then, confirmed by spraying a DPPH (0,2%) as spray detect agent, it spot was found to be bright yellow with a dark purple background. Thus, the spot 5 suspected of isoflavones, flavanones or flavonols. The spot 6 is suspected that the auron from flavonoid [17]. The thin layer chromatography can be seen in Figure 2.



A. spinosus



A. squamosa



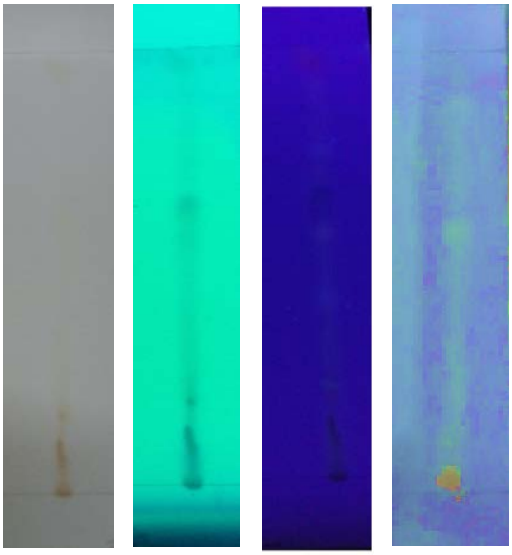
Ascorbic Acid

Figures 1. The linear regression curve of sample and reference drug

Table 4. The thin layer chromatography profile of *A. squamosa* ethanolic extract

Spot	Rf	Visual	UV 254nm	UV 366nm	$AlCl_3$ (366nm)	DPPH
1	0,19	Yellow	Brown	Yellow	Yellow	Yellow
2	0,36	-	-	Blue	Blue	Yellow
3	0,50	-	-	Blue	Blue	Yellow
4	0,60	-	Yellow	-	-	Yellow
5	0,71	-	-	Blue	Greenish-Yellow	Bright Yellow

6	0,88	Yellow	-	Yellow	Greenish-Yellow	Bright Yellow
7	0,94	-	Yellow	Pink	Pink	Yellow



a b c d

Figure 2. The tlc observed by (a) visible light ; (b) under UV 254 nm ; (c) under UV 366 nm ; (d) DPPH (0,2%)

The configuration, substitution, and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability. The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure. The B ring hydroxyl configuration is the most significant determinant of scavenging of ROS and RNS because it donates hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoids radical. Occurrence, position, structure, and total number of sugar moieties in flavonoid (flavonoids glycosides) play an important role in antioxidant activity. Aglycones are more potent antioxidants than their corresponding glycosides. The glycosides are usually weaker antioxidants than aglycones, bioavailability is sometimes enhanced by a glucose moiety. In the diet, flavonoid glycosidic moieties occur most frequently at the 3- or 7-position [12].

CONCLUSION

The results showed that both of ethanol extracts had antioxidant activity. Ethanol extract of custard apples leaves gave the highest antioxidant activity with IC_{50} value of 48,63 µg/mL, followed by spiny pigweed extract with IC_{50} value of 272,09 µg/mL. The extract custard apples had strongly active and the spiny pigweed extract had weak antioxidant activity. Then, the phytochemicals screening showed that the extracts contained alkaloid, polyphenolic tannin, flavonoid, and steroid. From qualitative using TLC, the suspected metabolite as antioxidant agent is flavonoid.

Confllict of interest : The authors declare that they have no conflict of interests.

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