ANTIOXIDANT ACTIVITY OF *JATROPHA Curcas* AND *JATROPHA Gossypifolia* BY DPPH METHOD

Aktivitas Antioksidan *Jatropha curcas* Dan *Jatropha gossypifolia* Dengan Metode DPPH

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ABSTRACT

Heart disease, cancer, diabetes, ischemia, and aging are diseases caused due to oxidative stress condition because the accumulation of free radicals. The negative effect of free radicals in the body can be resisted or scavenged by antioxidants. *Jatropa sp*. belongs to the family Euphorbiaceae contained alkaloids, tannins, flavonoids, steroid, saponins, and phenol compounds.This study has been detemined for an antioxidant activity both of *J.curcas* and *J.gossypifolia*. Extraction was performed by maceration methode. The ethanol extracts were vaporated using rotavapor. The antioxidant capacities were tested using DPPH assay. The results showed that *Jatropha curcas* had activity as an antioxidant with a very active intensity can be found in the parts of stem bark. Whereas in the *J.gossypifolia* plants that had an antioxidant activity with a very active intensity were the leaves and stem bark.
Key words : Antioxidant, *J.curcas*, *J.gossypifolia*, DPPH method.

ABSTRAK

Penyakit jantung, kanker, diabetes, iskemia, dan penuaan merupakan penyakit yang disebabkan akibat kondisi stres oksidatif yakni terdapat akumulasi radikal bebas. Pengaruh negatif radikal bebas didalam tubuh dapat ditangkal atau diredam oleh senyawa antioksidan. Jatropha sp. merupakan tanaman yang berasal dari familia Euphorbiaceae yang memiliki kandungan golongan senyawa alkaloida, tannin, flavonoida, steroida, saponin, dan fenol. Pada penelitian ini bertujuan untuk menentukan aktivitas antioksidan dati tanaman *J.curcas* dan *J.gossypifolia.* Proses ekstraksi menggunakan metode maserasi. Ekstak etanol dipekatkan mengunakan rotavapor. Kemampuan antioksidan diuji dengan mengunakan metode DPPH. Hasil menunjukkan bahwa pada tanaman *J.curcas,* memiliki aktivitas antioksidan dengan intensitas sangat aktif pada bagian kulit batang. Sedangkan pada tanaman *J.gossypifolia*, aktivitas antioksidan dengan intesitas sangat aktif pada bagian daun dan kulit batang.
Kata kunci : Antioksidan, *J.curcas*, *J.gossypifolia*, Metode DPPH.

INTRODUCTION

The oxidative stress is a condition where the amounts of free radicals in the body has exceeded the limits of body ability to neutralize the free radicals. This condition can lead to increased the oxidation processes in cells that may be associated with an overtly pathological conditions. Heart disease, cancer, diabetes, ischemia, and aging are some of the diseases that appear as a result of oxidative stress conditions (Valko *et al*., 2007). The condition appears when the number of free radicals in the body can not be neutralized by the body. The negative effect of free radicals in the body can be resisted or scavenged by antioxidants. Source of antioxidants were found in many plants are rich in phenolic compounds (flavonoids, phenolic acids, tocopherols), nitrogen compounds (alkaloids, chlorophyll derivate, amino acids, amines), derivatives of cinnamic acid, ascorbic acid and carotenoids (Pratt & Hudson, 1990; Rufino *et al*. 2010).

Jatropha sp. is a plant belongs to the family Euphorbiaceae that contained secondary metabolites include alkaloids, tannins, flavonoids, steroid, saponins and phenolic (Khafagy *et al*., 1977; Nwokocha *et al*., 2011; Gupta *et al*., 2011). According to research conducted by Rufino *et al*. (2010), the plants have an antioxidant activity, if it has metabolites of anthocyanins, carotenoids and phenolic

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compounds. So, based on the metabolites compound, *Jatropha sp*. plants have a potential as antioxidants.

To support its development as an antioxidant, we conducted a study of antioxidant activity of ethanolic extracts from leaves, fruits, stem bark and roots of *J.curcas* and *J.gossypifolia* were determined by using in vitro DPPH method. Some parts of the plant that have an antioxidant activity, were identified of their secondary metabolites by Thin Layer Chromatography method. The aim of this study was to provide scientific information about the mechanisms of antioxidants from both *J.curcas* and *J.gossypifolia*. plants as well as obtaining drug raw materials to be used as herbal products.

MATERIALS AND METHODS

This research was quantitative and qualitative. Bioassay guided extraction approach applied in this study, in order to obtain extracts which have been ability as an antioxidant.

Materials and Tools

The plant materials of *Jatropha curcas* and *Jatropha gossypifolia* were collected from from Kecamatan of Grati, Pasuruan, East Java. The plants were determined by UPT of Materia Medica, Batu, East Java.

Materials: Ascorbic acid (Merck); Ethanol 96% (technical) ; distilled water; methanol p.a; hexane p.a; chloroform p.a; diphenylpicryl-hydrazyl (DPPH); Dragendorf; Anisaldehyde-H_2SO_4, $FeCl_3$; H_2SO_4; KOH; TLC plates of Kiesel gel F254 Merck.

Instruments: Spectrophotometric UV-Vis (Shimadzu), rotary evaporator (Buchi), oven, analytical balance, extraction vessel, glassware, chamber, UV lamp.

Procedures

Preparation of Sample

The leaves, fruit, stem bark and roots of *J. curcas* L and *J.gossypifolia* L were dried in the oven at 40°C. The dried simplicia were milled to obtain the fine powders.

Extraction Method

The 100 g of powder leaves of *J.curcas* L plant. were macerated by using ethanol 96% as much as 1000 mL. Maceration process was conducted by stirring for 4 hours at a speed of 350 rpm. Then the filtrates and residues were separated. The residues were remacerated twice to the same treatment. The filtrates were collected and evaporated using rotary evaporator to obtain the thick extracts from the sample of *J.curcas* L leaves. The thick extracts were dried in an oven at 40°C. The extraction process from sample of stem bark, fruits, roots of *J.curcas* L and *J.gossypifolia* plants, also used the same extraction method as well as extraction the sample of *J. curcas* L leaves.

Determination of Antioxidant Activities

Into each test solution at concentration series, were added 1 ml solution of 0.4 mM DPPH. Then filled up with methanol to final volume 5.0 ml and homogenized. The sample solutions were incubated at 37°C in the dark place for a certain time. Absorbance was measured at wavelength of 516 nm by using spectrophotometric UV-VIS (Shimadzu). The experiment was performed for 3 times of replication at each concentration series. Positive control in this study used vitamin C.

The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using linear dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using

Table 1. The characteristics of powder of *J.curcas* and *J.gossypifolia*.

No	Samples	Organoleptic	Number of siever	Moisture content
1	*J.curcas* leaves	Dark green	60/40	7,94 %
2	*J.curcas* fruits	Light brown	40/20	2,76 %
3	*J.curcas* stem bark	Light brown	60/40	6,13 %
4	*J.curcas* roots	White	40/20	7,89 %
5	*J.gossypifolia* leaves	Green brownish	60/40	8,78 %
6	*J.gossypifolia* fruits	Brown	40/20	7,52%
7	*J.gossypifolia* stem bark	Light brown	60/40	6,06%
8	*J.gossypifolia* roots	White	40/20	5,27%

Table 2. Results of extraction of *J.curcas* and *J. gossypifolia*.

No	Samples	Organoleptic	Yield
1	*J.curcas* leaves	Dark green	9,86 %
2	*J.curcas* fruits	Brown greenish	7,50 %
3	*J.curcas* stem bark	Brown blackish	4,76 %
4	*J.curcas* roots	Dark brown	9,10 %
5	*J.gossypifolia* leaves	Green brownish	9,20 %
6	*J. gossypifolia* fruits	Brown blackish	7,40%
7	*J.gossypifolia* stem bark	Dark brown	12,17%
8	*J.gossypifolia* roots	Dark brown	9,03 %

following equation:

DPPH scavenging effect (%) or Percent inhibition

$$= \frac{A0-A1}{A0} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard.

RESULTS AND DISCUSSION

Dried leaves, fruits, stem bark and roots from *J.*curcas and *J.gossypifolia* plant were milled to obtain the fine powders with organoleptic characteristics were presented in Table 1. Powder of leaves and stem bark were finer than those of fruits and roots. The water content of different powder ranged between 2.76 to 8.78%. Characteristic of ethanolic extract that obtained from maceration process of *J.curcas* and *J.gossypifolia* plants presented in Table 2.

Table 3. Potential of antioxidant activity of Ascorbic acid, ethanolic extract of *J.curcas* and *J. gossypifolia*.

No	Samples	IC_{50} (µg/mL)
1	Ascorbic Acid	2,25 ± 0,32
2	*J.curcas* leaves	79,57 ±7,6
3	*J.curcas* fruits	420,98 ± 77,57
4	*J.curcas* stem bark	26,44 ± 4,99
5	*J.curcas* roots	58,86 ±1,38
6	*J.gossypifolia* leaves	31,32 ±1,72
7	*J. gossypifolia* fruits	65,27 ± 3,70
8	*J.gossypifolia* stem bark	10,79 ± 1,56
9	*J.gossypifolia* roots	98,63±2,60

Based on the determination of antioxidant activity in Ascobic acid, the ethanolic extract of *J.curcas* L and *J.gossypifolia* L plants with DPPH method resulted an average of IC_{50} values that presented in Table 3 and Figure 1.

Classification of the antioxidant activity from a test substance according to Blois (1958), i.e. if the value of IC_{50} <50 µg/mL had a very active intensity; 50-100 µg/mL had an active intensity; 101-250 µg/mL had a moderate intensity; 250-500 µg/mL had a weak intensity; > 500 µg/mL had the inactive intensity. Based on

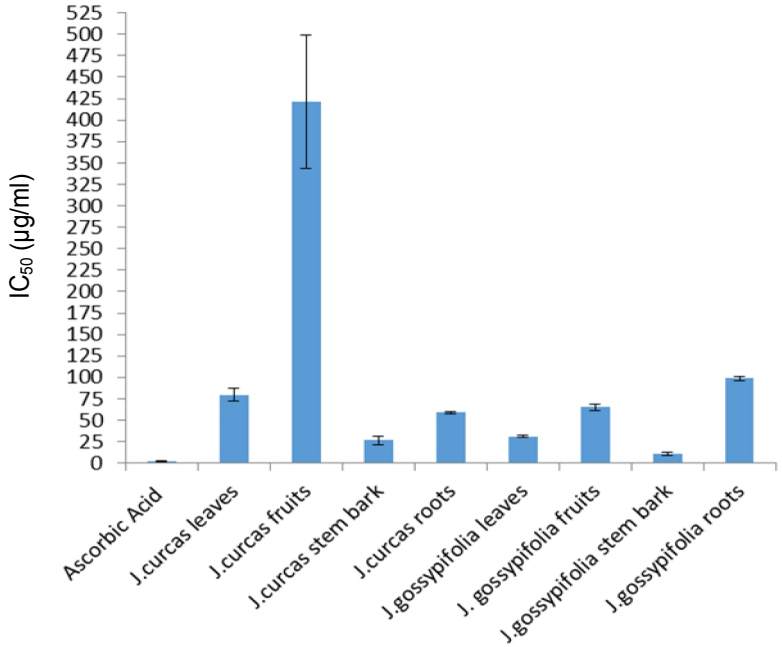

Figure 1. Antioxidant activity of Ascorbic acid, ethanolic extract of *J.curcas* and *J. Gossypifolia* by DPPH Assay.

the classification of Blois (1958), so the extracts that included of a very active intensity are the ethanolic extract of *J.curcas* L stem bark; the ethanolic extract of *J.gossypifolia* L leaves and stem bark that had IC_{50} values respectively by 26.44±4.99 µg/mL; 31.32±1.72 µg/mL; 10.79±1.56 µg/mL. Whereas the extracts that included of an active intensity are *J.curcas* L leaves and roots; *J.gossypifolia* L fruits and roots that had IC_{50} value respectively by 79.57±7.6 µg/mL; 58.86±1.38 µg/mL; 65.27±3.70 µg/mL; 98.63±2.60 µg/mL. The *J.curcas* L fruits had a weak intensity because the IC_{50} value was 420.98±77.57 µg/mL. Antioxidant activity of *J.curcas* and *J.gossypifolia was tested using DPPH has lower than Ascorbic Acid.*

CONCLUSION

Jatropha curcas L had activity as an antioxidant with a very active intensity can be found in the parts of stem bark. Whereas in the *J.gossypifolia* L plants that had an antioxidant activity with a very active intensity were the leaves and stem bark.

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