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Phytochemical screening and antioxidant activity from stem bark *Callicarapa arborea* Roxb

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ABSTRACT

Extraction of the bark of Callicarpa arborea Roxb begins using maceration method with methanol. Subsequently used partition method with a variety of solvent in accordance with the increase in solvent polarity that is : n-hexane, dikhloroform, ethyl acetate and n-butanol, sequentially separately. Results of phytochemical screening is phenolic, triterpenoids and coumarin compounds. Test the antioxidant activity using DPPH assay method against all extracts, giving the results: for hexane (IC50 =304.373, very weak), dichloromethana (IC50 =88.931, strong), ethyl acetate (IC50 = 4.578, very strong) and butanol extract (IC50 = 5.587, very strong).

Keywords: *Callicarpa arborea* Roxb; phytochemical screening; antioxidant activity.

INTRODUCTION

Callicarpa arborea Roxb included the Verbenaceae family is a small evergreen plant. The spread of this plant is in the Himalayan (Bhutan Khumaunto), India, Burma, South China, Indo-China, and Malaya [1]. . This plant in Bengkulu, Indonesia is known as "ketepung" and used as a traditional treatment for jaundice, while in India and Nepal used for fever, headache, stomachache, skin, and scorpion bites diseases [2].

Organic chemistry of natural materials plays a supportive field of bioindustry. Therefore, it is necessary to explore chemical compounds from plants itself [3]. This activity can provide a beneficial effect on the development of multiple plants for the production, preservation and tourism [4]. This study aimed to test the phytochemical screening and determining the value of antioxidant activity from Stem Bark *Callicarapa arborea* Roxb. This study is part of sustainable research.

Reactive oxygen species (ROS) such as hydroxyl radicals, singlet oxygen, and hydrogen peroxide is often produced as a byproduct of the reaction of the biological or exogenous factors. In the in vivo system, some of these ROS play a positive role as energy production, phagocytosis, cell growth regulation, and inter-signal, or the synthesis of biologically important compounds [5]. Oxidative stress occurs when free radicals or ROS exceed the antioxidant capacity of biological systems, they attack biological molecules such as lipids, proteins and nucleic acids that lead to tissue or cell injury. Oxidative stress has been associated with atherosclerotic diseases, cancer, diabetes, arthritis, reperfusion damage, and inflammation [6]. Many natural antioxidant compounds have the ability to dampen or change the ROS becomes stable compounds, such as phenolic compounds, nitrogen, terpenoids, vitamins, and other endogenous metabolites [7].

EXPERIMENTAL SECTION

2.1. Preparation

2.1.1. Chemical

Filter paper, destilat of n-hexane, dichloromethane, ethyl acetate, n-butanol, methanol, aquades and methanol p.a. Reagents for the phytochemical screening. DPPH (2,2-diphenyl-1-picrylhidrazyl), vitamine C and Thin layer chromatography (TLC).

2.1.2. Equipments

General chemistry glasswares, maceration flasks, separating funnel, rotary evaporator (Heidolph Laborota 4000), UV-visible spectrophotometer (Shimadzu UV -1700 PharmaSpec).

2.1.3. Plant

Plant used was taken from Park Rajolelo Forest, Bengkulu, Indonesia. The identification of plant was done in the laboratory of Biology Department, Faculty of Mathematics and Natural Science, University of Andalas. Fresh bark was dried at room temperature for 3 weeks. the sample is processed into powder by using a blender.

2.1.4. Extraction

Powdered sample (3,600 g) was macerated with methanol in erlenmeyer flasks for 24 hours while stirring a few times periodically, 6 repetitions. The filtrate was collected and filtered with a filter paper and then concentrated by using a rotary evaporator. The filtrate was collected and filtered with a filter paper and then concentrated by using a rotary evaporator at temperature of 50°C. Furthermore, the method of extraction solvents (partition) is used with a solvent hexane, DCM, EtOAc and butanol, respectively sequentially and separately. Each extract was separately collected and concentrated by using a rotary evaporator until dry. Dry extract was further treated to test the antioxidant activity.

2.2. Phytochemical Screening

phytochemical screening performed on all the extracts and uses methods or reagents suitable to detect all kinds of groups of compounds. Especially for the detection of phenolic compounds with $FeCl_3$ reagent; the other is flavonoids, triterpenoids, steroids, alkaloids, coumarin, saponins and tannins [8-10].

Terpenoids /Steroids Test (Liebermann-Burchard method)

A few drops of the extract were placed into a dropping plate, added a few drops of anhydride acetic acid and 1-2 drops of concentrated sulfuric acid. The occurrence of reddish pink indicates terpenoids and the purple color to blue or green indicates the presence of steroids.

Flavonoids Test (Synod method)

1 ml of extract incorporated in a test tube was added a few drops of concentrated hydrochloric acid and magnesium powder, shaked and heated for 5 minutes. The formation of red or orange color indicates the presence of flavonoids.

Coumarins test (NaOH method)

A few drops of extract were put in a dropping plate, added a few drops of 10% sodium hydroxide. The yellow color indicates coumarins.

Phenolics ($FeCl_3$ method)

A few drops of the extract were placed in the dropping plate, added a few drops of 5% ferry hydrochloric, stirred. The occurrence of red or dark red color indicates the presence of phenolics and black color bluish or bluish green indicates tannin compounds.

Saponins Test (foam method)

1 ml of extract incorporated in a test tube, was added 5 ml of water, shaked vigorously. Excessive foam formation and relatively stable showed saponin.

2.3. Antioxidant Activity with DPPH Method

DPPH method, the simple and inexpensive method, is based on the ability of antioxidants to inhibit free radicals DPPH by donating a hydrogen atom. PPH color change from purple to redish in this reaction, be used to determine the antioxidant activity of an antioxidant compounds [11]. This method uses a positive control for comparison or standard to determine the antioxidant activity of the sample. The principle is the reaction of hydrogen arrested by DPPH of antioxidant compounds and transformed into 1,1-diphenyl-2-pikrilhidrazin. In this study, ascorbic acid (vitamin C) was used as a control [12-14]. Antioxidant activity test used in accordance with such procedures are

already common, DPPH solution in methanol is used as the reference solution [15-17], a series of vitamin C concentration of the standard solution and the sample solution is modified.

DPPH solution (100 mg/mL) was made of in 250 ml of methanol. DPPH concentration on each seri of control solution and the sample solution was designed 25 mg/mL. Solution of vitamin C (1000 mg/mL) was made of in 10 ml of methanol. Solution series were designed by mixing ascorbic acid (1 ml of vitamin C + 1 ml DPPH + 2 ml of methanol) in 4 ml of a mixed solution, namely: 0.5; 1; 5; 10; 15; 25 and 50 ug/ml. The sample solution extract of n-hexane, dichloromethane (DCM), ethyl acetate, and butanol (1000 mg/mL) were made in 10 ml of methanol. Solution of sample series was designed with mixing (1 ml sample + 1 ml solution of DPPH + 2 ml of methanol) in 4 ml of a mixed solution, namely: 5; 10; 15; 25; 50; 100 and 150 pg/ml, in triplicate. Before measuring the absorbance of each member of the control solution series and sample solution, each solution was firstly incubated at room temperature and in the dark for 15 minutes. Measurements were made with a spectrophotometer at a wavelength of 518 nm. Absorbance values were transferred into percentage barriers to DPPH by the formula: [inhibition DPPH (%) = $(A_{blank} - A_{sample}/A_{blank})$ x 100 %]. Statistical analysis using Excel program.

DPPH absorbance value of 25 ug/ml at 518 nm was 0.538. Sample absorbance values were plotted against percent inhibition to obtain a linear equation. The IC_{50} value of the sample is the concentration of the sample required to inhibit 50% of the DPPH free radicals. The value was calculated from the linear regression equation "Y = aX + b" obtained through extrapolation of sample concentration (ug / mL) on the x-axis and barriers DPPH (%) on the y-axis. The smaller the IC_{50} value of a sample, the greater its antioxidant activity [18-19].

RESULTS AND DISCUSSION

2.1. Extraction and Phytochemical Screening

Extraction of the bark of Callicarpa arborea Roxb gave the following results: hexane 11.528 g (0.320%), DCM 8.220 g (0.220%), EtOAc 28.712 g (0.797%) and butanol Extracts 149.320 g (4.147%) compared to a sample of dry powder. These data indicate that the polar compounds content of the extract is relatively very much, followed by semi-polar compounds and so on for the non-polar compounds. Results of phytochemical screening can be seen at the Table 1 below. the level of antioxidant activity of an extract associated with the phenol content in the extract concerned.

Table 1. Results of Phytochemical screening of Solvent Variations from Stem Bark of *Callicarpa arborea* Roxb

Comp Class	hexane	DCM	EtOAc	Butanol
Triterpenoid	-	+	+	-
Steroid	-	-	-	-
Flavonoid	-	-	-	-
Coumarin	-	-	+	+
Phenolic	-	-	+	+
Saponin	-	-	-	-
Alkaloid	-	-	-	-

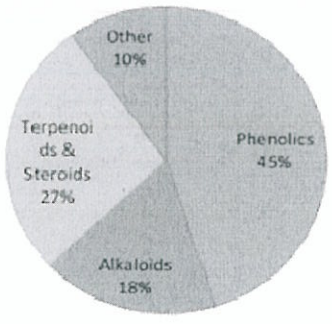

Figure 1. Pie chart representing the major groups of plant
Phytochemicals; source: M Saxena,IC Journal No 8192.

Type and sum of compounds from a plant is characteristic of the plant. Types of compounds contained in the stem bark of *Callicarpa arborea* Roxb relatively few. One group of compounds present in more than one solvent extraction. Phytochemical screening of extracts variation suggests that the phenolic compounds contained in EtOAc and butanol extracts, a positive reaction with FeCl3 shown by the red-black color. Triterpenoids compounds contained in of DCM and EtOAc extracts, while the coumarin compounds contained in of DCM, EtOAc extracts and butanol. All of this indicates that the polar properties of a group of compounds has a long range, so it is not

easy to separate from such a group of compounds in a solvent. Diversity compounds found in plants associated with the enzyme were also diverse. Different enzymes that produce compounds work in metabolism that occur in a plant. the polarity of a compound in a solvent and solvent-related properties of the same or almost the same.

Survey indicate that phenolics are the most numerous and diverse at sructure of plant phytocontituents (Figure 1 Pie chart) can be seen above [20].

3.2. Aktifitas Antioksidan

The results of the analysis of the antioxidant activity of samples are hexane (IC_{50}=304,373;very weak), DCM (IC_{50} = 88,946; strong), EtOAc (IC_{50} = 4,578; very strong) and butanol extract (IC_{50} = 5,587; very strong) and standard solution of vitamin C (IC_{50} = 4.74). Open to the fore that the series of concentration of the EtOAc extract, butanol and standards, should be lowered again, namely from 5-20 ug / mL, in order to show the real nature of the sample to bind DPPH. Therefore, the authors apply it to the linear regression curve in this research report. Linear regression charts for all of the extract (Figure 2-5) can be seen below.

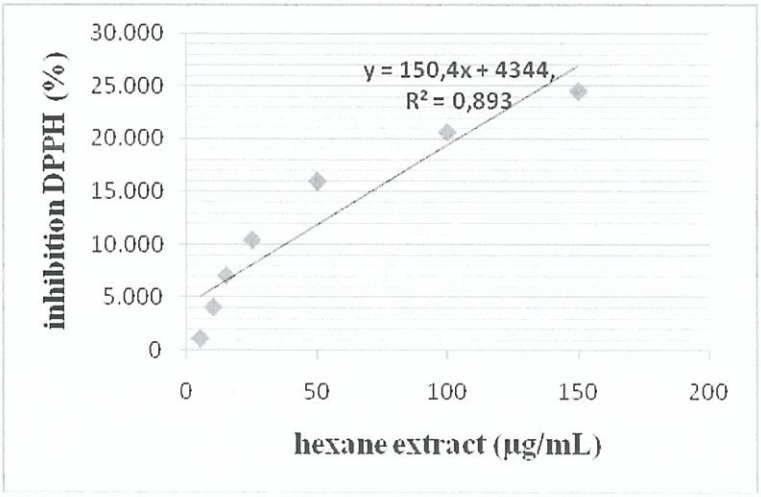

Figure 2. Linear regression chart of the antioxidant activity of hexane extract

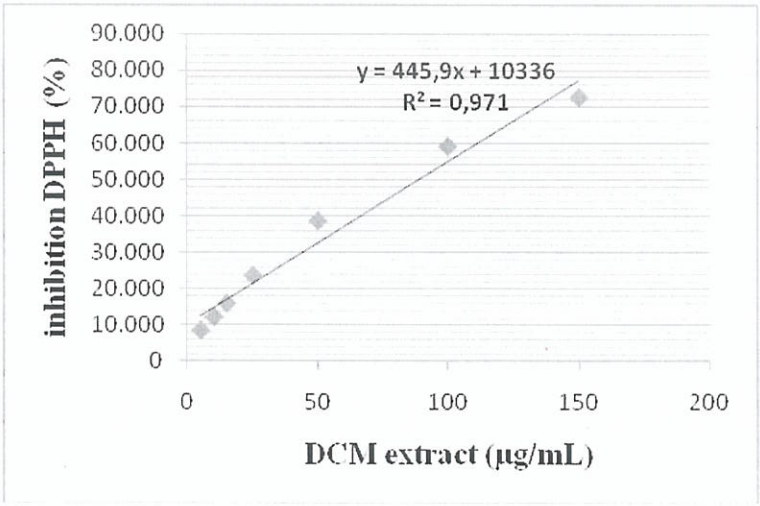

Figure 3. Linear regression chart of the antioxidant activity of DCM extract

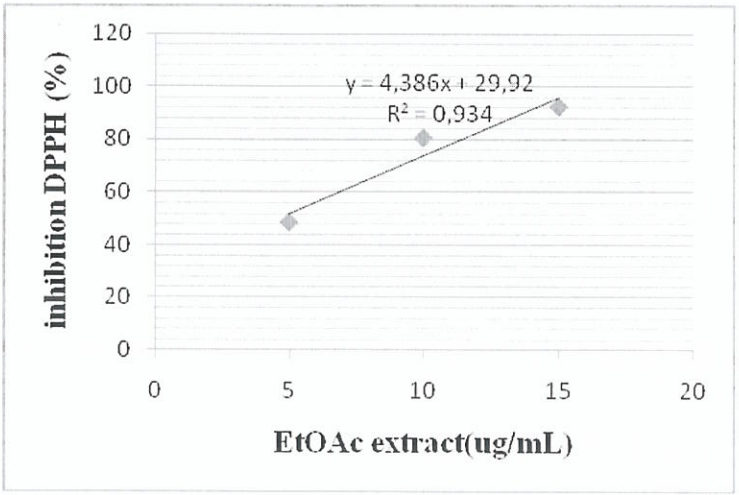

Figure 4. Linear regression chart of the antioxidant activity of EtOAcl extract

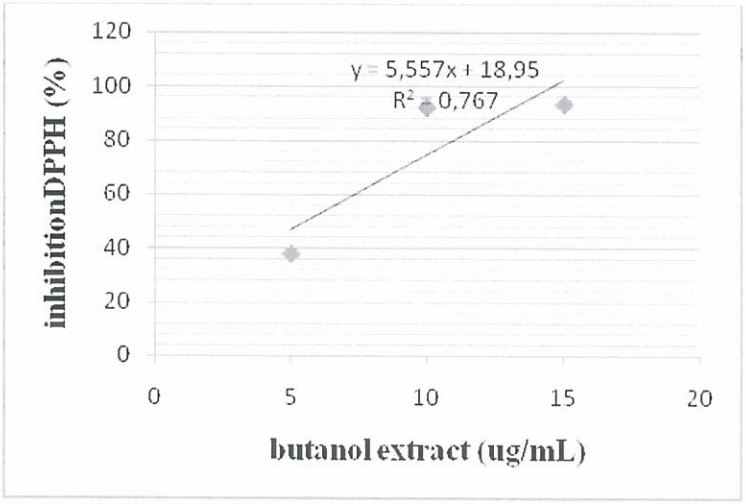

Figure 5. Linear regression chart of the antioxidant activity of butanol extract

EtOAc extract has the greatest antioxidant activity (IC50 small) of the extract another, even the antioxidant activity of the extract EtOAc larger than the standard used (vitamin C). The antioxidant activity of the extract butanol including large and other extracts decreased. It is strongly associated with the content of phenolic compounds in the extract, phenolic compounds in extracts of DCM and hexane is not detected, possibly due to little amounts.

Antioxidant activity assay of a sample is an oxidation-reduction reaction in which the sample will undergo oxidation and DPPH as the ROS is reduced. If the response is positive means that the sample contains a compound which has a functional group that can be oxidized, for example hydroxyl groups, aldehyde or a double bond and the other groups that can donate its electrons Phenolic compounds are compounds of large groups. Are simply the compounds formed by one or more hydroxyl groups attached to the aromatic rings, especially benzene. Phenolic compounds is the basic structure of all classes. Aromatic properties of the phenolic compounds to affect labile hydrogen atoms of hydroxyl. Sub-group of the phenolic compounds are: simple phenolics, phenolic acid linked, acetophenones, phenilacetic acid, cinamic, coumarins, chalcones, flavonoides, anthocianins, benzophenones, Quinones, stilbene, Tanine, polymers [21].

LC-MS technique has been successfully applied for the rapid separation and identification of the main components of the fractions of flowers and the rest of the plant Hypericum perforatum L. The compounds detected were representatives of three phenolic groups: flavonoids, naphthodianthrones and phloroglucinols. All fractions tests of antioxidant activity using DPPH method. The ability of activity varies, from very high to moderate. All samples showed the ability to bind DPPH radical, where most of them have IC50 values lower than the synthetic antioxidant BHT and BHA [22].

Ethanol extract of the roots, leaves and flowers of goat weed. The content of phenolic compounds and flavonoids were determined by spektorfotometer and provide quantitative results for phenolic and flavonoid compounds. DPPH method is one of them is used to test the antioxidant activity, suggesting that goat weed extract has the ability to scavenge free radicals and neutralize them. The antioxidant activity of the extracts of the leaves and flowers have a high ability, even flower extract whose activity exceeds the standards used (BHA). Extract obtained from the roots also showed antioxidant potency, although decreased when compared to other samples. The ability of the antioxidant activity of the extract relate to the amount of phenolic compounds and flavonoids [23].

Test three different solvents to determine the efficiency of their extraction from three species of blackberry: methanol, methanol-water heat and hot water. Determination of the content of phenolic compounds and phenolic acids used colorimetric method with a spectrophotometer. Total phenolics were tested by the method of Folin-Ciocalteau, which can estimate all flavonoids, anthocyanins and phenolics nonflavonoid, all phenolic compounds present in the extract. To test phenolic acids used Arnov's reagent. The content of phenolic compounds and phenolic acids showed the highest in methanol extracts, followed by hot water extract and hot methanol-water extract, consecutive. Antioxidants activity also showed association with quantity of compounds extracted, that is phenolic and phenolic acids [24].

CONCLUSION

Extraction of the bark of Callicarpa arborea Roxb gave the following results: hexane 11.528 g (0.320%), DCM 8.220 g (0.220%), EtOAc 28.712 g (0.797%) and butanol 149.320 g (4.147%) compared to a sample of dry powder. The phenolic compounds contained in extracts EtOAc and butanol. Triterpenoids compounds contained in of DCM and EtOAc extracts, while the coumarin compounds contained in extracts of DCM, EtOAc and butanol. The results of the analysis of the antioxidant activity of samples are hexane extract (IC_{50}=304,373; very weak), DCM (IC_{50} = 88,946; strong), EtOAc (IC_{50} = 4,578; very strong) and butanol extract (IC50 = 5,587; very strong) and standard solution of vitamin C (IC50 = 4.74).

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