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Phytochemical Screening and the Effect of Solvent Polarity on Antioxidant Activity of Kebiul Seed Extract (Caesalpinia bonduc (L) Roxb.) Using DPPH Method

Rina Desni Yetti*, Sindy Favorita, Rusdi, Zikra Azizah, Sestry Misfadhila, Ridho Asra, Boy Chandra, Gemmy Sarina, Meilinda Mustika, Henni Rosaini

Pharmacy, School of Pharmacy (STIFARM), Padang, Indonesia
E-mail : rinadesniyetti@gmail.com

ABSTRACT

Kebiul seeds have flavonoid compounds which are strong antioxidants so that they can prevent the formation of free radicals. The purpose of this research was to determine the chemical content and antioxidant activity of kebiul seed extract (*Caesalpinia bonduc* (L) Roxb.) based on the effect of solvent polarity. Kebiul seed extract was prepared using the maceration method using 96% ethanol, ethyl acetate and n-Hexane as solvents. Then phytochemical screening tests and antioxidant activity tests were carried out using the DPPH (*1,1-diphenyl-2- picrylhydrazil*) method. Ethanol extract can attract chemical compounds such as alkaloids, flavonoids, phenols, tannins, saponins and terpenoids. Meanwhile, ethyl acetate and n-Hexane extracts can attract chemical compounds such as alkaloids, saponins and steroids. The IC_{50} value of the 96% ethanol extract was 40.3619 µg/mL (very strong antioxidant <50 µg/mL), the ethyl acetate extract was 184.6667 µg/mL (moderate antioxidant 101-250 µg/mL) and the n-Hexane extract was 198.2069 µg/mL (moderate antioxidant 101-250 µg/mL). Can be conclude that 96% ethanol extract has the highest antioxidant activity with the lowest IC_{50}value.

Keywords : Kebiul Seeds; Maceration; Phytochemical Screening; DPPH

INTRODUCTION

The use of natural-sourced medicine in Indonesia is part of the culture and has been utilized by the community since centuries ago. However, in general, its safety and benefits or efficacy to health have not been fully supported by adequate research results. Given this and realizing that Indonesia as a country rich in medicinal plants and other natural-sourced materials, it is necessary to have a standard of these ingredients for public use in various purposes to achieve optimal health (*Ministry of Health of the Republic of Indonesia*, 2010).

One plant that can be used as medicine is kebiul seeds (*Caesalpinia bonduc* (L) Roxb.). Seedlings (*Caesalpinia bonduc* (L) Roxb.) belong to the family Fabaceae (Widodo *et al*., 2018). Traditionally seeded seed (*Caesalpinia bonduc* (L) Roxb.) can be used by the public as a drug for diabetes mellitus (Kannur *et al*., 2006), heat-lowering drugs, painkillers, anti-inflammatory drugs (Shukla et al., 2010), antifilaria (Gaur *et al.*, 2008), kidney stone drugs (Handayani & Yuliani, 2016), antibacterial and antifungal (Khan *et al*.,2011).

Kebiul seeds (*Caesalpinia bonduc* (L) Roxb.) contain chemical compounds namely alkaloids, flavonoids, saponins, triterpenoids (Dwitasari *et al.*, 2018), phytosterols, 20-24% fatty oil, starch, 2 filosterols (Sachan *et al.*, 2010). These flavonoid compounds are powerful antioxidants that can prevent the formation of free radicals (Sakihama *et al*., 2002). Antioxidants are compounds or chemical components that in certain levels or amounts can inhibit or slow down damage due to oxidation processes (Sayuti &Yenrina, 2015). A free radical is an unstable and highly reactive atom or molecule that has unpaired electrons in its outer orbital. Free radicals work by binding to molecules or cells in the body and are very dangerous because they can damage cells in the body and trigger disease (Badarinath *et al*.,2010).

One test to determine the antioxidant activity of free radical catchers is the DPPH (*1,1-Diphenyl-2- Picrylhydrazil*) method. TheDPPH (*1,1 Diphenyl-2-picrylhydrazyl*) method is one of the tests to determine the antioxidant activity of radical catchers. After reacting with antioxidant compounds, the DPPH will be reduced and the color will turn yellow, the change can be measured with a UV-Vis spectrophotometer (Sayuti & Yenrina, 2015).

The antioxidant activity test method against DPPH radicals (*1,1-diphenyl-2- picrylhydrazil*) was found to be the most effective and efficient among the three test methods used, while the FIC (*Ferrous IonChelating*) method was least effective and efficient due to its very low sensitivity and kelatnya power less than 20%. All three testmethods have a very high correlation (R>0.98), particularly between FRAP (*Ferric Reducing Antioxidant Power*) and DPPH (1,1-diphenyl- 2-picrylhydrazil) (Maesaroh *et al*., 2018).

Research conducted by Sachan *et al*., (2010) showed the antioxidant activity of the chloroform extract of castor seeds (*Caesalpinia bonduc* (L) Roxb.) with an IC_{50} value of 170 ±4.08 µg/mL, and the standard solution used was ascorbic acid with an IC_{50} value of 2.03 ± 0.16

µg/mL, the measurement was done spectrophotometry by measuring the uptake of each sample that had been reacted with a standard DPPH solution of 0.002% at a wavelength of 517 nm. Results obtained from the above research revealed the presence of antioxidant activity in the extract of chloroform castor seeds (*Caesalpinia bonduc* (L) Roxb.).

Antioxidant activity of castor seed ethanol extract (*Caesalpinia bonduc* (L) Roxb.) with an IC_{50} value of 74.73 µg/mL and a standard solution used ascorbic acid with an IC_{50} value of 26.68 µg/mL. The results obtained in this study clearly show that castor seeds (*Caesalpinia bonduc* (L) Roxb.) have significant potential for use as natural antioxidants (Shukla *et al*.,2009).

Based on the description above, researchers want to test on phytochemical screening and the effect of solvent polarity on antioxidant activity in castor seed extract (*Caesalpinia bonduc* (L) Roxb.) with the DPPHmethod (*1,1-difenil-2-pikrilhidazil*) using a UV- Vis spectrophotometer.

RESEARCH METHODS

Tools and materials

The tools used in the study are: Analytical Scales (Kern-ABC), Blender (Philips), Set of Rotary Evaporator Tools (Heidolph), UV-Vis Double Beam Spectrophotometry (UV-1700 PharmaSpec) and other glass tools.

Ingredients used: Dried Kebiul Fruit Seeds (*Caesalpinia bonduc* (L) Roxb.) refined, Ethanol 96% (Novalindo), Ethyl Acetate (Novalindo), n-Heksan (Novalindo), Aquadest (Novalindo), Sodium Chloride (NaCl) (10%), Iron(III) Chloride ($FeCl_3$), Sulfuric Acid (H_2SO_4) P, Anhydrous Acetic Acid $(CH_3CO)_2O$, Methanol (CH_3OH) p.a(PT.Bratachem),HydrochloricAcid (HCl)Magnesium,DPPH(*1,1Diphenyl-2- Picrilhydrazil*) (Sigma), Gallic Acid ($C_6H_8O_6$) (PT Bratachem),Mercury (II) Chloride P, Potassium Yodide P, Iodine P, Bismuth Nitrate P and Citric Acid.

WORKING PROCEDURE

Sampling

Samples were taken from Koto Gadang area, Padang Ganting Subdistrict, Tanah Datar Regency, West Sumatra Province.

Identification of Plants

Identification of Castor Seeds (*Caesalpinia bonduc* (L) Roxb.) conducted at Herbarium Andalas University (UNAND) Department of Biology Faculty of MIPA Andalas University.

Sample Preparation

The seedlings are washed on running tap water to remove the dirt that sticks, dried in the sun, broken from the outer skin and taken inside the seeds then mashed with a blender so that it is in the form of a finepowder.

Making Castor Seed Extract

Extraction of seed seed is done by maceration using ethanol solvents, ethyl acetate, and n- hexane. Weighed as much as 250 g of Kebiulseed powder then added solvent 1 Liter until simplisia submerged entirely. Extraction is carried out for 3x24 hours protected from light and stirring every 24 hours. On the 2nd and 3rd day the solvent is filtered for remassing so that 3 filtrates are obtained for each solvent. Filtrate is then evaluated with an evaporator rotary so that a thick extract of ethanol, ethyl acetate, and n- hexane from theseedlings.

Skrining Fitokimia Alkaloids

As much as 5 mL of seed extract (*Caesalpinia bonduc* (L) Roxb.) added 1 mL of 2N hydrochloric acid and 9 ml of water, heat over the water handler for 2 minutes, refrigerate and strain. The next mixture is divided. in 3different test tubes. Each test tube is dripped with 2 drops ofreagent.mayer in the first tube, in the second tube dripped 2 drops of dragendroff reagent and on the third tube dripped 2 drops of wegner reagent. The presence of alkaloid compounds if in the addition of mayer reagents formed white or yellow deposits, in the addition of dragendroff reagents formed reddish brown deposits and in the addition of wegner reagents formed reddish brown deposits (Ministry of Health of the Republic of Indonesia,1977).

Flavonoids

Steam to dry 1 mL of kebiul seed extract (*Caesalpinia bonduc* (L) Roxb.), the rest dissolved in 1 mL of 95% ethanol add 0.1 g of magnesium powder and 10 mL of hydrochloric acid P, in case of orange red to purple red, indicating the presence of flavonoids. If there is an orange yellow color then it indicates the presence of flavons, kalkon and auron (Ministry of Health of the Republic of Indonesia, 1977).

Saponins

Weigh 0.5 g of the extract, put it in a test tube add 10 mL of hot water, refrigerate and then shake firmly for 10 seconds. If a steady foam is formed for

not less than 10 minutes, as high as1 cm to 10 cm and at the addition of 1 drop of hydrochloric acid 2 N foam is not lost then the extract contains saponins (Ministry of Health of the Republic of Indonesia,1977).

Tannin

A total of 1 mL of kebiul seed extract (*Caesalpinia bonduc* (L) Roxb.) is added with 5 drops of 10% NaCl, then filtered. Filtrate obtained coupled with 3 drops of $FeCl_3$, if formed blue, blue - black and green indicates the presence of tannins (Tiwari *et al*.,2011).

Phenol

A total of 2 mL of kebiul seed extract (*Caesalpinia bonduc* (L) Roxb.) is added with 1-2 drops of iron solution (III) chloride 1%. When blue or purple is formed, it indicates the presence of phenolic compounds (Reiza *et al*., 2019).

Steroids/Terpenoids

As much as 1 mL of kebiul seed extract (*Caesalpinia bonduc* (L) Roxb.) added anhydrous acetic acid and concentrated H_2SO_4, positive results of steroid groups marked by changes in green or blue color and positive results of terpenoid groups if marked purple, orange or yellow color changes (Tukiran *et al*., 2016).

Test Antioxidant Activity with DPPH Method

DPPH Solution 30 µg/mL

Carefully weighed approximately 10 mg DPPH (BM 394.33). Then dissolved with methanol p.a up to 100 mL, then placed in a measuring pumpkin coated with aluminum foil. Simply apply the solvent to the limit mark then shake until homogeneous and obtained a solution of DPPH with a concentration of 100 µg / mL. Then diluted by 15 mL DPPH solution concentration of 100 µg / mL put in a measuring pumpkin 50 mL enough solvent to the limit mark then shake until homogeneous and obtained DPPH solution with a concentration of 30 µg / mL (Molyneux,2004).

Blanko Solution Creation optimization of DPPH Maksimun wavelength

3.8 mL solution DPPH 30 µg/mL into vial. Then added methanol p.a as much as 0.2 mL and homogenized and vial covered with aluminum foil. It is then incessed in a dark room for 30 minutes. Determine the absorption spectrum using a UV-Visible spketrofotometer at length waves are 400-800 nm and determine their maximum wavelength.

Making Galad Acid Comparison Solution

Weighed for 10 mg of gallic acid, put in a measuring gourd and then added methanol p.a to 100 mL, obtained a solution of 100 µg/mL of gallic acid. Nextmadeaseriesof concentrations of 4 µg/mL, 8 µg/mL, 12 µg/mL, 16 µg/mL and 20 µg/mL, by using a 100µg/mLmastersolutionof0.4mL,0.8mL,1.2 mL, 1.6 mL and 2.0 mL and then suffogged with methanol p.a to the 10 mL pumpkin limit mark. Then a 0.2 mL taken of each concentration is put in a vial and covered with allumunium foil, then add 3.8 mL of DPPH solution 30 µg/mL, inc silenced in a dark room for 30 minutes. Absorbance of various concentrations is measured using a UV-Visible spectrophotometer at a maximum wavelength of DPPH of 515.50 nm (Andayani *et al*., 2008).

Antioxidant Activity Testing

Ethanol Extract

Weighed ethanol extract as much as 100 mg, then dissolved with methanol p.a in the measuring pumpkin ad 100 mL, then obtained a concentration of 1000 µg/mL, thenpicked5.0 mL inserted into the measuring pumpkin 50 mL added methanol p.ad limit sign, so thatthe concentration of 100 µg/mL is obtained. Nextmadeaseriesofconcentrationsof40µg/mL,50µg/mL,60µg/mL,70µg/mLand80µg/mL, by 4 mL, 5 mL, 6 mL, 7 mL, and 8 mL from a concentration solution of 100 µg/mL, then suffogged with methanol p.a to the 10 mL measuring pumpkin limit mark. To determine the antioxidant activity of each taken concentration as much as 0.2 mL of sample solution and put it in a vial and cover with aluminum foil, then add 3.8 mL. DPPH solution 30 µg/mL. The mixture is homogenized and silenced for 30 minutes in dark places, uptake is measured with a UV- Visible spectrophotometer at a maximum wavelength of 515.50 nm (Andayani *et al*., 2008).

Ethyl Acetate Extract

Weighed ethyl acetate extract as much as 100 mg, then dissolved with methanol p.a in the measuring pumpkin ad 100 mL, then obtained a concentration of 1000 µg/mL. Furthermore, a series of concentrations of 150 µg/mL, 160 µg/mL, 170 µg/mL, 180 µg/mL and 190 µg/mL, by being picked up by 1.5 mL, 1.6 mL, 1.7 mL,1.8 mL, and 1.9 mL of a concentration of 1000 µg/mL, then sufficient with methanol p.a to the 10 mL pumpkin

limit mark. To determine the antioxidant activity of each concentration is taken as much as 0.2 mL of sample solution and put it in a vial and cover with aluminum foil, then add 3.8 mL solution DPPH 30 µg/mL. The mixture is homogenized and silenced for 30 minutes in dark places, uptake is measured by a UV-Visible spectrophotometer at a maximum wavelength of 515.50 nm (Andayani *et al*., 2008).

n-Hexan Extract

Weighed n-Hexan extract as much as 100 mg, then dissolved with methanol p.a in the measuring pumpkin ad 100 mL, then obtained a concentration of 1000 µg/mL. Furthermore, a series of concentrations of 160 µg/mL, 170 µg/mL, 180 µg/mL, 190 µg/mL and 200 µg/mL, by being pickled as much as 1.6 mL, 1.7 mL, 1.8 mL, 1.9 mL, and 2.0 mL of a concentration of 1000 µg/mL, then sufficient with methanol p.a until the pumpkin limit mark measured 10 mL. To determine the antioxidant activity of each taken concentration as much as 0.2 mL of sample solution and put it in a vial and cover with allumunium foil, then add 3.8 mL solution DPPH 30 µg/mL. The mixture is homogenized and silenced for 30 minutes in dark places, uptake is measured with a UV-Visible spectrophotometer at a maximum wavelength of 515.50 nm (Andayani *et al*., 2008).

RESULTS AND DISCUSSIONS

The seedlings were taken from Koto Gadang Village, Padang Ganting Subdistrict, Tanah Datar Regency, West Sumatra Province. Seedlings are taken directly from plantations that cultivate the seedling plants in Koto Gadang Village. The seedlings obtained were identified at the Herbarium of Andalas University, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Andalas University (UNAND) Padang, West Sumatra. The specimens proved that the sample used in this study was a plant (*Caesalpinia bonduc* (L) Roxb.) with the family*Leguminose.*

The manufacture of seed extract is done by the maceration method, this method was chosen because it is relatively simple and fast, but it can already provide the active substance simplisia with the maximum. The main advantage of this method is that it is not done by heating so that it can prevent damage or loss of active substances that want to be abstracted in compounds that are not heat resistant (Sa'adah & Nurhasnawati, 2015). Antioxidant content is the content of compounds that are not heat resistant so that the maceration method which is cold extraction will be more optimal in extracting antioxidant compounds (Sie, 2013).

In this study, the manufacture of extracts by maceration method was carried out with 3 solvents based on its polarity, namely ethanol, ethyl acetate and n-Hexane. The purpose of using three solvents with different polarities is to know yield, get active compounds and know the antioxidant activity of kebiul seed based on their level of polarity. The percentage of yield obtained from ethanol extract 96% is 12.5868%, the percentage of yield obtained from ethyl acetate extract is 11.5264%, the percentage of yield obtained from n-Hexan extract is 9.0704%.

After getting a thick extract, then the extract is characterized with the aim of seeing the quality of the extract obtained. Determination of organoleptis obtained ethanol extract 96% with brownish yellow color, bitter taste, bad smell with viscous extract, ethyl acetate extract with yellow color, bitter taste, bad smell with thick extract and extract n-Hexan with yellow color, bitter taste, bad smell with thick extract. The determination of organoleptis includes one of the specific parameters specified by the five senses and aims for simple and subjective early recognition (Ministry of Health of the Republic of Indonesia, 2000).

Then determine the phytochemical content in each extract. Qualitative phytochemical analysis is a method of initial analysis to examine the content of chemical compounds in the sample. Results obtained in ethanol extract 96% of seedlings showed positive results in alkaloids, flavonoids, saponins, tannins, phenols and terpenoids. Phytochemical screening results from ethyl acetate extract of castor seeds (*Caesalpinia bonduc*(L) Roxb.) showed positive results in alkaloids, saponins and steroids. Phytochemical screening results from n-Hexan extract of kebiul seed showed positive results in alkaloids, saponins andsteroids.

These flavonoid compounds are powerful antioxidants that can prevent the formation of free radicals (Sakihama *et al*., 2002). Flavonoids activity as antioxidants because they have clusters Hydroxyl can donate hydrogen atoms to free radical compounds and stabilize reactive oxygen compounds (ROS) and has a hydroxyl ketone group that can act as a metallated catalyst that becomes a lipid peroxidation catalyst (Rezaeizadeh, 2011).

The determination of the antioxidant activity test of the seed extract was conducted using the DPPH (*2,2-diphenyl-1-picrylhydrazil*) method analyzed with UV-Vis spectrophotometry. DPPH is a commonly used method as a radical to test antioxidant activity because of its stable nature in the form of free radicals and is a simple, fast, and inexpensive method (Bozin *et al*., 2008). The antioxidant activity of the sample was measured through measurements of the absorption intensity of each sample after adding DPPH using a UV-Vis spectrophotometer at a given wavelength. Based on this, before measuring antioxidant activity, first determine the maximum wavelength of solution *1,1-diphenyl-2- picrilhydrazil.*

The results of the experiment showed a maximum uptake of *1.1-diphenyl-2- picrilhydrazil* located at a wavelength of 515.50 nm with an absorbant of 0.656 nm. This means that the absorption measurement of the all extracts against DPPH free radicals is performed at a wavelength of 515.50nm.

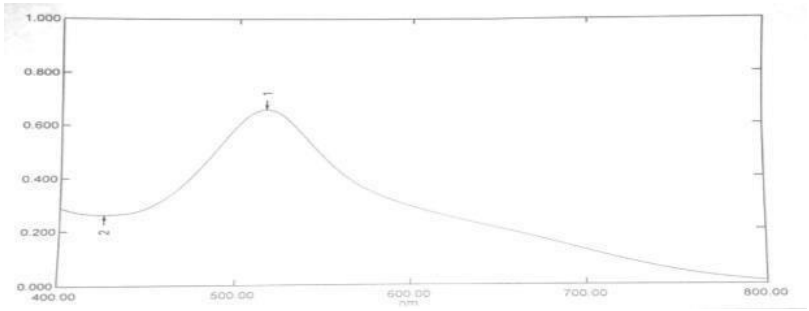

Figure 1. Maximum wavelength spectrum of DPPH solution 30 µg/mL

The size parameter used to indicate antioxidant activity is the value of inhibition concentration (IC_{50}) which is the concentration of an antioxidant substance that can cause 50% DPPH (*1,1-diphenyl-2-picryhydrazil*) to lose radical character or concentration of an antioxidant substance that provides a percentage (%) inhibition of 50%. Substances that have high antioxidant activity, will have a low IC_{50} value (Prasonto *et al*.,2017).

Testing the antioxidant activity of gallic acid, obtained the result of the solution absorbant 0.496; 0.423; 0.339; 0.265; 0.196.Viewed from the result of absorbant can be known that the greater the concentration of the sample, the smaller the absorbance value obtained, this is because the higher the antioxidant compounds that are able to reduce or ward off radicals in DPPH so that the percentage of inhibition will be greater (Bahriul *et al*., 2014). IC_{50} value or free radical antidote activity of 50% obtained from gallic acid of 12.8337 µg/mL. The results showed antioxidant activity with a very strong category due to IC_{50} values < 50 µg/mL (Rosidah &Tjitraresmi,2018).

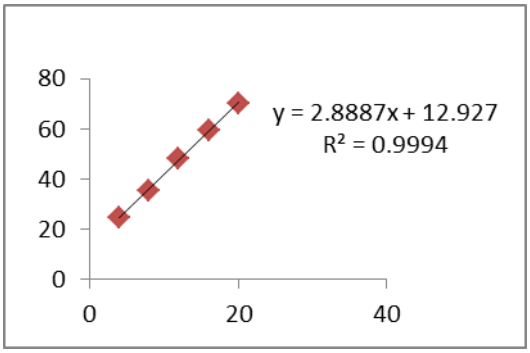

Figure 2. Galad acid extract calibration curve

In testing the antioxidant activity of ethanol extract 96% of kebiul seed, the result of absorbing a solution of 0.329; 0,311; 0,296; 0,280; 0.262. IC_{50} value or free radical antidote activity of 50% was obtained at 40.3619 µg/mL. The results showed antioxidant activity with a very strong category due to IC_{50} values < 50µg/mL (Rosidah & Tjitraresmi, 2018).

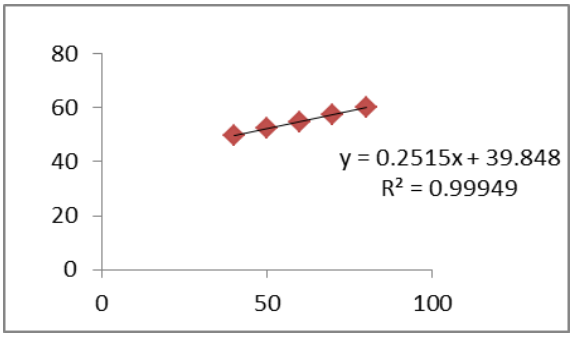

Figure 3. Calibration curve of ethanol 96% extract

In testing the antioxidant activity of n- Hexan extract from kebiul seed, a solution absorbantresultof0.655;0.578;0.490;0.400;0.309. IC_{50} value or free radical antidote activity of 50% was obtained at 198.2069 µg/mL. The results showed antioxidant activity in a moderate category due to IC_{50} values of 101-250 µg/mL (Rosidah & Tjitraresmi,2018).

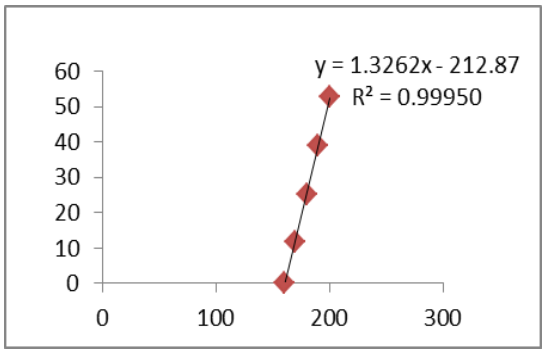

Figure 4. Calibration curve of n-Hexan extract

In testing the antioxidant activity of ethyl acetate extract from kebiul seed, a solution absorbant result of 0.599; 0.512;0.437; 0.361; 0.292. IC_{50} value or free radical antidote activity of 50% was obtained at 184.6666 µg/mL. The results showed antioxidant activity in a moderate category due toIC_{50} valuesof101-250µg/mL(Rosidah&Tjitraresmi, 2018).

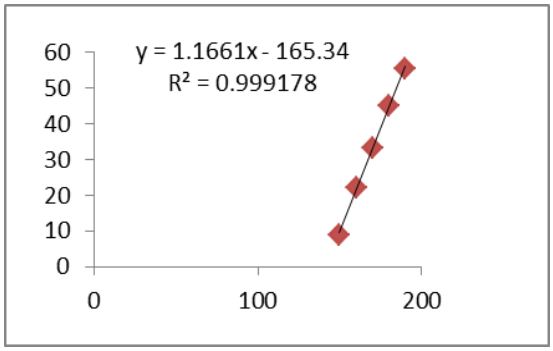

Figure 5. Calibration curve of ethyl acetate extract

From the value of IC_{50} can be seen the best antioxidant activity is ethanol extract 96% which is 40.3619 µg/mL and categorized very strongly in antioxidants. It is stated that very strong intensities have IC_{50} (<50µg/mL), strong(50-100µg/mL)values,moderate(101-250 µg/mL), weak (250-500 µg/mL) (Rosidah & Tjitraresmi, 2018). The effect of solvent polarity can affect the antioxidant levels of kebiul seed as seen from the value of IC_{50}, the polar a compound, the higher its antioxidant activity, because in polar compounds attract a lot of chemical content including chemical compounds that have strong antioxidant activity, namely flavonoids (Sakihama *et al*., 2002).

CONCLUSIONS

Based on research that has been done on phytochemical screening and the effect of solvent polarization on the antioxidant activity of castor seed extract (*Caesalpinia bonduc* (L) Roxb.) with the DPPH method, it can be concludedthat:

1. Solvent polarization affects the chemical content of kebiul seed extract (*Caesalpinia bonduc* (L) Roxb.), where ethanol extract 96% of kebiul seeds (*Caesalpinia bonduc* (L) Roxb.) attract the most chemical content compared to ethyl acetate extract and n- Hexan kebiul seeds (*Caesalpinia bonduc* (L) Roxb.).
2. Solvent polarization affects antioxidant activity in kebiul seed extract (*Caesalpinia bonduc* (L) Roxb.), where ethanol extract 96% of kebiul seeds (*Caesalpiniabonduc*
(L) Roxb.) has the highest antioxidant activity compared to ethyl acetate extract and n- Hexan kebiul seeds (*Caesalpinia bonduc* (L) Roxb.).

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