International Journal of Advances in Science Engineering and Technology, ISSN(p): 2321 –8991, ISSN(e): 2321 –9009
Volume-6, Issue-3, Jul.-2018, http://iraj.in

IDENTIFICATION AND ANTIOXIDANT ACTIVITY OF ALAMANDA LEAF (ALLAMANDA CATHARTICA L.) BASED ON SOLVENT VARIATIONS

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Abstract - Alamanda (Allamanda cathartica L.) included in Apocynaceae family. Empirically, people use alamanda leaf as anticancer. The purpose of this study was to determine the chemical content and activity level of antioxidant compounds from alamanda leaf extract (Allamanda cathartica L.) based on solvent variation. Extraction method used was maceration using n-hexane, ethanol and water solvent, and for infuse using water. Determination of antioxidant compound activity from alamanda leaf extract (Allamanda cathartica L.) using DPPH (2,2-diphenyl-1-picrylhydrazil) method. The results showed that alamanda leaf extract was positively contains Alkaloid, flavonoid, phenolic and saponin compounds. While the antioxidant activity of alamanda leaf (Alamandachatartica L) in water extract had strong antioxidant activity with IC_{50} value of 44,899 μg/mL, ethanol extract had weak antioxidant activity with IC_{50} value of 106,352 μg/mL, and for n-Hexane extract had very weak antioxidant activity with IC_{50} value of 164,063 μg/mL. while the quersetin reference standard has an IC_{50} of 9,112 μg/mL indicating strong antioxidant activity.

Index terms - Alamanda leaf (Allamanda catharticaL.), Antioxidant, DPPH

I. INTRODUCTION

Degenerative disease is caused by the accumulation of excessive free radicals in human body cells. These free radicals can damage the structure and function of biomolecules in cells that can lead to change in structure and function that give carcinogenic effects and even mutagenesis to normal cells (Harliansyah, 2001). Therefore, the body needs Antioxidants to prevent excessive free radicals. Efforts to utilize the plant as an alternative antioxidant from synthetic materials is needed. In general, plants are source of antioxidant compounds such as phenols, flavonoids, curcuminoids and organic acids scattered in various parts of plants such as roots, stems, skins, leaves, fruits, and seeds and flower parts (Lemmens, 2003).

One of the plants that has the potential as an antioxidant is alamanda (Allamanda catharticaL.). Alamanda leaves are empirically used as antidote (Widyaningrum, 2011) and as anticancer (Essiett and Udo, 2015). In the results of research conducted by Das and Bhatnagar (2017) methanol extract of Allamanda cathartica positively contains flavonoid compounds, saponins, cardiac glycosides, and tannins. Chloroform extract positively contains flavonoids, saponins and terpenoids. Based on research conducted by Rehan, Tasnuva, Farhana and Sharmin (2013), Allamanda cathartica leaves have antioxidant activity with IC_{50} in methanol extract is 167.40 ± 0.5μg/mL and at n-hexane fraction is 181.93 ± 1.21μg/mL. Based on research conducted by Amjad and Ghazala, Allamanda cathartica leaf methanol extract detected using ABTS method has a total antioxidant capacity is (5.43 ± 0.29 μM/g).
Based on these descriptions, the research conducted to identify and to test the antioxidant compound of alamanda leaf extract (Allamanda cathartica L.) based on solvent variation to add scientific data of the use of plants as natural antioxidants.

II. MATERIALS AND METHODS

A. Materials
The materials used are distilled water, AlCl3, DPPH (2,2- diphenyl-1-picryl-hydrazyl), ethanol, water extract of alamandaleaf (Allamanda cathartica L.), ethanol extract of Alamanda Leaf (Allamanda cathartica L.), n-Hexane Extract of Alamanda Leaf (Allamanda cathartica L.), FeCl3, filter sheet, Quercetin, TLC plate, Dragendorff reagent, capillary tubes, and Vanilin-sulfuric acid reagent.

B. Population and Sample
The population in this research is Alamanda (Allamanda cathartica L.) where the sample used isalamanda leaves obtained from KelurahanMalino, Kecamatan Tinggi Moncong, KabupatenGowa, South Sulawesi.

C. Work Procedures
1. **Processing materials**

Alamanda leaves (Allamanda cathartica L.) collected, then washed to remove other impurities that still attached to the sample. Then dry-sorted and cut into small pieces. After that the sample dried in a drying cupboard with temperature of less than 50°C. Then powdered and ready for extraction (Indartiyah et al, 2011 p38).

2. Extraction Method

a. Water solvent

Infusaof alamandaleaves(Allamanda cathartica L.) is prepared with25 grams of alamanda leaves powder inserted intoinfusa pot A thenaquadestadded as much

as 250 ml (until the material is completely submerged), infusa pot B is filled with water until the pot of Infusa A is partially submerged (infusapot A is closed), then heated at 90°C for 15 minutes, stir occasionally. Then filtered with a flannel cloth while hot (Yusriana et al., 2014 with modification). Filtrate that has been filtered, followed by evaporation using freeze dryer tool (Sari, 2010)

b. Ethanol solvent

The leaf powder of Alamanda (Allamanda cathartica L.) as much as 300 grams, put into a maceration container, then 2,000 ml of ethanol solvent was added until all the powder were submerged, the maceration process was carried out for 3 x 24 hours in a closed container while stirred occasionally, the resulting macerate is filtered. Liquid extracts were collected, then concentrated using rotary vacuum evaporator untilthick ethanolic extract obtained (Senja et al, 2014 with modification).

c. n-hexane Solvent

300 grams of leaf powder of Alamanda (Allamanda cathartica L.) was weighed, put in a maceration container, then 2 x 1000 mL n-hexane solvent added until all the powder submerged, the maceration process carried out for 3 x 24 hours in a closed container while stirred occasionaly, the resulting maserate is filtered. Liquid extracts were collected, then concentrated using rotary vacuum evaporator until thick n-hexane extract was obtained (Senja et al, 2014 with modification).

3. Identification of Chemical Content

a. Identification of alkaloids

The extract solution is spotted on the TLC plate, then eluted with eluent. After that sprayed using Dragendorff reagent. Observed under 254 nm and 366 nm UV lamps. After the plate is sprayed with the reagent Dragendorffit will show orange brown spots if positive (Harbone, 1987).

b. Identification of flavonoids

The extract solution was spotted on the TLC plate and eluted with appropriate eluent. Then it was observed under 254 nm and 366 nm UV lamps after being sprayed with $AlCl_3$. Flavonoids containing a conjugated aromatic system so that it would show strong absorption bands on UV light and visible light. In the analysis with TLC and appearance with $AlCl_3$ reagent, Flavnoid will show yellow spot and depending on its structure, the flavonoid will fluorescencewith yellow, blue or green spot under 366 nm UV (Harbone, 1987).

c. Identification of phenolics

The extract spotted on the TLC plate and eluted with appropriate eluent. Then observed the spots under UV 254 and 366 after it was sprayed with $FeCl_3$. Positive contains phenol if the stain is green, red, purple, blue or black strong (Harbone, 1987).

d. Identification of saponins

The extract was spotted on the TLC plate and eluted with an appropriate eluent. Then observed under UV254 and 366 lights and sprayed with vanillin-sulphate acid. Saponin glycosides when detected with vanilin-sulphate acid spray reagents will give blue to blue violet color sometimes red, yellow, dark blue, purple, green or brownish yellow (Harbone, 1987).

4. Qualitative Test Antioxidant Activity

Alamanda leaf extract (Allamanda chatartica L.) dissolved with 96% ethanol, then spotted on TLC plate using capillary pipe. The extracts that have been spotted on the TLC plate were eluted, observed under UV 366 and UV 254 nm. The TLC plate sprayed with DPPH then incubated for 30 minutes. Observe the color change that occur from the purple to yellow (Handayani, Ahmad &Sudir 2016).

5. Quantitative Test antioxidant activity

a. Making of DPPH solution

DPPH solution was made by weighing 5 mg of DPPH then dissolved with 100 mL methanol p.a in measuring flask and obtained DPPH solution with concentration 50 ppm (Brands William, 1995).

b. Determination maximum wavelength of DPPH

The test was done by taking 3.5 mL DPPH 50 ppm, add0.5 mL of methanol p.a and measured its absorbance at wavelength 400-700 nm (Syarif et at 2015, p 85).

c. Measurement of antioxidant activity of comparative sample (quercetin)

Making stock of 1000 ppm by weighing quercetin much as 10 mg then dissolved with methanol p.a and dihomogenkan then enough volume up to 10 mL, then dilution is done by taken 0,01 mL; 0.02 mL; 0.03 mL; 0.04; and 0,05 mL to make 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm, methanol to the final volume of 5 mL. The test was taken with 0.5 mL. Then each added 3.5 mL DPPH 50 ppm. Inserted in and incubated at 37 °C in dark space. Then its absorption by using UV-Vis spectrophotometer atmaximum wavelength. Testing was done by mixing 0.5 mL of sample solution from various concentrations. Then add 3.5 mL DPPH 50 ppm. The mixturethen homogenized and incubated at 37°C in the dark room. Then the absorption was measured using a UV-Vis spectrophotometer at maximum wavelength(Syarif et al 2015, p.86).

d. Measurement of antioxidant power of water extract, ethanol extract and n-hexane extract ofalamanda leaf (Allamanda cathartica L.)

Making stock of 1000 ppm by weighing extract much as 10 mg then dissolved with methanol p.a and dihomogenkan then enough volume up to 10 mL, then dilution is done by taken0,1 mL; 0,2 mL; 0.3 mL; 0.4 mL; and 0,5 mL to make 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm, methanol to the final volume of 5 mL. The test was taken with 0.5 mL. Then each added 3.5 mL DPPH 50 ppm. Inserted in and incubated at 37 ° C in dark space. Then its

absorption by using UV-Vis spectrophotometer at maximum wavelength. Testing was done by mixing 0.5 mL of sample solution from various concentrations. Then each added 3.5 mL DPPH 50 ppm. The mixture then homogenized and incubated at 37°C in the dark room. The absorption was measured using UV-Vis spectrophotometer at maximum wavelength. Same treatment was performed on quercetin as a reference standard. The percentage of DPPH inhibition was calculated by the equation (Syarif et al 2015, p.86):

$$\% \text{ inhibition} = \frac{(A-B)}{A} \times 100\%$$

Where: A = Absorption value of blank
B = Sample absorption value

Then the value of IC_{50} is calculated using the regression equation y= bx + a

III. RESULTS AND DISSCUSION

Antioxidants are electron-giving compounds. Antioxidants work by donating an electron to an oxidant compound so that the activity of the oxidant compound can be inhibited. Antioxidants stabilize free radicals by supplementing electron deficiencies possessing free radicals and inhibiting the occurrence of chain reactions from free radical formation (Malangngi, Sangi and Paendang 2012, p.6).

This study aims to determine the chemical content and determine the activity level of antioxidant compounds alamanda leaf extract (Allamanda chatartica L.) based on solvent variation. This research uses alamanda leaf (Allamanda chatartica L.). First sample was cleaned, then dried at room temperature so that the compound contained therein was not damaged, then powdered to easily pull off the compound in the extraction process.

After alamandaleaves has been powdered, then it's extracted using maceration method with ethanol and n-hexane solvent and extracted using infusa method with water solvent. The maceration method is chosen because the extraction does not use heating so that the changes of chemical compounds contained can be minimized. Selection of infusa method on water solvent, because by using infusa method the use of water solvent aims to get the polar active substances can be sought optimally (Yuliani and Dienna 2015, pp 1072).

The result of extraction from maseration method using ethanol and n-hexane solvent and infusa method using water solvent on alamandaleaf(Allamanda cathartica L.) sample obtained extract yield value which can be seen in table 1. The purpose of calculatingextract yield value is to know the amount of compound in a particular solvent but cannot determine the type of compound carried (Ukieyanna 2012, h.7)

Table 1. Yield value of water, ethanol, and n-hexane extract of alamanda leaf (Allamanda cathartica L.)

Solvent	Wight of Sample (g)	Amount of Solvent (mL)	Weight of Extract (g)	Rendemen of Extract (%)
Water	70	700	9,414	13,448
Ethanol 96%	300	2000	41,312	13,770
n-hexane	300	2000	5,257	1,752

Water solvents are used because alamanda plants contain many free radical inhibitor compounds such as phenolics and some other metabolite compounds that are rich in antioxidant activity. (Rizkayanti, Diah and Jura 2017, pp. 126). Percent rendamen water extract obtained from Alamanda leaves (Allamanda cathartica L.) amounted to 13.448%. The ethanol solvent is chosen because of its semi-polar properties which is capable of attracting all kinds of active substances, both polar and non-polar as well as good absorbance and low reactive toxicity. The ethanol solvent used is ethanol with 96% concentration because ethanol with such concentration can more easily penetrate into cells and have better extraction ability compared with low concentration ethanol (Arifin, Riyono and Elka 2010). Percent yield value ethanol extract obtained from Alamanda leaves (Allamanda cathartica L.) amounted to 13.770%. The n-hexane solvent is selected because it is non-polar, the choice of n-hexane solvent is based on its selectivity in extracting non-polar compounds, its safety level and its ease of evaporation (El munah 2013, p.34). The percentage of rendaments of n-hexane extract obtained from Alamanda leaves (Allamanda cathartica L.) amounted to 1.752%.

Water, ethanol and n-hexane extract of alamanda leaf (Allamanda cathartica L.) were further identified by TLC profile (Thin Layer Chromatography) to see the compound content contained in each extract. The TLC method was chosen because of some of its advantages, such as its versatility, speed, and sensitivity (Harbone 1987, p.13). The eluent used in ethanol and n-hexane extracts is n-hexane: ethyl acetate in the ratio (7: 3), whereas in the eluent water extract used is n-Butanol: acetic acid: water in the ratio (5: 1: 4). After that done the identification on each extract and obtained the results as in table 2.

Table 2. Results of phytochemical screening of water, ethanol and n-hexane extract Alamanda leaves (Allamanda cathartica L.)

Test	Extract			References
	Water	Ethanol	n-Hexane	
Alkaloids	-	-	+	Positive if there is orange brown spot (Harbone, 1987)
Flavonoids	+	+	-	fluorescent yellow, blue or green under 366 nm UV (Harbone, 1987)
Phenolic	+	+	+	Positive if there are green, red, purple, blue or black spot (Harbone, 1987)
Saponins	+	+	+	Positive if there are red, yellow, dark blue, purple, green or brownish yellow (Harbone, 1987)

Description: (+) positive contains test compound
(-) contains a negative test compound

Testing of antioxidant activity is done qualitatively and quantitatively. Which has antioxidant activity in reducing free radical. Extracts of ethanol and n-hexanealamandaleaf were then spotted on TLC plate and eluted with eluent n-hexane: ethyl acetate (7: 3) and water extract eluted with eluent n-Butanol: acetic acid: water withratio (6: 4: 2) then sprayed with DPPH solution.

The result of qualitative test showed that water, ethanol and n-hexane extract alamanda leaf (Allamanda cathartica L.) have potential as antioxidant, can be seen in table 3. The antioxidant compound reacts with the DPPH radical through the hydrogen atom donation mechanism and causes color decay from purple to yellow (Handayani, Ahmad and Sudir, 2014, p. 90).

Table 3. Results of Qualitative Test Antioxidant Activity

Extract	Qualitative Test of DPPH	Observation result
Water Extract	Yellow	+
Ethanol Extract	Yellow	+
n-Hexane Extract	Yellow	+

Description: (+) = indicates the presence of antioxidant activity

Furthermore, a quantitative test was conducted using UV-Vis spectrophotometer. The comparator used as a positive control is quercetin. The sample absorbance is measured at maximum wavelength in 516 nm.
After the measurements were calculated percent of IC_{50} inhibition, the parameters used for this DPPH radical capture test were expressed in IC_{50} (inhibition concentration) values, ie concentrations capable of inhibiting 50% DPPH (Wahdaningsih, Setyawati and Wahyuono 2011, p.158). According to Phongpaichit et al (2007), a compound is considered to be a very powerful antioxidant if the IC_{50} value is less than 10 µg/mL, strong if the IC50 value is between 10-50 µg/mL, while the IC_{50} value is between 50-100 µg/mL, weak if IC_{50} values range from 100-250 µg/mL and are inactive if IC_{50} is above 250 µg/mL.
In Table 4 and Table 5 can be seen the results of absorbance measurements, percentage of binding of DPPH, and comparation of IC_{50} value of water, ethanol and n-hexane extract from alamanda leaf and quercetin. Graphic of the relationship between each water extract, ethanol extract, n-hexane extract of alamanda leaf and quercetin comparing with % inhibition can be seen in Figure 3-6.

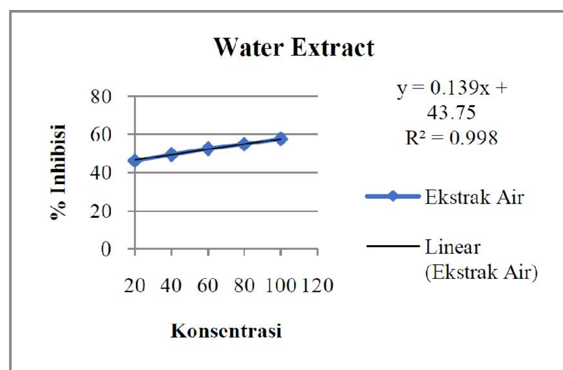

Figure 3. Graph of the relationship between water extract concentration of Alamanda leaf with % inhibition

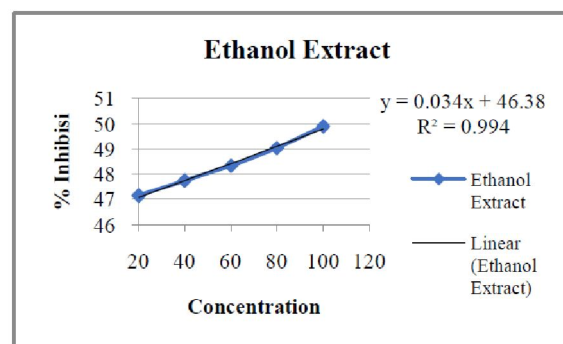

Figure 4. Graph of the relationship between ethanolic extract concentration of Alamanda leaf with % inhibition

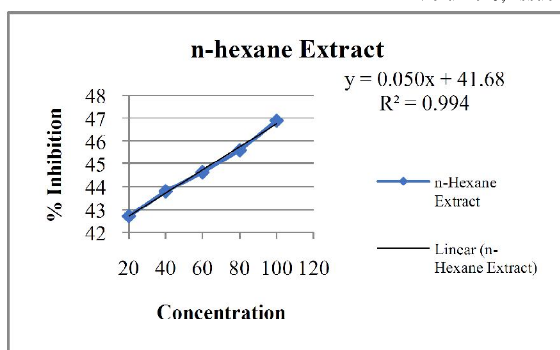

Figure 5. Graph of the relationship between n-hexane extract concentration of Alamanda leaf with% inhibition

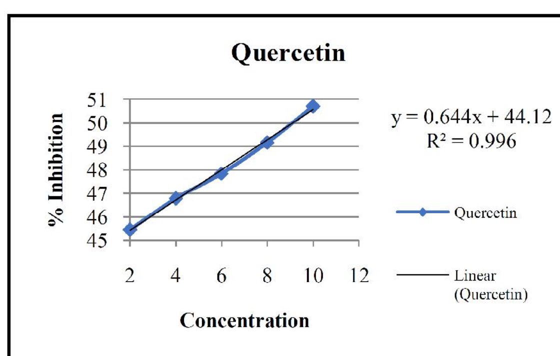

Figure 6. Graph of the relationship between quercetin concentration with % inhibition

Based on the results obtained in Table 4 and Table 5 the IC_{50} value of water extract from Alamanda leaf is qualifiedas strong antioxidant because IC_{50} value ranged from 50-100 µg/mL is 44.899 µg/mL, ethanol extract from Alamanda leaf included as weak antioxidant because IC_{50} value ranged >100-250 µg/mL of 106.352 µg/mL whereas n-hexane extract from Alamanda leaf is a weak antioxidant because its IC_{50} value obtained > 100-250 µg/mL is 164,063 µg/mL, and for quercetin as reference standard has IC_{50} value < 10 µg/mL is 9,112 µg/mL.

The result of statistical analysis using Statistical Product and Service Solution (SPSS) showed that there was no significant difference between % Inhibition of n-hexane extract and ethanol extract, and there was no significant difference between % Inhibition of ethanol extract and water extract, but between % Inhibition of n-hexane extract and water extracts there were a significant difference.

CONCLUSION

The antioxidant activity of Alamanda leaf (Alamandachatartica L) in water extract had strong antioxidant activity with IC_{50} value of 44.899 µg/mL, ethanol extract had weak antioxidant activity with IC_{50} value of 106,352 µg/mL, and for n-hexane extract had activity a very weak antioxidant with an IC_{50} value of 164,063 µg/mL. while the quersetin reference standard has an IC_{50} of 9,112 µg/mL indicating strong antioxidant activity

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