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ANTIOXIDANT ACTIVITIES OF SESEWANUA LEAF EXTRACTS (Clerodendrum fragrans [Vent.] Willd) USING DPAD (1,1-Diphenyl-2-Picrylhydrazyl) FREE RADICAL METHOD AND NITRATE OXIDE Benedicta I.Rumagit Department of Pharmacy, Poltekkes Kemenkes Manado, Manado, Indonesia Email: dicta.farmasi@gmail.com Adeanne C Wullur Department of Pharmacy, Poltekkes Kemenkes Manado, Manado, Indonesia Email: anne.wullur@gmail.com Donald E.

Kalonio Department of Pharmacy, Poltekkes Kemenkes Manado, Manado, Indonesia Email: donaldemilio.k@gmail.com Ab s tra ct — Free radicals are molecules that contain unpaired electrons so that they are unstable and highly reactive to other molecules. ROS / RNS radicals have physiological functions, but over-production of free radicals can initiate oxidative / nitrosative stress which contributes to a large number of diseases. The body has the ability to neutralize free radicals by forming endogenous antioxidants.

However, changes in environment, lifestyle and certain pathological conditions can cause a shift in the antioxidant balance of antioxidants. Because it is necessary to consume endogenous antioxidants, especially those sourced from natural ingredients. One plant that is thought to have antioxidant activity is the leaves of an individual (Clerodendrum fragrans [Vent.] Willd.).

This study aims to evaluate the antioxidant activity of ethanol extract, hexane fraction, female ethyl acetate fraction and leaf water fraction with DPPH and nitric oxide free radical capture methods and determine the 50% (Cinhibition concentration value50) free radical. This research is a laboratory experiment. The sample used is the leaves of women (Clerodendrum fragrans [Vent.]

Willd) obtained in East Malalayang I Village, Malalayang District, Manado City, North Sulawesi Province. Testing of antioxidant activity using DPPH (free radical capture method1,1-Diphenyl-2- Picrylhydrazyl)and nitric oxide. Data collected in the form of free radical inhibition percentage data were then analyzed using linear regression analysis to determine the inhibitory concentration of 50% (IC50) DPPH free radicals and nitric oxide.

Based on the results of the study it can be concluded that ethanol extract, hexane fraction, ethyl acetate fraction and fraction of Sesewanua leaf water have antioxidant activity through DPPH free antiradical activity, but are not active as anti radical NO. Keywords: Sesewanua Leaves, Antioxidants, DPPH, NO ? I. INTRODUCTION Free radicals are molecules that contain unpaired electrons so that they are unstable and highly reactive to other molecules (Lobo et al. 2010).

The human body produces free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) through endogenous metabolic processes, stress or from exogenous sources such as environmental pollution, cigarette smoke, radiation, chemical compounds including organic solvents, and fast food (Pourmorad et al. 2006; Lobo et al. 2010; Krishnaiah et al. 2011; Goggi & Malpathak 2017).

At low or moderate concentrations, ROS / RNS has physiological functions such as in the body's defense response to infectious agents, acting in thesignaling pathway and mitogenous cellular response (Valko et al. 2007). On the other hand, over-production of free radicals can initiate oxidative stress and nitrosative stress which can cause cell damage including membranes, lipids, proteins and DNA (Valko et al.

2007; Annapandian & Rajagopal 2017), and trigger a number of diseases such as inflammation related diseases (arthritis, vasculitis, glomerulonephritis, lupus), cardiovascular disorders, stroke, AIDS, gastric ulcer, hypertension, premature aging and neurological disorders (Alzheimer's and Parkinson's) (Lobo et al. 2010). Oxidative and nitrosative stress results from a disruption of the balance of prooxid production (ROS / RNS) with the antioxidant ability to neutralize it (Uys et al. 2011; Subedi et al. 2014). Normally, the body has the ability to neutralize free radicals by forming endogenous antioxidants (Iqbal et al.

2017) such as glutathione, catalase, superoxide dismutase, vitamin A, uric acid, and coenzyme Q10 (Carocho & Ferreira 2013). However, due to changes in the environment, lifestyle and certain pathological conditions can cause a shift in the antioxidant balance of antioxidants (Willcox et al. 2004). Therefore there is a need for exogenous antioxidant intake (Pourmorad et al. 2006).

Currently available natural and synthesized antioxidants that are widely used in the food, cosmetics, pharmaceutical industries and as therapeutic antioxidants (Lobo et al. 2010). Butylated hydroxytoluene (BHT) and BHA (butylated hydroxyanisole) are examples ofantioxidants synthetic that are often used, but their use is limited and reported to have some side effects (Branen 1975; Lobo et al. 2010; Krishnaiah et al. 2011; Carocho & Ferreira 2013; Subedi et al. 2014).

Along with the increased risk of dangerous diseases, there is a tendency for an increase in the use of antioxidants derived from natural ingredients (Lobo et al. 2010). Therefore the search for new antioxidants is more focused on natural sources (Subedi et al. 2014), especially those from plants. Leaves of Sesewanua (Clerodendrum fragrans [Vent.] Willd. Syn. Clerodendrum chinense [Osbeck] Mabb.) Are leaves of the genus Clerodendrum family Lamiaceae.

These plants are found in Sulawesi, Maluku, Kalimantan, Java, Sumatra, the Philippines, Peninsula Malaysia, Thailand and southern China (Leeratiwong et al. 2011; Wearn & Mabberley 2011). Female leaves are used as swelling (anti-inflammatory) by the people of North Sulawesi (Arini & Kinho 2015). There is a correlation between antioxidant activity and anti- inflammatory (Thakur et al. 2013; Hafiz et al. 2016).

Some plant species reported from the genus Clerodendrum have antioxidant activities such as C. colebrookianum (Rajlakshmi et al. 2003), C. trichotomum (Chae et al. 2006), C. infortunatum (Gouthamchandra et al. 2010), and C. paniculatum (Hafiz et al. 2016). This study aims to evaluate antioxidant activity and determine the value of 50% inhibition concentration (IC) of ethanol extract, hexane fraction, Sesewanua ethyl acetate fraction and leaf water fraction using DPPH free radical capture method and nitric oxide.

METHODS Sample collection The samples used were leaves of Sesewanua (Clerodendrum fragrans [Vent.] Willd) which were obtained in the East Malalayang I Village, Malalayang District, Manado City, North Sulawesi Province. The samples obtained were then identified / determined at the Biology Research Center-LIPI Bogor.

Extraction and Fractionation Ethanol extract of Sesewanua leaves was obtained by maceration using 70% ethanol solvents according to the method listed in Indonesian Herbal Pharmacopoeia (MOH 2008). The fractionation process was carried out using a liquid-liquid extraction method according to Zhao et al. (2009) with modifications to the type and amount of solvent used.

Ethanol extract was suspended in distilled water and then fractionated using n-hexane and ethyl acetate respectively. Phytochemical Compound Screening Phytochemical

compounds in alkaloid, phenolic, tannin, flavonoid, saponin, steroid and triterpenoid compounds were carried out using standard procedures listed in Harborne 1973 and Tiwari et al. 2011.

Testing of DPPH Free Radical Catching Activities Testing of DPPH free radical capture activity according to the methods listed in Elmastas et al. (2007) as follows: 1 ml of DPPH solution (0.1 mM in methanol) was added in 3 ml of the sample. The mixture was shaken vigorously and incubated at room temperature for 30 minutes, then the absorption was measured using a spectrophotometer at a wavelength of 517 nm.

The ability to capture DPPH radicals is calculated using the formula: DPPH scavenging effect (%) = [(A0 - A1 / A0) x 100] where A0 = absorption of the control solution and A1 = absorption of the test sample solution. Testing the Catching Activity of Nitric-Free Oxide Radicals Testing the capture activity of nitric oxide free radicals according to the method listed in Promega Corp. (2009) and Alam et al. (2013) with minor modifications, as follows: as much as 2 ml of 10 mM Sodium Nitropruside solution mixed with 0.5

ml phosphate saline buffer (pH 7.4) and add 0.5 ml of sample solution with various concentrations (0.1; 1; 10; 100 and 1000 ppm). As a control, 2 ml of 10 mM sodium nitropruside solution were mixed with 1 ml of phosphate saline buffer (pH 7.4). This mixture was incubated at 25oC for 2.5 hours. Nearing the end of the incubation time, moved as much as 50 µ l samples into 96-well flat-bottommicroplate.By using a multichannelpipettor,add 50 µ l 1% sulfanilamide solution into the well containing the sample solution and control, then incubated for 5-10 minutes at room temperature, protected from light. After the incubation period, 50was added µ l of the N-1-napthylethylenediamine dihydrochloride (NED) 0.1% solutionto all the wells and incubated again in 5-10 minutes, at room temperature, protected from light.

The solution will change immediately to purple / magenta and within 30 minutes measure absorption at a wavelength of 546 nm. The percentage of NO radical inhibition can be calculated by the formula: % inhibition of NO radical = (Control - Sample / Control) x 100 Data Analysis Data collected in the form of free radical inhibition percentage data were then analyzed using linear regression analysis to determine the value of 50% inhibition concentration (IC50) DPPH free radicals and nitric oxide.

RESULTS AND DISCUSSION Plant Determination The results of the determination of the Botanical Gardens Center for Conservation of Nature, Indonesian Institute of Sciences Number B-2498 / IPH.3 / KS / VII / 2018 show that the female samples used are Clerodendrum fragrans [Vent.] Willd. Syn. Clerodendrum chinense [Osbeck] Mabb. Extraction Results Extraction of 200 gr of female leaf powder with 70% ethanol was

obtained as much as 55.6 g with a percent yield of 27.8%.

Fractionation Results Results fractionation 25 grams of ethanol extract of the leaves sesewanua with liquid-liquid extraction method using a solvent consecutive n-heksan ethyl acetate and n-hexane fraction obtained 2.51 g; ethyl acetate fraction 4.76 g; and water fraction 17.63 g. Phytochemical Screening / ScreeningPhytochemical In Figure 1 can be observed under extracts and fractions of female leaves possess antioxidant activity through DPPH free radical capture.

From the picture and table, it can be seen that the ability of DPPH free radical capture extract and female leaf fraction have increased with increasing concentration. DPPH free radical capture ability of extract and fraction of female leaves shown by the inhibitory concentration value of 50% (IC50), respectively are ethyl acetate fraction> ethanol extract> n-hexane fraction> water fraction (Table 3). The ICvalue of50 ethyl acetate fraction (12.05 ppm) and ethanol extract (41.86 ppm) was included in the strong activity category, while the n- hexane fraction (52.51 ppm) and water fraction (52.55 ppm) were included in the category moderate activity (Phongpaichit et al.

2007) The results of this study are similar to some previous studies which reported that some plants of the genus Clerodendrum have antioxidant activity. Gouthamchandra et al. (2010) reported that ethanol, chloroform and petroleum ether extracts ofleaves C. infortunatum with a concentration of 250 ppm, had DPPH free radical capture activities of 92.6%; 52.2% and 16.7%. Hafiz et al.

(2016) also reported that the ethanol extract of the leaves of C. paniculatum had DPPH antioxidant activity. Some plants from the Lamiaceae family such as Leonurus cardiaca, Lamium album, Marrubium vulgare, Stachys officinalis, Lamium purpureum and Galeopsis speciosa show antioxidant activity (Krishnaiah et al. 2011). NO radicals synthesized in biological tissues through arginine metabolism into citrulline and radical formation NO.

In this study as a source of NO free radicals used Sodium Nitroprussida, which in aqueous solution at physiological pH (7.2) undergoes decomposition resulting in NO radicals. In aerobic conditions, NO radicals will bind to oxygen and form stable nitrates or nitrites, which quantitatively can be measured by the addition of Griess reagents (Tsikas 2005; Alam et al. 2013).

Sample Regretioni R2 IC50 (ppm) Ekstrak Etanol y = -0,0130x - 7,8326 0,4989 > 1000 Fraksi n-Heksan y = -0,0299x - 2,8781 0,9338 > 1000 Fraksi Etil Asetat y = -0,0161x - 6,0358 0,6036 > 1000 Fraksi Air y = -0,0071x - 5,1508 0,1735 > 1000 In Table 3 and

Figure 2 can be observed under extracts and fractions of leaves of women that have no antioxidant activity through free radical capture NO.

In Table 2, it can be seen that the ability of DPPH free radical capture extracts and fraction of leaves of women is shown by the value of 50% inhibition concentration (IC50)> 1000 ppm. The results of this study are not as expected, which may be caused by various factors, including reduction of nitrate to nitrite and diazotation reactions (Tsikas 2005). To find out this, further research is needed.

CONCLUSION Based on the results of the study it can be concluded that ethanol extract, hexane fraction, ethyl acetate fraction and fraction of female leaf water have antioxidant activity through DPPH free antiradical activity, but are not active as antiradical NO. The50% (ICinhibition concentration of50smallest)DPPH free radicals was indicated by ethyl acetate fraction (12.05 ppm) and included in the strong activity category.

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