INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

ANTI-OXIDANT ACTIVITY OF SESEWANUA (*Clerodendrum fragrans* [Vent.] Willd) LEAF EXTRACT AND FRACTION WITH 1,1-DiPHENYLl-2-PICRYLHYDRAZYL (DPPH) AND NITRATE-OXIDE FREE RADICAL SCAVENGING METHOD

Benedicta Irene Rumagit *, Adeanne Caroline Wullur, Donald Emilio Kalonio

Department of Pharmacy, Poltekkes Kemenkes Manado, Manado, North Sulawesi, Indonesia

*Corresponding Author Email: dicta.farmasi@gmail.com

Article Received on: 28/10/20 Approved for publication: 30/11/20

DOI: 10.7897/2230-8407.111199

ABSTRACT

Free radicals are molecules containing unpaired electrons so that they are not stable and very reactive to other molecules. ROS/RNS radicals have physiological function, but the overproduction of free radicals can initiate oxidative/nitrosative stress that contributes to a high number of diseases. Body has an ability to neutralize the free radicals by forming the endogenous antioxidant. Environmental changes, living style, certain pathological conditions can cause the shift of prooxidant-antioxidant equilibrium. Thus, endogenous antioxidant intake is needed, particularly that originating from natural materials. One of the plants believed to have antioxidant activity is sesewanua (*Clerodendrum fragrans* [Vent.] Willd.) leaf. This study was aimed to evaluate the antioxidant activity of ethanol extract, hexane fraction, ethyl acetate fraction, and water fraction of the sesewanua leaf using DPPH and nitrate-oxide free radical scavenging method. The study is a laboratory experiment. The sample was sesewanua (*Clerodendrum fragrans*) obtained from East Malalayang I village, Malalayang district, Manado city, North Sulawesi. The antioxidant activity testing utilized *1,1-Diphenyl-2-Picrylhydrazyl* (DPPH) and nitrate-oxide free radical scavenging method. Data included percent inhibition of free radicals and were analyzed using linear regression to determine 50% inhibition concentration (IC_{50}) of DPPH and nitrate-oxide free radicals. As conclusion, the ethanol extract, hexane fraction, ethyl acetate fraction, and water fraction of the sesewanua leaf had antioxidant activity through DPPH free antiradical activity, but not active as antiradical NO.

KEYWORDS: Sesewanua leaves, antioxidant, DPPH, NO

INTRODUCTION

Free radicals are molecules containing unpaired electrons so that they are not stable and very reactive to other molecules [1]. Human body produces free radicals, such as reactive oxygen species (ROS) dan reactive nitrogen species (RNS) through endogenous metabolic process, stress or exogenous factors, such as environmental pollution, cigarette smoke, radiation, chemicals including organic solvent, and fast food [1–4]. At low or moderate concentration, ROS/RNS has physiological function, such as in body defense response to the infectious agents, and in cellular signaling and mitogen response [5]. Overproduction of free radicals can initiate the oxidative stress and nitrosative stress that can make cell damages including cell membrane, lipid, protein, and DNA [5,6], and trigger a number of diseases, such as inflammation-related diseases (arthritis, vasculitis, glomerulonefritis, lupus), cardiovascular disturbance, stroke, AIDS, gastritis, hypertension, premature aging, and neurological disturbance [1].

Oxidative stress and nitrosative stress occur as a result of disturbance in the equilibrium between prooxidant (ROS/RNS) and the ability of the antioxidant to neutralize it [7,8]. Body normally has the ability to neutralize the free radicals by forming the endogenous antioxidant [9], such as glutation, catalase, superoxide dismutase, vitamin A, uric acid, and coenzym Q10 [10]. However, environmental change, lifestyle, certain pathological conditions can shift the prooxidant-antioxidant equilibrium [11], so that exogenous antioxidant intake is needed [2].

Nowadays, there are natural and synthetic antioxidants available that are widely used in food, cosmetics, drug industries, and as ***t****herapeutic antioxidants* [1]. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are synthetic antioxidant examples, but their usage is restricted and reported to have side effect [1,3,8,10,12]. In line with hazardous disease risk development, there is tendency to increase the use of antioxidant originated from natural materials [1]. Therefore, finding new antioxidants more focused on natural resources [8], especially those from plants.

Sesewanua (*Clerodendrum fragrans* [Vent.] Willd. Syn. *Clerodendrum chinense* [Osbeck] Mabb.) belongs to family Lamiaceae. This plant is found in Sulawesi, Moluccas, Kalimantan, Java, Sumatra, Philippine, Malaysia penninsula, Thailand, and south China [13,14]. Its leaves are used as anti-inflammation by North Sulawesi people [15]. There is correlation between antioxidant and anti-inflammation [16,17]. Several species of *Clerodendrum*, such as *C. colebrookianum* [18], *C. trichotomum* [19], *C. infortunatum* [20], and *C. paniculatum* [17] have been reported to have antioxidant.

This study aims to evaluate the antioxidant activity and determine 50% inhibition concentration (IC) of the ethanol extract, hexane fraction, ethyl acetate fraction, water fraction of the sesewanua leaves using DPPH and nitrate oxide free radical scavenging methods.

MATERIALS & METHODS

Sample collection

Sesewanua leaves were collected in East Malalayang I village, Malalayang district, Manado, North Sulawesi Province. The samples were identified in the Center for Plant Conservation Botanic Gradens of the Indonesian Science Institution.

Extraction and fractination

The ethanol extract of sesewanua leaves was obtained through maceration in 70% ethanol following the method described in Farmakope Herbal Indonesia[21]. Fractionation was done using the extraction method[22] with minor modification of type and amount of solvent. The ethanol extract was suspended in the distilled water and fractionated using *n*-hexane and ethyl acetate solvents.

Phytochemical screening

Phytochemical screening was done for alkaloid, phenolic, tannin, flavonoid, saponin, steroid and triterpenoid compounds [23, 24].

DPPH free radical scavenging activity test

DPPH free radical scavenging activity testing utilized the method described in Elmastas *et al*. [25] as follows: as much as 1 ml of DPPH solution(0.1 mM in methanol) was added into 3 mL of sample. The mixture was strongly stirred and incubated at room temperature for 30 min., then the absorbance was measured in spectrophotometer at 517 nm wavelength. The DPPH free radical scavenging capacity was estimated as follows:

$$\text{DPPH scavenging effect (\%)} = \left(\frac{Ao-A1}{Ao}\right) \text{x } 100$$

where A_0 is absorbance of control solution and A_1 is absorbance of tested sample solution.

Nitrate oxide free radical scavenging testing

This testing was carried out using the following method: As much as 2 ml of 10mM Sodium Nitroprusside was mixed with 0.5 ml of phosphate-buffered saline (pH 7.4) and added with 0.5 ml of sample in different concentrations (0.1, 1, 10, 100, and 1000 ppm). As control, 2 mL of 10mM Sodium Nitroprusside was mixed with one mL of phosphate-buffered saline (pH 7.4). This mixture was incubated at 25ºC for 2.5 hours. Approaching the end of incubation time, 50 µL of sample was moved into a 96-well flat-bottom microplate. Using a multichannel pipettor, 50µL of 1% sulphanilamide was put into the well containing sample and control solution, then incubated for 5-10 min. at room temperature protected from light. After the incubation period, the mixture was added with 50 µL of 0.1% N-1-napthylethylenediamine dihydrochloride (NED) solution into all wells and incubated for 5-10 min. at room temperature protected from light. The solution turned to purple color, and in 30 min., the absorbance was measured at 546 nm wavelength [26, 27]. Percent inhibition of NO radical was estimated as follows:

$$\%\ inhibition\ of\ NO\ radical = \frac{Control-Sample}{Control} \text{ x } 100$$

Data analysis

The free radical inhibition data were analyzed in linear regression to determine 50% inhibition concentration (IC_{50}) of DPPH and NO free radicals.

RESULTS

Plant identification

Species identification of the plant, according to Center for Plant Conservation Botanic Gardens of the Indonesian Science Institution numbered B-2498/IPH.3/KS/VII/2018, indicated that the sesewanua used in this study was *Clerodendrum fragrans* [Vent.] Willd. Syn. *Clerodendrum chinense* [Osbeck] Mabb.

Extraction product

Extraction of 200 g leaf powder of the sesewanua in 70% ethanol found 55.6 g extract or 27.8% rendement.

Fractionation product

The fractionation of 25 g ethanol extract of sesewanua leaf using liquid-liquid extraction in *n*-hexane and ethyl acetate solvents obtained 2.51 g hexane fraction, 4.76 g ethyl acetate fraction, and 17.63 g water fraction.

Phytochemical screening

Phytochemical compounds in ethanol extract, n-hexane fraction, ethyl acetate fraction, and water extract of sesewanua leaf are presented in Table 1.

Table 1. Phytochemical screening on extract and fraction of sesewanua leaf.

Phytochemical compounds	Ethanol extract	n-hexane fraction	Ethyl Acetate fraction	Water fraction
Phenol	+	-	+	+
Tannin	+	-	+	+
Flavonoid	+	+	+	+
Saponin	+	+	-	+
Triterpenoid	-	-	-	+
Steroid	+	+	+	-
Alkaloid	-	-	-	-

Note: " + " present; " – " absent

The present finding is in agreement with other previous reports [28, 29, 30] that *C. fragrans* growing or cultured in Thailand, China, and Egypt contains flavonoid, phenols, streoid, and terpenoid.

DPPH free radical scavenging activity

The relationship between % inhibition of DPPH free radical and series of concentrations of ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction of *C. fragrans* leaf is presented in Figure 1.

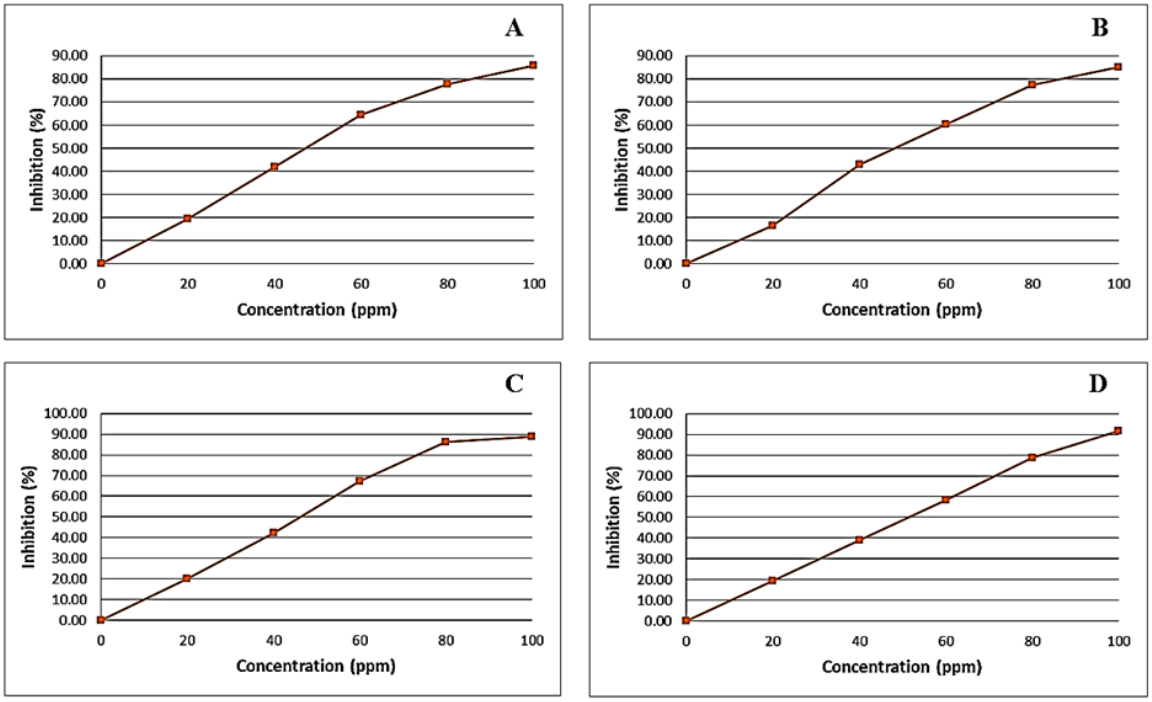

Figure 1. DPPH free radical scavenging activity of extract and fraction of *C. fragrans* leaf (A = ethanol extract; B = n-hexane fraction; C = ethyl acetate fraction; D = water fraction)

Nitrate oxide free radical scavenging activity

Relationship between % inhibition of NO radical and series of concentrations of ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction of *C. fragrans* leaf is presented in Figure 2.

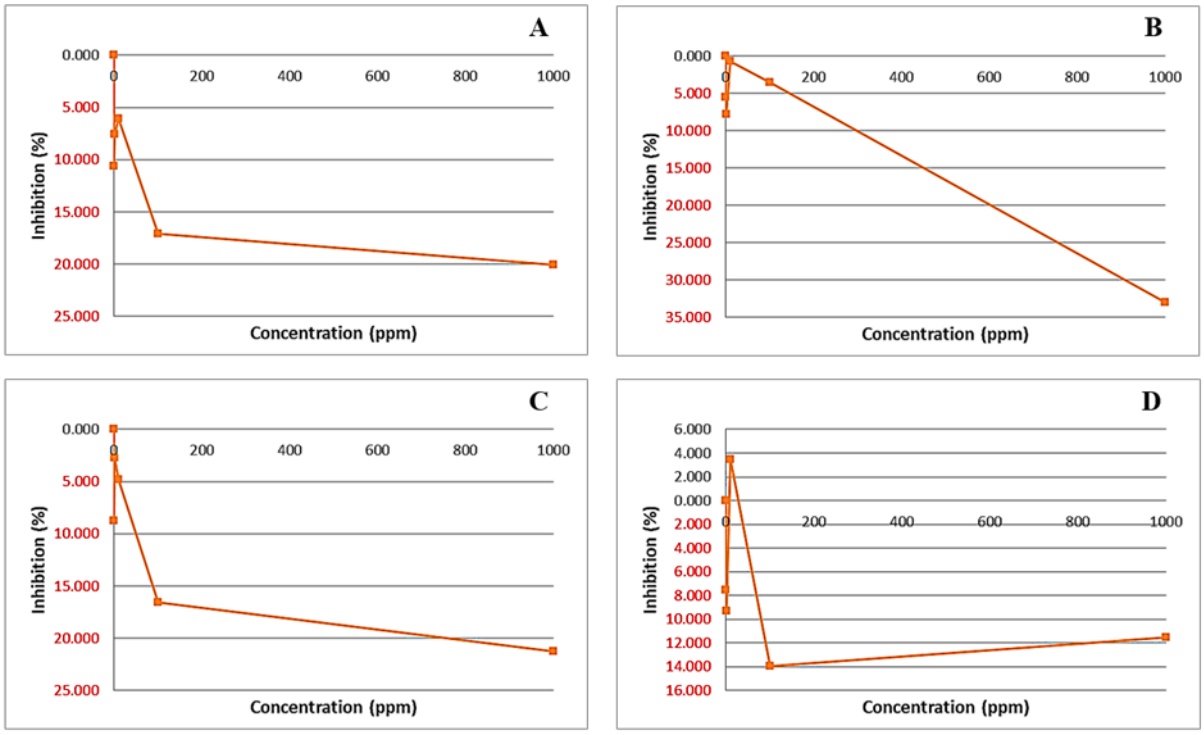

Figure 2. NO free radical scavenging activity of extract and fraction of *C. fragrans* leaf (A = ethanol extract; B = n-hexane fraction; C = ethyl acetate fraction; D = water fraction)

DISCUSSION

Figure 1 demonstrates that the extract and fraction of *C. fragans* leaf have antioxidant activity in scavenging the DPPH free radicals. The activity also increases with concentration. The scavenging ability of the extract and fraction indicated with 50% inhibition concentration (IC_{50}) shows that ethyl acetate fraction has the highest IC_{50}, then followed by that of ethanol extract, n-hexane fraction, and the lowest in water fraction (Table 3). The IC_{50} of ethyl acetate fraction, 12.05 ppm, and ethanol extract, 41.86 ppm, was categorized as having strong activity, while that of n-hexane fraction, 52.51 ppm, and water fraction, 52.55 ppm, belong to moderate activity category. [31]

The present study is also in line with several previous studies in other species of the same genus. Ethanol, chloroform, and petroleum ether extract of *C. infortunatum* leaf at the concentration of 250 ppm had DPPH free radical scavenging activity of 92.6%, 52.2%, and 16.7% [20]. The DPPH antioxidant compounds is found in the ethanol extract of *C. paniculatum* as well [17]. Several plants of family Lamiaceae, such as *Leonurus cardiaca, Lamium album, Marrubium vulgare, Stachys officinalis, Lamium purpureum*, and *Galeopsis speciosa*, have antioxidant activity [3].

NO radicals are biologically synthetized in the tissue through arginin metabolism to citrulline and free radical formation. In this study, as a source of NO free radicals, Sodium Nitroprussida was used, which in aqueous solution at physiological pH (7.2) undergoes decomposition to produce NO radicals. In aerobic condition, NO radicals will bond with oxygen and form stable nitrates or nitrites, that can be measured by addition of Griess reagent [27,32].

Figure 2 indicates that the extract and fraction of sesewanua leaves do not have antioxidant activity through NO free radical scavenging. The NO free radical scavenging ability of *C. fragans* leaf extract and fraction is indicated with 50% inhibition concentration (IC_{50}) of >1000 ppm. This result is not as expected and may be caused by various factors, such as nitrate reduction to nitrite and diazotization reaction [32]. Therefore, more detail study is needed.

ACKNOWLEDGEMENT

We would like to express our gratitude to the Poltekkes Kemenkes Manado, for the funding for us, so that this research can be carried out well.

REFERENCES

1. Lobo V, Patil A, Phatak A, Chandra N. Free Radicals, Antioxidants and Functional Foods: Impact on Human Health. Pharmacogn Rev. 2010; 4(8): 118–126.
2. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant Activity, Phenol and Flavonoid Contents of Some Selected Iranian Medicinal Plants. African J Biotechnol. 2006; 5(11):1142–1145.
3. Krishnaiah D, Sarbatly R, Nithyanandam R. A Review of The Antioxidant Potential of Medicinal Plant Species. Food Bioprod Process. 2011; 89: 217–233.
4. Goggi A, Malpathak N. Antioxidant Activities of Root, Stem and Leaves of Vernonia cinerea (L) Less. Free Radicals Antioxidants. 2017; 7(2):178–183.
5. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free Radicals and Antioxidants in Normal Physiological Functions and Human Disease. Int J Biochem Cell Biol. 2007; 39: 44–84.
6. Annapandian VM, Rajagopal SS. Phytochemical Evaluation and In vitro Antioxidant Activity of Various Solvent Extracts of Leucas aspera (Willd.) Link Leaves. Free Radicals Antioxidants. 2017; 7(2): 166–171.
7. Uys JD, Xiong Y, Townsend DM. Nitrosative Stress-Induced S-Glutathionylation of Protein Disulfide Isomerase. In: Conn PM, editor. Methods in Enzymology The Unfolded Protein Response and Cellular Stress, Part B. London: Elsevier Inc.; 2011; 321–332.
8. Subedi L, Timalsena S, Duwadi P, Thapa R, Paudel A, Parajuli K. Antioxidant Activity and Phenol and Flavonoid Contents of Eight Medicinal Plants from Western Nepal. J Tradit Chinese Med. 2014; 34(5): 584–590.
9. Iqbal S, Sivaraj C, Gunasekaran K. Antioxidant and Anticancer Activities of Methanol Extract of Seeds of Datura stramonium l. Free Radicals Antioxidants. 2017; 7(2): 184–189.
10. Carocho M, Ferreira ICFR. A Review on Antioxidants, Prooxidants and Related Controversy: Natural and Synthetic Compounds, Ccreening and Analysis Methodologies and Future Perspectives. Food Chem Toxicol. 2013; 51: 15–25.
11. Willcox JK, Ash SL, Catignani GL. Antioxidants and Prevention of Chronic Disease. Crit Rev Food Sci Nutr. 2004; 44: 275–295.
12. Branen AL. Toxicology and Biochemistry of Butylated Hydroxyanisole and Butylated Hydroxytoluene. J Am Oil Chem Soc. 1975; 52(2): 59–63.
13. Leeratiwong C, Chantaranothai P, Paton AJ. A Synopsis of the Genus Clerodendrum L. (Lamiaceae) in Thailand. Trop Nat Hist. 2011; 11(2): 177–211.
14. Wearn JA, Mabberley DJ. Clerodendrum (Lamiaceae) in Borneo. Syst Bot. 2011; 36(4): 1050–61.
15. Arini DI, Kinho J. Keragaman Tumbuhan Berkhasiat Obat Di Hutan Pantai Cagar Alam Tangkoko. J WASIAN. 2015; 2(1): 1–8.
16. Thakur R, Yadav K, Khadka KB. Study of Antioxidant, Antibacterial and Anti-Inflammatory Activity of Cinnamon (Cinamomum tamala), Ginger (Zingiber officinale) and Turmeric (Curcuma Longa). Am J Life Sci. 2013; 1(6): 273–277.
17. Hafiz I, Rosidah, Silalahi J. Antioxidant and Anti-Inflammatory Activity of Pagoda Leaves (Clerodendrum paniculatum L.) Ethanolic Extract in White Male Rats (Rattus norvegicus). Int J PharmTech Res. 2016; 9(5): 165–170.
18. Rajlakshmi D, Banerjee SK, Sood S, Maulik SK. In-Vitro and In-Vivo Antioxidant Activity of Different Extracts of The Leaves of Clerodendron colebrookianum Walp in The Rat. J Pharm Pharmacol. 2003; 55: 1681–1686.
19. Chae S, Kang KA, Kim JS, Hyun JW, Kang SS. Trichotomoside: A New Antioxidative Phenylpropanoid Glycoside from Clerodendron trichotomum. Chem Biodivers. 2006; 3:41–48.
20. Gouthamchandra K, Mahmood R, Manjunatha H. Free Radical Scavenging, Antioxidant Enzymes and Wound Healing Activities of Leaves Extracts from Clerodendrum infortunatum L. Environ Toxicol Pharmacol. 2010; 30: 11–18.
21. Depkes RI. Farmakope Herbal Indonesia. Edisi I. Jakarta: Departemen Kesehatan RI 2008; 187 pp.
22. Zhao S, Zhang L, Gao P, Shao Z. Isolation and Characterisation of The Isoflavones from Sprouted Chickpea Seeds. Food Chem. 2009; 114: 869–873.
23. Harborne JB. Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis. Chapman and Hall. London: Chapman and Hall; 1973; 278 p.
24. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical Screening and Extraction - A review. Int Pharm Sci. 2011; 1(1): 98–106.

25. Elmastas M, Isildak O, Turkekul I, Temur N. Determination of Antioxidant Activity and Antioxidant Compounds in Wild Edible Mushrooms. J Food Compos Anal. 2007; 20: 337–345.
26. Promega Corp. 2009 Griess Reagent System Protocol. Madison USA. 2009; p. 1-7 (5)
27. Alam MN, Bristi NJ, Rafiquzzaman M. Review on In Vivo and In Vitro Methods Evaluation of Antioxidant Activity. Saudi Pharm J. 2013; 21(2): 143–152.
28. Gao L, Wei X, He Y. Studies on Chemical Constituents in Leafs of Clerodendron fragrans. China J Chinese Mater Medica. 2003; 28(10): 948–951.
29. Kanchanapoom T, Chumsri P, Kasai R, Otsuka H, Yamasaki K. A New Iridoid Diglycoside from Clerodendrum chinense. J Asian Nat Prod Res. 2005; 7(3): 269–272.
30. Wahba HM, Abouzid SF, Sleem AA, Apers S, Pieters L, Shahat AA. Chemical and Biological Investigation of Some Clerodendrum species cultivated in Egypt. Pharm Biol. 2011; 49(1): 66–72.
31. Phongpaichit S, Nikom J, Rungjindamai N, Sakayaroj J, Hutadilok-Towatana N, Rukachaisirikul V, et al. Biological Activities of Extracts from Endophytic Fungi Isolated from Garcinia plants. FEMS Immunol Med Microbiol. 2007; 51(3): 517–525.
32. Tsikas D. Methods of Quantitative Analysis of the Nitric Oxide Metabolites Nitrite and Nitrate in Human Biological Fluids. Free Radic Res. 2005; 39(8): 797–815.

Cite this article as:

Benedicta Irene Rumagit *et al*. Anti-oxidant activity of Sesewanua (*Clerodendrum fragrans* [Vent.] Willd) leaf extract and fraction with 1,1-diphenyll-2-picrylhydrazyl (DPPH) and nitrate-oxide free radical scavenging method. Int. Res. J. Pharm. 2020;11(11):68-72.
http://dx.doi.org/10.7897/2230-8407.111199

Source of support: Poltekkes Kemenkes Manado, Manado, North Sulawesi, Indonesia, Conflict of interest: None Declared