International Journal of Public Health Science (IJPHS)
Vol.4, No.1, March 2015, pp. 21~26
ISSN: 2252-8806

Comparative Antioxidant Activity on the *Ficus benjamina* and *Annona reticulata* Leaves

Nyi Mekar Saptarini[1], Irma Erika Herawati[2]
[1]Faculty of Pharmacy, Padjadjaran University, Indonesia
[2]Department of Pharmacy, FMIPA, Al Ghifari University, Indonesia

Article Info

Article history:

Received Dec 12, 2014
Revised Jan 20, 2015
Accepted Feb 06, 2015

Keyword:

Annonareticulate
Antioxidant
Extract
Ficusbenjamina
Fraction

ABSTRACT

Antioxidants can prevent free radical formation. Natural antioxidants found in many plants, such as *Ficus benjamina* and *Annona reticulata*. The study aimed to compare the antioxidant activity of extracts and fractions of *Ficus benjamina* and *Annona reticulata* leaves against 1,1-diphenyl-2-picrilhydrazyl. The steps of this study consist of extraction, fractionation with n-hexane, ethyl acetate and water, phytochemical screening, antioxidant activity determination, and comparing the IC_{50} values. Percentage scavenging activity of the extracts and fractions against DPPH was calculated to determine the antioxidant activity. The IC_{50} value of *Ficus benjamina* was 127.86 ppm for ethanolic extract, 94.01 ppm for water fraction, 115.48 ppm for ethyl acetate fraction, and 335.50 ppm for n-hexane fraction. The IC_{50} value of *Annona reticulata* was 274.31 ppm for ethanolic extract, 211.42 ppm for water fraction, 367.91 ppm for ethyl acetate fraction, and 741.08 ppm for n-hexane fraction. The results showed that the *Ficus benjamina* water fraction was the best antioxidant compared to other extract and fraction.

Corresponding Author:

Nyi Mekar Saptarini,
Faculty of Pharmacy,
Universitas Padjadjaran,
Jl Raya Bandung Sumedang km 21, Jatinangor, West Java, Indonesia 45363.
Email: nyi.mekar@unpad.ac.id

1. INTRODUCTION

Free radicals defined as chemical species possessing unpaired electrons. These species responsible to many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [1]-[3]. The most effective compound to eliminate free radicals is antioxidants. Antioxidants prevent free radical formation by scavenging them or promoting their decomposition and suppressing such disorders [4]-[6]. There is a growing interest toward natural antioxidants. The idea of natural antioxidant in herbal resources can protect biology systems from oxidative stress [7]-[9]. *Ficus benjamina* (Moraceaae) and *Annona reticulata* (Annonaceae) are herbal resources that had antioxidant activity.

F.benjamina and *A. reticulate* have been used as traditional medicines. This is due sencondary metabolites. *F. benjamina* leaves contain tannins, carbohydrates, phytosterols, flavonoids, phenolics, saponins, oils and fats [10],[11]. The root barks, leaves and stems of *A.reticulata* contain isoquinoline alkaloids and flavonoids [12]. Phenolics, flavonoids, vitamins, and minerals (Cu, Mn, Zn, Se and Fe) are known as natural antioxidants[13]. This study aimed to compare the antioxidant activity of extracts and fractions of *F.benjamina* and *A. reticulata* leaves against 1,1-diphenyl-2-picrilhydrazyl (DPPH).

2. RESEARCH METHOD

2.1. Materials

F. benjamina and *A. reticulate* leaves obtained from Manoko, West Java, Indonesia. All chemicals with analytical grade are DPPH, vitamin C, ether, hydrochloric acid, sulfuric acid, ethanol, amyl alcohol, n-hexane, ethyl acetate, ammonia, chloroform, magnesium, Dragendorff and Mayer reagent, iron (III) chloride, gelatin, vanillin, and potassium hydroxide.

2.2. Samples Preparation

Simplicia macerated with 70% ethanol for 3 days. Each day, the solvent changed with the fresh one. All macerat collected and vaporated with rotary rotavapor. Dissolve 10 g of ethanolic extract with aquadest to obtain 100 mL solution, then done liquid liquid extraction with n-hexane and ethyl acetate, threetimes for each solvent. All fraction collected and vaporated. Phytochemical screening was conducted to simplicia, extract, and fraction with Fransworth methods[14].

2.3. Antioxidant Activity Determination

Dissolve 4 mg of DPPH with 96% ethanol in 100 mL volumetric flask (40 µg/mL). Dissolve 5 mg of vitamin C and 50 mg of samples (extract and fraction of *F. benjamina* and *A. reticulata*) with 96% ethanol, each in 100 mL volumetric flask, then diluted the solutions to prior concentration.

The DPPH radical was used for the determination of free radical-scavenging activity of the extracts and fractions. The modified method of Okada and Okada (1998) was employed[15].A portion (2 mL) of the different concentrations of extract, fraction or vitamin C, each in tube, was added with 3 mL of 40 µg/mL DPPH. The mixtures were vortexed and incubated in a dark chamber for 30 min, then the absorbancies were measured at 517 nm using spectrophotometer (*Ray* LEIGH).The blank was96% ethanol in place of sample. Percentage scavenging activity was calculated using this formula:

% of DPPH inhibition = [(Ab-Aa)/Ab] x 100

Where Aa and Ab are the absorbance values of the sample and the blank, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value.

3. RESULTS AND DISCUSSION

3.1. Samples Preparation

Maceration is a cold extraction method. These methods was conducted to maximal extraction of the entire secondary metabolites in the sample. *A. reticulata*leaves (6.94%) has more soluble secondary metabolites in 70% ethanol compared to *F. Benjamina* leaves (5.68%). Liquid liquid extraction was conducted to seperate the secondary metabolites based on its polarity[16]. Water fraction of *F.benjamina* and *A.reticulata* bigger than ethyl acetate and n-hexane fraction (Table 1). We concluded that the majority of secondary metabolites in both samples were the polar secondary metabolites.

Tabel 1. Fraction Rendement

Sample	*n*- Hexane (%)	Ethyl Acetate (%)	Water (%)
F. benjamina	4.66	11.92	82.00
A. reticulata	23.60	21.98	54.00

Table 2. Phytochemical Constituents of *F. benjamina*and *A. reticulata*

Sample	Group	Simplicia	Ethanolic Extract	*n*-Hexane Fraction	Ethyl Acetate Fraction	Water Fraction
F. benjamina	Alkaloid	-	-	-	-	-
	Polyphenolic	+	+	-	-	+
	Tannin	-	-	-	-	-
	Saponin	-	-	-	-	-
	Quinone	+	+	-	-	+
	Flavonoid	+	+	+	+	+
	Monoterpenoid / sesquiterpenoid	+	+	-	-	-
	Steroid and triterpenoid	-	-	-	-	-
A.reticulata	Alkaloid	-	-	-	-	-
	Polyphenolic	-	-	-	-	-
	Tannin	-	-	-	-	-
	Saponin	+	+	-	-	+
	Quinone	-	-	-	-	-
	Flavonoid	+	+	+	+	+
	Monoterpenoid / sesquiterpenoid	-	-	-	-	-
	Steroid and triterpenoid	-	-	-	-	-

+ = detected, - = undetected

Phytochemicals are synthesized by plant for self defense from pathogens and environmental stress. These phytochemicals can also used to cure several diseases. It can be stated that the phytochemicals are the compound that determined the medical potential of any plant. Phytochemical screening with color reaction method was conducted to determine the group of secondary metabolites in the sample. Phytochemical screening showed extract that has the same constituents as simplicia (Table 2). It's mean that maceration with 70% ethanol can extract all groups of secondary metabolites in simplicia.

All ethanolic extract and fraction of *F. benjamina* and *A. reticulata* contain flavonoids (Table 2). Jain *et al* (2013) was found that methanolic extract of *F. bejamina* leaves had high level of phenolic (4.006 mg gallic acid equivalence/g) and flavonoids (16.005 mg quercetin acid equivalence/g) [10]. Flavonoids are the phenolic compounds, which are synthesized by plants due to adaptation in response to biotic and abiotic stresses (infection, water stress, cold stress, and high visible light) [17]. Flavonoids inhibit the oxidation reaction through radical scavenging mechanisms by donating an electron to the unpaired electrons in free radicals. *In vitro*, flavonoids are potent inhibitor to lipid peroxidation, as acatcher of reactive oxygen or nitrogen species, and also able to inhibit the lipooxygenase and cyclooxygenase activity [18]-[20]. The antioxidant activity of phenolic compounds depend on their molecular structure, based on the availability of phenolic hydrogens, which result in the formation of phenoxyl radicals due to hydrogen donation[21].

3.2. Antioxidant Activity Determination

Table 3.Persentage of Inhibition Sample to DPPH Radicals

Sample		Concentration (ppm)	% inhibition	Sample		Concentration (ppm)	% inhibition
F. benjamina	Ethanolic extract	40	34.82	*A. reticulata*	Ethanolic extract	100	33.40
		80	38.03			150	34.76
		100	44.87			200	42.88
		120	48.71			250	45.14
		160	56.83			300	54.62
	Water fraction	40	35.04		Water fraction	50	31.60
		80	40.59			100	38.14
		100	46.15			150	42.66
		120	53.84			200	44.01
		160	87.07			250	57.78
	Ethyl acetate fraction	40	32.26		Ethyl acetate fraction	200	29.11
		80	36.96			250	32.50
		100	49.78			300	46.27
		120	55.34			350	48.08
		160	57.47			400	52.14
	n-hexane fraction	100	17.37		n-hexane fraction	200	31.60
		150	21.09			300	32.50
		200	21.58			400	39.50
		250	24.56			500	42.66
		300	51.86			600	44.01

Natural antioxidants from medicinal plants are a good choice to control oxidative stress. Because of natural origin, these compounds are usually non toxic. Antioxidants upon interaction with DPPH radicals transfer a proton to DPPH radicals by direct abstraction of phenol H- atoms and electron transfer process, thus neutralizing its free radical character, which produce DPPH-H (2,2-diphenyl-1-picrylhidrazyn), i.e DPPH with less reactivity.The DPPH radicals solution was purple, because the unpaired nitrogen electrons[22]. The absorbance of 40 ppm DPPH radicals solution was 0.443. These reaction was showed with color alteration from purple to yellow with absorbance reduction at 517 nm. The degree of discolouration indicates the scavenging potential of the antioxidants [23],[24]. Antioxidant activity were measured from reaction of the sample (extracts and fractions) and vitamin C solutions with DPPH solution, then percentage of DPPH inhibition were counted (Table 3).The IC_{50} value of extracts and fractions werecounted from the linear regression equation of the curve of concentrations versus% inhibition (Table 4). More smallerthe IC_{50}value, it'smeanmore higher antioxidant activity.The antioxidant activity was dose dependent manner.

Jain *et al* (2013) was determined the IC_{50} value for the methanolic extract of *F. benjamina* leaves was 59.07 ppm[10]. Jamkhande *et al* (2014) was determined the IC_{50} value for the methanolic extract of *A. reticulate* roots was 108.71 ppm[25]. These values lower than our results. These were due to differences in the solvent and the part of the plant which used in extraction, so that the content of secondary metabolites in the extracts was different.

Table4.The IC_{50} Value of Sample

Sample		Linear Regression Equation	R^2	IC_{50}
F.benjamina	Ethanolic extract	y=0.1918x+25.475	0.9638	127.86
	Water fraction	y=0.4233x+10.203	0.8515	94.01
	Ethyl acetate fraction	y=0.235x+22.86	0.8786	115.48
	n-hexane fraction	y=0.144x – 1.946	0.6720	335.50
A.reticulata	Ethanolic extract	y = 0.1056x + 21.032	0.9421	274.31
	Water fraction	y = 0.1165x + 25.369	0.9090	211.42
	Ethyl acetate fraction	y = 0.1233x + 4.636	0.9184	367.91
	n-hexane fraction	y = 0.0350x + 24.062	0.9320	741.08
Vitamin C		y=5.3525x+12.497	0.9149	7.00

Table 4 showed that the *F. benjamina* water fraction has the best antioxidant activity (IC_{50} 94.01 ppm) compared to the other extracts and fractions, both *F. benjamina* and *A. reticulata. F. benjamina* water fraction contains polyphenols, quinones, and flavonoids. All these structure having the hydroxyl group which can donate hydrogen to interact with DPPH radical to produce the DPPH-H.A compound is categorized as a very strong antioxidant when the IC_{50} value is less than 50 ppm, strong antioxidant if the IC_{50}value is 50-100 ppm, mild antioxidant if the IC_{50}value is 100-150 ppm, and weak antioxidant if the IC_{50} values is 150-200 ppm [22]. The *F. benjamina* water fraction categorized as strong antioxidant. The *F. Benjamina* ethanolic extract and ethyl acetate fraction categorized as mild antioxidant. The remnants are considered have no antioxidant activity.

The ratio of antioxidant activity ofthe IC_{50} value of *F. benjamina* and *A. reticulate* to vitamin C (7 ppm) was determined to compare the sample reactivity to DPPH radicals.The *F. benjamina* water fraction had the best ratio to vitamin C (Table 5). This means that the *F. benjamina* water fraction was potent antioxidant to be developed.

Table5.TheAntioxidant Activity Ratioof*F. benjamina* and *A. reticulata*toVitamin C

Sample		IC_{50} (ppm)	Ratio
F. benjamina	Ethanolic extract	127.86	1 :18.26
	Water fraction	94.01	1: 13.43
	Ethyl acetate fraction	115.48	1: 16.49
	n-hexane fraction	335.50	1: 46.57
A. reticulata	Ethanolic extract	274.31	1:39.18
	Water fraction	211.42	1:30.20
	Ethyl acetate fraction	367.91	1:52.55
	n-hexane fraction	741.08	1:105.86

4. CONCLUSION

The extract and fractions of the *A. reticulate* leaves are considered have no antioxidant activity. In *F. benjamina* leaves, the n-hexane fraction is considered have no antioxidant activity, ethanolic extract and ethyl acetate fraction are categorized as mild antioxidant, and the water fraction is categorized as strong antioxidant.

ACKNOWLEDGEMENTS

We thank Putri Fajrina Yuda and Rosmiati for their technical support.

REFERENCES

[1] Metodiewa D., Koska C.,"Reactive oxygen species and reactive nitrogen species: relevance to cyto(neuro)toxic events and neurologic disorders" An overview. *Neurotox Res*,vol. 1, pp. 197-233, 2000.
[2] Young I., Woodside J.,"Antioxidants in health and disease", *J Clin Pathol,*vol. 54, pp. 176-86, 2001.
[3] Heinecke J.,"Oxidative stress: new approaches to diagnosis and prognosis in atherosclerosis", *Am J Cardiol,*vol. 91, pp. 12A-6A, 2003.
[4] Maxwell S.,"Prospects for the use of antioxidant therapies",*Drugs,*vol. 49, pp. 345-61, 1995.
[5] Kaur C., Kapoor H.,"Antioxidant activity and total phenolic content of some Asian vegetables", *Int J Food Sci Tech,*vol. 37, pp. 153-62, 2002.
[6] Cesquini M., Torsoni M., Stoppa G., Ogo S.,"t-BuOH-induced oxidative damage in sickle red blood cells and the role of flavonoids", *Biomed Pharmacother,*vol. 57, pp. 124-9, 2003.
[7] Larson R.,"The antioxidants of higher plants", *Phytochem,*vol. 27, pp. 969-78, 1988.
[8] Gazzani G., Papetti A., Massolini G., Daglia M.,"Anti- and pro-oxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment", *J Agric Food Chem",*vol. 46, pp. 4118- 22, 1998.
[9] Velioglu Y., Mazza G., Gao L., Oomah B.,"Antioxidant activity and total phenolics in selected fruits, vegetables and grain products", *J Agric Food Chem,*vol. 46, pp. 4113-7, 1998.
[10] Jain A., Ojha V., Kumar G., Karthik L., Rao K.,"Phytochemical Composition and Antioxidant Activity of Methanolic Extract of Ficus benjamina (Moraceae) Leaves", *Research J Pharm and Tech,*vol/issue: 6(11), pp. 1184-90, 2003.
[11] Almahy H., Rahmani M., Sukari M., Ali A.,"The chemical constituents of Ficus benjamina Linn. and their biological activities", *Pertanika Journal of Science and Technology*,vol/issue: 11(1), pp. 73-81, 2003.
[12] Nadkarni K.,"Indian Materia Medica", Mumbai, India: Popular Prakashan,pp. 12-27, 2002.
[13] Saleh M., Clack S., Woodard B., Deolu-Sobogun S.,"Antioxidant and free radical scavenging activities of essential oils", *Ethnicity and Disease*,vol/issue: 20(1), pp. S1-78-82, 2010.
[14] Fransworth N.,"Biological and Phytochemycal Screening of Plants",*JPharm Sci,*vol/issue: 1(5), pp. 247-68, 1966.
[15] Okada Y., Okada M.,"Scavenging effect of soluble proteins in broad beans on free radicals and active oxygen species", *J Agric Food Chem,*vol. 46, pp. 401-6, 1998.
[16] Houghton P., Raman A.,"Laboratory Handbook for the Fractionation of Natural Extracts", London: Chapman & Hall, 1998.
[17] Pitchersky E., Gang D.,"Genetics and biochemistry of secondary metabolites in Plants: An evolutionary perspective", *Trends Plant Sci*,vol. 5, pp. 459-45, 2000.
[18] Gordon M.,"Food antioxidants", In: Hudson B, editor. The mechanism of antioxidant action in vitro. London, NY: Elsevier,pp. 50-80, 1990.
[19] Halliwell B., Gutteridge J.,"Free Radical in Biology and Medicine", New York: Oxford University Press, pp. 25-50, 2000.
[20] Okawa M., Kinjo J., Nohara T., Ono M.,"DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Radical Scavenging Activity of Flavonoids Obtained from Some Medicinal Plants", *Biol Pharm Bull*,vol/issue: 24(10), pp. 1202-5, 2001.
[21] Ramarathnam N., Ochi H., Takeuchi M.,"Natural Antioxidants; Chemistry, Health Effects and Application", In: Shahidi F, editor. Antioxidant defense system in vegetable extracts. Champaign IL: AOAC Press, pp. 76-87, 1997.
[22] Molyneux P.,"The Use of The Stable Free Radical Diphenylpicrilhydrazyl (DPPH) for Estimating Antioxidant Activity", *Songklanakarin J Sci Technol,*pp. 211-9, 2004.
[23] Foti M., Daquino C., Geraci C.,"Electron-transfer reaction of cinnamic acids and their methyl esters with the DPPH radical in alcoholic solutions", *J of Organic Chemistry*,vol/issue: 69(7), pp. 2309-14, 2004.
[24] Villaño D., Fernández-Pachón M., Moyá M., Troncoso A., García-Parrilla M.,"Radical scavenging ability of polyphenolic compounds towards DPPH free radical", *Talanta*,vol.71, pp. 230-5, 2007.
[25] Jamkhande P., Wattamwar A., Pekamwar S., Chandak P.,"Antioxidant, antimicrobial activity and in silico PASS prediction of *Annona reticulata* Linn. root extract", *Beni-Suef University J of Basic and Applied Science,*vol. 3, pp. 140-8, 2014.

BIOGRAPHIES OF AUTHORS

Full name	Nyi Mekar Saptarini
NIP	19761016 200604 2 001
Birth	Pandeglang, 16 Oktober 1976
Expertise	Pharmaceutical Analysis and Medicinal Chemistry
Occupation	Lecturer
Affiliation	Faculty of Pharmacy Universitas Padjadjaran
Office address	Jl. Raya Bandung-Sumedang Km.21,Jatinangor,West Java, 45363 Telp/Fax : 022-7796200/022-7796200
Email address	nyi.mekar @unpad.ac.id mkrnyi@gmail.com

Full name	Irma Erika Herawati
NIK	140090379/0.140
Birth	Bandung 7 Maret 1979
Expertise	Biology Pharmacy
Occupation	Lecturer
Affiliation	Departement of Pharmacy, FMIPA, Universitas Al-Ghifari
Office address	Jl. CisarantenKulon No.140 Soekarno-Hatta Bandung, West Java 40293 Telp/Fax : 022-7806223/ 022-7835813
Email address	irmaerika@yahoo.com