ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 07, Issue, 09, pp.14968-14971, September, 2017

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

ANTIOXIDANT ACTIVITY OF NUTMEG BARK (*MYRISTICA FRAGRANS* HOUTT)

[1,*]Mustanir, [2]Binawati Ginting and [3]Safrina

[1,2] Lecturer Chemistry Department of Mathematics and Natural Sciences Faculty, Syiah Kuala University, jl. Tanoh Abe No.3 Darussalam, Banda Aceh 23111

[3]Magister Scienes Student of Mathematics and Natural Sciences Faculty, Syiah Kuala University, Jl. Tgk, Chiek Pante Kulu No. 5 Darussalam, Banda Aceh, 23111,

ARTICLE INFO

Article History:

Received 18th June, 2017
Received in revised form
19th July, 2017
Accepted 24th August, 2017
Published online 30th September, 2017

Keywords:

Nutmeg bark (*Myristica fragrans* Houtt),
n-hexane extract,
DPPH,
Antioxidant activity.

ABSTRACT

This study would be focused on nutmeg bark (*Myristica fragrans* Houtt) isolation to observe antioxidant activity of nutmeg bark (NB). The result of phytochemicals screening test showed that metabolite secondary compound of NB were terpenoid and tanin. Antioxidant activity from the n-hexane extract of nutmeg bark (*n*-HENB) was conducted using the DPPH method. Antioxidant activity of *n*-HENB with DPPH at concentration of 25, 50 and 100 ppm is obtained IC_{50} 63.76 ppm. The results of column chromatography of *n*-HENB obtained 11 combined fraction, that were fraction of A to K. The results of antioxidant activity of IC_{50} with a very strong category was the fraction of H and K with 52.04 and 42.21 ppm, respectively. It showed that the fraction of the NB had a better inhibition due to the presence of some active compounds contained in fractions that can synergize in inhibiting free radicals, so that the IC_{50} value of the fraction is more powerful than the extract.

Corresponding author

Citation: Mustanir, Binawati Ginting and safrina. 2017. "Antioxidant Activity of Nutmeg Bark (*Myristica fragrans* Houtt)", *International Journal of Development Research*, 7, (09), 14968-14971.

INTRODUCTION

This studi was using natural ingredients as a drug that has antioxidant activity to be one of the targets of study, after the public concern about the side effects of synthetic antioxidants that make natural antioxidants as an alternative. The use of synthetic antioxidants began to be limited because the results of research have been conducted to suggest that synthetic antioxidants such as BHT (Butylated Hydroxy Toluene) that can be poison to experimental animals and carcinogenic (Ginting, *et al*., 2017). Nutmeg is a valuable spice that has been used for centuries around the world. In addition to its use in food and beverage additives, nutmeg has been used in traditional medicine for stomach and kidney disorders. Antioxidants, antimicrobials and central nervous system effects of nutmeg have also been widely reported in the literature. Nutmeg is a rich source of essential oils, triterpenes, and various types of phenolic compounds.

Many secondary nutmeg metabolites indicate biological activity that may support its use in traditional medicine (Lim, 2012, Abourashed and Abir, 2016). Many modifications of extraction methods and isolation of secondary metabolites were performed to obtain biologically active chemicals. It is called bioassay-guided isolation (Radwan *et al*., 2014; Eom *et al*., 2016; Acuna *et al*., 2016) which is a method that every stage of biological isolation test. In the extraction, solvent selection becomes very important. Extraction is a classic procedure for obtaining organic compounds (crude extracts) from dry plant tissue. The simplest method of isolation is the much-performed partition as the beginning of the extract purification. The partition uses two non-mixed solvents added to the extract. (Heinrich *et al*., 2010). In this study will focus on the isolation of nutmeg bark to determine secondary metabolite chemical compounds and observe the potential of antioxidant activity in the nutmeg bark. The value of IC_{50} indicates the potential of a compound as cytotoxic.

The larger the IC_{50}'s value so the compounds are less toxic. Separation of active antioxidant compounds in this study will use n-hexane extract of nutmeg bark, so that in this research will be isolated active compounds as antioxidants from hexane extract of nutmeg bark (bioassay guided or each isolation step will be guided by biological tests include test Antioxidant activity with DPPH). The pure isolate obtained will be elucidated by its interpretive structure spectral data of UV, FTIR, ^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC, HMBC, COZY and MS.

METHODOLOGY

Population and Sample

The population and samples in this study is NB from Paya Peulumat village, East Labuhan Haji subdistrict, South Aceh district.

Apparatus and chemicals

The equipment used in this research were a blender, flask, beakers, flask, beaker, funnel, analytical balance, test tubes, tube racks, maceration set, vacuum rotary evaporator set, oven, set of Gravity chromatography column, distillation equipment set, pipette, micropipette. For analytical instruments used the UV-visible (Shimadzu Model of UV-160A). The chemicals used in this study are: n - heksana , methanol, ethyl acetate, ammonia, chloroform, HCl, $FeCl_3$, boric acid, acetic acid, acetone, citric acid, ether, Bourchard Liberman reagent (glacial acetic acid - $H_2SO_{4(P)}$), Mayer reagent (tetra potassium iodo merkurat), Dragendorf reagent (Bi $(NO_3)_3$) and Wagner reagent (I_2 KI). Chemicals which was used to anti-oxidant test were methanol pro analysis, DPPH and vitamin C.

Procudures

Sample Preparation

The NB is cleaned and dried in the open air indoors so it is not exposed to direct sunlight. Extraction of NB begins with sample preparation by drying and milling using a blender until get fine powder.

The NB Extraction

The NB powder of 10 kg, macerated with methanol for 24 hours. After that, filtered and obtained the fraction of methanol and residue. The residue is further macerated with methanol until get the clear filtrate. The methanol filtrate was concentrated using a rotary evaporator to obtain methanol extract. The methanol extract is partitioned with ethyl acetate and separated from ethyl acetate and methanol layer. The methanol layer was evaporated and obtained a concentrated extract of methanol. The methanol extract was obtained then partitioned with n-hexane, to obtain a methanol and a n-hexane layer. The n-hexane layer was evaporated and obtained a concentrated of *n*-HENB. The *n*-HENB then tested for antioxidant activity, isolation and purification.

Isolation and Purification of *n*-HENB

The *n*-HENB of 30 g in gravity column chromatography with 60G silica gel, 400 g of *n*-HENB as stationary phase and n-hexane motion phase: ethyl acetate determined from thin layer chromatography (gradient elution). Each fraction of 50 mL was collected. Each fraction obtained was analyzed by thin layer chromatography (TLC) and fractions that having similar stain patterns will be combined and tested for their antioxidants. Furthermore, the active fraction obtained was recromatographed, and the appearance of a single stain from the TLC analysis and carried out by TLC or High Performance Liquid Chromatography (HPLC). Then it was recrystallized with acetone and n-hexane to get crystals form. The crystals obtained were each tested for antioxidant activity. The pure isolate obtained was took the data spectra by UV, IR, 1D and 2D NMR (proton and carbon) and MS.

Antioxidant Activity Analysis

Preparation of DPPH solution 0.4 mM

DPPH powder is weighed with carefully as much as 7.9 mg, then dissolved with methanol in 50 mL flask, immediately cover with aluminum foil then homogenized. Store the solution in a dark bottle and always made a fresh one when it will be used.

Preparation Of NB Extract Solution And Vitamin C

To make the total concentration variations of extracts first, made a 500 ppm mother liquor by dissolving 5 mg of total extract into ethanol and musb be sufficient until 10 mL. Furthermore, from the mother liquor made variations in concentrations of 25, 50 and 100 ppm of solution again for antioxidant activity test. As a comparison test the antioxidant activity against vitamin C, because vitamin C is a compound that has a very high antioxidant activity. The mother liquor of vitamin C is made by dissolving 3 mg of vitamin C with methanol until the volume is exactly 5 mL. Further diluted to 3; 6; 9; 12 and 15 ppm. Furthermore, homogenized with a *vortex mixer* and incubated for 30 minutes at 30 ° C, measured absorbance at wavelength of 517 nm.

Blanko Solution Test

A total of 1 mL of 0.4 mM DPPH solution and its volume was 5 mL with methanol in the reaction tube (covered with aluminum foil), then homogenized with a vortex mixer and incubated for 30 min at 37 ° C. Further will be measured uptake at a maximum wavelength of 517 nm.

Antoxidant Activity Total Test of NB Extract Solution and Vitamin C

25 ppm concentration extract as much as 250 µL, 50 ppm extract as much as 500 µL and 100 ppm extract as much as 1000 µL, each added a solution of DPPH 0.4 mM as much as 1 mL then the volume is 5 mL with methanol and the container is covered with aluminum foil.Further homogenized using a vortex mixer and incubated for 30 min at 30 °C. Read more uptake at 517 nm wavelength (Ramaswamy, *et al*., 2011).

Calculation of IC_{50}

IC_{50} value is the concentration of antioxidants in ppm (mg / mL) which is able to inhibit 50% of free radicals. Values of IC_{50} is obtained from the intersection of the lines between the 50% inhibitory concentration axis, and then inserted into the equation $Y = a + bX$ where $Y = 50$ and X showed IC_{50} values. The percentage of inhibition is calculated by the following formula:

$$antioxidant\ power = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}}\ x\ 100\%$$

Equation 1. (Saeed, *et al*., 2012)

Where:

Antioxidant power (IC_{50}) : Concentrations of antioxidant compounds that inhibit 50% of the formation of DPPH compounds (µg / ml)

$Abs_{control}$: Absorbance of control solution (synthetic antioxidant Trolox or vitamin C)

Abs_{sample} : Absorbance of sample solution

RESULT AND DISCUSSION

Phytochemicals Profile of *n*-HENB

Secondary metabolites extraction of NB performed with maceration process for 24 hours. Maceration process using methanol so that all components of compounds that are polar and non-polar can be extracted properly. Maceration is done to obtain a clear filtrate. Methanol filtrate concentrated by evaporation using a rotary evaporator to obtain methanol extract. Concentrated methanol extract obtained partitioned with ethyl acetate to remove chlorophyll and fatty substances. Methanol layer which has split into ethyl acetate layer was partitioned with *n* -hexane, to attract the compounds that are non-polar in order to obtain extracts of *n* -hexane. The extract was obtained then tested for phytochemical content.

Harbone (1987) describes the phytochemical analysis process is part of pharmacognosy science which studies methods or means of analysis of chemical constituents contained in the plant or animal as a whole or its parts, including the way of isolation or separation. The content of phytochemicals in a sample is seen based on reactions that show positive results with certain reagents. Treatment to test for the class of chemical compounds contained in the extract in accordance with the procedures of Harborne (1987) which consist of flavonoids, alkaloids, steroids, terpenoids, and saponins. The test results phytochemical *n* -hexane bark extract of nutmeg can be seen in Table 1. The positive result of secondary metabolite compounds is shown by terpenoids and tannins. In the study conducted Ginting, *et al*., (2017) showed positive results in terpenoid compounds also in *n*-HENB. Identification of terpenoids showed positive results using Lieberman-Buchard reagent (acetic anhydride-concentrated H_2SO_4) which gives a red color when the solution is dripped on the plate drops.

Table 1. Results of phytochemical test *of n*-HENB

Secondary Metabolite Type		*n*-HENB	
		Author	Ginting, *et al* (2017)
1.	Alkaloids		
-	Dragendorf	-	-
-	Wagner	-	-
-	Mayer	-	-
2.	Steroids	-	-
3.	Terpenoids	+	+
4.	Flavonoids	-	-
5.	Saponin	-	-
6.	Tanin	+	Not tested

(+) Contains secondary metabolite compounds
(-) Does not contains secondary metabolite compounds

The principle of the reaction in the reaction mechanism of terpenoids test is condensation or release H_2O and incorporation carbocation. This reaction begins with a process of acetylation of a hydroxyl group using acetic acid anhydride. The acetyl group is a group that will loose to form a double bond. Furthermore, the release of hydrogen and electron groups, resulting in double bonds move to show the appearance of red color (Ginting, *et al*., 2017). Identification of tannins in the NB showed positive results carried out by reacting the sample with a reagent of iron (III) chloride ($FeCl_3$) to give a dark green color. The addition of the extract with 1% $FeCl_3$ in water causing blackish green color because of tannin in the bark of nutmeg will react with Fe^{3+} ions to form complex compounds (Harbone, 1987).

Antioxidant Activity of *n*-HENB

Tests conducted to determine the antioxidant activity IC_{50} value of materials or samples. The antioxidant antioxidant examination was performed by DPPH method by reacting the sample with DPPH solution and measured at wavelength of 517 nm. The use of DPPH selected as the process was simple, easy, did not last long, and has a high sensitivity (Ginting, *et al*., 2017; Saeed, *et al*, 2012).

Table 2. Antioxidant activity test of *n*-HENB and fractions

fractions	Concentration (ppm)	Inhibition (%)	IC_{50} (PPM)
NB	25	15.42	63.76
	50	34.23	
	100	85.05	
A	25	23.48	496
	50	24.77	
	100	27.69	
B	25	25.47	106.39
	50	44.86	
	100	45.79	
C	25	23.13	114.17
	50	26.64	
	100	45.79	
D	25	27.57	137.73
	50	28.27	
	100	43.57	
E	25	19.39	247.59
	50	24.07	
	100	29.79	
F	25	24.65	133.32
	50	37.73	
	100	41.59	
G	25	8.18	114.67
	50	11.68	
	100	44.39	
H	25	28.62	58.04
	50	36.80	
	100	85.28	
I	25	26.4	90.90
	50	32.59	
	100	54.09	
J	25	18.11	300
	50	20.44	
	100	26.75	
K	25	49.07	42.21
	50	49.18	
	100	57.71	

Antioxidants that examined in the compounds has function as inhibitors of oxidative damage to biological macromolecules due to oxidation of free radicals or so-called *reactive oxygen species* (ROS). All these radicals have in common that have one or more unpaired electrons and potentially cause damage to living cells (Akinboro, *et al*., 2011).

Examination of antioxidant activity was performed on the *n*-HENB and fractions of partition results. It was measured their absorbance in concentration of 25, 50 and 100 ppm and antioxidant activity assay results are shown in Table 2. The antioxidant activity of the *n*-HENB shows the value of 63.76 ppm. These results are in line with tests performed by Ginting *et al*. (2017) on NB extracts of 63.817 ppm. Each fraction obtained from thin layer chromatography (TLC) and fraction which showed the same pattern of stain were combined then examined its antioxidant activity. IC_{50}'s value obtained from the plot between the concentration of the extract to the value of the percentage inhibition (calculated based on the equation 1) were obtained as shown in Figure 1.

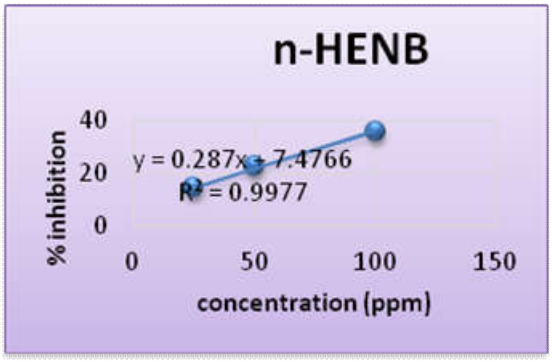

Figure 1. Line equation of *n*-HENB

Based on Figure 1, we get the equation of line is $y = 0.287x + 7.476$. Calculation of IC_{50} done by substituting the value of y with a score of 50 in order to obtain the value of x as the value of the IC_{50} on the *n*-HENB is 63.76 ppm. The *n*-HENB that has been tested its antioxidant activity then partitioned and also its results will be tested. IC_{50} 's value partition results are shown in Table 2. The calculation of IC_{50} values for all fractions calculated same as *n*-HENB. From Table 2 can be seen, the fraction of H and K fractions showed IC_{50} values were very low compared to other fractions are 52.04 and 42.21 ppm, respectively. With IC_{50} values are low indicates that the fraction of H and K have antioxidant activity better than other fractions.The lower the IC_{50} value, the better its antioxidant activity (Filbert, *et al*., 2014). Values of IC_{50} (inhibition concentration) if referring to the category of Gessler, *et al*., (1994) is as shown in Table 3.

.

Table 3. Value Categories of IC_{50}

Range of IC_{50}	Category
≤ 10 ppm	Very good
10-50 ppm	Good
≥ 50 ppm	Not Good

Based on the category analysis, the fraction of K provides a good antioxidant activity value of 42, 21 ppm.

Conclusion

Based on research that has been done, then it can be knotted right that the NB are secondary metabolites, which showed positive results are terpenoids and tannins. Antioxidant activity on *n*-HENB shows the IC_{50} value 63.76 ppm. In fractions showed antioxidant activity is better than the extract that is the fraction of H and K with IC_{50} values are 52.04 and 42.21 ppm, respectively.

Conflict of Interest

The author declare that there is no conflict of interest with the parties concerned in this study.

Acknowledgment

The author would like to thank the Ministry of Technology Research and High Education of Indonesia for research funding through the Lecturer Research Grant in 2016.

REFERENCES

Abourashed, E. A., dan Abir, T. E., 2016. Chemical Diversity And Pharmacological Significance Of The Secondary Metabolites Of Nutmeg (Myristica Fragrans Houtt), Department of Pharmaceutical Sciences, *College of Pharmacy, Chicago State University, Phytochemical Review*, Vol. 15, Issue 6, pp.1035-1056.

Acuña, U.Munoz., P. Blanco, S. Matthew, Esperanza J., Carcache de Blanco, 2016. New Acyclic bis-phenylpropanoid and Neolignans From Myristica Fragrans Houtt., *Exhibiting PARP-1 and Nf-κb Inhibitory Effects, Food Chemistry Journal*, pp. 269-271.

Akinboro, A , Kamaruzzaman, M. B, Asmawi, M. Z, Sulaiman, S. F, Sofiman, O. H, 2011. Antioxidans in Aqueous extract of Mirystica fragrans (Houtt) Suppress Mitosis and Cyclophosphamide-induce chromosomal aberrations in Allium cepa L. Cells, *Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)*. ISSN 1673-1581 (Print); ISSN 1862-1783.

Eom, H. J, H. R. Kang, H. K. Kim, E. B. Jung, H. B. Park, Ki Hyun, Kim, Ki Sung Ka, 2016. Bioactivity-guided Isolation of Antioxidant Triterpenoids From Betula Platyphylla Var. Japonica Bark, Biorganic Chemistry Journal, Volume 66, pp. 97-101.

Filbert, H. S. J. Koleangan, Max. R. J.R., V. S., Kamu, 2014. Penentuan Aktivitas Antioksidan Berdasarkan Nilai IC_{50} Ekstrak Metanol dan Fraksi Hasil Partisinya Pada Kulit Biji Pinang Yaki, Jurnal FMIPA, Unsrat, Manado.

Gessler, M. C., Nkunya, L.B. Mwasumbi, M. Heinrich and M. Toner, 1994. *Screening tanzanian medical plants antimalarial activity*, Acta Trop. 56:65-77.

Ginting , B, Mustanir, H. Helwati, L. Septa Desiyana, Eralisa, R. Mujahid, 2017. *Antioxidant Activity of N-Hexane Extract Of Nutmeg Plants From South Aceh Provine, Jurnal Natural* Vol. 17, No. 1.

Harborne, J.B, 1987. Metode Fitokimia : Penuntun Cara Modern Menganalisis Tumbuhan, Penerbit ITB, Bandung.

Heinrich, M., J. Barnes, S. Gibbon, A. M. Williamson, 2010, Farmakognosi dan Fitoterapi, (Fundamentals of Phamacognosy and Phytotherapi), Penerbit buku Kedokteran EGC, Jakarta.

Lim, T. K., 2012. Myristica Fragrans, Edible Medicinal And Non-Medicinal Plants: Volume 3, Fruits.

Radwan, M. M., N. Tabanca, D. E. Wedge, A. H. Tarawneh, S. J. Cutler, 2014. Antifungal Compounds Fromturmeric And Nutmeg With Activity Against Plant Pathogens, Fitoterapia Journal.

Saeed, N., M.R. Khan, dan M. Shabbir, 2012. Antioxidant Activity, Total Phenolic And Total Flavonoid Contente Of Whole Plant Extracts Torilis Leptophylla L, Research Article Bio Med Central, Department of Biochemistry, Islamabad University.

****