



ISOLATION OF ESSENSIAL OIL OF NUTMEG (*Myristica fragrans* Houtt) and ANTIOXIDANT ACTIVITY TEST WITH DPPH

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Abstract. The essential oil of the nutmeg plant (*Myristica fragrans* Houtt) has been obtained from roots, bark, fruit, mace and seeds using Stahl steam distillation. Each essential oil have tested for antioxidant activity with DPPH. Antioxidant activity of essential oil from each nutmeg plant to DPPH with concentration 25, 50 and 100 ppm. Each essential oil did not show strong antioxidant activity but the result of nutmeg isolation had strong antioxidant activity with IC₅₀ that was 80,555 ppm. Based on GC-MS analysis of essential oil of nutmeg isolate obtained *myristicin* compound with 96.52% area and time Retention 22.127. *Myristicin* is one of the main components of essential oils of nutmeg plants that play an important role as antioxidants.

Keywords: Nutmeg plant (*Myristica fragrans* Houtt), Essential Oil, DPPH, Antioxidant

I. INTRODUCTION

Indonesia is a prosperous country in various types of plants that can be used as medicines (herbal medicine). Active compounds in plants are generally contained in the form of secondary metabolites such as alkaloids, steroids, terpenoids, flavonoids, coumarins, tannins, saponins and so forth. Secondary metabolites can serve as a self-defense against plants from outside attacks such as viruses, fungi and bacteria [1]. One of the plants that could potentially be medicinal is the nutmeg plant (*Myristica fragrans* Houtt). Nutmeg plants grow a lot in the region of West and South Aceh. Nutmeg is known as a spice plant that has economic value and multipurpose because every part of the plant can be utilized in various industries. Besides being used as flavoring spice, nutmeg is also used as a medicinal material because it has properties as antioxidants, antimicrobial [2] and antifungals [3]. Nutmeg plant is one of the essential oil producing plants that known as nutmeg oil [4]. Essential oil is one of the multi benefit vegetable oils [5]. The basic material of this oil is obtained from various parts of plants such as leaves, flowers, fruits, seeds, seed shell, stems, roots or rhizomes. One of the main

characteristics of volatile oil is its volatile and distinctive flavor. The essential oil of nutmeg known in the world market is oil which is processed from mace and nutmeg [6]. The nutmeg oil contains 20 types of compound components with main composition is β -pinen 22.69%, α -pinen 14.06% and α -thujen 13.93% and shows antimicrobial and anticancer activity [7]. The essential oil of nutmeg seed has also been reported to have antioxidant, antimicrobial and antifungal activity [2] and has a strong antioxidant properties [6] the presence of antioxidant activity from the nutmeg mace [8], mace and nutmeg flowers can also be used as a powerful antibacterial and antioxidant [9,8]. Antioxidants are compounds that can inhibit the rate of oxidation or neutralize free radicals. All of these radicals have in common that have one or more unpaired electrons and potentially cause damage to living cells [10]. These unpaired electrons cause free radicals to be highly reactive compounds to the cells of the body by binding to electrons of cell molecules. In everyday life, we can not be clear from the free radical compounds. Foods that are either fried or burnt, excessive sun exposure, cigarette smoke, motor vehicle fumes, certain drugs, toxins and air pollution are some sources of free radical compounds. In a previous study reported

that the extract ethyl acetate of nutmeg leaves had antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, while the leaf nutmeg methanol extract is inactive [11]. Further phytochemical test of methanol extract of nutmeg leaf contains alkaloid, flavonoid, terpenoid and tannin, while ethyl acetate extract contains flavonoid compound and has antifungal activity against *Candida albicans* [12]. The isolation result of methanol extract of nutmeg contains flavonoid compound type *dihydrocaemferol* or *3,5,7,4'-tetrahydroxy dihydroflavonol* which is active as an antioxidant with IC_{50} 9,75 ppm [13] and also has anticancer with activity IC_{50} 5,3209 μ g/mL [14]. Research on essential oils on the stem and root of the nutmeg especially from Aceh Province has not been reported. Likewise, the isolation of chemical components and antioxidant activity test part of the plant. Therefore it is necessary to isolate parts of nutmeg plants such as fruit, mace, seeds, roots and stem bark by steam distillation method and activity test of antioxidants using the free radical DPPH method. Essential oils of the most actively planted parts were isolated and determined by structure based on data analysis *Gas Chromatography – Mass Spectrometry* (GC-MS).

II. METHODOLOGY

Equipment used in this research is Stahl steam distillation device, condenser, vial bottle, measuring cup and flask, separating funnel, analytical scales, test tube, oven, Kromatografi Gravity Column equipment, eyedropper, micro pipettes. For instrument used in the form GC-MS QP2000A spectrometer 70 eV and UV visible (Model Shimadzu UV-160A). The materials used in this study are: technical *n*-hexane, diethyl ether, DMSO, reagent Sulfuric Acid ($Ce(SO_4)_2$), Na_2SO_4 anhydrous, methanol p.a, DPPH and vitamin C. Sample used in this research is part of nutmeg plant (*Myristica fragrans* Houtt) which includes roots, bark, fruit (nutmeg), mace and seeds obtained from the village of Kampung Paya, North Klau District, South Aceh district in August of 2016. Results of determination in LIPI Cibinong states that the plants used in this study is *Myristica fragrans* Houtt (pala), family Myristicaceae. Determination of this plant is done.

Distillation of essential oils from plant samples

A total of 500 g of nutmeg samples were fed into distillation flask, aquadest added 1000 ml until all the flesh of nutmeg submerged then string up the distillation equipment (Stahl

equipment) and oil temperature 120°C. Distilled for 3 h is calculated after boiling water, the oil obtained is accommodated into the erlenmeyer and then added Na_2SO_4 anhydrous to separate water and oil. The same treatment is done to obtain essential oils on bark, roots, seeds and mace. Essential oils are then tested for antioxidant activation. The essential oil obtained is divided into two parts for the antioxidant activity test and analysis of its components with GC-MS.

Antioxidant test

Antioxidant activity test can be done by making solution of DPPH 0,4 mM. DPPH powder (BM 394.32 g/mol) was weighed as much as 7.9 mg, subsequently dissolved with methanol in 50 mL flask, then homogenized. To make a variation of the total concentration of each essential oil of nutmeg plants (root, bark, fruit, mace and seeds) previously made main solution 500 ppm by dissolving each essential oil 5 mg into methanol until volume reaches 10 mL. Furthermore, from the main solution be made variations in solution concentration of 25, 50 and 100 ppm (Table 1).

Table 1 Variation concentration of antioxidant activity test on essential oil

Concentration (ppm)	The main solution taken (μ L)	DPPH solution (mL)	Information
25	250	1	Volume be appointed to 5 mL
50	500	1	
100	1000	1	

Table 2 Variation concentration of antioxidant activity test on Vitamin C

Concentration (ppm)	The main solution taken (μ L)	DPPH solution (mL)	Information
3	25	1	Volume be appointed to 5 mL with methanol
6	50	1	
9	75	1	
12	100	1	
15	125	1	

As a comparative do the test of antioxidant activity of vitamin C. The main vitamin C solution is made by dissolving 3 mg of vitamin C dissolved with methanol until the volume is exactly 5 mL, further diluted to 3, 6, 9, 12 and 15 ppm (Table 2). Further homogenized with vortex mixer and incubated for 30 min at 37°C, measured uptake at a wavelength of 517 nm [15]. The blank solution test, 1 mL of 0.4 mM DPPH solution and volume of 5 mL with methanol in a test tube (which is covered with aluminum foil), then homogenized with a vortex mixer and incubated for 30 min at 37°C, further measured uptake at wavelength 517 nm using UV-Vis instrument. Antioxidant test of essential oils of nutmeg and vitamin C.

Essential oil at 25 ppm concentration of 250 μ L, 50 ppm concentration of 500 μ L and 100 ppm concentration of 1000 μ L, each added 1 mL of DPPH 0.4 mM and volume of 5 mL with methanol. Further homogenized using a vortex mixer and incubated for 30 min at 37°C then read the uptake on $\lambda = 517$ nm [16]. IC_{50} value is the concentration of antioxidants in ppm (μ g/mL) which is able to inhibit 50% of free radicals. IC_{50} value obtained from the intersection of the line between 50% inhibitory power with the concentration axis, then included in the equation $Y = a + bX$ which is $Y = 50$ and X value shows IC_{50} . The percentage of inhibition is calculated by the Eq (1).

$$\%inhibition = \frac{blankabsorption - sampleabsorption}{blankabsorption} \times 100\% \quad (1)$$

Essential oil is declared active if IC_{50} value is less than 100 ppm (μ g / mL).

III. RESULT DAN DISCUSSION

The method that can be used to obtain essential oil of nutmeg is generally by water distillation method. Water distillation has the advantage of being used to distill powdered material, as it can coagulate if distilled by steam distillation [17]. A wet nutmeg cultivated sample of 500 g distilled water steam to extract essential nutmeg oil components contained in the sample. The advantage of water steam distillation is the presence of hydrodifusi events where water vapor will enter into the cellular tissue of the plant resulting in the rupture of the cell wall of the plant so that the oil contained in it will be pushed out. The mixture of water steam and essential oil will flow into the condenser so that condensation occurs and distillate is produced. Essential oils that still contain water molecules are dried by adding anhydrous Na_2SO_4 . The function of addition of anhydrous Na_2SO_4 to bind the water still contained in the oil, to obtain essential oil of nutmeg no longer contain water.

Phytochemical test

Fresh part of the nutmeg cultivars was analyzed phytochemically to determine the secondary metabolite content contained in the plant, while terpenoid test was performed. The results of phytochemical tests on seeds, mace, fruit, bark and root of nutmeg plants contain secondary terpenoid metabolite compounds. The main chemical components contained in essential oils are generally monoterpen compounds. Secondary metabolites or chemical components

in a part of the plant are generally present in other parts of the plant [18].

Results of Antioxidant Activity Test

Antioxidant activity is expressed by % DPPH inhibition of antioxidant ability to damp free radical of DPPH. The antioxidant activity test was performed on λ_{max} that is 517 nm. The results of antioxidal activity of root essential oils, bark, fruit, mace and nutmeg seeds can be seen in Table 3. The result of antioxidant activity test in Table 3 shows that essential oil of fuli and fruit has IC_{50} value stronger than other nutmeg parts such as seeds, roots and bark. The IC_{50} value from each essential oil of mace and fruit is 185,943 ppm and 221,036 ppm, respectively. The world's known essential oil is derived from nutmeg seed and nutmeg mace, while the nutmeg flesh is rarely processed into essential oil [19]. Then it can be proposed that the fruits essential oil can be isolated further because not many have isolated. The curve of inhibition relationship to the concentration of root essential oils, bark, fruit, mace and nutmeg seeds can be seen in the curve of Figure 1.

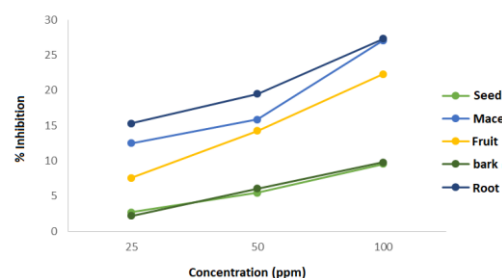


Figure 1 Curve of inhibition relationship to the concentration of essential oils of nutmeg plants.

GC-MS analysis of root essential oil obtained 49 components of compound, 32 component stem bark, 33 pieces of fruit, 27 components and nutmeg nut obtained 6 components of compound. The main components of the five parts of the nutmeg plant are shown in Table 4. Compounds contained in root essential oils, bark, fruit, mace and seeds of GC data each contain *Myristicin*, *Terpineol-4*, *Alpha-Terpinol*, *Dodecanoic acid*, *Torreyol*, *Palmitin*, *Safrol* with varying amounts. In the essential oils of fruits contain compounds *Myristicin*, *Alpha-Terpineol*, *Terpineol-4*, *safrole*, *Terpinene 1-ol* with consecutive amounts 34.85, 33,00, 14,56, 2,38 and 1,86% while on the other part of the essential oil is less. The terpinene-4-ol content in nutmeg oil produced is higher when compared to oil from the mace of nutmeg (9,04 %).

Table 3 Value data of inhibition and IC₅₀ value essential oil of nutmeg plants

Essential oil sample	Absorbance control (DPPH)	Concentration (ppm)	Avg. absorbance	% Inhibition	IC ₅₀ value (ppm)
Root	0,856	25	0,725	15,30	241,493
		50	0,689	19,51	
		100	0,622	27,34	
Stem Bark	0,856	25	0,837	2,22	497,270
		50	0,804	6,07	
		100	0,772	9,81	
Fruit	0,856	25	0,791	7,59	221,036
		50	0,734	14,25	
		100	0,665	22,31	
Mace	0,856	25	0,749	12,50	185,943
		50	0,720	15,89	
		100	0,624	27,10	
Seed	0,856	25	0,833	2,69	520,356
		50	0,809	5,49	
		100	0,774	9,58	
Vitamin C		3	0,356	58,41	-2,055
		6	0,236	72,43	
		9	0,129	84,93	
		12	0,099	88,43	
		15	0,091	89,37	

Table 4 GC-MS analysis of essential oil

No	Retention Time	compound	% Area	Similarity
GC-MS Data of Nutmeg Essential Oil				
1	26.415	Myristicin	34.85	81
2	16.856	Alpha-Terpineol	33	97
3	15.639	Terpineol-4	14.56	95
4	19.059	1,3-Benzodioxole, 5-(2-propenyl-(CAS) safrole	2.38	94
5	13.93	Terpinene 1-ol	1.86	92
GC-MS Data of Nutmeg seed Essential Oil				
1	33.763	2,3-dihydroxypropyl ester	30.33	84
2	39.006	Dodecanoic acid, ethenyl ester	19.06	82
3	37.541	Dodecanoic acid (2,2-dimethyl-1,3-dioxolan-4-yl) methyl ester	17.44	82
4	34.497	Hexadecanoic acid, methyl ester (CAS) methyl palmitate	10.58	93
5	25.35	Myristicin	11.19	85
GC-MS Data of Nutmeg skin essential oil				
1	16.008	Alpha-Terpinol	27.73	95
2	25.559	Myristicin	19.94	83
3	27.088	(+) Spathulenol	6.54	92
4	28.543	Torreyol	2.85	89
5	21.384	Nerylacetate	2.03	87
GC-MS Data of Nutmeg root essential oil				
1	25.68	Myristicin	30.47	82
2	27.174	(+) Spathulenol	10.18	92
3	28.593	Torreyol	5.29	87
4	28.253	(-)-Caryophyllene oxide	4.05	84
5	27.657	Cyclohexanol, 2-methylene-3-(1-methylethenyl)-,acetate, cis-	3.98	80
GC-MS Data of Nutmeg mace essential oil				
1	25.9	Myristicin	52.23	80
2	27.421	Dodecanoic acid (CAS) lauric acid	13.16	93
3	15.385	Terpineol-4	9.04	94
4	18.82	Safrol	3.48	93
5	33.782	Palmitin, 2-mono-	3.26	83

Terpinene-4-ol has strong antimicrobial activity, anti-inflammatory and antifungal activity. These compounds are used as flavors, perfumes, and medications (such as treating sinus, bronchial, and throat infections, and others).

Isolation and Purification of Nutmeg Fruits

Fractionation of nutmeg essential oil to separate its components was done by column chromatography using silica gel 60 mesh silicone phase and *n*-hexane motion phase: diethyl ether (9:1) gradient elution. The

fractions obtained were analyzed by Thin Layer Chromatography (TLC) to see the stain patterns of each fraction on the TLC plate with eluent *n*-hexane: diethyl ether (9:1). The same stain and R_f patterns serve as a basis for combining multiple fractions. Of the 140 fractions, 13 fractions were obtained as in Table 5.

Table 5 Chromatographic results of nutmeg essential oil column

No	Faction Group	Fraction Weight (mg)	Color
1	MFMAbu 1 (1-3)	0,9	Clear
2	MFMAbu 2 (4-5)	25,9	Pale Yellow
3	MFMAbu 3 (6-7)	0,7	Clear
4	MFMAbu 4 (8-10)	0,7	Clear
5	MFMAbu 5 (11-14)	0,3	Clear
6	MFMAbu 6 (15-18)	0,2	Clear
7	MFMAbu 4 (19-28)	0,8	Clear
8	MFMAbu 5 (29-37)	0,5	Clear
9	MFMAbu 6 (38-40)	0,8	Clear
10	MFMAbu 7 (41-47)	0,2	Clear
11	MFMAbu 8 (48-56)	0,6	Clear
12	MFMAbu 9 (57-83)	0,9	Clear
13	MFMAbu 10 (84-140)	0,9	Clear

Based on the results of GC-MS data from MFMAbu 2 containing compound components that is *Myristicin*. *Myristicin*, isoeugenol and eugenol compound have strong antioxidant activity [2], therefore the compounds having antioxidant activity in the MFMAbu 2 fraction are thought to contain the compound. The curve of inhibition % relationship to the concentration of root essential oils, bark, fruit, mace and nutmeg seeds can be seen in Figure 2. The result of the fraction test of MFMAbu 2 has IC₅₀ value that is 80,555 ppm which has strong antioxidant activity and has a single stain pattern, then purified by means of preparative TLC using *n*-hexane and diethyl ether, to obtain a pure compound weighing 25.9 mg. Furthermore, MFMAbu 2 compound was done two-dimensional TLC (Figure 3). The TLC results can show that the fraction of MFMAbu 2 is purely visible from one resulting stain. The fraction of MFMAbu 2 obtained was a pale yellow liquid weighing 25.9 mg. MFMAbu 2 Fraction.

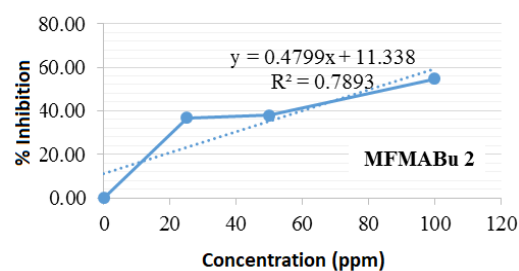


Figure 2 Correlation Curve Inhibition on MFMAbu Concentration

Analyze MFMAbu 2 by GC-MS

Chromatographic column results of the fruit oil atsri with eluen *n*-hexane: diethyl ether 9:1. The fraction of MFMAbu 2 has a greater amount than the other fractions weighing 25.9 mg. The MFMAbu 2 fraction was further analyzed by GC-MS spectroscopy. The component analysis of Fraction MFMAbu 2 using GC-MS produces the main component that is *myristicin* with a 96.52% area while the 4% was detected as a solvent compound and a group of fatty acids. *Myristicin* is one of the main components of nutmeg plants essential oils that have an important role as antioxidants [20, 21, 2]. The *myristicin* compounds contained in the active isolate of persimmon leaf (*Diospyros kaki* Thunb.) has antioxidant activity with IC₅₀ value 100,0 µg/mL [22]. *Myristicin* has activity as antioxidant [23], *myristicin* is classified in flavonols from flavonoid compounds which are phenolic compounds that functionate as antioxidants [24]. One important component in nutmeg is *myristicin* which has activity as a hepatoprotector [25]. *Myristicin* is a psychoactive drug, functionate as an anticholinergic [26]. The *myristicin* compound is a volatile compound and has a peculiar smell of nutmeg and has great assassinate power against insect larvae and can increase mental activity or as a psychoactive or psychotropic substance.

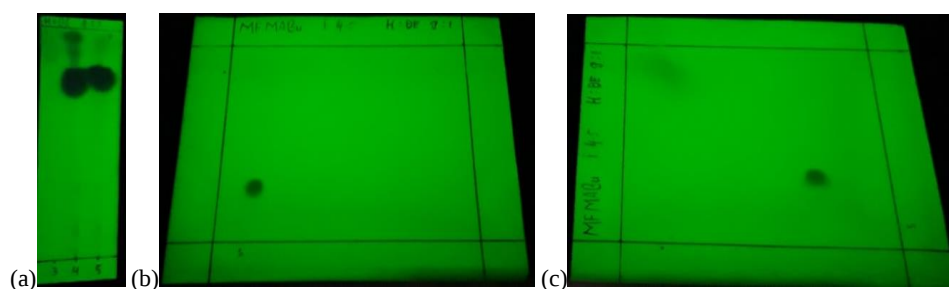


Figure 3 (a) TLC Chromatogram Fractions 4 and 5 Nutmeg with Eluen *n*-hexane: diethyl ether (9: 1) (b) Two-dimensional TLC chromatogram compound 4-5 Nutmeg with Eluen *n*-hexane: diethyl ether (9:1), first elution (c) Chromatogram compound 4-5 Nutmeg, second elution

CONCLUSION

Antioxidant activity results of essential oil of mace and fruit have IC₅₀ value stronger than other nutmeg parts such as seeds, roots and bark. GC-MS analysis of root essential oil obtained 49 components of compound, 32 component of stem bark, 33 component of fruit, 27 components and nutmeg nut obtained 6 components of compound. Fraction analysis of MFMA Bu 2 using GC-MS produces a major component of *myristicin* with a 96.52% area.

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