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Sunscreen and Antioxidant Potential of Myristicin in Nutmeg Essential Oils (*Myristica fragrans*)

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Abstract- objectives: **Nutmeg is a native Indonesian plant which is widely exported in the form of nutmeg and nutmeg essential oil. Exploration of nutmeg essential oils content and its activities continues to be carried out. Nutmeg essential oil contained 8-11% of myristicin, and this compound has alkenylbenzenes and ether groups. The present study was designed to evaluate nutmeg essential oil, nutmeg essential oil without myristicin and myristicin in-vitro activity as UV-B protector and DPPH antioxidant agent by UV-Vis spectrophotometer. Nutmeg essential oil, nutmeg essential oil without myristicin and myristicin were identified by GC-Ms. Myristicin was isolated with fractional distillation at 423 K, 25 mmHg and identified the structure by MS, IR and ^{1}H-NMR spectrometer. The result showed that myristicin is compound which is the most contributed in nutmeg essential for UV-B protection, it has shown with myristicin give better SPF value than nutmeg essential oil, which is 19.44 to 4.98, and nutmeg essential oil without myristicin give 0,71, similarly to antioxidant activity, myristicin is the main compound that causes antioxidant effects in nutmeg essential oil, it's indicated by the IC_{50} value of myristicin which is better than nutmeg essential oil and nutmeg essential oil without myristicin, which is 189; 3,181 and 33,254 ppm respectively.**

Keywords: myristicin, nutmeg essential oils, sunscreen, antioxidant

I. INTRODUCTION

Nutmeg is a native plant of Indonesia which is an export commodity. Nutmeg exported in the form of seed and essential oils. The essential oils of nutmeg is known to have antibacterial activity [1], antioxidants [2], anti-inflammation [3], analgesic [3], antipyretic activity [3], anti-thrombotic [3] and anti-diarrheal [3]. Nutmeg essential oil has a main myristicin compound, 4-terpineol, safrole, sabinene, α-pinene, and δ-limonene [4]. Myristicin contained in nutmeg oil is considered to have toxicity effects [5] thus considered as compounds that give adverse effects. Based on the composition of compounds contained in nutmeg essential oil, myristicin is the only compound that has an aromatic group with a composition that is quite high. Other high composition compounds do not have conjugated double bonds (chromophores) and ausochrome groups, so myristicin is thought to be the compound with the highest contribution to the ability of nutmeg essential oil in the role as an absorbent of UV light and as an antioxidant.

The novelty of this research is the study of compounds in nutmeg essential oils that act as sunscreens and antioxidants. This study aimed to separate myristicin from nutmeg essential oils and to determine antioxidant and sunscreen activity of nutmeg essential oils, essential oils of nutmeg without myristicin and myristicin.

II. MATERIALS AND METHODS

A. Materials

Nutmeg essential oils obtained from Caringin, Bogor, West Java. Separation of myristicin from nutmeg essential oils, DPPH (e-merck), ethanol (e-merck), ethylhexyl methoxycinnamate (e-merck).

B. Methods

1) Myristicin separation and Analysis of compound.

The separation submitted with fractional distillation at 423 K, 25 mmHg, so that there's three samples: nutmeg essential oils (MP), nutmeg essential oils without myristicin (MPTM), and myristicin (M). All samples analyzed by GC-MS (QP-2010 Plus, Shimadzu), myristicin in structural analysis using IR (IR Prestige-21, Shimadzu) and ^{1}H-NMR (400 MHz Agilent).

2) Antioxidant activity test

All samples tested with DPPH with a UV-Vis spectrophotometer to determine the IC_{50} value.

$$\% \text{ I} = \frac{A \text{ blank} - A \text{ sample}}{A \text{ blank}} \times 100 \quad (1)$$

A blank is absorbance of blank and A sample is the absorbance of the sample. Concentrate on giving 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition versus concentration of sample [6].

3) Sunscreen test

All samples tested at 290-320 nm. The equation of Mansur calculates the uptake of each sample. By processing absorbance data from positive samples, ethylhexyl methoxycinnamate into the Mansur equation, so that obtained a value of the Correction Factor (CF), Equation 2 [7].

$$SPF = CF \times \sum_{290}^{320} EE\ (\lambda) \times I\ (\lambda) \times A\ (\lambda) \quad (2)[7]$$

Information:

EE = spectrum of erythemal effects (Table 6)
I = intensity of the light spectrum (Table 6)
A = uptake of sunscreen products
CF = correction factor

TABLE 1: THE COMPOUNDS OF NUTMEG ESSENTIAL OILS, NUTMEG ESSENTIAL OIL WITHOUT MYRISTICIN, MYRISTICIN

No.	Retention time (minute)	Compounds	Compositions (%)		
			Nutmeg essential oil	Nutmeg essential oil without myristicin	Myristicin
1	9661	α-pinene	1:41	1.66	-
2	10 008	α-pinene	20.96	24.66	-
3	10 557	Camphene	0:29	0:34	-
4	11 548	Sabinene	26.62	31.32	-
5	11 720	β-pinene	15:10	17.76	-
6	12 079	Myrcene	2:08	2:45	-
7	12 583	4- (Bromomethyl) Cyclohexene	0:04	-	-
8	12 698	α-phellandrene	0:50	0:59	-
9	12 773	β-ocimene	0:52	0.61	-
10	13,099	alpha-terpinene	2:22	2.61	-
11	13 407	1-methyl-2- (1-methylethyl) benzene	0:31	0:36	-
12	13 582	Limonene	5:24	6:16	-
13	14 630	γ-terpinene	3:27	3.85	-
14	15,100	Sabinenehydrate	0:17	-	-
15	15 580	α-terpinolene	1:00	1:25	-
16	16,200	Linalool	0:15	-	-
17	17 010	1-terpineol	0:12	-	-
18	18 913	4-terpineol	3.71	4:36	0.92
19	19 431	α-terpineol	0:46	0:58	-
20	22 080	bornyl acetate	0:09	-	-
21	22 383	Safrole	1:15	1:44	0.98
22	22 649	Amylanisole	0:43	-	-
23	23 923	Myrtanylacetate	0:31	-	2:21
24	24 241	Eugenol	0:09	-	0.64
25	24 771	α-Copaene	0:46	-	1:18
26	25 496	Methyleugenol	0:09	-	0.71
27	26 038	β-caryophyllene	0:07	-	-
28	26 316	α-bergamotene	0:10	-	0.72
29	28 320	β-bisabolene	0:04	-	-
30	28 632	β-cadinene	0:06	-	0:43
31	28 858	Myristicin	12.94	-	92.13

III. RESULTS

The GC-Ms analysis results from nutmeg essential oils and separation results are present in **Table I.** Nutmeg essential oils containing 31 compounds with myristicin content of 12.94%. Myristicin separation from nutmeg essential oils carried out up to 92.13% of purity.

Myristicin structure analysis with IR results is present in **Table 2.**

TABLE 2.: THE RESULTS OF FUNCTIONAL GROUP ANALYSIS BY FT-IR.

Wavenumber (cm^{-1})	Bond type
3070	= CH (Csp2)
2893	-CH (Csp3)
1627 and 1504	(C = C) aromatic
1435	-CH2-
1357	-CH3
1128	-COC-

Myristicin structure analysis with ^{1}H-NMR results showed the chemical shifts: 3.28 ppm (m, $-CH_2-$), 3.87 ppm (s, $-CH_3$), 5.04 ppm (t, $=CH_2$), 5.90 ppm (m, -CH=), 5.91 ppm (s, $-CH_2-$), 6.34 ppm(s, Ar-H), and 6.37 ppm(s, Ar-H).

The result of antioxidant activity using DPPH: nutmeg essential oils has 3.181 ppm, nutmeg essential oil without myristicin has 33.254 ppm and myristicin has 189 ppm of IC_{50}.

Sunscreen activity in vitro test carried out by UV spectrophotometry by reading the absorbance, then calculated with the equation Mansur SPF value for protection against UV-B (320-290 nm). The result of analysis give SPF of nutmeg essential oils, nutmeg essential oilwithout myristicin and myristicin are 4.98, 0.71 and 19.44, respectively. For more details, can be seen in Table 5.

IV. DISCUSSION

A. Myristicin separation and Analysis of compound

The GC-MS analysis results showed that nutmeg essential oil used in this study had 31 compounds with myristicin levels of 12.94%. Nutmeg essential oil of Balik, Penang, Malaysia has 37 compounds containing 6.8% myristicin [8] as a comparison. Myristicin separation from nutmeg essential oils successfully carried out up to 92.13% of purity, while the

essential oils of nutmeg without myristicin contain 16 compounds.

Compared to research that has done before, the results of myristicin separation from nutmeg essential oil that we have done have better results which are shown by the higher purity of myristicin based on GC Chromatogram. Isolation by column chromatography myristicin has been done and the results obtained myristicin purity of 79.47% [9] and also by the method of steam distillation at 15 mmHg 418 K for 3 hours obtained myristicin purity of 84.44% [10]. Myristicin higher purity of our study could be due to the content of nutmeg essential oil that we use a reasonably long vegrux column and the appropriate heating process. There are nine compounds present in the separation results, the presence of other compounds in myristicin separation results shows that the compound contained has a similar boiling point, making it difficult to separate using this method.

Myristicin IR (table II) and ^{1}H NMR (table III) spectra data indicate a suitable functional group with the structure of myristicin. The results of the analysis with ^{1}H NMR are in accordance with the structure of Miristisin and have similarities with previous studies from Valente et al. [12], as in Table III.

B. Antioxidant activity test

The potential of antioxidants was assessed by looking at the ability of the sample to react with DPPH radicals. The antioxidant value illustrated from IC_{50} is lower than IC_{50} of nutmeg essential oil from Pamalayan Cisewu, Garut grading, that has 3.16% from the previous study [1] of antioxidant IC_{50}, as shown in Table IV.

From the test results can be seen that antioxidant of nutmeg essential oils affected by the presence of myristicin, the IC_{50} value of the myristicin is 189 ppm, which is much stronger than the essential oil of nutmeg without myristicin (IC_{50} = 33.254 ppm and nutmeg essential oil (IC_{50} = 3.181 ppm). This is proof that myristicin is a compound that plays a crucial role in nutmeg essential oil antioxidant activity. IC_{50} DPPH nutmeg essential oil of Pamalayan Cisewu, Garut grading 22.22% myristicin amounted to 3.16% [2], this shows the correlation between this study with previous research. The weak antioxidant activity of nutmeg essential oil without myristicin may be caused by its constituent compounds that have no aromatic group, as it was previously known that the aromatic compounds contained in nutmeg essential oil were myristicin, while other aromatic compounds, although there are, can only be found in small amounts, so it doesn't have a significant impact.

TABLE 3: 1H NMR ANALYSIS RESULTS IN COMPARISON WITH ANOTHER RESEARCH

chemical shifts, δ data	chemical shifts, δ comparison
3.28	3.32
3.87	3.92
5.04	5.10
5.90	5.94
5.91	5.96
6.34	6.42

[a] The comparison was using 500MHz NMR [11]

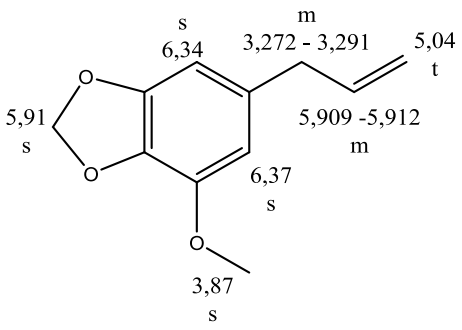

Figure 1. Some protons chemical shifts, δ, ppm of myristicin analysis using ^{1}H-NMR

TABLE 4: ANTIOXIDANT POTENTIAL OF TEST SAMPLE WHEN TESTED USING DPPH

Samples	IC50 (ppm) in DPPH
Nutmeg essential oils *	3.16 %
Nutmeg essential oils	3.181 ppm
Nutmeg essential oils without myristicin	33.254 ppm
Myristicin	189 ppm

[b] * comparison data from another research [1]

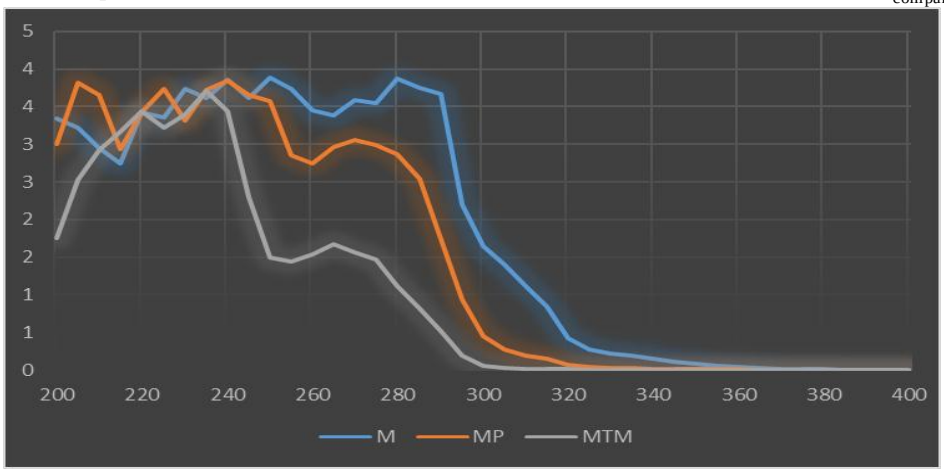

Figure 2. Absorbance graph of nutmeg essential oils (MP), nutmeg essential oils without myristicin (MTM) and myristicin (M)

The antioxidants mechanism, among others, as a giver of H atoms, so that they can join with free radicals and form more stable conditions [10]. Myristicin exhibit antioxidant activity due to the presence of the -CH_2 group in the chain of alyl, chains attached to these aromatic groups make CH_2 acidic because ions are stabilized by conjugation with the aromatic. System of the end acidic of the structure thought to contribute to antioxidant activity and work synergistically. It just the same mechanism of DPPH free radical inhibition with phenol group, are the H-radical bound to oxygen atoms which are more electronegative so that the H atom can bind free radicals from DPPH [11]. In this case, myristicin with CH_2 that is bound to the aromatic system can release H (acid) so that it can react with DPPH. Myristicin ability in releasing H (acid), was given an effect on the strength of myristicin as an antioxidant.

TABLE 5: THE RESULTS OF THE CALCULATION OF THE SPF VALUE OF NUTMEG OIL, NUTMEG OIL WITHOUT MYRISTICIN, AND MYRISTICIN.

Samples	SPF
Nutmeg essential oils	4.98
Nutmeg essential oils without myristicin	0.71
Myristicin	19.44
Positive control	50

C. Sunscreen test

The measurement results show that myristicin has a dominant role in the absorption of light in nutmeg essential oils. Myristicin shows more excellent absorption than the nutmeg essential oils (MP) and nutmeg essential oil without myristicin (MPTM). The majority compounds in nutmeg essential oil are monoterpenes, oxygenated monoterpenes, and sesquiterpenes which is a saturated hydrocarbon or do not have conjugated double bonds causing more nutmeg essential oil has a significant absorption in the region 200-285 nm (UV-C) as in Figure 1. The absorption of nutmeg essential oil and nutmeg essential oil without myristicin is more excellent at short wavelengths caused by compounds that do not have chromophore and ausochrome groups. While a good sunscreen is a substance that has absorption at 290-330 nm (UV-B) [13].

Myristicin has better absorption at UV-B than MP and MPTM, this is because myristicin has chromophores and autochromes, aromatic and methylenedioxy groups. The presence of conjugated double bonds will make the absorption of compounds at longer wavelengths. This makes myristicin have better absorption at 290-320 nm. The amount of light absorption by a molecule is influenced by the molar extension coefficient of the compound [14], this can cause the higher absorption ability of myristicin compared to MP and MPTM.

TABLE 6: EE X I VALUE AT WAVELENGTHS FROM 250 TO 350 NM. ACCORDING TO THE UNITED STATES FOOD DRUG ADMINISTRATION (FDA), EFFECTIVENESS

Wavelength (λ nm)	EE x I
290	0,0150
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,0839
320	0,0180
TOTAL	**1**

From the test results obtained that with the same ratio of concentrations in nutmeg oil, nutmeg oil without myristicin, and myristicin the highest SPF results were myristicin with an SPF value of 19.44, as shown in table 4. The SPF value of myristicin was higher than the SPF value of the Alpinia galanga rhizome extract showing a low value of 0.69 ± 0.13 [15]. Myristicin has a chromophore group than nutmeg or nutmeg oil without myristicin. Based on the SPF value, nutmeg oil without myristicin is classified as minimal protection, nutmeg oil is classified as moderate protection, whereas myristicin classified as extra protection. Based on these results, it informed that all three samples have potential antioxidant effectiveness.

V. CONCLUSION

The test results obtained that myristicin is a compound that contributes as an antioxidant in nutmeg essential oil with an IC50 value of 189 ppm, myristicin is also the compound with the most significant contribution to the absorption of UV-B rays with an SPF value of 19.44. Based on the SPF value, the nutmeg oils without myristicin classified as minimal protection, nutmeg essential oil classified as moderate protection, whereas myristicin classified as extra protection.

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REFERENCES

[1] Wibowo, DP, Febriana, Y., Riasari, H., & Auilifa, DL, "Essential Oil Composition, Antioxidant and Antibacterial Activities of Nutmeg (Myristica fragrans Houtt) from Garut," West Java. Indonesian Journal of Pharmaceutical Science and Technology, vol 5 (3), pp 82, 2018. https://doi.org/10.24198/ijpst.v5i3.16030

[2] Piaru, SP, Mahmud, R., Abdul Majid, AMS, and Mahmoud Nassar, ZD, "Antioxidant and antiangiogenic activities of the essential oils of Myristica fragrans and Morinda citrifolia," Asian Pacific Journal of Tropical Medicine, 2012. https://doi.org/10.1016/S1995-7645(12)60042-X

[3] Olajide, OA, Makinde, JM, & Awe, SO, "Evaluation Of Pharmacological Properties Of Nutmeg Oil In Rats And Mice," Pharmaceutical Biology, vol 38 (5), pp. 385-390, 2003. https://doi.org/10.1076/phbi.38.5.385.5976

[4] Hidayah, N. 'Aini, Ansory, HM, Princess, PKK, and Nilawati, A, "Analysis of Essential Oil Compounds Fuli Pala By GC-MS and Antibacterial Activity Test Against Escherichia coli and Staphylococcus

aureus. Farmaseutik Magazine," vol 13 (2), 2018. 56.https://doi.org/10.22146/farmaseutik.v13i2.40915

[5] Hallström, H., & Thuvander, A., "Toxicological evaluation of myristicin. Natural Toxins," vol 5 (5), pp. 186-192. 1998. https://doi.org/10.1002/19970505nt3

[6] Regina A, Yovita L, and Maimunah, "Penentuan Aktivitas Antioksidan, Kadar Asam Fenolat Total dan Likopen Pada Buah Tomat (Solanum Lycopersicum L.), DIKTI Depdiknas RI. Fakultas Farmasi University of Andalas: Padang, 2003

[7] Mansur JS, Breder MN, Mansur MC, Aulay RD, " Determination of Sun protection factor by spectrophotometry," An Bras Dermatol vol 61, pp. 121, 1995

[8] Piaru, SP, Mahmud, R., Abdul Majid, AMS, Ismail, S., & Man, CN, "Chemical composition, antioxidant and cytotoxicity activities of the essential oils of Myristica fragrans and Morinda citrifolia," Journal of the Science of Food and Agriculture, 2012. https://doi.org/10.1002/jsfa.4613

[9] SINABARIBA MELY YANTI SILALAHI, "Myristicin fractionation of essential oil pala (Myristica fragrans Houtt) as the slimming aromatherapy in vivo" Skripsi DEPARTEMEN KIMIA FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM IPB (2013).

[10] Suprihatin, D., Ketaren, S., Ngudiwaluyo, S., Friyadi, and A., & Assessment and Application of Technology, B., Myristicin isolation of oil nutmeg (Myristica fragrans), Department of Industrial Technology of Agriculture, Faculty of Agricultural Technology-IPB 2), vol 17 (1), pp. 23-28, 1998

[11] Diniatik, D., Suparman, S., Anggraeni, D., & Amar, I. (2016). UJI ANTIOKSIDAN EKSTRAK ETANOL DAUN DAN KULIT BATANG MANGGIS Garcinia mangostana L. *Pharmaciana*, *6*(1). https://doi.org/10.12928/pharmaciana.v6i1.3314.

[12] Valente Vania Maria Moreira, Gulab Newandram Jham, Carolina Marangon Jardim, Onkar Dev Dhingra, and Ion Ghiviriga, "Major Antifungals in Nutmeg Essential Oil against Aspergillus flavus and A. Ochraceus" Journal of Food Research; Vol. 4, No. 1, 2015 DOI:10.5539/jfr.v4n1p51

[13] Goswami, P. K., Samant, M., & Srivastava, R. (2013). Scholars Academic Journal of Pharmacy (SAJP) Natural Sunscreen Agents : A Review Scholars Academic Journal of Pharmacy (SAJP) Review Article Natural Sunscreen Agents : A Review. (December).

[14] Soeratri W., 1993,Studi Proteksi Radiasi UV Sinar Matahari Tahap 1 : Studi Efektivitas Protektor Kimia, Lembaga Penelitian Universitas Airlangga, Surabaya.

[15] Nelli Karina, "Penentuan Nilai Sun Protection Factor (SPF) Ekstrak dan Fraksi Rimpang Lengkuas (Alpinia galanga) Sebagai Tabir Surya Dengan Metode Spektrofotometri UV-Vis," Program Study Farmasi Fakultas Kedokteran Universitas Tanjungpura: Pontianak, 2015