NVEO
Natural Volatiles & Essential Oils

Comparison Of Chemical Compositions Of Seed And Mace Nutmeg (Myristica Fragrans) Essential Oils From Amboina Island, Moluccas, Indonesia And Their Antioxidant Activities

Mario Rowan Sohilait[1,2] , Hanoch Julianus Sohilait[1,2*] and Healthy Kainama[3]

[1]Department of Chemistry, Faculty of Mathematics and Science, Pattimura University, Ambon, Indonesia.

[2]Organic Chemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Science, Pattimura University, Ambon, Indonesia.

[3]Department of Chemistry Education, Faculty of Training and Science Education, Pattimura University, Ambon, Indonesia.

Abstract

The essential oils of seed and mace nutmeg (Myristica fragrans Houtt) were obtained by steam distillation. The components are characterized by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS), respectively. In total, nine components were identified in seed oil and ten components were identified in mace oil. The main components in seed oil are linalool (10.696 ± 0.187%), α-terpineol (13.384 ± 0.808%) and myristicin (61.197 ± 0.613%). While, the main components in mace oil are α-Pinene (13.975 ± 0.159%), sabinene (26.407± 0.456%), β-myrene (14.193± 0.429%), safrole (6.493± 0.326%) and myristicin (27.279 ± 0.735%). The antioxidant activity was identified using both DPPH and ABTS methods. In addition, this effect may be specific due to the chemical components of the oil. The seed oils displayed high DPPH free radical-scavenging activity with IC_{50} value of 2.48±0.42 µg/mL and 2.80±0.24 µg /ml respectively, and the ABTS free radical-scavenging activity with IC_{50} value of 1.09±0.25 µg/mL and 2.92±0.59 µg /ml in mace oil when compared with synthetic antioxidants BHT (1,03±0.10µg/mL) and (1.52±0.11µg/mL).

Keywords: Essential oil, seed and mace, Myristica fragrans, chemical composition, antioxidant

Introduction

The nutmeg tree (Myristica fragrans Houtt) is a plant originating from Moluccas (the Spice Islands). Futhe more, it is now cultivated every where such as in Indonesia, Caribbean islands, India, and other tropical countries. The local name of these trees from Moluccas Islands is pala. The trunk of the tree can grow as height as 8 to 12 meters, on altitudes of 5.0-1000.0 m above the sea level in Amboina Island. It produces seed and mace. The seed of the plant is known as "nutmeg" and the arillus of the seed is called "mace". Both seed and mace contain many volatile oils (Jukic et al, 2006). Dried seed and mace are used as a spice as well as distilled oil. Moreover, the essential oil of the seed and mace has been widely used as a spice and also is well known for its medicinal properties. It is active against bacteria (Piaru, et al, 2012; Shafiei et al, 2012**)**, anti-fungal (Valente et al, 2011; Valente et al, 2015), and antioxidant (Piaru et al 2011; Han et al, 2017). A comparison of these published results reveals a great variability in the chemical compositions of the nutmeg essential oils. Forty components were identified in seed essential oil from West Sumatra and the main compounds in seed oil are alpha-pinene (16.16%), sabinene (11.07%), beta-pinene (12.26%), 4-terpineol (10.39%), and myristicin (15.61%). The main compounds in mace oil are alpha-pinene (19.77%), sabinene (12.45%), beta-pinene (14.77%,) and myristicin (13.83%) respectively, (Saputro et al, 2016)**.** Seven-ten compounds in Ref. (Jukic, 2006) were identified of seed nutmeg oil which the main compounds are alpha-pinene (17.2%), beta-pinene (23.9%), and myristicin (16.2%) with DPPH scavenging activities with an IC_{50} of 22 g/L . The antioxidant activity on the DPPH method of the essential oil of nutmeg has a difference from an earlier reports (IC_{50}= 136 µgmL^{-1}), (Piaru et al, 2012)**.** On the other hand, Muchtaridi (2010) reported thirty-two compounds in the essential oil of nutmeg with major compounds are sabinene (21.38%), 4-terpineol (13.92%), and myristicin (13.57%). thirty-seven compounds in seed oil with only one major which is terpineol-4 (21.3 %) (Piaru et al**,** 2012). Based on a deep literature study, it is indicated that there are no previous reports on the comparison of chemical compositions of seed and mace nutmeg (Myristica fragrans) essential oils from Amboina Island, Moluccas, Indonesia and their antioxidant activities.

Materials and Methods

Plant Material

Nutmeg seed and mace were collected from Allang village, Amboina Island in September 2019.

Isolation of nutmeg Essential Oil from seed and mace

The dry plant material from seed (1000 g) and mace (600 g) of nutmeg was treated with a steam-distilled using steam distiller for 6 hours to obtain the essential oil. The essential oil of nutmeg was separated from water and dried by over anhydrous sodium sulfate (Na_2SO_4) and cool-stored in a brown bottle for further analysis.

Gas Chromatography (GC) Analysis

GC analysis of the nutmeg oil was performed on a Shimadzu QP-2010, equipped with an FID and Rtx-5 using a fused silica capillary column (30 m x 0.25 mm ID, film thickness 1.0 µm). The oven temperature was from 60^{0}C for 5 minutes and programmed heating from 60 to 270^{0}C for 5 minutes. It was conducted with injector temperature 260^{0}C, and detector temperature 270^{0}C, the pressure of carrier nitrogen gas at inlet 100 psi, split 40 and volume of 0.5 µL.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis of nutmeg oil was performed using Shimadzu QP-2010 Plus at 70 eV and 320 ^{0}C with autosampler, a system equipped with Rtx-5 fused silica capillary column (30 m x 0.25 mm ID, film thickness 1.0 µm). The oven temperature was from 60 ^{0}C for 5 minutes, then programmed heating from 80 to 280 ^{0}C at a rate of 15 ^{0}C for 3 minutes, injector temperature 270 °C. The carrier gas (H_2) purge flow rate was 1.14 mL/min. Spectra were scanned from 30 to 500 m/z, split ratio 40 under the ion source temperature 210 ^{0}C. The spectrum of the unknown component was compared with that of known components stored in the Wiley 7 library and Kovats Index (Adam, 2007). Name, molecular weight and structure of the component of the test materials were ascertained.

DPPH and ABTS radical scavenging activity

The DPPH and ABTS assay were performed according to the method described by Sohilait et al, 2019. BHT was used positive controls.
The percentage of radical scavenging activities was calculated as follows:
scavenging (%) = (A control- A sample)/A control ×100. (eq. 1)

The result was expressed as IC_{50}, the concentration of the extract to scavenge 50% of the DPPH and ABTS radical scavenging activities.

Results and Discussion

Performance of seed and mace of nutmeg essential oil

The essential oils of seed and mace were distilled from the dry plant material inside the equipment of the steam distillation. Average yields of seed and mace of nutmeg essential oils were calculated toward dry material. The results of nutmeg oil from steam distillation revealed that from seed have yield (3.8% v/w) and mace (7.8%) respectively.

Chemical composition of seed and mace essential oils

The nutmeg of seed and mace essential oils of nutmeg, Myristica fragrans, were analyzed by GC and GC-MS. Nine components of seed oil and teen components of mace oil were identified and quantified, as shown in Table 1, Figure 1, and Figure 2, according to their elution order on the Rtx-5 column. The major components of seed -oil are linalool (10.696 ± 0.187%), α-terpineol (13.384 ± 0.808%) and Myristicin (61.197 ± 0.613%). While the main compounds in mace-oil as shown in Fig. 3 are α-Pinene (13.975 ± 0.159%), sabinene (26.407± 0.456%), β-myrene (14.193± 0.429%), safrole (6.493± 0.326%) and myristicin (27.279 ± 0.735%). The composition differed between both oils was myristicin content. A higher content of myristicin in seed oil was found. However, a much higher contain of sabinene in mace oil was observed. The compounds are linalool and α-terpineol present in seed oil but not found in mace oil. In addition the compounds are α-pinene and β-myrene present in mace oil but not found in seed oil (Table 1).

Table 1. Chemicals comparison of seed and mace from Myristica fragrans Houtt.

No.	RT (min)	KI[a]	Compounds	Formula	MW	Seed (%)[b]	Mace (%)[b]
1	7.161	9.30	α-Thujune (MH)	$C_{10}H_{16}$	136	-	1.128 ± 0.01
2	7.333	9.39	α-Pinene (MH)	$C_{10}H_{16}$	136	-	13.975 ± 0.159
3	8.264	975	Sabinene (MH)	$C_{10}H_{16}$	136	5.685 ± 0.149	26.407± 0.456
4	8.658	990	β-myrene (MH)	$C_{10}H_{16}$	136	-	14.193± 0.429
5	8.325	979	β- pinene (MH)	$C_{10}H_{16}$	136	1.840 ± 0.062	-
6	9.051	1011	Δ-3-carene (MH)	$C_{10}H_{16}$	136	-	1.754 ± 0.019
7	9.340	1024	Cymene(MH)	$C_{10}H_{14}$	132	2.592 ± 0.039	2.189 ± 0.046
8	9.420	1029	Limonene (MH)	$C_{10}H_{16}$	136	1.343 ± 0.053	2.616 ± 0.093
9	10.724	1072	Linalool (OM)	$C_{10}H_{18}O$	154	10.696 ± 0.187	-
10	12.006	1177	4-Terpineol (OM)	$C_{10}H_{18}O$	154	1.307 ± 0.022	3.977 ± 0.096

11	12.204	1188	α-terpineol (OM)	$C_{10}H_{18}O$	154	13.384 ± 0.808	-
12	13.579	1287	Safrole (AC)	$C_{10}H_{10}O_2$	162	0.911± 0.263	6.493± 0.326
13	16.392	1518	Myristicin (AC)	$C_{11}H_{12}O_3$	192	61.197 ± 0.613	27.279 ± 0.735
Total identified (%)						98.883	100

[a]Kovats index literature, [b] Concentration of compounds base on GC-FID peaks (Fig.1 and Fig.2), MH: Monoterpene hydrocarbon; OM: Oxygenated Monoterpene; AC: Aromatic compounds, -Not detected

On the other hand, minor components detected in the essential oil in about 2% were cymene (2.592%), and sabinene (5.685 %) in seed oil, but the minor component in mace oil are cymene (2.189 %), limonene (2.616%), (4-terpineolol (3.977%) and safrole (6.493%). In contrast, monoterpene hydrocarbons made up a minor fraction in the oil which constituted 1.3-1.8% in seed oil and (1.1-1.7%) of the total oil, respectively. The essential oils of both seed and mace in the present study (Maya et al, 2004) consisted of myristicin (13.23%), elemicin (11.03% and sabinene (24.82%) in seed oil and myristicin (12.75%), elemicin (10.83% and sabinene (24.33%) in mace oil. According to Saputro et al, (2016), the chemical compounds of seed and mace nutmeg oil originated from west Sumatra are 40 components, which are alpha pinene (16.16%), sabinene(11.07), Beta pinene (12.26%), 4-terpineol (10.39%), and myristicin (15.61%) in seed oil and alpha-pinene (16.16%), sabinene(12.45%), beta-pinene (14.77%), 4-terpineol 7.87%) and myristicin (15.61%) in mace oil and alpha-pinene (19.77%), sabinene(12.45%), beta-pinene (14.77%), 4-terpineol 7.87%) and myristicin (13.83%), respectively.

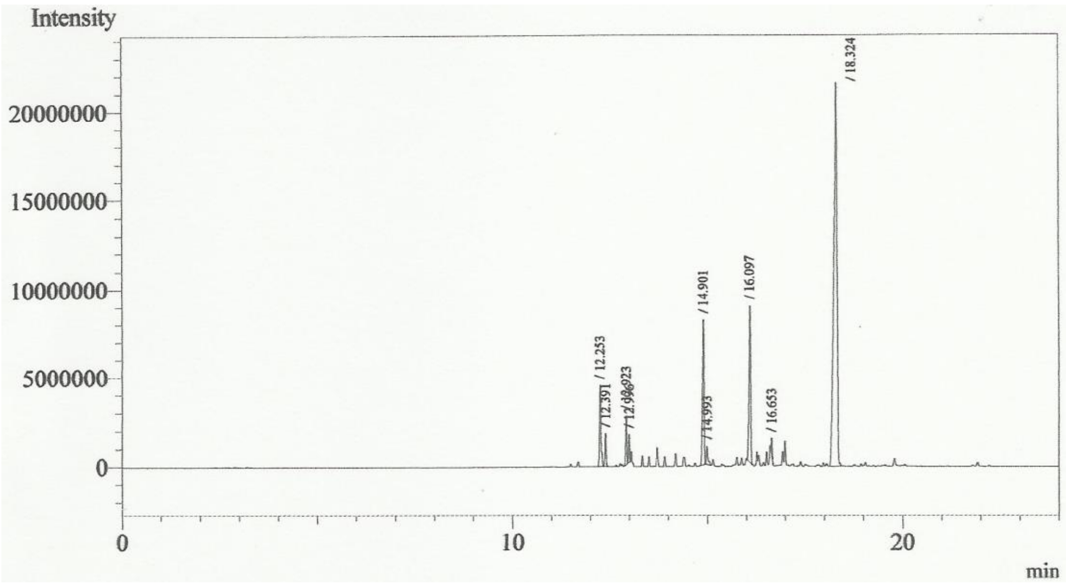

Figure 1 . A typical gas chromatogram of the seed nutmeg essential oil

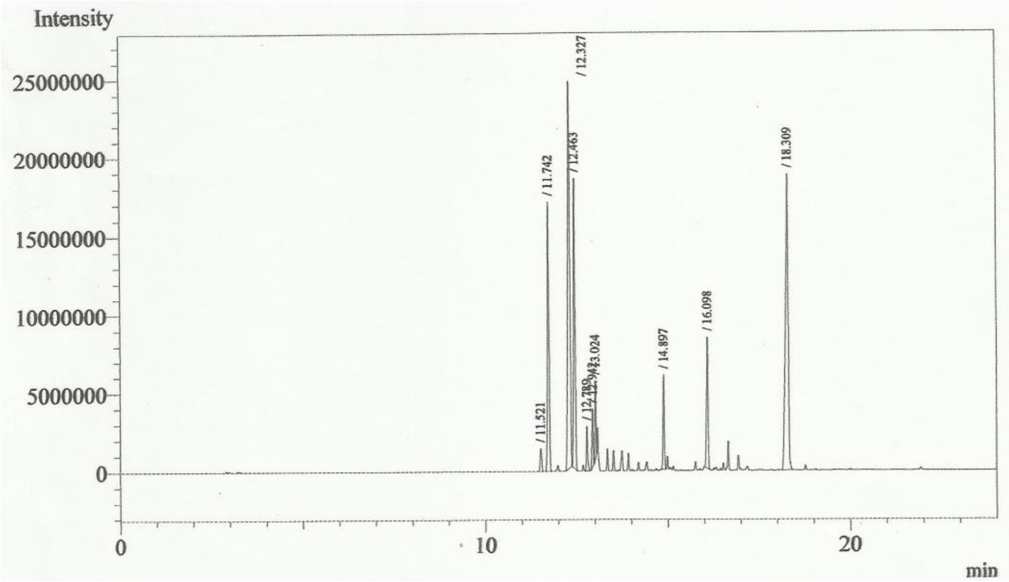

Figure 2. A typical chromatogram GC-FID of the mace nutmeg essential oil.

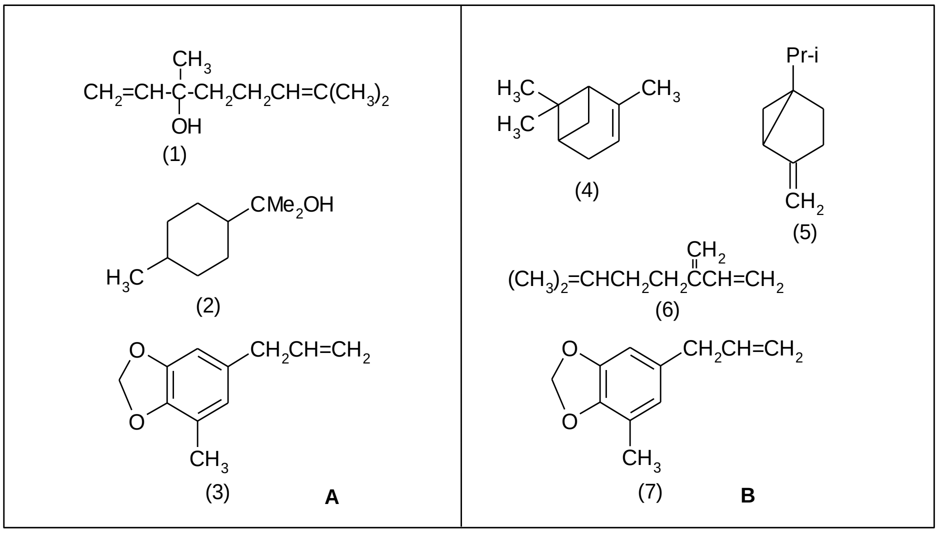

Figure 3. Major components of nutmeg seeds oil (A) (1) linalool, (2) α-terpineol, (3) myristicin, and Mace oil (B) (4) α-Pinene, (5) sabinene, (6) β-myrene, and (7) myristicin.

A typical gas chromatogram of Amboina Island nutmeg oil is shown in Figure 1 (oil from seed) and Figure 2 (oil from mace). This study reveals that the nutmeg essential oils from seed and mace compounds have differences from the earlier reports (Muchtaridi et al., 2016; Saputro et al, (2016); Jukic et al, 2006; Piaru et al, (2012); Schenk & Lampar sky, 2004). The differences in the essential oil composition could be due to the different environmental and genetic factors, a group compound from biosynthesis path way and nutritional soil of the plants, which may influence the oil composition (Salleh et al; 2016a, 2021b).

Antioxidant activities assays

The antioxidant activities of seed and mace nutmeg essential oil were evaluated by DPPH and ABTS assays. According to both methods, the results revealed that seed and mace nutmeg essential oil had antioxidant activities (Figure 4). The used positive control in this research was BHT. The inhibition concentration, IC_{50} was listed in Table 2. The free radical scavenging activity of the essential oil on the DPPH scavenging was observed at 19.97 µg/mL to 0.62 µg/mL concentration. The percentage of inhibition of DPPH from seed oil and mace oil was as high as (90.82±0.42%) and (96.49 ± 0.10%) (Figure 5), respectively. .

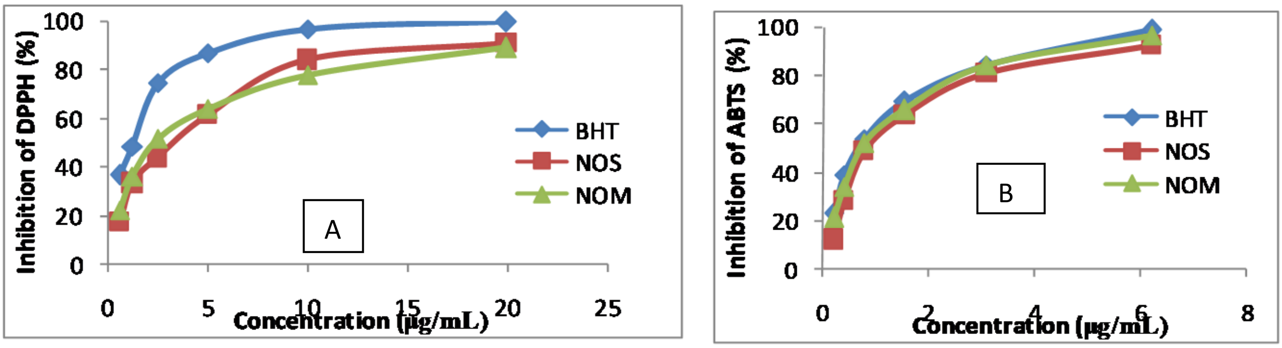

Figure 4. Free radical scavenging activities of DPPH (A) and ABTS (B) in Nutmeg oil.

The nutmeg essential oil exhibited strong DPPH and ABTS scavenging activities with an IC_{50} values of 2.48±0.42 µg/mL and 1.09±0.25 µg/mL in seed oil (Fig. 4A). On the others side the scavenging activities with an IC_{50} value 2.80±0.24 µg/mL µg/mL and 2.92±0.59 µg/mL were obtained in mace oil (Fig. 4 B), respectively. These IC_{50} values of (1,03±0.10 µg/ml) in seed oil and (1.52±0.11µg/ml) in mace oil were compared according to the standard BHT.

Table 2. IC_{50} value of Nutmeg oil from Amboina Island

Sample	IC_{50} value (µg/mL) nutmeg oil	
	DPPH	ABTS
NOS	2,48±0,42	1,09±0,25
NOM	2,80±0,24	2,92±0,59
BHT (positive control)	1,03±0,10	1,52±0,11

Abbreviation; NOS: nutmeg oil from seed, NOM: nutmeg oil from Mace

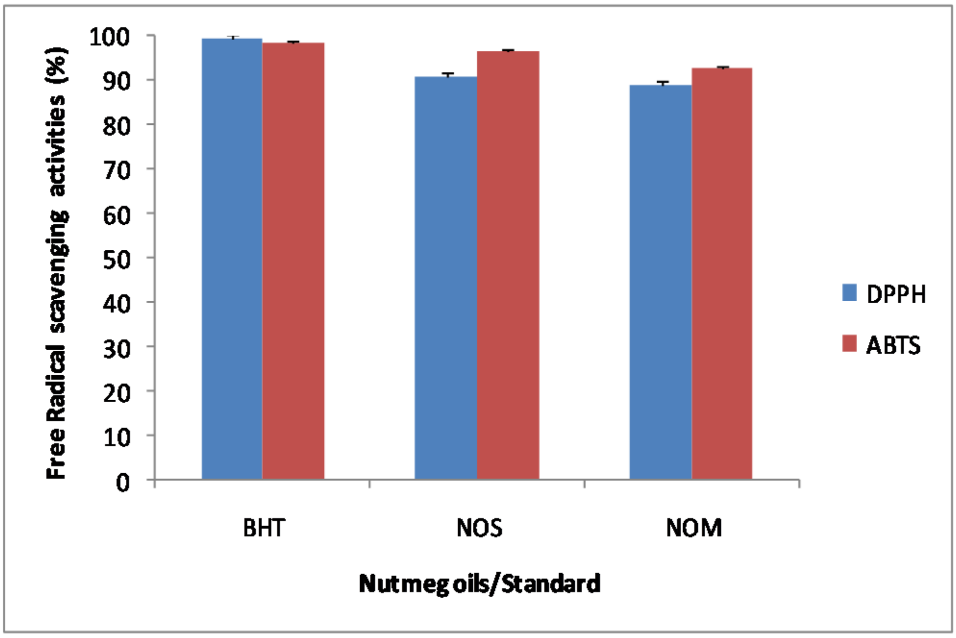

Figure 5. Inhibition in high concentration of DPPH (19.97± 0.24μg/mL) and ABTS (6.19±0.10μg/mL). Data of mean triplicated experiment with standard deviation (SD).

The nutmeg essential oils in our study had composition similar to previously analyzed samples with the main differences. The antioxidant difference between both seed and mace oils was on the significant myristicin content in seed oil about 2.24 times higher than that in mace oil.

Conclusion

The essential oils of seed and mace nutmeg from Amboina Island isolated by steam distillation method and its chemical composition were determined by GC and GC-MS. The result is nine components in seed oil and ten component in mace oil. The major components of seed oil are linalool (10.696 ± 0.187%), α-terpineol (13.384 ± 0.808%) and Myristicin (61.197 ± 0.613%). While, mace oil had the major components of α-Pinene (13.975 ± 0.159%), sabinene (26.407± 0.456%), β-myrene (14.193± 0.429%), safrole (6.493± 0.326%) and myristicin (27.279 ± 0.735%). Seed essential oil showed higher antioxidants than mace essential oil.

ACKNOWLEDGMENT

We thank the Faculty of Mathematics and Science, Pattimura University Ambon for providing the necessary infrastructure and financial support to conduct this study.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

Adams, R. P. (2007). Identification of essential oil components by gas chromatography/mass spectroscopy. Carol Stream (IL): Allured. Publishing Corporation.

Han. F; Ma, G.Q, Yang, M; Yan, L; Xiong, W; Shu, J. C; Zhao, Z. D; Xu, H.L. (2017). Chemical composition and antioxidant activities of essential oils from different parts of the oregano; J. Zhejiang Univ-Sci B (Biomed & Biotechnol), 18(1):79-84

Jukic, M; Politeo, O and Milo. M. (2006). Chemical Composition and Antioxidant Effect of Free Volatile Aglycones from Nutmeg (Myristica fragrans Houtt.) Compared to Its Essential Oil, CROATICA CHEMICA ACTA CCACAA **79** (2) 209-214.

Maya,K. M; Sacharia, T. J and Krishnanoorthy B. (2004). Chemical Composition of Essential oils of Nutmeg (Myristica fragrans, Houtt) accessions, Journal of Spices and Aromatic Crops, Vol 13 (2), 135-139,

Muchtaridi; Subarnas, A; Apriyantono, A;and Mustarichie, R. (2010). Identification of Compounds in the Essential Oil of Nutmeg Seeds (Myristica fragrans Houtt.) That Inhibit Locomotor Activity in Mice, Int. J. Mol. Sci. 11, 4771-4781

Ozaki, Y; Soedigdo, S, Wattimena, Y.R. (1989). Antiinflammatory Effect of Mace, Arill of Myristica fragrans, HOUTT, Japan, J, Pharmacol,49, 155-163.

Piaru,S. P; Mahmud, R; ,Majid, A.M.S.A, Ismail, S; b and Manc, N. C. (2012). Chemical composition, antioxidant and cytotoxicity activities of the essential oils of Myristica fragrans and Morinda citrifolia, Journal of the Science Food and Agricultur; **92**: 593–597.

Salleh, W. M. N. H. W., & Ahmad, F. (2016). Antioxidant and anti-inflammatory activities of essential oils of ctinodaphne macrophylla and A. pruinosa (Lauraceae). Natural Product Communications, 11(6), 853-855

Salleh,W.M.N.H.W; Khamis, Sh; Kassim, H and Alene Tawang, A, (2021 b), Essential oil composition of Strychnos axillaris Colebr. (Loganiaceae), Nat. Volatiles & Essent. Oils, 8(1): 13-17

Schenk, H.P and Lamparsky, D, (1981). Analysis of nutmeg oil using Chromatography Metods, Journal of Chromatography, 204, 391-395.

Saputro, M. A; Andarwulan, N and Didah Nur Faridah, D.N. (2016). Physical characterization and essential oil properties of West Sumatra mace and nutmeg seed (Myristica fragrans Houtt) at different ages at Journal of Pharmacognosy and Phytochemistry, 5(6): 371-376

Shafiei, Z; Shuhairi,N. N; Yap, N. F. S; Sibungkil, C. A. H; and Latip, J; (2012). Antibacterial Activity of Myristica fragrans against Oral Pathogens, Evidence-Based Complementary and Alternative Medicine Volume 2012, Article ID 825362, 7 pages doi:10.1155/2012/825362

Sohilait, H. J; Kainama, H, (2019). Free Radical Scavenging Activity of Essential Oil of Eugenia caryophylata from Amboina Island and Derivatives of Eugenol, Open Chemistryt., 2019; 17: 422–428

Valente, V. M. M; JHAM, G. N; Dhingra, O. D, and Ghiviriga, I; (2011[a]) Composition and antifungal activity of Brazilian Myristica fragrans Houtt essential oil, Journal of Food Safety **31;** 197–202

Valente,V. M. M; Jham, G. N; Jardim,C. M, Onkar Dev Dhingra, O. D; & Ion Ghiviriga, I; (2015 [b]) , Major Antifungals in Nutmeg Essential Oil against Aspergillus flavus and A. ochraceus, Journal of Food Research; Vol. 4, No. 1; 2015, 51-57.