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Manuscript Number: J Young Pharm, 2017; 9(4): 483-486

Manuscript title: The Isolation of Myristicin from Nutmeg Oil by Sequences Distillation

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Young Pharmacists

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Contents Inside

Editorial

Z Does Electromagnetic Radiation affect drug stability?

Original Article

- Molecular Dynamics Simulation of SIRT1 Inhibitor from Indonesian Herbal Database
- Pharmacophore-Based Virtual Screening from Indonesian Herbal Database to Finding New Inhibitor of HDAC4 and HDAC7
- Synthesis and Pain Inhibition Activity of the Analogs of 1-Allyl-3-Benzoylthiourea for New Analgesic Lead Compound Discovery
 - Green Synthesis of Silver Nanoparticles Using Hydnocarpus pentandra Leaf Extract: In-vitro Cyto-Toxicity Studies Against MCF-7 Cell Line
 - The Isolation of Myristicin from Nutmeg Oil by Sequences Distillation
- Formulation Optimization and Performance
 Evaluation of Papain and Clove Oil based
 Chemo-mechanical Caries Removing Gel
 - Formulation and in vitro Penetration Study of Transfersomes Gel Containing Gotu Kola Leaves Extract (Centella asiatica L. Urban)
 - Formulation and Characterization of pH based Stimuli Sensitive based Hydrogels for the Treatment of Ocular Infection
- Formulation and Characterization of Rice Bran Oil in Alginate Microcapsules
- Gel Formulation Containing Microcapsules of Grape Seed Oil (Vitis vinifera L.) for Skin Moisturizer Read more...

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Editor-in-Chief : Dr. S Parasuraman, Ph.D ISSN : [ISSN: Print -0975-1483, Online - 0975-1505] Frequency : Quarterly (4 issues/year)

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The Isolation of Myristicin from Nutmeg Oil by Sequences Distillation

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ABSTRACT

Background: Nutmeg oil is used in Asia as a traditional medicine for a stormach cramps, diarrhaa, and insomnia. It's quality need to be improved by increasing myristicin content. **Objectives:** This study aimed to increase the myristicin content of nutmeg oil by sequences distillations. **Methods:** Three different conditions of distillations were applied sequentially. The first, second and third distillation were at 110 °C (0.2 bars) 105 °C (1 bar), and 145 °C (0.2 bars) respectively. Myristicin contents determined by get content of the original nutmeg oil, the first, the second, and the third distillations were 5.84%, were the applied sequences distillation with original oil. **Conclusion:** The use of sequences distillation with original (0.1 **Conclusion:** The use of sequences distillation with original (0.1 **Conclusion:** The use of sequences distillation with original (0.1 **Conclusion:** The use of sequences distillation with original (0.1 **Conclusion:** The use of sequences distillation with original (0.1 **Conclusion:** The use of sequences distillation with original (0.1 **Conclusion:** The use of sequences distillation with original (0.1 **Conclusion:** The use of sequences distillation with original (0.1 **Conclusion:** The use of sequences distillation with the tradient or the original (0.1 **Conclusion:** The use of sequences distillation with the tradient or the original (0.1 **Conclusion:** The use of sequences distillation with the tradient or the original (0.1 **Conclusion:** The use of sequences distillation with the tradient or the original (0.1 **Conclusion:** The use of sequences distillation with the tradient or the original (0.1 **Conclusion:** The use of sequences distillation with the tradient or the original (0.1 **Conclusion:** The use of sequences distillation with the tradient or the original (0.1 **Conclusion:** The use of sequences distillation with the tradient or the original (0.1 **Conclusion:** The use of sequences distillation with the tradient or the original (0.1 **Conclusion:** The use of sequences distil

a different condition can increase the myristicin content from the original nutrineg oil.

Key words: Nutrieg oil, Myristicin, Sequences distillation, GC-MS. Correspondence

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INTRODUCTION

The seed and mace of nutmeg are the product of the same plant that is Myristica fragrams Houtt from the Myristicaceae family. This plant is originally from the Moluccas and now is widely grown in eastern India, the Caribbean, and Grenada. Since a long time ago this nutmeg has been used as a spice and, medicine.¹ Myristicin is psychoactive substance and one of the main constituents of nutmeg oil. Myristicin content in plant organs varies, depending on growing and storage condition.²

Myristicin

Myristicin (4-methoxy-6-(2-propenyl)1,3-benzodioxole) is a major compound of the essential oil of seeds and mace nutmeg.¹ Nutmeg poisoning is rare but should be considered in drug abuse.³The increased of the use of myristicin as a hallucinogen is because it is cheap and this often causes fatal cases of poisoning due to myristicin.⁴ We can also find myristicin in fennel, celery, and parsley plants.⁶ Myristicin is also potential as an effective chemoprotective agent against cancer and hepatoprotective activity.⁶⁷ Myristicin is a derivative of phenylpropanoid compounds, in the form of a clear liquid, not soluble in water but soluble in organic solvents. It smells like spices, and the aroma is sharp and easily vaporized. Myristicin is the primary aromatic compound of the volatile of nutmeg oil, derived from the dried ripe seed of *M. fragrans*. Nutmeg from the Moluccas contain 5.57-13.76% myristicin.⁸

There are various ways to isolate myristicin, among others; distillation, extraction, and column chromatography? The method that is most widely used to separate myristicin is by distillation. The advantage of this simple method is that it doesn't need organic solvents and it has high concentration result.¹⁰Nutrneg oil has the various compound with different boiling points. Distillation is the process of separating the compounds of a mixture comprising two or more liquids based on having different vapor pressure or boiling points. The essential oil compounds are not stable at high temperatures. To obtain the high-grade nutmeg oil, it is necessary to conduct distillation at low temperature under certain pressure. According to Guenther (1987), at 15 mm Hg, the boiling point of myristicin is 149.5 °C. The aim of this distillation process is to find the appropriate temperature for obtaining the highest concentration of myristicin in the nutmeg oil. The distillation pressure used in this distillation was applied to decrease the boiling point of myristicin significantly to prevent the decomposition on myristicin.¹¹

GCMS is the coupling of the gas chromatograph with the mass spectrometer. The often use of GCMS is because it is a well-established technique to analyze the essential oils. This analysis often used is due to the development of easy-to-handle robust system concerning sensitivity and relatively low cost. The complex compounds can be analyzed in detail by spectral interpretation of the separated compounds because of the on-line coupling of a chromatographic device to a spectrometer.¹²

The sequences distillation isolated myristicin and separated the components by different boiling points. In this case, it is important to know the boiling point of each compound.

MATERIAL AND METHODS

Material

In this research, we obtained the nutrneg oil from Balittro (Balai Penelitian Tanaman Rempah dan Obat) Bogor, West Java.

Method

The Sequences Distillations Method

The sequences distillations in three stages at different temperature and vacuum/pressures are as diagramed in Figure 2. The products of each step were named isolate (1), isolate (2), and isolate (3) respectively. The

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms. analyses of the nutmeg oil sample and the isolates is by Gas chromatography-mass spectrophotometry (GC-MS).

Distillation (1)

The distillation of one hundred and fifty ml nutmeg oil is for approximately 1 h of 0.2 bars at 110 °C. The volume of the isolate (1) and distillate (1) is measured and then is tested by GC-MS.

Distillation (2)

The distillation of twenty ml isolates (1) mixed with 200 ml of 5% Na Cl solution is for 2 h of 1 bar at 105 °C.

The volume of the isolate (2) and distillate (2) is measured and then tested by GC-MS.

Distillation (3)

One hundred and fifty ml isolate (2) is distilled approximately for 1 h of 0.2 bars at 145 °C. The volume of the residue and isolate (3) is measured and then tested by GC-MS.

Gas chromatography-mass spectrophotometry (GC-MS)

The GCMS Agilent Model 6890 equipped with Selective Mass Detector 5973 analyzed the myristicin and other compounds. The capillary column HP SMS (60-meter x 0.25 mm x 0.25 µm) was directly coupled to the mass spectrophotometer. The carrier gas was helium (1 ml/min). The program used was 4 min isothermal at 100°C then 4°C/min to 350°C. The Wiley 9 N11L Mass Spectral Library identified the spectra.

Characterization

The quality of nutrneg oil purchased from Balittro (Balai Penelitian Tanaman Rempah dan Obat) Bogor, West Java needs to be tested, to know the percentage of its purity, color, density, and solubility in alcohol.

RESULTS AND DISCUSSION

In this research, the sequences distillations of myristicin from nutmeg oil by using two types of distillation, as follows: distillation of mixed liquid without water vapor (fractional vacuum distillation) and distillation from a mixture of a non-mixed liquid with water vapor. The difference of boiling points compounds of nutmeg oil is the base of distillation. The compounds having a low boiling point will evaporate first, whereas the compounds having high boiling points will evaporate at the end of the distillation.

The nutrneg oil from Bogor-West Java (Balitro) tested by GCMS contains 55 compounds with different retention times and levels. Twenty-one compounds have more than 0.3% concentration as shown in Table 1. The main compounds in the oil were a-terpineol (15.42%), 4-terpineol (10.77%), myristicin (12.93%), a-terpinolene (9.77%), and a-terpinen (6.09%). This research showed that the nutmeg oil has a high quality because of its a high percentage of active compounds. On distillation (1), nutmeg oil from Bogor distilled at 0.2 bars and temperature of 110 °C for approximately 1 h produces distillate (1) and isolate (1). Terpene compounds having a low boiling point will evaporate first. Therefore, these compounds are called the light fraction of nutmeg oil. This terpene is the dominant compound about 80% of nutmeg oil. The range of the terpenes boiling point compounds is about 150-180 °C at 1 bar. At a pressure below 1 bar, the boiling point range will become lower. Myristicin is one of the terpene-o compound having the high boiling point, so it evaporates at the end of the distillation. The order of the evaporation of the distillation is shown in Figure 2. GC-MS analyzed the compounds in distillate and isolate. Distillate (1) contains the compounds which have a lower boiling point than myristicin such as ß pinene, dl-limonene, 1,8-cineole, camphene, and α-terpinolene.

On distillation (1), nutmeg oil from Bogor distilled at a pressure of 0.2 bars and a temperature of 110 °C for approximately 1 h produces

Journal of Young Pharmacists, Vol 10, Issue 1, Jan-Mar, 2018

distillate (1) and isolate (1). The volume of the isolate (1) is 80.9 ml that containing 17.50% myristicin. At this step, there is an increase myristicin level of nutmeg oil from 12.93% to 17.50%. There are still other compounds besides the myristicin, such as a-terpineol, diethyl phthalate, 4-terpineol, a-terpinolene and phenol, 2,6-dimethoxy-4-(2-propenyl). We must remove the other compound except for myristicin. The results obtained from the distillation (1) will become the material for distillation (2) with NaCl solution.

On distillation (2), we distill the mixed isolate (1) with 5% Na Cl solution at 105 °C of 1 bar for 2 h. The addition of Na Cl solution into the system is intended to increase the boiling point of water because only Na Cl will dissolve in the water. The principle used in this process is the purification of the compounds by utilizing the other compounds which have a lower boiling point. In this case, the distillation process follows the Dalton laws for partial pressure, stating that if two or more gas or steam that does not react at a constant temperature, each gas will produce the same pressure whether it is alone or mixed. This law means that the temperature of the boiling point will be lower than the boiling point of a volatile compound. In this research, the used of Na Cl solution is because it has a lower boiling point than that of the other compounds. Thus, the boiling point of the mixture will be lower than that of the boiling point of a Na Cl solution at normal temperature. The process of steam distillation is very beneficial because it can reduce the destruction of the material due to its contacts with heat (Guenther, 1987).9 The measurement of the densities is in Table 3. The high density of the isolate is because of the decline of terpene contents in the oil and causes the remains in the heavy isolate fraction.

Table 1: The chemical compositions of nutmeg oil.

e 1: The chemical compositions of hutine	g on.
Compounds	Contents (%)
Monoterpene hydrocarbons	
β-ptnene	0.50
a-terpinene	6.09
p-cymene	4.11
dl-ltmonene	4.73
y-terpinene	4.05
a-terpinolene	9.77
Oxides	
1,8-Cincole	3.53
Sesquiterpene hydrocarbons	
a-copaene	1.05
Caryophyllene	0.55
Aromatics	
Safrole	1.94
Eugenol	0.33
trans-Isoeugenol	0.51
Myristicin	12.93
Elemicin	0.36
Monoterpene alcohols	
D-fenchyl alcohol	2.84
Terpinene 1-ol	4.35
β-Terpineol	2.42
Isoborneol	1.37
4-terpineol	10.77
a-Terpineol	15.42
Esters	
Bornyl acetate	0.31

The next compound that will evaporate is terpene-o compounds that have higher boiling points than that of the others. Myristicin has a high boiling point, so it evaporates at the end of the distillation process. The order of the evaporation of the distillation is in Figure 2. On distillation (2) with additional Na Cl solution into the system is to increase the boiling point of water so that the light compounds will evaporate and increase the level of myristicin in an isolate (2). Distillation (2) produces isolate containing 54.21% myristicin, 31.58% diethyl phthalate and 4.01% phenol,2,6-dimethoxy-4-(2-propenyl). The boiling point of diethyl phthalate at 1 bar is 299 °C while the boiling point of myristicin is 276.5 °C and the boiling point of phenol2,6-dimethoxy-4-(2-propenyl) is 296.51 °C. If we want to get myristicin with higher purity, it is needed to be isolated from diethyl phthalate and phenol2,6-dimethoxy-4-(2propenyl) by distillation (3).

Distillation (3) is done approximately for 1 h at 0.2 bars and temperature of 145 °C. Since the boiling point of myristicin is lower than the boiling point of diethyl phthalate, the myristicin is removed into a distillate (3) while the diethyl phthalate is still in the residue. The vacuum pressure used is to lower the boiling point of myristicin. The decrease of the boiling point is significant to prevent the decomposition of myristicin. This temperature distillation at 145 °C is due to reduce the compounds of higher terpenes and to increase the levels of myristicin. The range of the densities of the isolates is in Table 3. The results indicate that the higher the temperature of the distillation the weigher the density is. The process of distillation (3) is to improve the levels of myristicin by separating it from the light fractions of nutrneg oil. Thus, the isolation or purification of myristicin is easier and faster.

Table 4 provides different characteristics of nutmeg oil and isolates. This table indicates that there are considerable changes in characteristics of the oil after distillation at 145 °C. This increase is due to the decline of the light fraction or the terpene compounds in the oil. Also, the reduced levels of terpenes in oil affect the higher density of oil. In this case, the lower pressure is done to reduce the polymerization that causes the increase of long carbon chains. In general, the efficiency of tools, time and temperature of distillation influenced the result of fraction randemen. The higher the efficiency of the device the higher the result is.

Overall, the concentration of myristicin increased at each stage of distillation, starting from nutmeg oil in distillation (1) to distillation (2). Increased levels of myristicin can be seen in Table 4. Distillation (1) produces 17.50% myristicin, distillation (2) produce 54.21% myristicin, and distillation (3) produce 83.45% myristicin.

Isolate (3) contains 83.45% myristicin and 4.01% phenol,2,6-dimethoxy-4-(2-propenyl). Phenol,2,6-dimethoxy-4-(2-propenyl) is used as food additives. This material is used at the maximum of 5 ppm, to add the scent of smoke and to preserve the food (meat, fish, etc.).

Table 2 : The analysis of GCMS.					
Compounds	Retention time (min.)	Nutmeg oll (%)	Isolate 1 (%)	Isolate 2 (%)	Isolate 3 (%)
a-terpineol	10.171	15.42	12.81	-	-
myristicin	14.309	12.93	17.50	54.21	83.45
diethyl phthalate	14.967	4.56	8.67	31.58	0.17
4-terpineol	9.977	10.77	9	-	-
a-terptnolene	8.787	9.77	7.83	0.17	0.17
a-terpinene	7.972	5.48	-	-	-
phenol, 2,6-dtmethoxy-4-(2-propenyl)	15.012	0.39	1.06	4.01	4.01

ble 3 : The characterization	on of nutmeg oil and is	olate 1,2,3		
Characteristics	Nutmeg oil	Isolate (1)	Isolate (2)	Isolate (3)
Color	Light yellow	Dark yellow	Light brown	Colorless
Density	0.913	1.12	1.15	1.15
Odor	Aromatic	Over powering	Over powering	Light
Solubility	1:1	1:1	1:1	1:1
tn alcohol				

Table 4 : The increase	ng levels of myristici	n			
Stage	Material	Myristicin (%, w/v)	Volume (ml)	Yield (g)	Percentage (%)
	Nutmeg of	12.93	150	19.35	100
Distillation 1	Isolate1	17.50	80.9	14.16	73
Distillation 2	Isolate 2	54.21	24	13.01	67
Distillation 3	Isolate 3	83.45	7	5.84	30

Journal of Young Pharmacists, Vol 10, Issue 1, Jan-Mar, 2018

Sudradjat et al.: The isolation of myristicin

Compound	Retention time (minute)	Area (%)	Molecular Weight	Boiling point (°C)	Molecule Structure
Myristicin	14.309	83.45	192.2	276.5	CTCH,
Phenol, 2,6-tmethoxy-4-(2- propenyl)	15.102	4.01	194.23	296.51	He J

Note : The other compound does not mention above are lower than 4%

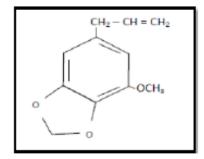


Figure 1 : The molecular structure of myristicin.

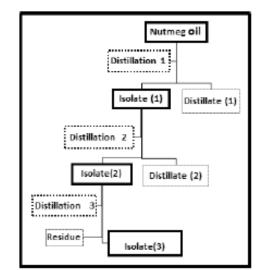


Figure 2: The sequences distillation diagram.

CONCLUSION

The isolation of myristicin from nutrneg oil by sequences distillation according to the GC-MS analysis increase from 5.84% to 83.45% concentration.

ACKNOWLEDGMENT

This study was supported by Directorate of Research and Community Service (DRPM) Universitas Indonesia via a grant "Hibah Publikasi Internasional Terindeks Untuk Tugas Akhir Mahasiswa Universitas Indonesia (Pitta)" 2017.

CONFLICT OF INTEREST

There is no conflict of interest.

ABBREVIATION USED

GC-MS: Gas chromatography-mass spectrophotometry; h: hour; Na Cl: Sodium Chloride.

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Article History: Submission Date : 31-07-2017 ; Revised Date : 21-09-2017; Acceptance Date : 04-11-2017. Cite this article: Sudradjat SE, Timotius KH, Mun'im A, Anwar E. The Isolation of Myristicin from Nutmeg Oil by Sequences Distillation. J Young Pharm. 2018;10(1):20-3.