EQUILIBRIUM JOURNAL OF CHEMICAL ENGINEERING
*Homepage:*https://jurnal.uns.ac.id/equilibrium

The Use of Soxhlet Techniques in Essential Oil Extraction from Anise Seeds (*Pimpinella anisum*)

Arifah Sukasri, Wahyu Budi Utomo*, Rahmiah Sjafruddin, Muhammad Nursam
Department of Chemical Engineering, Politeknik Negeri Ujung Pandang, Makassar, Indonesia 90245
*Corresponding author: wahyubudiutomo@poliupg.ac.id

DOI: *https://dx.doi.org/10.20961/equilibrium.v7i1.73410*

Article History
Received: 02-05-2023, Accepted: 20-05-2023, Published: 23-05-2023

Keywords:
anise seeds,
essential oil,
soxhlet,
GC-MS

ABSTRACT. Anise seeds can be processed into essential oils by steam distillation and solvent extraction. This research aims to produce a high oil yield by determining the optimum conditions of extraction, extracting anise seeds according to optimum conditions, producing anise oil with good quality based on 90% alcohol solubility analysis, analyzing the acid number, and conducting GC-MS analysis. The Activity Method was carried out by determining the optimum conditions of extraction on the effect of the variation in the ratio between anise seed powder and petroleum ether. Furthermore, based on optimal conditions, extraction was carried out at a temperature of 60°C, 5 hours with 5 repetitions. The results of essential oils were analyzed including solubility analysis, oil acid number, and GC-MS. The optimal extraction conditions were obtained at the ratio of anise seeds and petroleum ether 1:12.5 with an average oil yield of 4.72%. The results of the analysis showed that anise oil had good quality based on the oil solubility in 90% alcohol with a 1:7 ratio. The acid number of anise seed oil was obtained at 1.14444. GC-MS analysis showed that anise seed oil contained estragole, anethole, limonene, fenchone, and anis ketone.

1. INTRODUCTION

Many types of plants contain essential oils whose utilization is not optimal. Essential oils are found in parts of various plants, ranging from leaves, seeds, flowers, and stems [1]. One of the plants which contain essential oils is the anise plant found in the seeds. Anise seeds are known to give a distinctive aroma and smell. The main component contained in anise oil is trans-anethole around 75-90% [2]. Anise seeds can be processed into essential oils by steam distillation [3], solvent extraction [4], and hydrodistillation by pre-drying [5].

The method used in previous research to produce anise oil is a steam distillation method that requires quite a lot of material but the resulting oil yield is small. Based on research [3], anise seed vapor distillation of as much as 200 g produced 0.6% oil yield. In Hardjono's Book entitled Essential Oil Chemistry [6], a distillation of 500 g of anise seeds yields 2.6% yield. While research [5] stated that anise seed extraction produced 2.62% oil yield by hydrodistillation and preliminary drying of the shade drying method. Several other methods can be used to obtain essential oils, namely maceration, solvent extraction, supercritical CO_2 extraction, cold pressing, and enfleuration method. The last method, enfleuration, is extraction of essential oils from flowers with the help of fat [7].

From some of these methods, solvent extraction can be an option because it can be produced using fewer materials but produces a higher oil yield. The results of research [4], showed that the extraction of 50 g of anise seeds with petroleum ether resulted in an oil yield of 34.95%. Extraction of essential oils is carried out using appropriate solvents, which are generally hydrocarbons. Distillation is carried out to concentrate the resulting solution. Then, the pure alcohol is added to extract oil from the concentrate. The last stage is the evaporation of alcohol so that pure essential oils are produced [7]. The Soxhlet method is widely an option because the solvent used can be recovered after extraction and used for subsequent extraction [8].

The need for essential oil is quite increasing because of its great benefits. Potential essential oils that can be developed include anise oil, ginger oil, lime leaf oil, cinnamon, cardamom, and candy oil [3]. As a main component in anise seed essential oil, anethole has the ability to give a sweet characteristic and a distinctive aroma. Previous studies have shown that other benefits of anise seed essential oil include antioxidant, antibacterial, anti-inflammatory, analgesic, stimulant, and many others [2].

Petroleum ether is used in solvent extraction because it is a non-polar and charged solvent that can easily enter the anise seed. Petroleum ether has a low boiling point so it speeds up the extraction time. With a high solvent extraction capacity, petroleum ether does not affect the oil's chemical properties [9]. The purpose of this study is to do soxhlet extraction of anise seed with petroleum ether to process the anise seeds into essential oils with good quality and high yield, analyze the number of acids on the anise oil produced, and find out the main components of anise oil with GC-MS analysis.

2. MATERIALS AND METHODS

2.1 Tools and Materials

The tools used in this study were a beaker, funnel, round base flask, GC-MS instrument (GCMS-QP2010 Ultra Shimadzu), separate funnel, Erlenmeyer, measuring pipette, volume pipette, hot plate, series of soxhlet extraction equipment and rotary evaporator (Buchi). The ingredients used in this activity were a sample of 1 kg of anise seeds obtained from West Java; petroleum ether from Merck; phenolphthalein indicator from Merck; KOH from Merck; alcohol; and distilled water.

2.2 Initial Treatment

A kg of anise seeds was mashed to powder/powder using a blender, then dried in the oven for 15 minutes at 105 ± 5 °C.

2.3 Determination of Optimum Extraction Conditions

The first step was the determination of the effect of variation in the ratio between anise seed weight powder (g) and petroleum ether (mL) respectively (1:24; 1:11.5; 1:7.3; 1:5.25 and 1:4) at a temperature of 60°C and an extraction time of 5 hours. Based on the result, we got the optimum yield result ratio. Then, we pretended to analyze the further 5 approximate ratios near the previous optimum result ratio.

2.4 Extraction According to Optimum Conditions

Anise seed powder was weighed as much as 20 g then wrapped in filter paper and then the package was dried in the oven for 15 minutes at 105 ± 5 °C. Anise seed powder was put into the Soxhlet tool and added 250 mL of petroleum ether and then a heater was installed whose temperature was set at 60 °C and extracted for 5 hours. The extracted mixture was separated by adding 100 mL KOH 0.05N to the separation funnel. A mixture of oil and petroleum ether was accommodated into the Erlenmeyer. The addition of KOH after extraction is carried out for the saponification process so that the wax fraction can be separated from the oil.

2.5 Anise Oil Extraction Evaporation

A mixture of oil and petroleum ether was incorporated into a round base flask. Buchii's rotary evaporator was turned on, all cables were connected to their respective switches. First, the cooler was turned on by pressing the On/Off button for power and On/Off for vacuum, waiting for a while until the temperature showed the standard temperature of 25 °C. The temperature is then set by pressing the set button then the temperature is set to 4 °C by pressing the Up/Down button. Once the temperature was set, a round base flask containing a mixture of oil and petroleum ether was installed on the drive rotor and distillate flask. Vaseline was applied around the connecting part of the two objects and a clip was used to strengthen the joint. The water suppressor was turned on by pressing the On/Off button and the temperature was set to 60 °C by pressing the Set and Up/Down buttons. The rotary evaporator was turned on by pressing the On/Off button and the rotation speed was set to 2 rad/second by turning the player button. Then the vacuum pump was turned on.

2.6 Calculation

2.6.1 Calculation of oil yield

Analysis of yield was carried out by calculating the ratio of the weight of anise oil produced with the weight of anise seed powder used and multiplied by 100%.

$$\text{Yield} = \frac{\text{a}-\text{b}}{\text{Anise seed weight (g)}} \times 100\% \qquad (1)$$

Where:

a = oil weight (g) + empty erlenmeyer weight (g)

b = empty erlenmeyer weight (g)

2.6.2 Calculation of oil acid number

According to Guenther [10], the maximum limit of anise seed oil acid number is 1.33. The number of acids is determined by the formula below:

$$\text{Acid number} = \frac{\text{Volume of KOH} \times 56{,}1 \times \text{M KOH}}{\text{Anise seed weight (g)}} \quad (2)$$

Where: 56.1 = Molecular Weight of KOH (g/mol)

2.7 Analysis

2.7.1 Solubility Analysis in 90% Alcohol [4]

A total of 1 mL of oil samples were put in a test tube and then 90% alcohol was added 1 to 7 mL gradually. At each alcohol addition was whipped and its clarity was observed.

2.7.2 Acid determination

A total of 2.5 g of oil samples were put into Erlenmeyer, then 15 mL of alcohol and 3 drops of phenolphthalein 1% was added. Then it was titrated with 0.1 N KOH. The addition of good alkaline drops during titration was approximately 30 drops per minute. The contents of the pumpkin should be shaken during the titration. A pink color that does not disappear in 10 seconds indicates the endpoint of the titration.

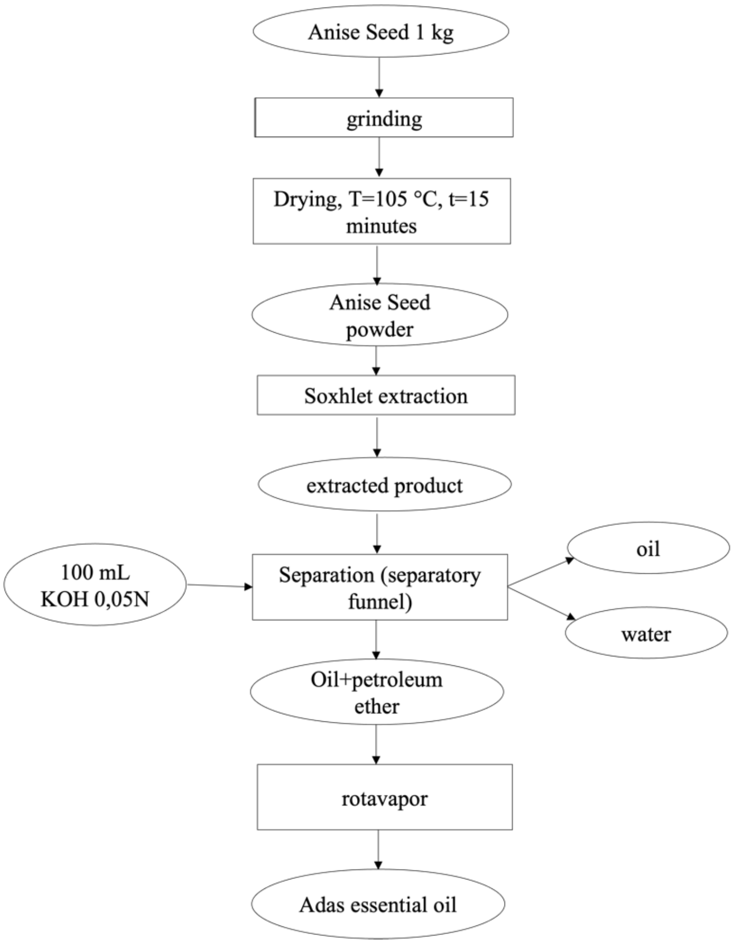

Figure 1. Anise Seed Oil Extraction Process

2.7.3 Oil component analysis

Oil was piped as much as 2 drops into a test tube and 3 mL of ethanol was added. Then, it was sonicated for 10 minutes. The sample was inserted into the GC-MS vial, and as much as 1 μL of the sample was injected automatically. The instrument was equipped with a split/splitless injector, using an RTX_R-1 column (30 m × 0.25mmID × 0.25 μm) and a mass spectrometer detector. Helium was used as a carrier gas on a 1.25 mL/min flow path. Injectors and detectors were set at 260 °C. The chromatographic condition for separation was an initial temperature of 50 °C, increased to 200 °C at an isothermal temperature increase of 200 °C and held for 4 minutes. The heating rate was 2°C/min to 240 °C and held for 10 minutes.

3. RESULTS AND DISCUSSION

3.1 Soxhlet Extraction

In this activity, the anise seed oil extraction method applied was the soxhlet method aiming to separate oil and some components from anise seeds using petroleum ether solvent. Soxhlet extraction was an effective method of extracting oil and producing a high yield [11]. In soxhlet extraction, there is continuous extraction where the sample was repeatedly contemplated with solvent, with a relatively constant amount of solvent. Extraction was carried out in hot conditions and maintained temperatures so it was faster when compared to maceration [12]. Petroleum ether used as solvent has a boiling point range between 30°C to 70°C. This substance has stable and volatile properties so it is very good to use in the extraction process. This solvent use is very beneficial because it is selective in dissolving substances and this process produces small amounts of wax, albumin, and dyes [10].

3.2 Determination of Optimum Extraction Conditions

In this activity, the determination of the optimum conditions of extraction was carried out aiming to obtain a high oil yield by extracting the variation in the ratio of anise seed powder with solvent (petroleum ether). From the highest yield, another variation in the ratio of anise seed powder was carried out with the addition of the amount of solvent (petroleum ether). The comparison of materials and solvents can affect the yield number [13].

3.2.1 The effect of the ratio of anise seed powder and solvent on yield

Based on the results of this study, a comparison effect was obtained between the weight of anise seed powder (g) and petroleum ether (mL) successively (1:24; 1:11.5; 1:7.3; 1:5.25 and 1:4) at a temperature of 60 °C and an extraction time of 5 hours as presented in Figure 2.

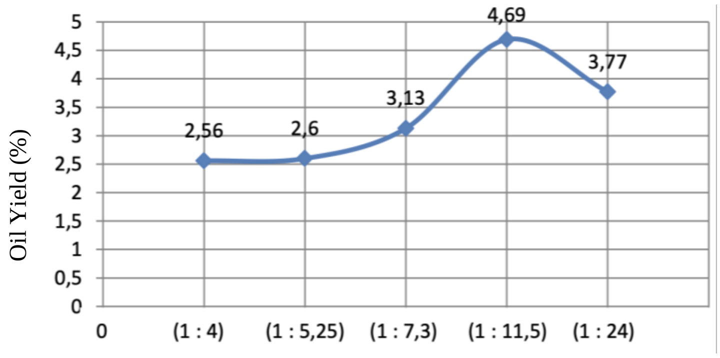

Figure 2. The Relationship of the Weight of Anise Seed Powder with the Amount of Petroleum Ether to Oil Yield

Based on Figure 2, in the ratio of anise seed powder extraction (g) and solvent (mL) 1:24, a 3.77% oil yield was obtained. The ratio of extraction of anise seed powder (g) and solvent (mL) 1:11.5 showed an increase in the oil yield value to 4.69%. Extraction of the ratio between anise seed powder (g) and solvent (mL) successively (1:7.3; 1:5.25 and 1:4) decreased the yield of oil successively to 3.13%, 2.6%, and 2.56%. The change in the yield value was influenced due to the comparison of the amount of solvent and anise seed powder. The amount of solvents affected the contact area between anise seed powder and solvent. The greater the solvent was, the greater the diffusion speed of the solvents to raise anise seed powder. It caused the oil yield to increase. The less solvent used will reduce the diffusion speed between solvent and anise seed powder because the solvent has a limit on the ability to dissolve anise seed powder (saturation limit) so the oil yield obtained is getting less and less. In solvents 1:24 there was a decrease in yield. According to research [14], this is due to the solvent experiencing saturation, where the increase in the amount of solvent no longer increases the yield.

3.2.2 The Effect of Adding Petroleum Ether on Yield

Based on step 3.2.1, the highest yield result was obtained in the condition of the ratio of anise seed powder (g) and solvent (mL) 1:11.5 so the condition should be used as a basic comparison for further extraction. Extraction was carried out by the ratio of anise seed powder (g) and the addition of the amount of petroleum ether (mL) consecutively (1:11.5; 1:11.75; 1:12; 1:12.25 and 1:12.5) at 60 °C and a time of 5 hours. The extraction results were presented in Figure 3.

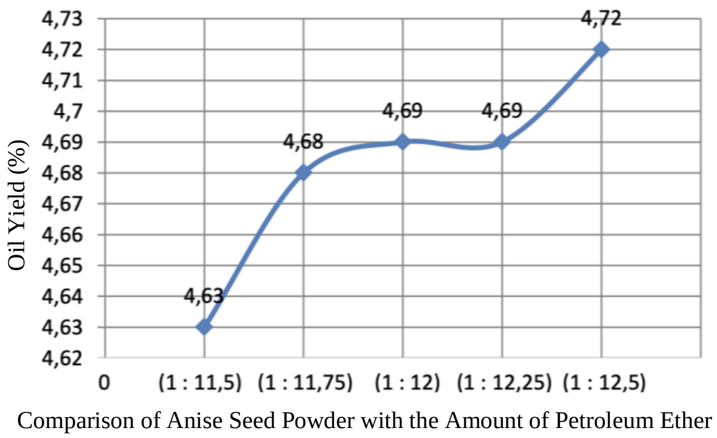

Figure 3. The Comparative Relationship of Seed Powder Weight with the Increase in the Amount of Petroleum Ether to Oil Yield.

Figure 3 showed the oil yield obtained in the treatment of variations in the amount of solvent (petroleum ether). The highest oil yield was obtained at the ratio between anise seed powder (g) and solvent (mL) 1:12.5. The increase in the amount of solvent significantly greatly affects the yield. Previous research [15] said that an excess amount of solvent will cause the distribution of particles in the solvent to spread so that the contact surface is wider. This resulted in more and more yield being obtained. Several factors affect the effectiveness of extraction including the type and amount of solvent, powder size, extraction method, extraction time, and solvent concentration [16].

3.2.3 Extraction of Anise Seed Powder According to Optimum Conditions

Extraction was carried out at a temperature of 60 °C, for 5 hours, with a ratio of anise seed powder (g) and the amount of petroleum ether (mL) 1:12.5. Through 5 repetitions, results were obtained according to Table 1.

Table 1. Anise Seed Oil yield

Sample No.	Comparison of Materials (g) and Solvents (mL)	Oil Yield (%)
1	1: 12.5	4.72
2	1: 12.5	4.79
3	1: 12.5	4.72
4	1: 12.5	4.68
5	1: 12.5	4.70
Average		4.72

Extraction of anise seed powder with petroleum ether obtained oil yield in samples of 1, 2, 3, 4, and 5 respectively 4.72%, 4.79%, 4.72%, 4.68%, and 4.70% with an average percentage of 4.72%. Petroleum ether has been widely used in the extraction of fats and oils from the seeds of fruit crops [8]. The use of petroleum ether as a solvent is very beneficial because it is selective in dissolving substances. Petroleum ether has a high solvent extraction capacity and doesn’t affect the chemical properties of oil [17]. However, the oil products obtained from this process produce a small amount of wax fraction that affects the resulting product. This wax fraction at room temperature changes its form to solid so it is necessary to do a soaping process with KOH to separate the wax fraction from the oil. In addition, there is a considerable loss of solvent during the extraction process due to the evaporation of low boiling point oil and the flammable properties of petroleum ether.

3.3 Oil Quality Analysis of Extracted Oil

3.3.1 Solubility of Anise Oil in Alcohol 90%

The solubility of alcohol is the ability of essential oils to dissolve in various alcohol concentrations. The quality of essential oils improves with time dissolving [18]. Our research showed that anise oil dissolved in 90% alcohol had a fairly good solubility rate. A 1 mL of anise seed oil in 3 mL of alcohol only dissolved briefly and when it was allowed to stand for a few minutes, a lump of oil appeared deposited at the bottom of the test tube. After the addition of 7 mL of alcohol, the oil was perfectly mixed and looked clear. This is because the main constituent component of anise oil is anethole [4]. Although included in the group of oxygenated hydrocarbons, anethole is not easily soluble in alcohol because anethole and safrole have low solubility in alcohol [10]. Dissolving 1 mL of anise oil requires 3 mL to 7 mL of alcohol.

3.3.2 Analysis of Anise Oil Acid Number

In determining the number of acids, KOH 0.1 N was used as a titrant solution because if concentrated KOH is used, a number of ester compounds in the oil will be soiled even in a cold state. Most essential oils contain small amounts of free acids. The amount of free acid is usually expressed as the number of acids and is rarely counted in acid percent. A previous study by Park et al. [19] stated that the number of acids is defined as the number of milligrams of KOH needed to neutralize free acids in 1 g of oil.

Acid number analysis can provide a single parameter used for the acidity ratio. The acidic number of essential oil increases following its shelf life, especially if the oil storage method is not good. Processes such as aldehyde oxidation and ester hydrolysis will increase the number of acids. Oils that have been drained and protected from air and sunlight have a relatively smaller number of free acids [10].

According to Guenther [10], the maximum limit of the number of anise seed oil acids is 1.33. The results of the analysis of the number of anise seed oil acids obtained from the study were 1.14444. Our research showed that the number of oil acids obtained didn’t exceed the set maximum limit. The storage of oil samples in sealed glass containers was protected from air and light so the relatively smaller acid numbers are obtained and met the established limits. Research by Salim et al. [20] found that the acid number of anise oil was 1.29. This difference in yield may be due to climate, cultural practices, and post-harvest factors.

Table 2. Comparison of oil characteristics from several research

Research by	Acid number	Solubility in the alcohol 90%
Damayanti and Setyawan [21]	n.a.	Soluble 1:3
Kojong et al. [4]	2.81	Soluble 1:3, perfectly soluble 1:7
Salim et al. [20]	1.29	n.a.
Yadav et al. [22]	2.55	n.a.
Albulushi et al. [23]	0.3927	Clearly soluble at any volume

n.a.: not available provided at the paper.

3.3.3 Analysis of Anise Oil Components with GC-MS

The chemical composition of anise seeds varies depending on the variety and place of growth. But, in general, the main component of anise oil is anethole. According to [6], the main components of anise seeds identified from the results of the analysis with GC-MS are limonene, fenchone, estragole, anisaldehyde, and anethole. Based on the results of the analysis with GC-MS in our study, 5 (five) main compositions could be identified as anise oil constituents. Identification was done by matching each peak with Wiley's database to determine the type of compound.

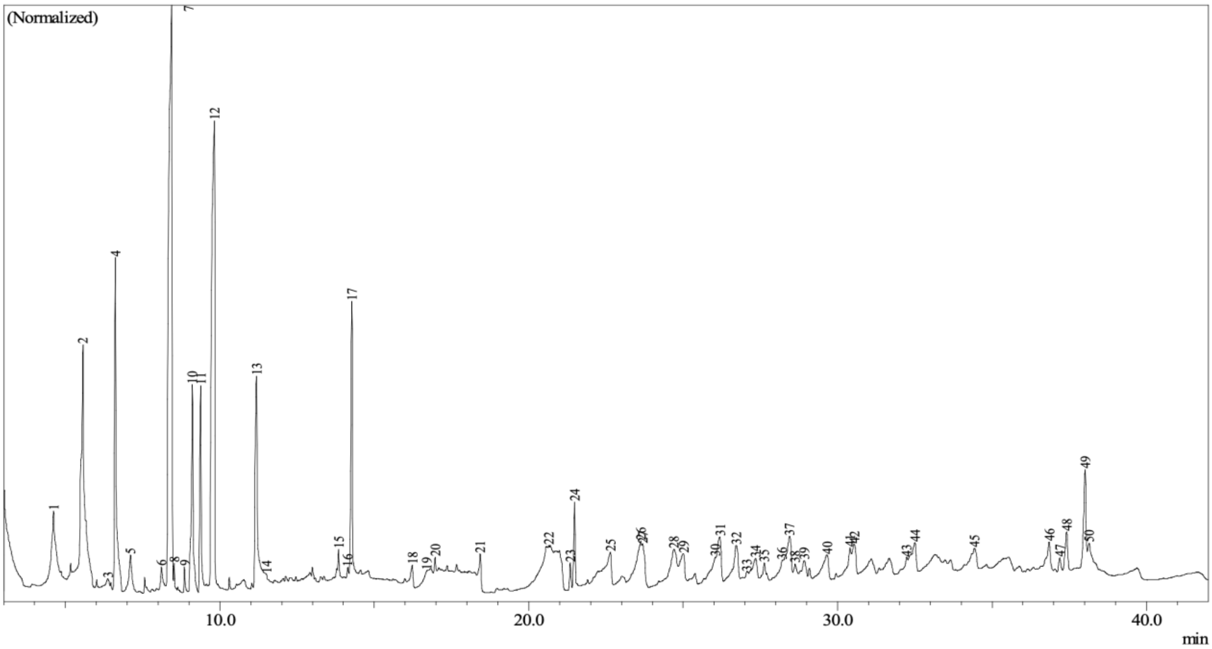

Figure 3. GC-MS Chromatogram of Anise Seed Oil from this study

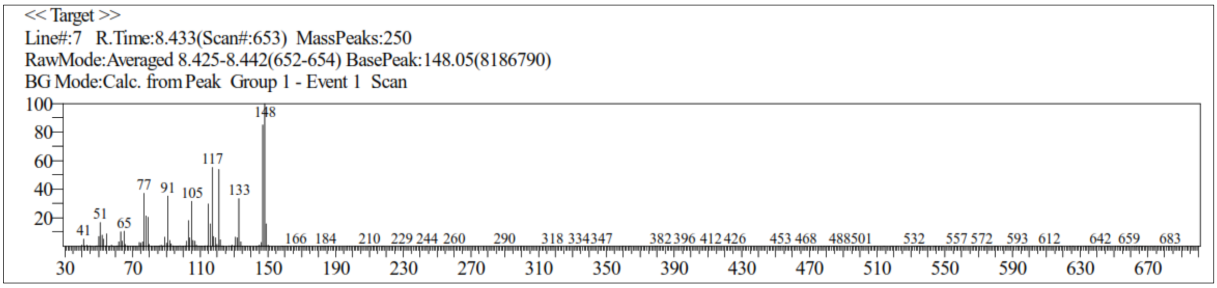

Figure 4a. Chromatogram of the Top Number 7 Anise Oil from this study

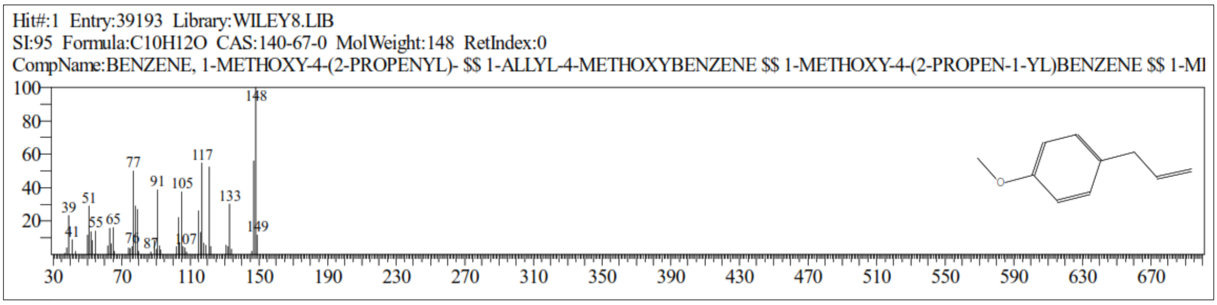

Figure 4b. Wiley's Database Reference Chromatogram no.7

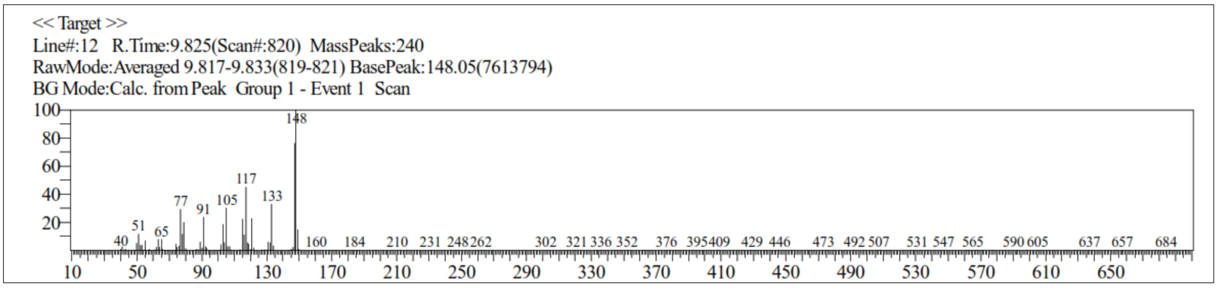

Figure 4c. Chromatogram of the Top Number 12 Anise Oil from this study

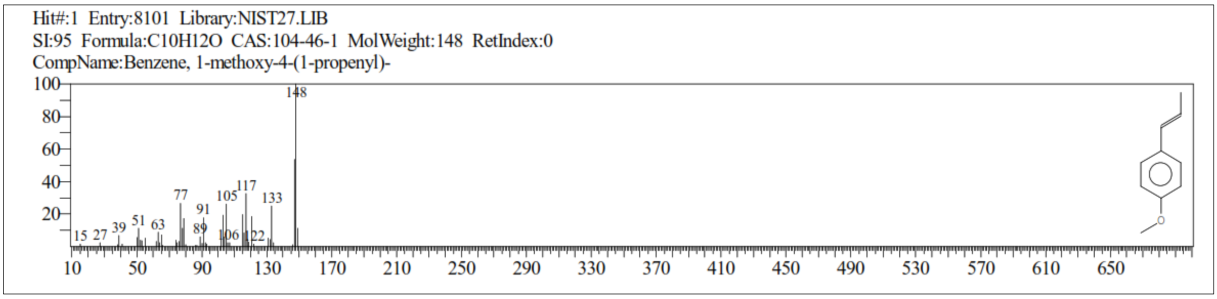

Figure 4d. Wiley's Database Reference Chromatogram no.12

Figure 4a was the chromatogram of the top no.7 anise oil, while 4b was Wiley's database reference chromatogram (Benzene, 1-methoxy-4-(2-propenyl). It has a price of m/z of 148 which refers to estragole compounds. Figure 4c was the chromatogram of the top no.12 anise oil, while 4d was Wiley's database reference chromatogram (Benzene, 1-methoxy-4-(1-propenyl). It has a price of m/z of 148 which refers to anethole compounds. Here are 5 identified main components identified from the GC-MS chromatogram:

1) Estragole (benzene, 1-methoxy-4-(2-propenyl)) is the most dominant component (peak numbers 7). The peak of the estragole compound appeared in 8.43 minutes with an area of 20.22%.
2) Anethole (benzene, 1-methoxy-4-(1-propenyl)) is the second abundant compound if anise seed oil seen at peaks numbered 12 that appear in the 9.82 minutes with an area of 15.01%
3) Limonents (cyclohexene, 1-methyl-4-(1-methyl ethenyl)) seen at peaks numbered 2 that appear in the 5.56 minutes with an area of 7.75%.
4) Fenchone (Bicyclo [2.2.1] heptan-2-one, 1,3,3-trimethyl-) seen at peak number 4 which appeared in the 6.65 minutes with an area of 6.14%.
5) Anis ketone (2-Propanone, 1-(4-methoxyphenyl)-) seen at peak number 13 which appears in the 11.18 minute with an area of 5.32%.

The results of the GC-MS analysis of anise oil contained the main components of estragole and other components, namely anethole, limonene, fenchone, and anis ketone. This is in accordance with the research by Saibi et al. [24], Ullah et al. [25], and Ozcan and Chalchat [26] that the components of aniseed oil were trans-anethole, estragole, and himachalane. Previous research conducted by Anastasopoulou et al. [27] also supports this result that the constituent components of anise seeds are anethole and limonents, and also another research by Sharifi et al. [28] where the constituent components of anise seeds are anethole, limonents, anisaldehyde.

4. CONCLUSION

The result of determining the optimum extraction condition was at a temperature of 60°C, 5 hours with a ratio of anise seed powder (g) and amount of petroleum ether (mL) 1:12.5. The yield of anise seed oil extraction with petroleum ether based on optimum conditions, with 5 repetitions of extraction obtained successive oil yield of 4.72%; 4.79%; 4.72%; 4.68% and 4.70% with an average percentage of 4.72%. The results of the analysis showed that anise oil had good quality based on the solubility of oil in 90% alcohol with a 1:7 ratio. The acid number of anise seed oil was obtained at 1.14444. GC-MS analysis showed that anise seed oil contained estragole, anethole, limonene, fenchone, and anis ketone. It was hoped that further research would be in order to be able to conduct a complete analysis of physicochemical properties.

ACKNOWLEDGMENT

We would like to acknowledge and express our deepest gratitude to all those who made this work possible. We also would like to give special thanks to the Chemical Engineering Department of the State Polytechnic of Ujung Pandang.

REFERENCES

[1] M. Zubair, S. Shahzad, A. Hussain, R.A. Pradhan, M. Arshad, A. Ullah, “Current Trends in the Utilization of Essential Oils for Polysaccharide-and Protein-Derived Food Packaging Materials,” *Polymers (Basel)*. 14 (2022). https://doi.org/10.3390/polym14061146.

[2] W. Sun, M.H. Shahrajabian, Q. Cheng, “Anise (Pimpinella anisum L .), a dominant spice and traditional medicinal herb for both food and medicinal purposes,” *Cogent Biol*. 5 1673688 (2019). https://doi.org/10.1080/23312025.2019.1673688.

[3] A.H. Prakosa, I.D. Pamungkas, “Pengaruh Waktu pada Penyulingan Minyak Biji dan Daun Adas dengan Metode Uap dan Air,” *Jurnal Teknologi Kimia Dan Industri*. 2 14–17 (2013). https://ejournal3.undip.ac.id/index.php/jtki/article/view/2598 (accessed May 2, 2023).

[4] V.Ch.O. Kojong, M.S. Sangi, J. Pontoh, “Uji Kualitas Minyak Biji Adas (Foeniculum vulgare) yang diperoleh dengan Metode Soxhletasi,” *Jurnal FMIPA UNSRAT Online*. 2 124–127 (2013). https://doi.org/https://doi.org/10.35799/jm.2.2.2013.3031.

[5] I. Bettaieb Rebey, S. Bourgou, S. Ben Kaab, W. Aidi Wannes, R. Ksouri, M. Saidani Tounsi, M.L. Fauconnier, “On the effect of initial drying techniques on essential oil composition, phenolic compound and antioxidant properties of anise (Pimpinella anisum L.) seeds,” *Journal of Food Measurement and Characterization*. 14 220–228 (2020). https://doi.org/10.1007/s11694-019-00284-4.

[6] H. Sastrohamidjojo, Kimia Minyak Atsiri, 2021. https://books.google.co.id/books?hl=en&lr=&id=5nMZEAAAQBAJ (accessed May 2, 2023).

[7] M.A. Hanif, S. Nisar, G.S. Khan, Z. Mushtaq, M. Zubair, Essential Oils, in: Essential Oil Research, Springer International Publishing, Cham, 2019: pp. 3–17. https://doi.org/10.1007/978-3-030-16546-8_1.

[8] M.R. Norazlina, Y.S. Tan, M. Hasmadi, M.H.A. Jahurul, “Effect of solvent pre-treatment on the physicochemical, thermal profiles and morphological behavior of Mangifera pajang seed fat,” *Heliyon*. 7 (2021). https://doi.org/10.1016/j.heliyon.2021.e08073.

[9] A. Verma, A. Sharma, P.K. Rai, “Impact of soxhlet extraction method on oil yield and antioxidant potential of Brassica juncea,” *J Pharmacogn Phytochem*. 8 (2019).

[10] E. Guenther, The Essential Oils: History-Origin in Plants Production-Analysis, New York, 1948.

[11] S. Zain, T. Herwanto, S. Harnesa Putri, “Antioxidant Activity in Oil Seeds Moringa (Moringa oleifera l.) Soxhletation Method with using Solvents N-hexane, Methanol and Ethanol),” *Jurnal Teknotan*. 10 (2016).

[12] M.A. López-Bascón-Bascon, M.D. Luque de Castro, Soxhlet extraction, in: Liquid-Phase Extraction, Elsevier, 2019: pp. 327–354. https://doi.org/10.1016/B978-0-12-816911-7.00011-6.

[13] G.A.K.F. Yudharini, A.A.P.A. Suryawan, N.M. Wartini, “Pengaruh Perbandingan Bahan dengan Pelarut dan Lama Ekstraksi terhadap Rendemen dan Karakteristik Ekstrak Pewarna dari Buah Pandan (Pandanus tectorius),” *Jurnal REKAYASA DAN MANAJEMEN AGROINDUSTRI*. 4 36–46 (2016).

[14] A. Noviyanty, C. Anggriani Salingkat, “The Effect of Solvent Ratio to the Quality of Extracts from the Red Dragon Fruit Peel (Hylocereus polyrhizus),” *KOVALEN*. 5 280–289 (2019).

[15] Y.L. Maslukhah, T.D. Widyaningsih, E. Waziiroh, N. Wijayanti, F.H. Sriherfyna, “Influence Factor of Black Cincau (Mesona palustris BL) Extraction in Pilot Plant Scale: A Review,” *Jurnal Pangan Dan Agroindustri*. 4 245–252 (2016).

[16] M.R.R. Rahardhian, B.T. Murti, D. Wigati, R. Suharsanti, C.N. Putri, “Solvent concentration effect on total flavonoid and total phenolic contents of Averrhoa bilimbi leaf extract Antioxidant Activity, Total Flavonoid Content, Total Phenolic Content View project Biosensors for theranostics View project,” *Pharmaciana*. 9 137–144 (2019). https://doi.org/https://doi.org/10.12928/pharmaciana.v9i1.8793.

[17] A. Verma, A. Sharma, Meghna, P.K. Rai, “IndiaImpact of soxhlet extraction method on oil yield and antioxidant potential of Brassica juncea,” *J Pharmacogn Phytochem*. 8 1134–1137 (2019). https://doi.org/10.32474/aoics.2018.02.000141.

[18] M.A. Arrayyan, B. Dwiloka, D.S. Susanti, “The Effect of Vegetable Fat Enfleurage Concentration to Antioxidant Activity and Physical Characteristic of Basil Essential Oil (Ocimum americanum L.),” *Jurnal Teknologi Pangan*. 3 221–227 (2019). www.ejournal-s1.undip.ac.id/index.php/tekpangan.

[19] L.K.E. Park, J. Liu, S. Yiacoumi, A.P. Borole, C. Tsouris, “Contribution of acidic components to the total acid number (TAN) of bio-oil,” *Fuel*. 200 171–181 (2017). https://doi.org/10.1016/j.fuel.2017.03.022.

[20] E.R.A. Salim, “Histology, Phytochemistry and Bacterial Activity of Anise (pimpinella anisum l.) Seed and Essential Oil,” *Journal of Bacteriology & Mycology: Open Access*. 3 (2016). https://doi.org/10.15406/jbmoa.2016.03.00070.

[21] A. Damayanti, E. Setyawan, “Essential Oil Extraction of Fennel Seed (Foeniculum vulgare) Using Steam Distillation,” *International Journal of Science and Engineering*. 3 (2012). https://doi.org/10.12777/ijse.3.2.12-14.

[22] S.D. Yadav, O.K. Mahadwad, S. Kshirsagar, V.A. Gite, Extraction and Characterization Study of Aniseed Oil, in: 2nd International Conference on Multidisciplinary Research & Practice, 2015: pp. 48–50.

[23] S.M.A. Albulushi, H. Al Saidi, N. Amaresh, A. Mullaicharam, "Study of physicochemical properties, antibacterial and GC-MS analysis of essential oil of the aniseed (Pimpinella anisum Linn.) in Oman, 2(4), 24-33.," *Research and Reviews: Journal of Pharmacognosy and Phytochemistry*. 2 24–33 (2014). https://www.researchgate.net/publication/267506328.

[24] S. Saibi, M. Belhadj, E.H. Benyoussef, "Essential Oil Composition of Pimpinella anisum from Algeria," *Analytical Chemistry Letters*. 2 (2012). https://doi.org/10.1080/22297928.2012.10662624.

[25] H. Ullah, A. Mahmood, B. Honermeier, "Essential oil and composition of anise (Pimpinella anisum L.) with varying seed rates and row spacing," *Pak J Bot*. 46 (2014).

[26] M.M. Özcan, J.C. Chalchat, "Chemical composition and antifungal effect of anise (Pimpinella anisum L.) fruit oil at ripening stage," *Ann Microbiol*. 56 (2006). https://doi.org/10.1007/BF03175031.

[27] E. Anastasopoulou, K. Graikou, C. Ganos, G. Calapai, I. Chinou, "Pimpinella anisum seeds essential oil from Lesvos island: Effect of hydrodistillation time, comparison of its aromatic profile with other samples of the Greek market. Safe use," *Food and Chemical Toxicology*. 135 (2020). https://doi.org/10.1016/j.fct.2019.110875.

[28] R. Sharifi, H. Kiani, M. Farzaneh, M. Ahmadzadeh, "Chemical Composition of Essential Oils of Iranian Pimpinella anisum L. and Foeniculum vulgare Miller and their Antifungal Activity Against Postharvest Pathogens," *Journal of Essential Oil-Bearing Plants*. 11 514–522 (2008). https://doi.org/10.1080/0972060X.2008.10643660.