Indonesian Journal of Pharmacy

VOL 35 (3) 2024: 425–436 | RESEARCH ARTICLE

Isolation of Nutmeg Essential Oil (*Myristica fragrans* houtt) From Aceh Indonesia and Their Antioxidant and Antibacterial Activities

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Article Info

Submitted: 01-06-2023
Revised: 20-12-2023
Accepted: 13-01-2024

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ABSTRACT

This study aims to isolate the nutmeg essential oil (*Myristica fragrans* Houtt) from Aceh Indonesia and evaluate their biological activity. The antioxidant activity of the nutmeg essential oils (*Myristica fragrans* Houtt) was determined by DPPH (1,1-difenil-2-pikrihidrazil) assay. The DPPH assay showed that arillus essential oil (AMFH) has the highest antioxidant activity with the IC_{50} values was 216.695 ppm. The chemical composition of the AMFH was performed by Gas Chromatography-Mass Spectroscopy (GC-MS), and the results showed that AMFH contains Terpinen-4-ol (11.20%), α-Terpineol (1.83%), Safrole (5.10%) and Myristicin (27.80%). The AMFH was isolated by column chromatography and four fractions were obtained, namely AMFH1; AMFH2; AMFH3; and AMFH4 fractions. The DPPH assay showed that the AMFH3 fraction showed strong antioxidant activity with the IC_{50} values of 59.329 ppm. The AMFH3 fraction was then re-isolated by using column chromatography to obtain four sub fractions namely AMFH3A; AMFH3B; AMFH3C; and AMFH3D. The DPPH assay showed that the sub fraction of AMFH3A has the highest antioxidant activity with the IC_{50} values of 98.993 ppm. The antibacterial activity of AMFH3A sub fractions was evaluated by Kirby Bauer-Disc diffusion method, and the results showed that AMFH3A sub fraction showed activity against *Staphylococcus epidermidis* and *Staphylococcus aureus* with the diameter inhibition of 14.28 mm and 12.26 mm respectively. The chemical composition of the AMFH3A sub fraction was performed by GC-MS, and the results showed that AMFH3A sub fraction contains Terpinen-4-ol (14.37%), α-Terpineol (2.16%), Safrole (7.36%) and Myristicin (54.77%).

Keywords: *Myristica fragrans* Houtt, nutmeg essential oil, antioxidant activity, antibacterial activity

INTRODUCTION

The essential oils are secondary metabolites produced by medicinal or aromatic plants. Essential oils generally produced in small yields, usually ranging from 5-10%. Essential oils are volatile, and generally in liquid form and colourless at room temperature, poor soluble in water, easily soluble in alcohol or in other organic

solvents (Falleh *et al*, 2020). Essential oils are known showed excellent their biological activities including antioxidant, antiparasitic, antimicrobial, antifungal, antiviral, antimycotic and insecticidal. Their biological activities generally related to the function of bioactive secondary metabolites such as terpenes, hydrocarbon, alkaloids and flavonoids (Ju *et al*, 2022; Tétédé Rodrigue *et al*, 2023).

Myristica fragrans (*M*. *fragrans*) belongs to the Myristicaceae family, and commonly known as Nutmeg. The essential oil that produced by this plant called nutmeg essential oil. Scientifically, the seed of this plant composed of starch, protein, fat, mucilage, fixed oil, and volatile oil (Luciana *et al*, 2021; Barceloux, 2008). Luciana *et al*, (2021) reported that nutmeg essential oil obtained from distillation process produced 21 compounds with the main components were γ-terpinene (8.5%), Sabinene (9.1%), α-pinene (10.5%), and β-pinene (26%). Luciana *et al* also showed that distilled nutmeg oil had insecticidal activity against *Musca domestica* and *Chrysomya albiceps* with LC_{50} values of 2.02 ± 0.56, and 8.57 ± 2.41, respectively.

Ethnomedical, it was reported that *M. fragrans* can be used as improving digestion, good for spleen and sore throats, anti-allergic and analgesic, insulin resistance, prevent inflammation, liver tonic, uterine tonic and cardiotonic, sedative, aphrodiasic and hypolipidemic (Neetu & Surender, 2022; Gottardi *et al*, 2016). *M. fragrans* not only provides a pleasant aromatic aroma, but traditionally can also be used to treat digestive problems, diarrhea and nausea. Pharmacologically, *M. fragrans* has anti-inflammatory, antidiarrheal and antidiabetic effects (Matulyte *et al*, 2020; Zhipeng *et al*, 2020). Zhipeng also reported that seed of Myristica fragrans commonly contains fat, protein and starch, while it essential oil particularly contains myristicin. Myristicin is an aromatic compound that plays a role in the aroma of nutmeg oil. Myristicin has a hallucinogenic effect due to its structural similarity to 3-methoxy-4,5-methylenedioxyamphetamine (MMDA) (Zhipeng *et al*, 2020).

Sathya *et al*, (2020) stated that ethyl acetate extract and aryl methanolic extract of *M. fragrans* showed excellent antioxidant activity by in vitro assay. The aril (mace) extract of *M. fragrans* also showed good anticholinesterase and α-glucosidase inhibitory activities, so it can be used as the treatment of Alzheimer's disease. Manh *et al*, (2020) reported that *M. fragrans* contain metabolites of Lignans, Neolignans, Diphenyl alkanes, Phenylpropanoids, and Terpenoids. *M*. *fragrans* is also reported to contain Alkaloids, Flavonoids, and Phenolic compounds. *M*. *fragrans* also contains Myristic acid, Myristicin, Safrole, Terpineol, Elemicin, Quercetin, Camphene, Eugenol, and Isoeugenol. The previous studies reported that the *n*-hexane extract of nutmeg root had an antioxidant activity with an IC_{50} value of 99.76 ppm. (2E)-5(2z.4E)-Hexa-2,4,-dio-Zyl)-2propylcyclohexanol ($C_{18}H_{30}O_4$) compound from an *n*-Hexane extract of nutmeg root was also reported to have anticancer activity against the MCF-7 cell line with the IC_{50} value of 10.75 ppm (Ginting *et al*, 2018; Ginting *et al*, 2021). Wahidah *et al*, (2022) reported that the elimicin compound isolated from the ethanolic seed extract of *M. fragrans* showed high antioxidant activity. Wahidah also showed that the elimicin compound has antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumonia* and *Staphylococcus aureus* with MIC_{50} values ranging from 31.25-62.5 µg/mL. Elimicin was also reported to have antifungal activity against *Candida tropicalis*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium chrysogenum* and *Trichophyton rubrum*.

Nutmeg essential oil was reported rich in Terpenes. This compound has been shown to possess anti-inflammatory, antioxidant, antibacterial, analgesic, hepatoprotective, and cardioprotective (Matulyte *et al*, 2020). Rokas *et al*, (2021) stated that the essential oil of *M. fragrans* has a potential to reduce cell viability, proliferation, and colony formation ability of Novikoff hepatoma cells. Nutmeg essential oil also contains of 4-Terpineol, Myristicin, Safrole and Methyl eugenol. These compounds are main components of nutmeg essential oil, which are carcinogenic (Al-Malahmeh Amer *et al*. 2017; Daniela *et al*, 2022). Nutmeg oil and its fractions were reported to have hepatoprotective, antioxidant, anticancer, antimicrobial, antidiabetic and aphrodisiac properties (Kumari *et al*, 2021). The active metabolite compounds contained in nutmeg and nutmeg essential oil such as Licarin B and Myristicin were reported have anticancer effects on skin carcinoma (Kunnumakkara *et al*, 2018; Perez-Ortiz *et al*, 2020) and oral cancer (oral cell line) (Cinthura *et al*, 2017). There are few reports that discuss to isolate the nutmeg essential oils from Aceh Indonesia. Hence, there is a need to isolate the active compounds from the essential oils of *M. fragrans* and evaluate their biological

activity such as antioxidant, and antibacterial activity.

MATERIALS AND METHODS

The DPPH (1,1-difenil-2-pikrihidrazil) and Mueller Hinton Agar (MHA) were purchase from Merck. Anhydrous Sodium Sulphate (Sigma Aldrich), Methanol, Silica Gel G_{60} (Merck), the solvents of *n*-Hexane, Ethyl Acetate, and Ethanol were purchase from Rudang Jaya Medan, Thin Layer Chromatography, TLC (Merck), Vancomycin, and Amoxicillin were provided from our Laboratory at Department of Pharmacy, Universitas Syiah Kuala.

Methods

Plant material

The flesh of fruit, seed, and arillus of *M. fragrans* were obtained from Blangpidie, Southwest Aceh Regency, Indonesia. Voucher specimen (1815) *M. fragrans* Houtt was authenticated by botanist, Dr. Joeni Setijjo Rahajoe and deposited at the Herbarium Bogoriense, Research Centre for Biology, Indonesian Institute of Sciences, Bogor, Indonesia.

Sample Preparation

Each parts of flesh of fruit, seed, and arillus of *M. fragrans* Houtt were thoroughly cleaned, dried at room temperature and cut into small pieces. Each component was kept in an air-tight container at room temperature until further use.

Extraction of essential oil

Each component of the flesh of fruit, seed, and arillus of *M. fragrans* were submitted to water distillation for 6 hours. The extracted essential oils were dried over anhydrous sodium sulphate. The addition of the anhydrous sodium sulphate is to dry the extract. It is the sodium sulphate that will absorb any water, and it will clump together. The percentage yield was then determined for each part of the oil. Furthermore, the essential oils obtained were then evaluated for the antioxidant activity using DPPH Assay method. The oil with the higher antioxidant activity was then used for further analysis.

DPPH Assay

For all antioxidant radical reactions, DPPH (1,1-difenil-2-pikrihidrazil) was dissolved in methanol (MeOH) to a concentration of 0.4 mM. In addition, 1 mL DPPH and 5 mL MeOH were added to the test tube with 100; 50; 25; 12.5; and 6.25 ppm concentrations of the essential oil of DPPH (1,1-difenil-2-pikrihidrazil) of *M. fragrans* Houtt. After 30 minutes, the mixture was homogenized with a vortex for a few seconds, and the absorbance was measured every test for 30 min at a wavelength of 517 nm. Three measurements were taken, and the average absorption value for each concentration was recorded. Positive controls for ascorbic acid received the same treatment (Ginting *et al*, 2021).

Isolation of the active nutmeg essential oil

A thirty-gram sample of the most active essential oil was separated from its constituents using gravity column chromatography with a silica gel G_{60} as the stationary phase and *n*-Hexane and Ethyl acetate gradient elution system with a 9.5:0.5 ratio as a mobile phase. The eluent was determined by thin layer chromatography (TLC). The active nutmeg essential oil was separated by its components and each fraction was collected in a test tube. The fraction with the identical spot pattern were collected and evaporated by using a rotary evaporator vacuum.

FT-IR analysis

The resulting of the oils were analysed to determine the functional group of each oil using Fourier-Transform Infrared Spectroscopy (FT-IR spectroscopy). The FT-IR spectrum analysis was performed at wave numbers 400-4000 cm^{-1} (Khairan *et al*, 2023).

GC-MS analysis

The GC–MS analysis of the samples were conducted using TG-SQC system qualification column (*Thermo Scientific™* TRACE 1310 GC) in tandem with *Thermo Scientific™* ISQ LT *Single Quadrupole Mass Spectrometer. Autosampler* is used *Thermo Scientific™ TriPlus™* RSH *Autosampler* with column HP-5MS, dan TG-WAXMS with 15 m in length × 0.25 mm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by Single Quadrupole Mass Spectrometer. Pure Helium gas (99.995%) was used as the carrier gas with a flow rate of 1 mL/min. Maximum temperature of 330/350°C. The relative quantity of the chemical compounds presented in each sample was expressed as a percentage based on the peak area produced in the chromatogram (Ginting *et al*, 2018; Ginting *et al*, 2021).

Antibacterial Activity

The Kirby Bauer-Disc diffusion method was used to determine the antibacterial activity of the sample. Nineteen grams of Mueller Hinton Agar (MHA) medium was added to 500 mL of sterile distilled water. The medium was subsequently heated to boil using magnetic stirring. The media was then sterilized in an autoclave at 121°C for 15 minutes. The media (25 mL) was then poured into a petri dish and allowed to solidify. By using a cotton swab, a suspension of *Staphylococcus aureus* or *Staphylococcus epidermidis* was applied to the solidified MHA media. The paper disc that had previously been soaked in a positive control solution (Vancomycin 30 µg or Amoxicillin 25 µg) and the sample were then placed in the petri dish. The diameter of the inhibition formed from the samples after incubating the media at 37°C for 24 h was then measured. The inhibition zone formed on the media was measured to determine antibacterial activity of the samples (Khairan *et al*, 2023).

RESULTS AND DISCUSSION

Essential Oils Distillation

Each component of the fruit (FMFH), seed (SMFH), and arillus (AMFH) of *M. fragrans* were distilled to obtain the fruit, seed, and arillus essentials oils. The results showed the percentage of the yield of the essential oils obtained from the fruit (FMFH), seed (SMFH), and arillus (AMFH) were 0.03%; 1.5%; and 3.5% respectively. The results revealed that AMFH has the highest rendement with the percentage rendement of the oil of 3.926%. The strongest aroma of the oil was produced by AMFH.

Antioxidant Activity of the Essentials Oils

The antioxidant activity of each essential oils of the *M. fragrans* Houtt (Figure 1 and Table I). The essential oils concentrations employed for the antioxidant activity assay were 25; 50; and 100 ppm. The essential oil derived from the arillus part (AMFH) has the highest antioxidant activity with IC_{50} values of 216.69 ppm (Table I). The result obtained in this study is significantly more active than research conducted by Yuniasih & Rachmawati (2021) that was 299.69 ppm. (Suthagar *et al*, 2012a) mentioned that nutmeg oil effectively inhibited the oxidation of linoleic acid at 88.68±0.1%. Suthagar also showed that nutmeg oil showed reducing power with the EC_{50} value was 181.4 ppm. Meanwhile, Ginting *et al*, (2021) reported that the bark extract of *M. fragrans* Houtt exhibited a good antioxidant activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, with the IC_{50} value was 99.76 ppm.

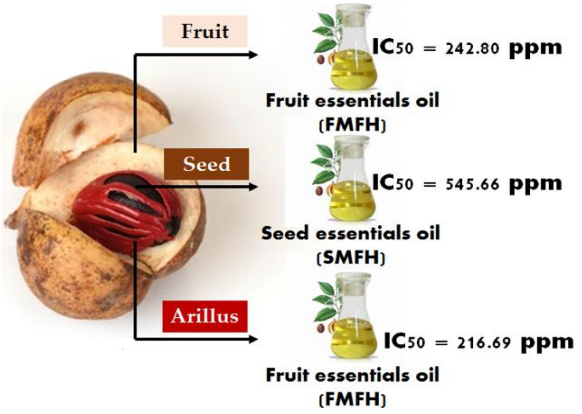

Figure 1. The diagram of antioxidant activity of flesh of fruit (FMFH), seed (SMFH), and arillus (AMFH) essential oil from *Myristica fragrans* Houtt (MFH).

Table I. Percent inhibition and antioxidant activity of nutmeg essential oils using the DPPH assay.

Conc.	Percent Inhibition (%)			
(ppm)	AA[a]	FoFEO[b]	SEO[c]	AEO[d]
25	30.3±1.0	79.1±0.8	83.3±1.0	74.9±1.2
50	68.9±1.5	73.4±1.0	80.9±1.2	72.0±1.9
100	96.3±0.8	66.5±1.5	77.4±1.3	62.4±0.9
IC_{50}	**3.9±1.1**	**242.8±1.1**	**545.7±1.2**	**216.7±1.3**

Note: a: Ascorbic Acid or Vitamin C (AA); b: Flesh of Fruit Essential Oil (FoFEO); c: Seed Essential Oil (SEO); d: Arillus Essential Oil (AEO).

Fractionation of the Essential Oil from Arillus (AMFH)

The antioxidant activity assay (Table IA)z revealed that the essential oil from arillus (AMFH) has the highest antioxidant activity. The AMFH was then isolated using column chromatography, using silica gel G_{60} as the stationary phase and *n*-Hexane : Ethyl acetate in a ratio of 9.5:0.5 was used as a mobile phase, and the result showed that AMFH produce 255 fractions. The fractions were then monitored by thin layer chromatography (TLC) to determine the spot pattern and the Rf values of each fraction. The fractions with the same of spot and Rf values were collected and evaporated using rotary evaporator to obtain the fractions of AMFH. In this analysis, we have found four fractions

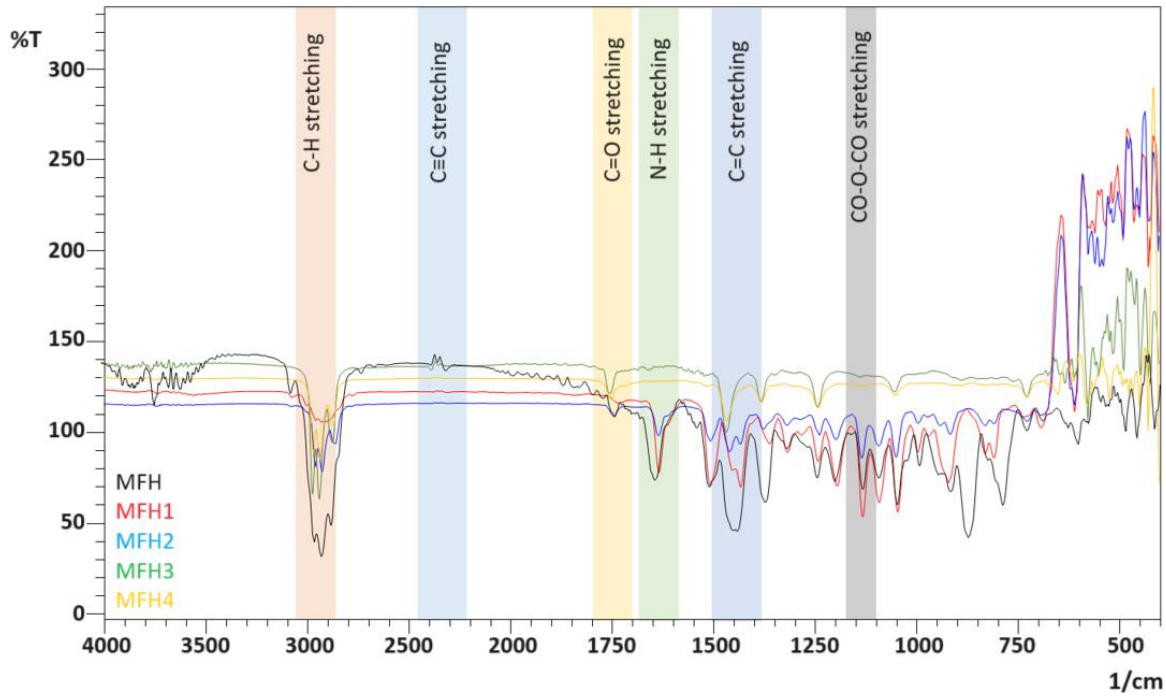

Figure 2. The spectrum FT-IR of AMFH and its fractions (AMFH1; AMFH2; AMFH3; and AMFH4)

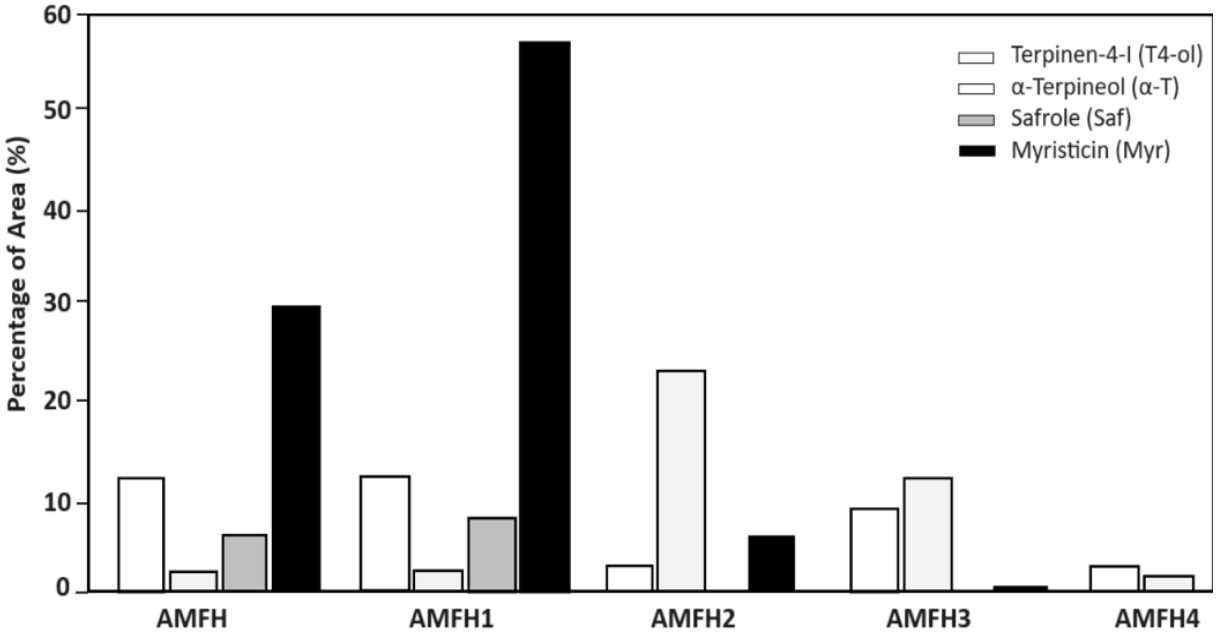

Figure 3. The percentage area of Terpinen-4-ol (T-4-ol), α-Terpineol (α-T), Safrole (Saf), and Myristicin (Myr) in MFH and its fractions by GC-MS analysis.

namely AMFH1 fraction (fractions 1-110), AMFH2 fraction (fractions 111-130), AMFH3 fraction (fractions 131-200), and AMFH4 fraction (fractions 201-255). After evaporated, we have founded that AMFH1; AMFH2; AMFH3; and AMFH4 fractions have weight of 434.14 mg; 56.56 mg; 11.04 mg; and 09.03 mg respectively. The results also revealed that AMFH1; AMFH2; AMFH3; and AMFH4 fractions have the Rf values of 0.25; 0.38; 0.53; and 0.86, respectively.

FT-IR Analysis

The Fourier-transform infrared spectrum (FTIR) analysis was performed to determine the functional group of AMFH and its fractions (AMFH1; AMFH2; AMFH3; and AMFH4) (Figure 2).

The AMFH and it fractions showed strong absorption band at range 3000-2700 cm^{-1}, these indicated the presences of C-H stretching from Alkyne, Alkene, Alkane, and Aldehyde (Figure 2). Ginting *et al*, (2021) reported that the characteristic peaks at 2926-2856 cm^{-1} indicates the presence C-H stretching. Horison *et al*, (2019) also reported that the band arising at 3000-2700 cm^{-1} are contributed by the stretching vibration of $-CH_2$ (2924 cm^{-1}) and $-CH_3$ (2857 cm^{-1}) are from nutmeg essential oil.

The AMFH and its fractions showed weak absorption band at range 2260-2222 cm^{-1} indicated the presence of C≡N stretching (Figure 2). The weak absorption peak at range of 1750-1706 cm^{-1} corresponds to C=O stretching from functional groups of Lactone or Carboxylic acid. The medium characteristic peak at range 1650-1580 cm^{-1} indicates the presence of N-H stretching from Amine. The medium peak at range 1500-1400 cm^{-1} corresponds to C=C stretching from alkene and conjugated alkene. Meanwhile, the medium peak in the region of 1050-1040 cm^{-1} corresponds to CO-O-CO stretching from Anhydride.

GC-MS Analysis of the AMFH and Its Fractions

The GC-MS analysis showed that AMFH and its fractions (AMFH1; AMFH2; AMFH3; and AMFH4) contain 33; 36; 45; 56; and 68 compounds respectively. The GC-MS analysis also showed that AMFH and its fractions contain four main bioactives compound i.e. Terpinen-4-ol (T-4-ol), α-Terpineol (α-T), Safrole (Saf), and Myristicin (Myr) with the percentage area of 11.20%; 1.83%; 5.10%; and 27.80% respectively. The GC-MS analysis also revealed that the main compound of the AMFH and its fractions is Myristicin, where AMFH1 fraction contain the highest Myristicin compound with the percentage area of 52.69% followed by AMFH; AMFH2; AMFH3; and AMFH4 respectively. The percentage area of Terpinen-4-ol (T-4-ol), α-Terpineol (α-T), Safrole (Saf), and Myristicin (Myr) in AMFH and its fractions by GC-MS analysis (Figure 3).

Myristicin was reported to play a role in the antioxidant and antimicrobial activity of *Myristica fragrans* Houtt (Gupta *et al*, 2013). Morita *et al*, (2003) also reported that Myristicin possesses extraordinarily potent hepatoprotective activity. They are also able to suppress the superoxide production by agonist-stimulated monocytes but not neutrophils (Touyz, 2000). α-Terpineol also exerts an anti-proliferative effect. Therefore, it can be used in the prevention or even treatment of cancer. Besides that, α-Terpineol also exerted cytostatic activities against six human cancer cell lines, such as prostate, breast, lung, leukemia and ovarian, especially against breast adenocarcinoma (MCF-7) and chronic myeloid leukemia (K-562). Both α-Terpineol and Terpinen-4-ol are the most important commercial products and they occur in a large number of essential oils (Bauer & Garbe, 2001; Bauer & Garbe, 1985).

Interestingly, Safrole is present at AMFH and fractions of AMFH1 with the percentage of relative area are 5.1% and 7.9% respectively, but not detected in fractionsA MFH2, AMFH3, and AMFH4. Safrole is a Phenylpropanoid have a potent cytotoxic activity against two breast cancer cell lines (MCF-7 and MDA-MB231 cells lines). Safrole has been found to inhibit angiogenesis and to arrest the growth and induce death of human tumor cells *in vitro* (Du *et al*, 2005). Based on the previous studies, some of the constituents revealed by GC–MS are biologically active compounds. They were proven to possess pharmacologic activities which may contribute to the healing potential of the plant. α-Terpineol, Terpinen-4-ol, and Safrole were proven to exhibit antioxidant and antinociceptive effects (Eid & Hawash, 2021; Bauer & Garbe, 2001).

Antioxidant Activity of the AMFH and Its Fractions

To examine the antioxidant activity of the four fractions arising from the separation of the active components using a gravity chromatography column, the antioxidant activity of the arillus essential oil sub-fraction was determined. The antioxidant activity was measured, and Ascorbic acid or Vitamin C was used as a positive control. Determination of antioxidant activity was carried out by DPPH free radical reduction method (1,1-Diphenyl-2-picrylhydrazyl) using a UV-Vis spectrophotometer. This method was elected because the process is faster, easier, cheaper, sensitive, and the method is reproducible. Determination of the antioxidant activity of Ascorbic acid as a positive control and AMFH and its fractions begins with determining the maximum wavelength of DPPH. The maximum wavelength is determined by drawing a curve

depicting the relationship between the solution's absorbance and the wavelength. The maximum wavelength of DPPH employed in this assay was 517 nm. Antioxidant activity against DPPH radical scavenging occurs due to its ability to donate hydrogen atoms. Free radical scavenging activity is strongly influenced by the Hydroxyl group of a test sample (Amarowicz *et al*, 2004). The antioxidant activity of AMFH and its fractions by using the DPPH assay method (Table IIA). The results showed that AMFH3 fraction had the highest antioxidant activity, with the IC_{50} value of 59.32 ppm. The AMFH2 and AMFH4 fractions, on the other hand, produced the lowest antioxidant activity, with IC_{50} values of 730.26 ppm and 402.025 ppm, respectively. Meanwhile, the AMFH1 fraction produced moderate antioxidant activity with an IC_{50} value of 101.23 ppm. Ginting *et al*, (2021) stated that the *n*-Hexane extract of *M. fragrans* Houtt root exhibited an IC_{50} value of 99.76 ppm when tested using the DPPH method.

Re-isolation of the AMFH3 Fraction

The AMFH3 fraction was re-isolated by column chromatography using *n*-Hexane and Ethyl acetate with a ratio of 9.5:0.5 as a mobile phase. Based on the spot pattern and Rf value, AMFH3 fraction produce four sub-fractions i.e. AMFH3A; AMFH3B; AMFH3C; and AMFH3D.

The AMFH3A sub-fraction produced the highest fraction by weight of 34.41 mg, followed by sub-fraction of AMFH3B; AMFH3D; and AMFH3C (Table IIB). The AMFH3A sub-fraction with the highest by weight produced a fine-yellow sub-fraction with the Rf value of 0.16.

The Antioxidant Activity of AMFH3 and its Sub-fractions

In this assay, we also determined the antioxidant activity of the sub-fraction using DPPH assay method. The antioxidant activity of the sub-fractions (Table II) with the Ascorbic acid or Vitamin C as a positive control. The results show that sub-fraction AMFH3A produced the highest antioxidant activity with the IC_{50} value was 98.9 ppm when tested using the DPPH method. Meanwhile the sub fractions of AMFH3B to AMFH3D exhibited conformable IC_{50} value which range between 242 to 246 ppm. For these sub-fractions, we also determined the antimicrobial activity against *S. aureus* and *S. epidermidis.*

Antibacterial Activity of the AMFH3 and its Sub-fractions

The antimicrobial activity of the AMFH3 sub-fractions were performed against *S.aureus* and *S. epidermidis*. The Kirby Bauer-Disc diffusion method was used to determine the antibacterial activity of the AMFH3 sub-fractions. The diameter inhibition zone of the AMFH3 sub-fractions against *S. aureus* and *S. epidermidis* (Figure 4).

In this assay, we used Vancomycin (30 µg) and Amoxicillin (25 µg) as positive control for *S. aureus* and *S. epidermidis* respectively. The positive control of Vancomycin and Amoxicillin showed inhibited activity against *S. aureus* and *S. epidermidis* with diameter inhibitions of 16.73 mm and 15.80 mm respectively. In this assay Dimethyl sulfoxide (DMSO) was used as a negative control. As a solvent, DMSO proved to be safe, which offered no interference with the results obtained in the experiment. The results also showed that AMFH3A sub-fraction showed slightly higher active against *S. epidermidis* than *S. aureus* with the diameter inhibition of 14.28 mm and 12.26 mm respectively. Meanwhile, the sub-fractions of AMFH3B; AMFH3C; and AMFH3D showed poor activity against both that Gram-positive bacterium.

GC-MS Analysis of AMFH3A Subfractions

To determine which compounds played a role in the antioxidant and antibacterial activity, the AMFH3A sub-fraction was analyzed for the chemical components using GC-MS spectroscopy. The GC-MS analysis showed that the AMFH3A sub-fraction contain 31 compounds among others are Terpinen-4-ol (T-4-ol), α-Terpineol (α-T), Safrole (Saf), and Myristicin (Myr) (Figure 5). The GC-MS analysis also showed that the main component of the MFH3A sub-fraction was Myristicin (peak 25), followed by Terpinen-4-ol (peak 6); Safrole (peak 13); and α-Terpineol (peak 8) with the percentage area of 54.77%; 14.37%; 7.36%; and 2.16% respectively.

Myristicin belongs to the family of the Allylbenzenes, also known as Alkenylbenzenes or Phenylpropenes. In baverage such as cola drinks, Myristicin is used as a flavoring agent, and in complementary medicine, Myristicin is used to treat some healths such as anxiety, rheumatism, stomach cramps, and nausea. Nutmeg essential oil has been reported to contain about 4% Myristicin, and some researchers reported about 4%-12%, it varies depending on geographical origin (Martin *et al*., 2018; Randerath *et al*, 1993).

Table II. Percent inhibition and antioxidant activity of the fractions and sub-fractions using the DPPH assay.

A.Percent inhibition and antioxidant activity of AMFH and its fractions

Concentration	**Inhibition Concentration (%)**					
(ppm)	**Ascorbic Acid**	**AMFH**	**AMFH1**	**AMFH2**	**AMFH3**	**AMFH4**
25	30.3±1.2	4.6±0.9	21.5±0.9	4.4±3.5	26.4±2.3	7.4±2.3
50	68.9±2.1	4.9±1.1	27.1±0.8	5.7±2.4	46.1±0.9	10.8±0.8
100	96.3±2.3	9.6±1.5	48.8±1.1	8.5±3.1	78.8±1.1	13.5±0.5
IC_{50} (ppm)	**3.9±1.9**	**605.2±1.2**	**101.2±1.0**	**730.3±3.0**	**59.3±1.1**	**402.0±1.2**

B.Percent inhibition and antioxidant activity of fraction of AMFH3 and its sub-fractions

Concentration	**Inhibition Concentration (%)**					
(ppm)	**Ascorbic Acid**	**AMFH3**	**AMFH3A**	**AMFH3B**	**AMFH3C**	**AMFH3D**
25	76.5±1.1	26.4±1.3	55.0±0.9	68.5±2.5	65.6±0.9	76.2±0.3
50	88.5±1.3	46.1±1.5	60.0±1.2	68.6±2.5	66.8±0.6	77.2±1.1
100	94.3±1.2	78.8±1.7	60.0±1.3	69.0±1.6	69.1±0.7	78.4±0.9
IC_{50} (ppm)	**1.2±1.2**	**59.3±1.5**	**98.9±1.2**	**242.6±2.2**	**246.7±0.8**	**246.4±0.9**

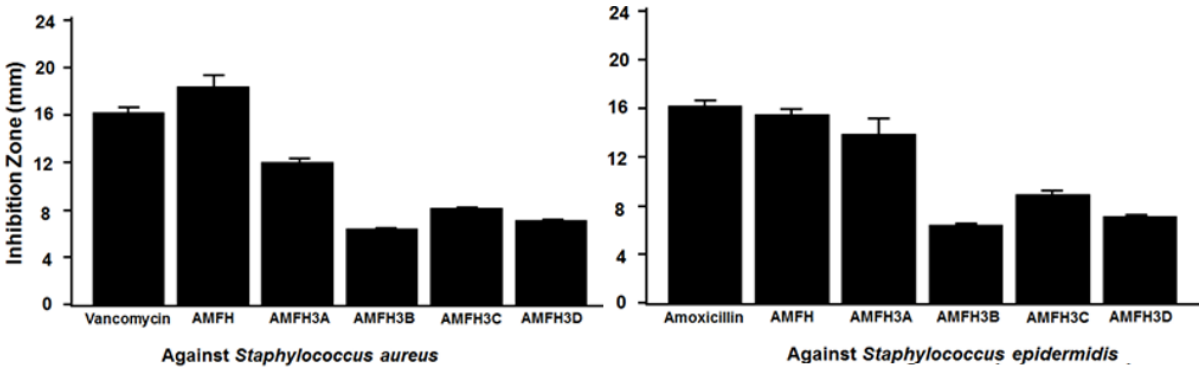

Figure 4. Antibacterial activity of AMFH, and its subfractions against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Vancomycin and amoxicillin were used as positive controls, and dimethyl sulfoxide (DMSO) 2.5% as the negative control.

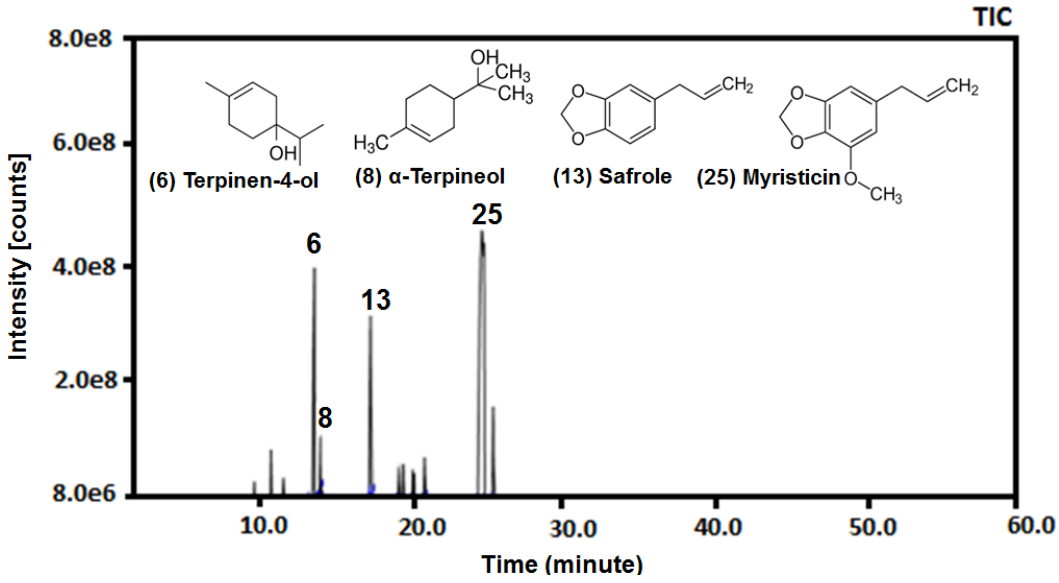

Figure 5. The GC-MS spectrum of MFH3A sub-fraction

Nutmeg essential oil origin from Nigeria contains about 27.43% Myristicin (Okiki *et al*, 2023), Grenada, Spain contain about 1.88% Myristicin (Rokas *et al*, 2021), Brazil contain about 0.76% Myristicin (Luciana *et al*, 2021), Kalimantan contain about 18.44% Myristicin (Guntur *et al*, 2019), Maluku contain about 13.95% Myristicin (Yuniasih *et al*, 2023), and Sulawesi contain about 13.73% Myristicin (Syarifah et al, 2017). Meanwhile, our research reported that Nutmeg essential oil origin from Blang Pidie, Aceh contain about 54.77% of Myristicin.

Myristicin and Terpinen-4-ol were known play a role in the antioxidant and antimicrobial activity of *Myristica fragrans*. Safrole is known to have activity as an antioxidant, anticancer and antitumor (Du *et al*, 2005; Eid & Hawash, 2021; Bauer & Garbe, 2001). The α-Terpineol (α-T) compound is known to show an antioxidant activity, and is able to give an anti-proliferative effect. Therefore, α-Terpineol (α-T) can be used in the prevention or even treatment of cancer (Touyz, 2000; Bauer & Garbe, 2001; Held *et al*, 2007).

Based on the antibacterial and antioxidant activity aboved, it was shown that the AMFH3A sub-fraction produced a highest activity compared to the AMFH3B; AMFH3C; and AMFH3D sub-fractions. Based on this finding, the compounds of the Myristicin, Terpinen-4-ol, Safrole, and α-Terpineol from the fractions and the sub-fractions of *Myristica fragrans* were play in important role in the antioxidant and antibacterial activity.

CONCLUSION

The DPPH assay showed that AMFH has the highest antioxidant activity with the IC_{50} values of 216.69 ppm. The column chromatography analysis showed that AMFH obtained four fractions namely AMFH1; AMFH2; AMFH3, and AMFH4. The GC-MS analysis showed that Myristicin, Terpinen-4-ol, dan α-Terpineol were present in AMFH and its fractions. The DPPH assay showed that AMFH3 fraction showed excellent antioxidant activity with the IC_{50} values of 59.32 ppm. However, this activity is still poor compared with the Ascorbic acid (a positive control) with the IC_{50} values of 3.86 ppm. The AMFH3 fraction was isolated, and four sub-fractions were obtained, i.e. AMFH3A; AMFH3B; AMFH3C; and AMFH3D. The results of the antioxidant activity test using the DPPH assay showed that the AMFH3A sub-fraction produced the highest antioxidant activity with an IC_{50} value of 98.99 ± 0.98 ppm.

The antibacterial activity showed that the AMFH3A sub-fraction showed slightly higher activity against *Staphylococcus epidermidis* compared to *Staphylococcus aureus* with the inhibition diameter of 14.28 mm and 12.26 mm respectively. GC-MS analysis was performed on the AMFH3A sub-fraction, and the results showed that the AMFH3A sub-fraction contains Myristicin followed by Terpinen-4-ol, Safrole, and α-Terpineol with the percentage area of 54.77%; 14.37%; 7.36%; and 2.16% respectively.

AUTHOR CONTRIBUTION

Khairan Khairan designing experiments, assisting in experiment, visualizing data, compiling, editing and finishing paper. Syaifullah Muhammad designing experiments, visualizing data and compiling paper. Binawati Ginting designing and assiting experiments, and visualizing data. All authors have read and approved the final manuscript.

ACKNOWLEDGMENTS

The authors would like to thank the Universitas Syiah Kuala for the supporting the research. The author also would like to thank The Ministry of Technology Research and High Education of Indonesia (RISTEKBRIN) for research funding through Prioritas Riset Nasional Grant (PRN BOPTN) in 2021.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Al-Malahmeh AJ, Al-Ajlouni A, Wesseling S, Soffers AE, Al-Subeihi A, Kiwamoto R, Vervoort J, Rietjens IM. Physiologically based kinetic modeling of the bioactivation of myristicin. Arch Toxicol. 2017 Feb;91(2):713-734. doi: 10.1007/s00204-016-1752-5. Epub 2016 Jun 22. PMID: 27334372; PMCID: PMC5306082.

Amarowicz, R. B., Pegg, P., Rahimi-Moghaddam, B. B., & Weil, J.A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem*. 84, 551-562. https://doi.org/10.1016/S0308814603)00278-4.

Barceloux, D.G., (2008). Medical Toxicology of Natural Substances: Foods, Fungi, Medicinal Herbs, Toxic Plants, and Venomous Animals.

John Wiley & Sons Inc, New, York, USA, pp. 373–379.

Bauer, K., & Garbe, D. (1985). *Common Fragrance and Odor Materials*. VCH Verlagsgesellschaft: Weinheim

Bauer, K., Garbe, D., & Surburg, H. (2001). Common *Fragrance and Flavor materials: Preparations, properties, and uses*, 4th ed., Wiley, New York, 2001.

Cinthura, C., Gayathri, R., Priya, V., (2017). Genotoxicity analysis of mace (Myristica Fragrans) on oral cancer cell line by DNA fragmentation. J. Pharmaceut. Sci. Res. 9, 205.

Daniela Lanari, Claudia Zadra, Francesca Negro, Rima Njem, Maria Carla Marcotullio. (2022) Influence of choline chloride-based NADES on the composition of *Myristica fragrans* Houtt. essential oil, Heliyon 8, e09531.

Du, A.Y., Zhao, B., Yin, D., Zhang, S. & Miao, J. (2005). Discovery of a novel small molecule, 1-ethoxy-3-(3,4-methylenedioxyphenyl)-2propanol,that induces apoptosis in A549 human lung cancer cells. *Bioorg. Med. Chem*., 13, 4176-4183.

Eid, A.A., & Hawash, M. (2021). Biological evaluation of Safrole oil and Safrole oil Nanoemulgel as antioxidant, antidiabetic, antibacterial, antifungal and anticancer *BMC Complementary Medicine and Therapies*, 21:159. https://doi.org/10.1186/s12906-021 03324-z

Falleh, H., Jemaa, M. B., Saada, M., & Ksouri, R. (2020). Essential oils: A promising eco-friendly food preservative. Food Chemistry, 330,Article127268.10.1016/j.foodchem.2020.127268.

Ginting, B., Maira, R., Mustanir, Helmi, H., Desiyana, L. S., & Mujahid, R. (2018). Isolation of Essensial Oil Of Nutmeg (*Myristica fragrans* Houtt) and Antioxidant Activity Test With DPPH. *Jurnal Natural,* vol. 18 (1).DOI 10.24815/jn.v18i1.6604.

Ginting, B., Mustanir, Nurdin, Maulidna, Murniati, & Safrina. (2021). Evaluation of Antioxidant and Anticancer Activity of Myristica fragrans Houtt. Bark. *Pharmacogn J*, 13 (3): 780-786.DOI:10.5530/pj.2021.13.99.

Gottardi, D., Bukvicki, D., Prasad, S., Tyagi, A.K., (2016). Beneficial effects of spices in food preservation and safety. Front. Microbiol. 7, 1394.https://doi.org/10.3389/fmicb.2016.01394.

Guntur, Harlia, Ajuk Sapar. (2019). Identifikasi Komponen Minyak Atsiri Daging Buah Pala (Myristica Fraghans Houtt.) Asal Pulau Lemukutan Kalimantan Barat Dan Uji Aktivitas Antiinflamasi Dengan Metode Stabilisasi Membran Rbcs, Al-Kimia, Volume 7 Nomor 2.

Gupta, A.D., Vipin, K.B., Vikash, B., & Nishi, M. (2013). Chemistry, antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans* Houtt), *Journal of Genetic Engineering and Biotechnology*, 11, 25–31.https://doi.org/10.1016/j.jgeb.2012.12.001

Held S., Schieberle P., & Somoza V. (2007). Characterization of alpha-terpineol as an anti-inflammatory component of orange juice by in vitro studies using oral buccal cells, *J. Agric. Food Chem*., 55(20), 8040-8046.https://doi.org/10.1021/jf071691m.

Helen M. P. A., Vargheese, T. A., Kumari, J. J. J., Abiramy, M. R., Sajina, N., & Jaya, S. S. (2012). Phytochemical Analysis and Anticancer Activity of Essential Oil from *Myristica fragrans*. *International Journal of Current Pharmaceutical Review and Research,* 2(4), 188-198. https://doi.org/10.1016/j.bmc.2005.04.031.

Horison, R., Sulaiman, F.O., Alfredo, D. and Wardana, A.A., (2019), Physical characteristics of nanoemulsion from chitosan/nutmeg seed oil and evaluation of its coating against microbial growth on strawberry, Food Research, 1-7

Ju, J., Guo, Y., Cheng, Y., & Yaoc, W. (2022). Analysis of the synergistic antifungal mechanism of small molecular combinations of essential oils at the molecular level. Industrial Crops and Products, 188, Article 115612. 10.1016/j.indcrop.2022.115612.

Khairan, K., Hasan, M., Idroes, R., & Diah, M. (2023). Fabrication and Evaluation of Polyvinyl Alcohol/Corn Starch/Patchouli Oil Hydrogel Films Loaded with Silver Nanoparticles Biosynthesized in *Pogostemon cablin* Benth Leaves' Extract. *Molecule MDPI*, 28, 2020. https://doi.org/10.3390/molecules28052020

Kumari I, H. Kaurav, G. Chaudhary, (2021). Myristica fragrans (Jaiphal): a significant medicinal herbal plant, Int. J. Res. App. Sci.

Biotechnol. 8 (2), 213–224, doi:10.31033/ijrasb.8.2.27.

Kunnumakkara, A.B., Sailo, B.L., Banik, K., Harsha, C., Prasad, S., Gupta, S.C., Aggarwal, B.B., (2018). Chronic diseases, inflammation, and spices: how are they linked? J. Transl. Med. 16, 1–25. https://doi.org/10.1186/s12967-018-1381-2.

Luciana F. Cossetin, Eduarda M.T. Santi, Quelen I. Garlet, Antˆonio F.I.M. Matos, Tiago P. De Souza b, Luiza Loebens, Berta M. Heinzmann, Sílvia G. Monteiro, (2021). Comparing the efficacy of nutmeg essential oil and a chemical pesticide against *Musca domestica* and *Chrysomya albiceps* for selecting a new insecticide agent against synantropic vectors, Experimental Parasitology 225, 108104.

Manh, T.H., Ngoc Khanh, V., Thu Huong, T., Jeong , AK., Mi, H.W., & Byung, S.M. (2020). Phytochemical and pharmacological properties of *Myristica fragrans* Houtt.: an updated review. *Archives of Pharmacal Research* volume 43, pages1067–1092. https://doi.org/10.1007/s12272-020-01285-4.

Martins, C, J. Rueff, A.S. Rodrigues. (2018), Myristicin from: Handbook of Foodborne Diseases CRC Press Accessed on: 19 Sep 2023https://www.routledgehandbooks.com/doi/10.1201/b22030-103.

Matulyte I, A. Jekabsone, L. Jankauskaite, P. Zavistanaviciute, V. Sakiene, E. Bartkiene, M. Ruzauskas, D.M. Kopustinskiene, A. Santini, J. Bernatoniene, (2020). The Essential Oil and Hydrolats from Myristica fragrans Seeds with Magnesium Aluminometasilicate as Excipient: Antioxidant, Antibacterial, and Anti-inflammatory Activity, Foods 9 (1), https://doi.org/10.3390/ foods9010037.

Morita, T., Keiko, J., Hirokazu, K., Yasushi, A., Hiroyuki, S., Takahiro, I., & Kimio, S. (2003). Hepatoprotective effect of myristicin from nutmeg (Myristica fragrans) on lipopolysaccharide/d-galactosamine-induced liver injury. *J Agric Food Chem*. 51(6):1560-5.doi: 10.1021/jf020946n.

Neetu Singh, Surender Singh Yadav, (2022). Ethnomedicinal uses of Indian spices used for cancer treatment: A treatise on structure-activity relationship and signaling pathways, Current Research in Food Science 5. 1845–1872.

Perez-Ortiz, J.M., Galan-Moya, E.M., de la Cruz-Morcillo, M.A., Rodriguez, J.F., Gracia, I., Garcia, M.T., Redondo-Calvo, F.J., (2020). Cost effective use of a thiosulfinate-enriched Allium sativum extract in combination with chemotherapy in colon cancer. Int. J. Math. Stat.21,2766.https://doi.org/10.3390/ijms21082766.

Pius A. Okiki, Chizobam P. Nwobi, Oghenerobor B. Akpor, Ezekiel Adewole, Richard D. Agbana, (2023). Assessment of nutritional and medicinal properties of nutmeg, Scientific African 19, e01548.

Randerath K, Putman KL, Randerath E. (1993). Flavor constituents in cola drinks induce hepatic DNA adducts in adult and fetal mice. Biochem Biophys Res Commun 1993;192(1):61–8.

Rokas Mickus, Gintar Janˇciuk˙, Vytautas Raˇskeviˇcius, Valeryia Mikalayeva, Inga Matulyt, Mindaugas Marksa, Kęstutis Maci¯unas, Jurga Bernatonien, Vytenis Arvydas Skeberdis, (2021). The effect of nutmeg essential oil constituents on Novikoff hepatoma cell viability and communication through Cx43 gap junctions. Biomedicine & Pharmacotherapy, 135, 111229.

Sarifah Nurjanah, Indira Lanti Putri, and Dwi Pretti Sugiarti, (2017), "Antibacterial Activity of Nutmeg Oil" in 2nd International Conference on Sustainable Agriculture and Food Security: A Comprehensive Approach, KnE Life Sciences, pages 563–569. DOI 10.18502/kls.v2i6.1076

Sathya, S, N.R. Amarasinghe, L. Jayasinghe, H. Araya, Y. Fujimoto, (2020). Enzyme inhibitors from the aril of Myristica fragrans, South African Journal of Botany, 130, 172-176.

Suthagar, P.P., Roziahanim, M., Amin, M.S., Abdul Majid, Zeyad Daoud, M.N. (2012a). Antioxidant and antiangiogenic activities of the essential oils of *Myristica fragrans* and *Morinda citrifolia*, *Asian Pacific Journal of Tropical Medicine*, 5, 4, 294-298. https://doi.org/10.1016/S1995-7645(12)60042-X.

Suthagar, P.P., Roziahanim, M., Wei, C.L., & Shanmugapriya, P. (2012b). Anti-parasitic Activity of *Myristica fragrans* Houtt. Essential Oil against *Toxoplasma gondii* Parasite, *APCBEE Procedia,* 2, 92-96.

https://doi.org/10.1016/j.apcbee.2012.06.017.

TétédéRodrigue Christian Konfo, Fowe Michelle Carole Djouhou, Yaya Alain Koudoro, Edwige Dahouenon-Ahoussi, Félicien Avlessi, Codjo Koko Dominique Sohounhloue, Jesus Simal-Gandara, (2023). Essential oils as natural antioxidants for the control of food preservation, Food Chemistry Advances 2 (2023) 100312.

Thuong, P.T., Hung, T.M., Khoi, N.M., Nhung, H.T.M., Chinh, N.T., Quy, N.T. (2014). Cytotoxic and anti-tumor activities of lignans from the seeds of Vietnamese nutmeg *Myristica fragrans*. *Arch Pharmacal Res*. 37(3):399-403. doi: 10.1007/s12272-013-0185-4.

Touyz R.M. (2000). Oxidative stress and vascular damage in hypertension, *Curr. Hypertens. Rep*., 2000, 2(1), 98-105. DOI: 10.1007/s11906-000-0066-3.

Wahidah H. Al-Qahtani, Yuvaraj Dinakarkumar, Selvaraj Arokiyaraj, Vigneshwar Saravanakumar, Jothi Ramalingam Rajabathar, Kowsalya Arjun, P.K. Gayathri, Jimmy Nelson Appaturi, (2022). Phyto-chemical and biological activity of Myristica fragrans, an ayurvedic medicinal plant in Southern India and its ingredient analysis, Saudi Journal of Biological Sciences 29, 3815–3821.

Yuniasih Mulyani Jubeliene Taihuttu; Rachmawati Dwi Agustin, (2023). Antioxidant and antibacterial activity of nutmeg oil (Myristica fragrans Houtt) from Ambon Moluccas, AIP Conference Proceedings 2588, 020012, https://doi.org/10.1063/5.0113251.

Yuniasih, M., Jubeliene, T., & Rachmawati, D. A. (2021). Antioxidant and antibacterial activity of nutmeg oil (Myristica fragrans Houtt) from Ambon Moluccas, *The 7th International Conference on Basic Sciences* 2021 (ICBS 2021). *AIP Conf. Proc*. 2588, 020012-1–020012-4; https://doi.org/10.1063/5.0113251.

Zhipeng Cao, Wei Xia, Xiaoyu Zhang, Huiya Yuan, Dawei Guan, (2020). Lina Gao. Hepatotoxicity of nutmeg: A pilot study based on metabolomics, Biomedicine & Pharmacotherapy, 131, 110780.