PHYTOCHEMICAL PROFILES, ANTIOXIDANT, AND ANTICANCER ACTIVITIES FROM LEAVES AND SEEDS EXTRACT OF *Myristica fragrans*

Ariyanti Saputri[1], Sofa Fajriah[1*], Antonius Herry Cahyana[2*], Devi Kasih Putria[3], Ziyan Saputra[1,4], Marissa Angelina[1]

[1]Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency Republic of Indonesia, Jl Raya Serpong Puspiptek, South Tangerang 15314, Banten, Indonesia

[2]Department of Chemistry, Faculty of Science of Mathematics, University of Indonesia, Jl Lingkar Pondok Cina, Depok 16424, West Java, Indonesia

[3]Department of Chemistry, Faculty of Science of Mathematics, Singaperbangsa Karawang University, Jl. HS. Ronggo Waluyo, Karawang 41363, West Java, Indonesia

[4]Department of Chemistry Education, Faculty of Mathematics and Natural Sciences Education, Indonesian University of Education, Jl. Setia Budi No. 229, Bandung 40154, West Java, Indonesia

[*]Email: herrykim@ui.ac.id and sofa001@brin.go.id

Received 01 October 2024
Accepted 26 November 2024

Abstract

Nutmeg (*Myristica fragrans*) is a plant that grows naturally in Indonesia and widely used in the food and industrial sectors. Nutmeg seeds have been widely studied because they have a variety of compounds with good activities, but research on the leaves still needs to be completed. Therefore, this research compared nutmeg leaves and seed extracts' secondary metabolite content and bioactivity. This study aims to determine the phytochemical contents of nutmeg leaves and seed extracts by phytochemical screening and GC-MS analysis, as well as its potential as an antioxidant and anticancer. The antioxidant activity test uses the DPPH (1,1-diphenyl-2-picrylhydrazyl) method measured at a wavelength of 517 nm. Meanwhile, the anticancer activity test uses MCF-7 cells with the MTT assay method. Nine compounds (lipid, phenylpropanoid, lignan, terpenoid, and quinone groups) were identified by GC-MS analysis in the leaf extracts. Meanwhile, seed extract has 23 compounds from the lipid, fatty acid, phenylpropanoid, lignan, and terpenoid groups. The antioxidant activity of nutmeg leaves and seed extracts was carried out using the DPPH method, while the cytotoxic activity against MCF-7 cells used the MTT method. Nutmeg leaves have more potent antioxidant activity than nutmeg seeds, with IC_{50} values of 17.80 µg/mL and 53.01 µg/mL, respectively. The cytotoxic activity test on MCF-7 cells also showed that the inhibition value of nutmeg extract (26%) was higher than that of nutmeg seeds (22.73%). This research shows that nutmeg leaf extract has the potential to carry out further research regarding the bioactivity of its compounds.

***Keywords**: antioxidant, cytotoxicity, MCF-7 cells, nutmeg, phytochemical*

Introduction

Indonesia has abundant biodiversity; various types of plants are spread across Indonesia's land and waters. These plants have various benefits, including the community's use of traditional medicine. One of the widely used plants is the nutmeg (*Myristica fragrans*). This plant comes from the Myristicaceae family, comprising 15 genus and 250 species. This plant grows in the islands of Indonesia, including Maluku, North Sulawesi, West Sumatra, and West Java (Anggoro, 2023). This plant is used as a medicine for stomachaches, headaches, diarrhea, nausea, vomiting, fever, stimulating appetite, and treating flatulence (Kuete, 2017). Literature

studies show that the nutmeg plant contains secondary metabolites from the flavonoid, saponin, and alkaloid groups (Cao *et al*., 2013). The machilin D compound isolated from nutmeg seeds has significant activity in suppressing iNOS mRNA expression in LPS-stimulated RAW26.7 cells and inhibiting NO production and synthesis (Cao *et al*., 2015). n-hexane extract from the roots of the nutmeg plant also produces the compound 6'-methyl-(7–hydroxy-8-methylbut-9-en)-3,2'-dimethoxybiphenyl-4,5-diol.Bioactivity testing was carried out on this compound, and it produced moderate anti-cancer activity against MCF-7 cells. Meanwhile, testing of DPPH activity shows that this compound has good antioxidant activity with an IC_{50} value of 12.67 µg/mL (Ginting *et al*., 2020). This literature shows that the nutmeg plant has various secondary metabolites with various exciting activities, especially in the nutmeg seed. However, studies on the parts of nutmeg leaves are still very limited. Several studies have investigated nutmeg's potential as an antioxidant, with most focusing on its essential oil. This oil, primarily derived from nutmeg's seeds and mace, has been the subject of significant research (Weerakoon *et al*., 2021; Li *et al*., 2020; Piaru *et al*., 2011). However, the research on the potential of nutmeg leaf extract is still in its infancy. In particular, the potential of nutmeg leaf extract macerated with ethanol solvent against MCF-7 breast cancer cells is a promising area that warrants urgent attention. For this reason, this research will compare the activities of the leaves and nutmeg seeds.

Antioxidant testing can be carried out using several methods, including the DPPH, ABTS, and FRAP. DPPH testing is the method most widely used to determine the antioxidant activity of an extract or compound (Rohmah, 2022). From a comparative study of antioxidant activity test methods conducted by Maesaroh *et al*. (2018) it is known that the DPPH method is the most effective and efficient compared to the FRAP and FIC test methods for ascorbic acid, gallic acid and quercetin (Maesaroh *et al*., 2018). Thus, in this research, the process of testing the antioxidant activity of nutmeg leaves and seed extracts was carried out using the DPPH method. Meanwhile, the cytotoxic activity of leaves and seed extracts was determined on MCF-7 breast cancer cells using the MTT method.

The results of this research can provide information about the potential of nutmeg leaves and seeds and determine which parts have better activity so that further research can be carried out. The broader impact of this research will be to provide information on potential plants originating from Indonesia and develop research into natural-based medicinal raw materials.

Research Methods

The plant materials that will be examined in this research are samples of leaves and seeds from the nutmeg (*Myristica fragrans*) originating from Sukabumi, West Java. The staff of the Herbarium Bogoriensis, Bogor, Indonesia identified the species identity of the plant samples. The selected leaves and seed samples were cleaned of dirt, dried in the oven at 50 °C, and then crushed until smooth. Extraction was carried out using the maceration method and ethanol as a solvent. Usually, extraction is carried out three times, 24 hours a day, for each plant sample to achieve the maximum amount of extract. The combined ethanol extract is then dried by evaporation at low pressure (Ginting *et al*., 2017). The extract was used for phytochemical screening, identification using GC-MS analysis, antioxidant and cytotoxic activity test.

Materials

The materials used in this research included samples of nutmeg leaves and

seeds, solvents such as 96% ethanol (Fulltime, China), methanol (Fulltime, China), n-hexane (Fulltime, China), chloroform (Fulltime, China), ethyl acetate (Fulltime, China), concentrated HCl (Fulltime, China), H_2SO_4 (Merck, Germany), distilled water, Mg powder, and $FeCl_3$ (Merck, Germany). Apart from that, various phytochemical screening reagents are also used, namely Bouchardat's reagent, Mayer's reagent and Dragendorff's reagent. Materials used for antioxidant and cytotoxic activity tests include DPPH reagent (1,1-diphenyl-2-picrylhydrazyl) (Merck, Germany), MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Merck, Germany), FBS (Fetal Bovine Serum) (Corning, U.S), MCF-7 cell culture, DMSO (Dimethyl Sulfoxide) (Merck, Germany), DMEM (Dulbecco's Modified Eagle Medium) (Merck, Germany) and PBS (Phosphoric Buffer Solution) media (Merck, Germany).

Instrumentation

The GC-MS analysis of the nutmeg extract was performed using the Agilent 7890B series coupled with a mass spectrophotometer detector of 5977A equipped with an Agilent DB-5MS Ultra Inert capillary column. Meanwhile, the antioxidant test was immediately measured using a UV-Vis spectrometer (Agilent, Cary 60 UV-Vis) at a wavelength of 517 nm.

Procedure

1) Phytochemical Screening

Phytochemical screening was carried out to determine the secondary metabolite content contained in nutmeg extract (Fajriah and Megawati, 2015). The formation of a specific precipitate or colour change based on the test mark positive results from each test. Observation data is assessed with a value of (+1) if the test result is positive and (+2) if the test result is faster and more concentrated reaction than other tests. Meanwhile, the sign (-) is if there are no positive results from the test sample.

Alkaloid test, each sample was dissolved in 9 mL of water and 1 mL of 2 N HCl then heated for 2 minutes in a water bath. Next, the solution was cooled and filtered to obtain the filtrate of each sample. The filtrate was tested on three reagents: Bouchardat's reagent, Meyer's reagent, and Dragendorff's reagent. Positive results from the three reagents contained a brown precipitate, a white or pale yellow precipitate and a brownish orange precipitate. Flavonoid test, sample was dissolved in 4 mL of ethanol then 0.5 grams of Mg powder and 2 mL of 2 N HCl were added. The mixture was left for 1 minute, and ten drops of concentrated HCl were added. The colour change to bright red after being left for a few minutes indicates the presence of flavonoids.

Saponin test, sample was put into a test tube containing 4 mL of hot water, then cooled and shaken quickly. After 10 minutes, the solution was observed, and one drop of 2 N HCl was added. Stable foam of more than 1 cm indicated that the sample was positive for saponin. Tannin test, sample was added to 15 mL of hot water and boiled on a hot plate for several minutes. After boiling, the mixture is cooled and filtered to obtain the filtrate. Next, three drops of $FeCl_3$ were added to the filtrate. The result was positive if the colour changed to greenish purple. Terpenoid test, sample was dissolved in 2 mL of chloroform and ten drops of anhydrous acetic acid were added and stirred. Next, two drops of concentrated sulfuric acid are slowly added to the solution. Positive results occur when the solution changes colour to blue or green.

2) Extract Identification Using GC-MS Analysis

The GC-MS analysis of the nutmeg extract was performed using the Agilent 7890B series combined with a 5977A mass spectrophotometer detector and an Agilent DB-5MS Ultra Inert capillary column (30 mx 250 μm, id 0.25 μm, composed of (5%-phenyl)-methylpolysiloxane). Helium served as the gas carrier, flowing at a 1 mL/min rate. The extraction sample was introduced into the GC-MS system in splitless mode at the 250 °C GC inlet. The oven's initial temperature was established at 40 °C for 1 minute, afterwards escalating to 300 °C at a progression of 10 °C/min, and maintained at 300 ^{o}C for an additional 4 minutes. The temperatures of the transfer line, MS source, and MS quadrupole were respectively set at 270, 230, and 150 °C. An electron ionization system operating at 70 eV energy within a mass range of 30-600 AMU facilitated the detection of GC-MS. Identification of the chemical components was achieved by matching the test sample's mass spectral data against the NIST 17 library database (National Institute of Standards and Technology, Mass Spectra Libraries) (Okselni *et al*., 2024).

3) Antioxidant Activity Test

Antioxidant activity or the ability of a compound to capture free radicals was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) test (Nikolic *et al*., 2021). Four mg each of nutmeg leaves and nutmeg seed extract was dissolved in 4 mL methanol. Next, 750 μL of DPPH reagent was added to the tube, which had been dissolved in 0.1 mM methanol. The mixture was then shaken until homogeneous and left to stand for 30 minutes at room temperature in a dark room. This treatment was also carried out on the blank (methanol) and standard (quercetin) solution. After incubation, the absorbance of the solution was immediately measured using a UV-Vis spectrometer (Agilent, Cary 60 UV-Vis) at a wavelength of 517 nm. From the absorbance value, the inhibition percentage can be determined to determine the antioxidant activity of the test sample using Equation (1).

$$\text{Inhibition percentage (\%)} = \frac{\text{Radical solution absorbance} - \text{Sample absorbance}}{\text{Radical solution absorbance}} \times 100\% \quad (1)$$

4) Cytotoxic Activity Test

Cell culture was carried out using 10% DMEM (Dulbecco's Modified Eagle Medium)-FBS (Fetal Bovine Serum) culture medium and incubated at 37 ^{o}C with 5% CO_2. The samples were dissolved in DMSO (Dimethyl Sulfoxide) solvent with 200, 100, 50, and 25 μg/mL concentrations. Cytotoxic activity was carried out using the MTT test by Khoerunisah *et al*. (2022) with slight modifications. MCF-7 cells were seeded in 96 well plates at 1.5×10^4 cells/well and incubated for 24 hours (Khoerunisah *et al*., 2022). Then, the culture medium was discarded, replaced with sample solutions of various concentrations, and incubated again for 48 hours. After 48 hours, the sample solution was discarded, replaced with 10% MTT, and then incubated for 2 hours. After 2 hours, the MTT solution was discarded and replaced by DMSO solvent to dissolve the formazan crystals. The condition

of the cells in the well plate can be observed in a microscope and measured using a microplate reader at 570 nm. Then, the average value was calculated from the absorbance data obtained to determine the % cell viability. The Equation (2) is the formula for calculating % cell viability. Then, for the % inhibition value of the test sample, the Equation (3) can be used.

$$\text{Cell Viability (\%)} = \frac{\text{Sample absorbance} - \text{Medium absorbance}}{\text{Control absorbance} - \text{Medium absorbance}} \times 100\% \quad (2)$$

$$\text{Inhibition (\%)} = \frac{\text{Control absorbance} - \text{Sampel absorbance}}{\text{Control absorbance}} \times 100\% \quad (3)$$

Results and Discussion

The dry powder of nutmeg leaves and seeds was extracted by maceration to produce ethanol extract of the leaves and seeds. The dry extract was obtained after the solvent was evaporated using a rotary evaporator and dried in an oven at 50 °C.

Phytochemical screening

Phytochemical screening of nutmeg leaves and seeds was carried out by conducting an alkaloid test using Dragendorff, Mayer and Burchardart reagents. Apart from that, tests were also carried out to determine flavonoids, tannins, terpenoids and saponins. The results of this test can be used to initially screen the secondary metabolite content of nutmeg leaves, seed, and fruit extracts. The phytochemical screening test (Table 1) showed that the seeds produced a positive reaction in the alkaloid test using Dragendorff and Burchardart reagents. Meanwhile, brown and brownish-orange deposits did not form on the leaves when tested with Dragendorff and Buchardart reagents. All extracts produced positive reactions in the flavonoid test, but the leaves had better results than the seeds and fruit. In addition, in the tannin test, only the nutmeg leaves produced a positive reaction, which was indicated by a greenish-purple colour change when $FeCl_3$ was dropped. The leaves and seed test samples produce a dark blue to green colour change in the terpenoid test, indicating terpenoids' presence. Meanwhile, only the seeds can form stable foam up to 1 cm high for the saponin test. The stable foam on the leaves is only 0.5 cm high; this indicates that the leaves do not contain saponin. Therefore, from the test results, the secondary metabolite content can be compared between the leaves and nutmeg seeds. The similarity in the test results of the leaves and seeds showed flavonoid and terpenoid contents in both parts. Meanwhile, several differences in test results include alkaloid content identified in the seeds and none in the leaves. Apart from that, the tannin content is only contained in the leaves and saponin in the seeds.

Chemical composition

The chemical composition of the extract obtained was then identified using GC-MS chromatography. The resulting data is a GC chromatogram, which is evaluated for peak area with a similarity index (SI) of ≥ 95%. The compound content contained in these extracts is presented in the graph below. In Figure 1. the secondary metabolite content of the nutmeg leaves is shown. These compounds come from various secondary metabolites, including fatty esters, phenylpropanoids, lignans, terpenoids and quinones. Meanwhile, in the nutmeg seed, there are groups of fatty esters and fatty acids, phenylpropanoids, lignans and terpenoids, which can be seen in Figure 2.

Table 1. Phytochemical screening test results of nutmeg leaves and seeds

Compound Group	Reactants	Observation	Leaves	Seeds
Alkaloids	Dragendorff	Orange-brown precipitate	-	+1
	Mayer	White-yellow precipitate	-	-
	Burchadart	Brown precipitate	-	+1
Flavonoids	HCl 2N + Mg powder	Red or red-orange	+2	+1
Tannins	Aquades + FeCl3 1%	Greenish purple	+1	-
Terpenoid	CH_3COOH dan H_2SO_4	Dark blue to green	+1	+1
Saponins	Heated, shaked + HCl 2 N	Stable foam	-	+2

The compounds contained in nutmeg leaves and seeds consist of several groups of secondary metabolites. The compound with the most significant content in both parts comes from the fatty ester group. A long hydrocarbon chain with an ester group characterizes the structure of fatty esters. Some compounds also have a double bond on one of the carbon atoms in the cis position because, naturally, the fat structure has a cis geometry due to enzymatic reactions (Chatgilialoglu *et al*., 2014).

The fatty ester group compounds with the most significant content in the leaves are heptadecanoic acid, methyl ester 12.68% (**1**); hexadecanoic acid, methyl ester 1.51% (**2**); 9-Octadecenoic acid (Z)-, methyl ester 0.58% (**3**) and methyl stearate 0.38% (**4**). Meanwhile, the seeds contain cis-13-octadecenoic acid, methyl ester 26% (**5**); (**1**) 10.41%; (**2**) 2.26%; methyl tetradecanoate 1.21% (**6**); (**4**) 0.75%; (E)-9-octadecenoic acid ethyl ester 0.66% (**7**); methyl 18-methylnonadecanoate 0.18% (**8**); 7-hexadecenoic acid, methyl ester, (Z)- 0.14% (**9**); docosanoic acid, methyl ester 0.1% (**10**) and hexadecanoic acid, ethyl ester 0.1% (**11**). Several fatty acid compounds were only identified in the seeds, namely 9-octadecenoic acid (E) 0.83% (**12**) and n-hexadecanoic acid 0.42% (**13**). The structure of fatty acids is similar to that of fatty esters; it is just that there are different functional groups in the two fats. Fatty acids have a -COOH functional group, while fatty esters have a -COOR functional group at the base of the carbon chain (Macabuhay *et al*., 2021).

These two parts of nutmeg also contain the second most prominent compound after fatty esters from the phenylpropanoid group. The structural characteristics of phenylpropanoids are characterized by a phenyl ring (C_6) bound to a propanoid chain (C_3). The phenylpropanoid group compounds identified from the GC-MS results of nutmeg leaves were 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl) 8.47% (**14**). Meanwhile, several compounds in nutmeg include (**14**) 13.8%; phenol 2,6-dimethoxy-4-(2-propenyl) 2.36% (**15**); phenol, 2-methoxy-4-(1-propenyl) 1.59% (**16**); benzene, 1,2,3-trimethoxy-5-(2-propenyl) 0.55% (**17**); eugenol 0.13% (**18**); benzene, 1,2-dimethoxy-4-propenyl-, (Z) 0.08% (**19**) and methyleugenol 0.04% (**20**).

Phenylpropanoids are also divided into subgroups, distinguished by their chemical structure and function. These subgroups include phenylic acid, coumarin, flavonoids, lignin and lignans. Flavonoids generally have a phenyl ring structure (C_6) linked to a popanoid group (C_3), which is linked back to a phenyl ring (C_6) (Rahim *et al*., 2023). Various literature also shows that flavonoids have good antioxidant activity (Hassanpour *et al*., 2022). The flavonoid group compound from nutmeg leaves, 3,5,7,4'-tetrahydroxy-dihydroflavonol, has good antioxidant activity with an IC_{50} value of 9.75 µg/mL (Ginting *et al*., 2016). In the

previous phytochemical test, flavonoid content was seen in the leaves and nutmeg; however, in the results of the GC-MS test, this flavonoid subgroup was not identified. This could be because the flavonoid group compounds do not evaporate quickly and are polar due to the large number of -OH groups, so more of them are retained in the column (Awouafack *et al*., 2017).

The following compound identified came from the lignan group. Lignans have a variety of promising bioactivities, such as antifungal, antidiabetic, hepatoprotective, and antiobesity (Weerakoon *et al*., 2021; Yakaiah *et al*., 2019). The lignan group compounds identified in the leaves were (S)-5-Allyl-1,3-dimethoxy-2-((1-(3,4,5-trimethoxyphenyl) propan-2-yl) oxy) benzene 2.22% (**21**) and (1S,2R)-2-(4-allyl-2,6-Dimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl) propan-1-ol-rel 0.94% (**22**). Meanwhile, nutmeg seeds contain licarin A 7.34% (**23**); [1,1'-Biphenyl]-2,2'-diol, 3,3'-dimethoxy-5,5'-di-2-propenyl 0.27% (**24**) and (1S,2R)-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)propan-1-ol-rel 0.06% (**22**).

The last secondary metabolite identified in the leaves and seeds of nutmeg comes from the terpenoid group. The GC-MS results identified diterpenoid compounds in nutmeg leaves, namely tocopherol 0.59% (**25**) and colavenol acetate 0.2% (**26**) in nutmeg seeds. Apart from the secondary metabolites previously described, nutmeg leaves also contain benzoquinone derivative compounds, namely 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione 0.38% (**27**). This compound was also identified in the essential oil from the bark of Cordia sebestena, which has higher antioxidant activity than the test standard (Adeosun *et al*., 2013). In the phytochemical test, there were positive results for alkaloids in the seeds, and when compared with the GC-MS results, the presence of alkaloid compounds was also confirmed. However, these compounds are not displayed on the GC-MS graph because the SI of the three compounds is below 95%.

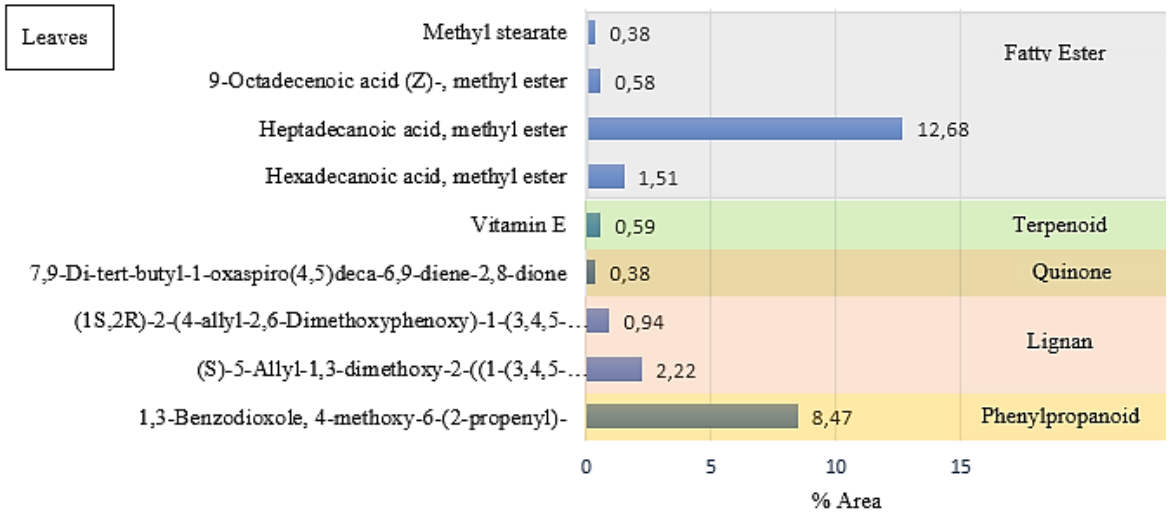


Figure 1. GC-MS analysis of chemical composition in nutmeg leaves

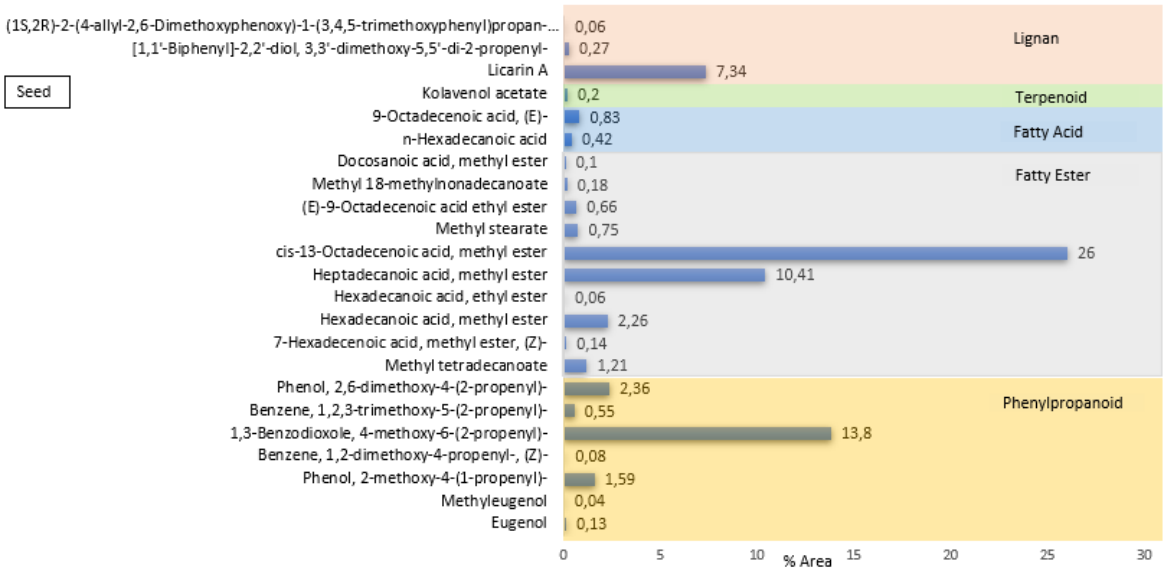


Figure 2. GC-MS analysis of chemical composition in nutmeg seeds

Table 2. Structure of compounds contained in nutmeg leaves

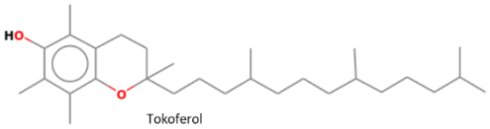
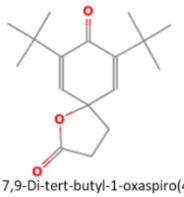
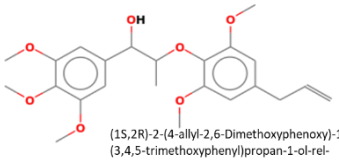
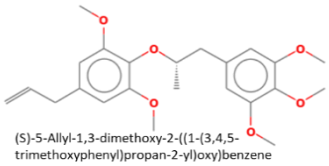
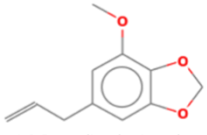
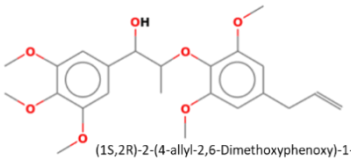
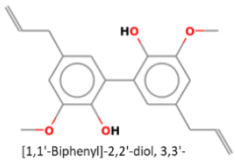
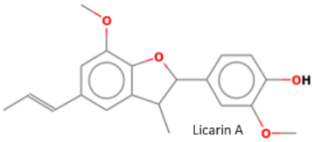
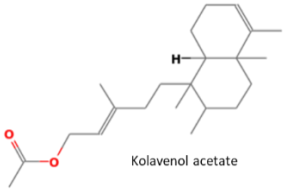
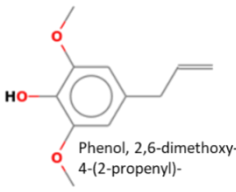
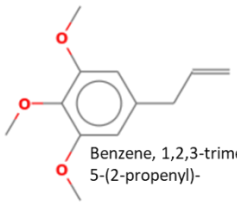
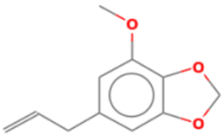
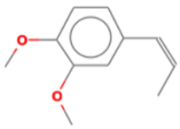
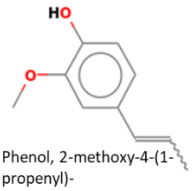
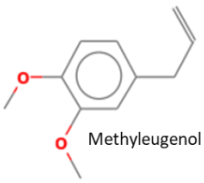
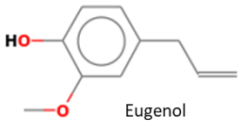
No	Group Compound	Structure
1	Terpenoid	 Tokoferol **(25)**
2	Quinone	 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione **(27)**
3	Lignan	 (1S,2R)-2-(4-allyl-2,6-Dimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)propan-1-ol-rel- **(22)**  (S)-5-Allyl-1,3-dimethoxy-2-((1-(3,4,5-trimethoxyphenyl)propan-2-yl)oxy)benzene **(21)**
4	Phenylpropanoid	 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)- **(14)**

Table 3. Structure of compounds contained in nutmeg seeds

No	Group Compound	Structure
1	Lignan	 (1S,2R)-2-(4-allyl-2,6-Dimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)propan-1-ol-rel- **(22)**  [1,1'-Biphenyl]-2,2'-diol, 3,3'-dimethoxy-5,5'-di-2-propenyl- **(24)**  Licarin A **(23)**
2	Terpenoid	 Kolavenol acetate **(26)**
3	Phenylpropanoid	 Phenol, 2,6-dimethoxy-4-(2-propenyl)- **(15)**  Benzene, 1,2,3-trimethoxy-5-(2-propenyl)- **(17)**  1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)- **(14)**  Benzene, 1,2-dimethoxy-4-propenyl-, (Z)- **(19)**  Phenol, 2-methoxy-4-(1-propenyl)- **(16)**  Methyleugenol **(20)**  Eugenol **(18)**

Antioxidant activity

The antioxidant activity of leaves and seed extracts was tested using the DPPH method. A compound has very strong antioxidant activity if the IC_{50} value is <50 µg/mL, strong (50–100 µg/mL), medium (100–150 µg/mL) and weak (151–200 µg/mL) (Panjaitan and Yuliana, 2022; Handayani *et al*., 2014). Leaves extracts have very strong antioxidant activity with an IC_{50} value of 17.80 µg/mL, while the leaves have strong antioxidant activity with an IC_{50} value of 53.01 µg/mL. From the test results, it can be seen that the leaves have better antioxidant activity than the seeds. The presence of flavonoids can also cause this antioxidant activity. Flavonoids can act as

antioxidants because the structure has many hydroxyl groups and conjugated double bonds to neutralize free radicals (Hassanpour *et al*., 2022). Previous phytochemical screening data shows that nutmeg leaves have a higher flavonoid content than the seeds. Therefore, the difference in IC_{50} values in these two parts can also be influenced by the number of flavonoids in each extract according to the results of the previous phytochemical screening.

In addition, the acetone extract of nutmeg leaves showed potential antioxidant activity with TPC values (895.12±44.24 mg GAE /g of leaves), FRAP (715.78±51.09 mg of Trolox /g of leaves), DPPH (65.56±0.93 mg of Ascorbic acid /g of leaves), ABTS (31.67±0.49 mg of Trolox equivalent/g of leaves) and Ferrous Ion chelating (10.87±1.85 mg of EDTA/g of leaves). These results also show that the acetone extract of nutmeg leaves has great potential to be developed further into a pure compound (Adibuduge and Senevirathne, 2023). In addition to acetone extract, methanol extract from nutmeg leaves was also reported to have an IC50 value of 36.31 µg/mL with an IC50 reference (vit. C) of 3.657 µg/mL using the DPPH method (Nurmilasari *et al*., 2017).

Table 4. Antioxidant activity of leaves extract, seeds extract and reference compound using the DPPH method (n=3)

Rep.	Seed		Leaves		Quercetin	
	Inhibition at conc. 100 µg/mL (%)	**IC50 (µg/mL)**	**Inhibition at conc. 25 µg/mL (%)**	**IC50 (µg/mL)**	**Inhibition at conc. 5 µg/mL (%)**	**IC50 (µg/mL)**
I	62.82	55.95	65.21	18.94	65.58	3.41
II	69.54	50.08	67.89	16.66	64.81	3.35
III	65.27	53.01	88.14	17.78	66.68	3.34
		53.01 ±2.93		17.8 ±1.14		3.36 ±0.04

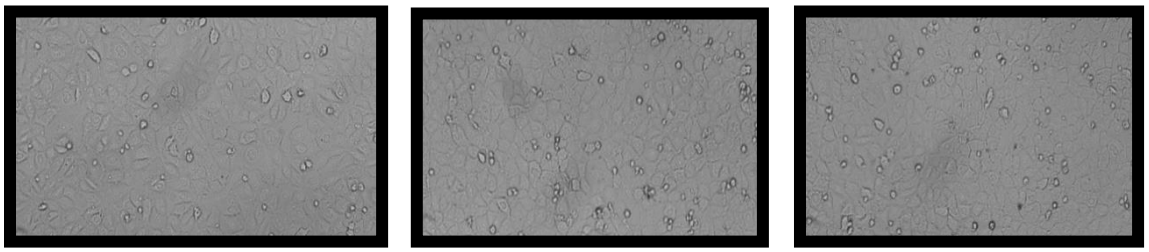
*Data are mean±SD, n=3. One-way ANOVA test showed significant differences between groups ($p < 0.05$). Tukey's post-hoc test identified that all pairwise comparisons showed significant differences ($p < 0.05$).

Cytotoxic activity

Evaluation of the inhibitory activity of nutmeg leaves and seed extracts was carried out on MCF-7 breast cancer cells with a sample concentration of 200 µg/mL using the MTT test method. This test aims to compare the effectiveness of nutmeg leaves and seed extracts in inhibiting the growth of MCF-7 cancer cells. This inhibition process occurs due to the cytotoxic properties of the extract, which can stop the development of MCF-7 cancer (Sirait *et al*., 2019). The measurement data can determine cell viability values and sample inhibition capabilities. Cell viability is the ability of cells to survive and continue to function normally, where the higher the cell viability value, the more cells will live under normal conditions. Meanwhile, inhibition refers to the sample's ability to inhibit cell growth, so the more significant the inhibition value, the better the sample's ability to kill living cells. In other words, % inhibition indicates how toxic a sample is to test cells (MCF-7) (Ghauri *et al*., 2021).

Table 5. Cytotoxic activity of nutmeg leaves and seed extracts (n=3)

Extract	Absorbance				Cell Viability (%)	Inhibition (%)
	I	II	III	Mean		
Leaves	0.507	0.565	0.56	0.544	67.7	26.85
Seeds	0.58	0.575	0.569	0.574	72.6	22.73
Control (-)	0.736	0.701	0.794	0.743	100	-



(a) (b) (c)

Figure 3. Morphology of MCF-7 cells in (a) control, (b) leaves extract, and (c) seed extract

The image above is of the morphology of MCF-7 cancer cells in the well-plate in the cytotoxic test of nutmeg leaves and seed extracts. Image (a) shows the cell condition without adding a sample, image (b) shows the cell condition with nutmeg leaf extract and image (c) with nutmeg seed extract. Dark-colored spots characterize dead cancer cells, and the shape of the cells becomes irregular due to rupture, while grey spots with precise cell shapes indicate that cancer cells are alive.

In the negative control, almost all cells were seen to be alive, indicating that there was no inhibitory process on cell growth. This is because the medium in the negative control does not contain compounds that can kill cancer cells. Meanwhile, in the nutmeg leaves extract sample, a more significant number of dead cells were seen compared to the cells with the addition of nutmeg seed extract. This was also confirmed by the higher inhibition data from leaf extract compared to nutmeg seed extract (Angelina *et al*., 2024).

In this test, MCF-7 cell inhibition was 26.85% in the leaf extract and 22.73% in the seed extract. These results indicate that nutmeg leaves have better inhibitory activity than nutmeg seeds. This result also correlates with the antioxidant test results, where the leaves have better antioxidant activity. Research on the cytotoxic activity of nutmeg has been studied in its essential oil. Nutmeg essential oil has been reported to have cytotoxic activity against human colorectal cancer (HCT-116) and human breast cancer (MCF-7) cell lines with IC_{50} values of 78.61 and 66.45 µg/mL, respectively (Piaru *et al*., 2011).

Conclusions

Nutmeg leaves and seed extracts contain various compounds with similar secondary metabolite groups. However, nutmeg leaf extract has better antioxidant and cytotoxic activity than nutmeg seed extract. This is confirmed by the IC_{50} and % inhibition values for nutmeg leaves, which are better than those of nutmeg seeds. Apart from that, the flavonoid content is also thought to play a role in determining this activity. In the phytochemical screening test, nutmeg extract had a more responsive flavonoid formation reaction than nutmeg seed extract. In this research, it can be concluded that nutmeg leaf extract has more potential for advanced testing than nutmeg seed extract.

Acknowledgment

The authors would like to thank the DBR Program of the National Research and Innovation Agency (BRIN) for funding the research and providing guidance from staff and promoters at the Center for Research on Pharmaceutical Materials and Traditional Medicines, BRIN, and the University of Indonesia.

Conflict of Interest

The authors have no conflict of interest.

Author Contributions

AS: Investigation and Manuscript Drafting; SF: Data Curation and Manuscript Review; AHC: Conceptualization and Supervision.

References

Kuete, V., 2017. Chapter 23 - *Myristica fragrans*: A review, medicinal spices and vegetables from africa. *Medicinal Spices and Vegetables from Africa*, 497-512.

Cao, G. Y., Yang, X. W., Xu, W., Li, F., 2013. New inhibitors of nitric oxide production from the seeds of myristica fragrans. *Food and Chemical Toxicology*, 62, 167–171.

Cao, G. Y., Xu, W., Yang, X.W., Gonzales, F.J., Li, F., 2015. New neolignans from the seeds of myristica fragrans that inhibit nitric oxide production. *Food Chemistry*, 173, 231-237.

Ginting, B., Saidi, N., Murniana., Mustanir., Maulidna., Simanjuntak, P., 2020. Lignan compound isolated from n-hexane extract *Myristica fragrans* houtt root as antioxidant and antitumor activities against MCF-7 cell lines data. *Data in Brief*, 31.

Maesaroh, K., Kurnia, D., Anshori, J.A., 2018. Perbandingan metode uji aktivitas antioksidan DPPH, FRAP dan FIC terhadap asam askorbat, asam galat dan kuersetin. *Chimica et Natura Acta*, 6(2), 93-100.

Ginting, B., Mustanir., Helwati, H., Desiyana, L.S., Eralisa., Mujahi, R., 2017. Antioxidant activity of n-hexane extract of nutmeg plants from south aceh province. *Jurnal Natural*, 17(1), 39-44.

Fajriah, S. and Megawati., 2015. Penalisan fitokimia dan uji toksisitas dari daun *Myristica fatua* Houtt. *Chimica et Natura Acta*, 3(3), 116-119.

Okselni, T., Tasfiyati, A.N., Juliadmi, D., Handayani, UF., Saputri, A., Dewi, R. T., Septama, A. W., 2024. An extraction technique to reach the effective radical scavenging constituents from the leaves of Indonesian malva nut tree (Scaphium macropodum): A comparative study of maceration, heat-assisted, ultrasound-assisted, and microwave-assisted extraction. *Biocatalysis and Agricultural Biotechnology*, 55, 1-11.

Nikolic, V., Nikolic, L., Dinic, A., Gajic, I., Urosevic, M., Stanojevic, L., Stanojevic. J., Danilovic, B., 2021. Chemical composition, antioxidant and antimicrobial activity of nutmeg (*Myristica fragrans* Houtt.) seed essential oil. *Journal of Essential Oil Bearing Plants*, 24(2), 218-227.

Khoerunisah, M.S., Marissa Angelina, Kasiyati., 2022. Cytotoxic test of cassia alata l. leaves ethanol extracts, fractions, and main compounds against MCF-7 cells. *Indones. J. Cancer Chemoprevent*, 13(3), 144-151.

Chatgilialoglu, C., Carla Ferreri, Michele Melchiorre, Anna Sansone, and Armida Torreggiani, 2014. Lipid geometrical isomerism: from chemistry to biology and diagnostics. *Chemical Reviews*, 114, 255-284.

Macabuhay, A., Borjana Arsova, Robert Walker, Alexander Johnson, Michelle Watt and Ute Roessner, 2021. Modulators or facilitators? Roles of lipids inplant root–microbe

interactions. *Trends in Plant Science*, 21(8), 1-11.

Rahim, M.A., Xuebin Zhang and Nicola Busatto, 2023. Editorial: Phenylpropanoid biosynthesis in plants. *Frontiers in Plant Science*, 14, 1-3.

Hassanpour, S. H., Alireza Doroudi, 2022. Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. *Avicenna Journal of Phytomedicine*, 13(4), 354-376.

Ginting, B., Lamek Marpaung, Tonel Barus dan Partomuan Simanjutak, 2016. Isolation and identification of flavonoid compound from nutmeg leaves (*Myristica fragrans* Houtt). *Asian Journal of Chemistry*, 28(1), 199-202.

Awouafack, M. D., Pierre Tane and Hiroyuki Morita, 2017. Isolation and structure characterization of flavonoids, flavonoids - from biosynthesis to human health. *InTech*.

Weerakoon, T., W. P. R. T. Perera., Harindu Rajapaksha., Janitha A Liyanage, 2021. A review on bioactive compounds and pharmacological activities of *Myristica fragrans* as a medicinal plant. *International Journal of Innovation Scientific Research and Review*, 3(6), 1316-1320.

Yakaiah, V., Dakshinamoorthi, A., & Kavimani, S., 2019. Effect of *Myristica fragrans* extract on total body composition in cafeteria diet induced obese rats. *Bioinformation*, 15(9), 657.

Adeosun, C. B., Sinmisola Olaseinde, A.O. Opeifa, Olubunmi Atolani, 2013. Essential oil from the stem bark of cordia sebestena scavenges free radicals. *Journal of Acute Medicine*, 3, 138-141.

Handayani, V., Aktsar Roskiana Ahmad, Miswati Sudir, 2014. Uji aktivitas antioksidan ekstrak metanol bunga dan daun patikala (Etlingera elatior (Jack) R.M.Sm) menggunakan metode DPPH. *Pharmacy Science Research*, 1(2), 86-93.

Adibuduge, Y., and Senevirathne, M., 2023. Potential of Nutmeg (Myristica fragrans Houtt) Leaf Extracts as a Source of Functional Ingredients with Antibacterial, Antifungal and Antioxidant Activities. *Journal of Agricultural Sciences (Sri Lanka)*, 18(2), 221–236.

Anggoro, N., 2019. Mengenal Pala. Mengenal tanaman pala. Pusat Penyuluhan Pertanian RI-Kementrian Pertanian. http://cybex.pertanian.go.id/artikel/87545/mengenal-tanaman-pala/

Nurmilasari, N., Ginting, B. and Helwati, H., 2017. Isolation of antioxidant compounds of methanol extract of nutmeg leaves (Myristica Fragrans Houtt). *Journal Natural*, 17(1), 49-57.

Angelina, M., Marya Salfia Khoerunisah, Kasiyatib, Alfian Mahardika Forentin, Muhammad Anwar Djaelani, 2024. Synergistic cytotoxicity effect by combination of n-hexane fraction of the herbs (*Peperomia pellucida*) with doxorubicin against breast cancer cells (MCF-7). *South African Journal of Botany*, 170, 260-270.

Piaru, S. P., Roziahanim Mahmud, Amin Malik Shah Abdul Majid, Sabariah Ismail, Che Nin Man, 2011. Chemical composition, antioxidant and cytotoxicity activities of the essential oils of Myristica fragrans and Morinda citrifolia. *Journal of the Science of Food and Agriculture*, 92 (3): 593-597.

Sirait, P. S., Iriani Setyaningsih, dan Kustiariyah Tarman, 2019. Aktivitas antikanker ekstrak spirulina yang dikultur pada media walne dan media organik. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 22(1), 50-59.

Ghauri, M. A., Liaqat Iqbal, Ali Raza, Uzma Hayat, Naveel Atif and Aqeel Javeed, 2021. In vivo anti-inflammatory, antipyretic, analgesic activity and in vitro anti-proliferative activity of aqueous methanolicextract of *Euphorbia granulata* Forssk. *Future Journal of Pharmaceutical Sciences*, 7(34), 2-10.

Rohmah, J., 2022. Antioxidant activities using DPPH, FIC, FRAP, and ABTS methods from ethanolic extract of lempuyang gajah rhizome (*Zingiber zerumbet* (l.) Roscoeex Sm.). *Jurnal Kimia Riset*, 7 (2), 152-166.

Panjaitan, R. S. and Windri Yuliana, 2022. Antioxidant and toxicity activities of *Gracilaria gracilis* methanol extract based on different extraction methods. *Jurnal Kimia Riset*, 7 (2), 141-151.