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Research Article

# Evaluation of Antioxidant and Anticancer Activity of *Myristica fragrans* Houtt. Bark

**Binawati Ginting[1,*], Mustanir[1], Nurdin[1], Maulidna[2], Murniana[1], Safrina[3]**

**ABSTRACT**

This study aims to evaluate the antioxidant and anticancer activity of secondary metabolite compounds from *Myristica fragrans* Houtt. (nutmeg) bark using n-hexane extract based on DPPH radical scavenging and microculture tetrazolium salt (MTT) assay. The chemical structural analysis using NMR, FTIR, and LC-MS spectroscopy confirmed and identified the structure of isolated compound namely (2E)-5(2z.4E)-hexa-2,4,-dio-zyl)-2propylcyclohexanol ($C_{18}H_{30}O_4$) for the first time which is corresponding for the excellent antioxidant and anticancer activity against MCF-7 cell lines with the $IC_{50}$ value of 99.76 and 10.75 ppm, respectively.

**Key words**: *Nutmeg (Myristica fragrans Houtt)*, Bark, n-Hexane extract, Antioxidant, Anticancer.

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## INTRODUCTION

Myristica fragrans Houtt., a plant species native to Indonesia mainly spread in coastal areas and tropical region widely known as nutmeg for its use in the production of spices.[1,2] The nutmeg bark is a part of an unexplored nutmeg which is supposedly containing secondary metabolite compounds.[3,4] The extract of nutmeg plant parts from its roots, seeds, fruit, and bark exhibits good antithrombotic, antimicrobial, psychostimulant and antioxidant.[5-7] Besides, the nutmeg bark contains secondary metabolite compounds with high potential as an antioxidant and anticancer treatment. Additionally, the isolation of triterpenoid antioxidant compounds from the bark has also been reported from *Betula platyphylla var. japonica* by bioassay-guided fractionation.[8] Several studies reported the potential of anticancer activity from the extract nutmeg.[9,10] In addition, the anticancer activity of nutmeg seeds has been investigated to several human cancer cells such as H1299, H358, H460, Hela, HepG2, KPL4, RD, and MDCK.[11] Previously, phytochemical investigation of Myristica fragrans stem bark extract using polar solvent was investigated extensively and found lignan and neolignan compound.[12] However, extraction using non-polar solvent such as n-hexane and its antioxidant and anticancer activity of isolated nutmeg bark has not yet been explored so far. Herein, in this work the isolation of *Myristica fragrans* of *n*-Hexane Bark (MFHB) was carried out. Based on FTIR, NMR and LC-MS analysis further confirm the isolated compound of MFHB such as propyl (2E)-5(2z.4E)-hexa-2,4,-dio-zyl)-2-propylcyclohexanol ($C_{18}H_{30}O_4$) was determined for the first time and demonstrated strong antioxidant and anticancer activity.

## MATERIALS AND METHODS

Nutmeg barks were collected from Paya Peulumat village. East Labuhan Haji subdistrict. South Aceh district on November 2019. Nutmeg bark around 1 kg was cleaned with tap water and distilled water to remove the dirt, followed by oven dried for overnight and milled using a laboratory blender to obtain fine powder. Subsequently, the nutmeg bark powder was macerated with methanol for 24 h. The methanol extract was obtained then partitioned with n-hexane. to obtain a methanol and a n-hexane layer. The n-hexane layer was evaporated and obtained a concentrated of n-hexane nutmeg bark. The n-hexane of nutmeg bark extract was then isolated and purified. 30 g nutmeg bark n-hexane extract was isolated in gravity column chromatography with 60G silica gel, where 400 g of n-hexane of nutmeg bark extract as stationary phase and n-hexane motion phase: ethyl acetate determined from thin layer chromatography (gradient elution). Each fraction of 50 mL was collected and analyzed using thin layer chromatography (TLC) and fractions with similar stain patterns were combined and further analyzed. In addition, the active fraction was recromatographed and the appearance of a single stain from the TLC analysis and carried out by TLC or High Performance Liquid Chromatography (HPLC). Finally, it was recrystallized with acetone and n-hexane to obtain a crystals form.

MFHB was analyzed for hydrogen donation or radical scavenging ability using stable DPPH radical scavenging assay. The initial concentration of DPPH radical was kept at 0.4 mM for all the antioxidant radical reactions. Each concentration of 25, 50 and 100 ppm of extract was added into test tube with 1 mL of DPPH and 5 mL of methanol. After 30 minute of homogenization in vortex mixer, absorbance was recorded at 517 nm.

The anticancer activity of n-hexane extract of nutmeg bark with various concentrations of 25, 50, 100 and 200 ppm was carried out, where doxorubicin was used as a positive control. The preparation of MCF-7 cells was grown with concentrations of 5000 cells in 100 µl of growth media.[13] The extract was added after the cells has reached confluent of 50% for 24 h. The MTT assay test was carried out after 3 days of aging,

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then 5 mg/mL was added into MTT per well and was incubated for 4 h at a temperature of 37 °C with the addition of formazan in ethanol. To analyze the anticancer activity, the absorbance peaks of extracted solution were monitored at a wavelength of 595 nm using Versamax ELISA microplate reader at the Center for Animal Studies. Primate Research Center – Institut Pertanian Bogor.

The chemical composition and structure of isolated compound was determined by NMR data ($^{1}$H and $^{13}$C-on JEOL JNM-FX400 spectrometer; 500 MHz for $^{1}$H-NMR; 125 MHz for $^{13}$C-NMR). Fourier transform infrared spectroscopy (FTIR) data was performed using a Perkin Elmer 1600 Series FT-IR spectrometer. LC-MS mass spectra were characterized by the Shimadzu GC-MS QP2000A spectrometer, 70 eV and an Automass Thermofinnigan was used for HRESI$^{+}$ measurement.

## RESULTS AND DISCUSSION

Fourier-transform infrared spectrum (FTIR) was carried out to investigate the functional groups of MFHB. Figure 1a exhibits the strong absorption band at range of 3247–3309 cm$^{-1}$ can be ascribed to the stretching of the –OH groups.[14] The characteristic peaks at 2926–2856 cm$^{-1}$ indicates the presence C-H stretching, meanwhile in the region of 1700–1900 cm$^{-1}$ and 1454 cm$^{-1}$ corresponds to C=O and C=C groups, respectively. The column chromatography of n-hexane nutmeg bark demonstrated that 11 fractions was found and labeled as MFHB A to K. The pure compound then tested the antioxidant activity resulting in $IC_{50}$ of 99.76 ppm based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (Table 1).

The microculture tetrazolium salt (MTT) assay was carried out to evaluate the anticancer activity of MFHB isolated compound. Figure 1b shows that anticancer activity of compound H with $IC_{50}$ value of 10.75 ppm indicates the strongest active anticancer activity among all fraction. In general, the cytotoxic activity of extract compounds on cancer cells can be classified as a highly active ($IC_{50}$ <10 ppm), moderate ($IC_{50}$ of 10 – 100 ppm) and less activity ($IC_{50}$ of 100 – 500 ppm).[15] Accordingly, the order from the lowest to highest anticancer activity are G, J, A and I compound with an $IC_{50}$ value of 12.79, 17.19, 30.39, and 37.71 ppm, respectively as summarized in Tables 2 and 3. In comparison to the nutmeg skin extract was $IC_{50}$ value of 22.62 ppm.[4]

To elucidate the chemical structure of isolated compound, NMR analysis was employed. The $^{1}$H NMR spectra of the isolated compounds exhibit the type and number of protons present in the terpenoid compound as shown in Figure 2.

At $^{1}$H-NMR spectra indicates there are three proton methyl ($CH_3$). that is in region $\delta_H$ 0.96 (d) and $\delta_H$ 1.02 (s) and $\delta_H$ 0.88 (d). A carbonyl group (CO) and six cluster of methyl (CH) contained in the region $\delta_H$ 4.10 (d), $\delta_H$ 5.36 (d) $\delta_H$ 5.38 (d), $\delta_H$ 5.31 ( d), $\delta_H$ 2.27 (d) and $\delta_H$ 1.04 (d). There are two groups of hodroxyl (C-OH) and six methylene groups ($CH_2$) present in the region $\delta_H$ 4.03 (d), $\delta_H$ 1.60 (d) $\delta_H$ 2.33 (d), $\delta_H$ 1.29 (d), $\delta_H$ 1.30 (d) and $\delta_H$ 1.06 (d).

Moreover, the $^{13}$C NMR spectra were also performed in Figure 3. In the MF*n*HB based sample, there are 18 carbon atoms in the MF*n*HB compound. A quaternary carbon (C) contained in H 142.3 δ area (s) and three carbon methyl ($CH_3$) are shown in the area of $\delta_C$ 14.3 (d), $\delta_C$ 19.2 (d), $\delta_C$ 19.8 (s). Besides, six methyl carbon (CH) are also shown in the area of $\delta_C$ 65.7 (d), $\delta_C$ 130.1 (d), $\delta_C$ 130.8 (d), $\delta_C$ 121.5 (d), $\delta_C$ 34 , 0 (d) and $\delta_C$ 21.8 (d). There is also a carbonyl group (CO) in the area of $\delta_C$ 173.4 (s) and two hydroxyl groups in the area of $\delta_C$ 65.7 $\delta_C$ 71.6. Furthermore, six methylene groups ($CH_2$) are shown in the area of $\delta_C$ 68.0 (d), $\delta_C$ 25.2 (d), $\delta_C$ 34.3 (d), $\delta_C$ 23.3 (d), $\delta_C$ 32.6 (d) and $\delta_C$ 38.2 (d). Furthermore, 2D NMR hetero multiple bond connectivity (HMBC) spectra displayed the presence of a proton relationship with carbon more than one bond apart as shown in Figure 4. The HMBC spectra of the isolated compound exhibit a proton linkage with carbon space more than one bond. Ordinate axis in the HMBC spectrum plotted with core chemical shifts of carbon ($^{13}$C) and the plotted axis with proton chemical shift ($^{1}$H). In the HMBC spectrum MFHB compound on proton signals at $\delta_H$ 2.27 ppm when a vertical line drawn from the signal, obtained by relationships at $\delta_C$ 34.0 ppm. The vertical line drawn from the signal obtained $\delta_H$ 1.30 ppm spot at $\delta_C$ 32.6 ppm and others.

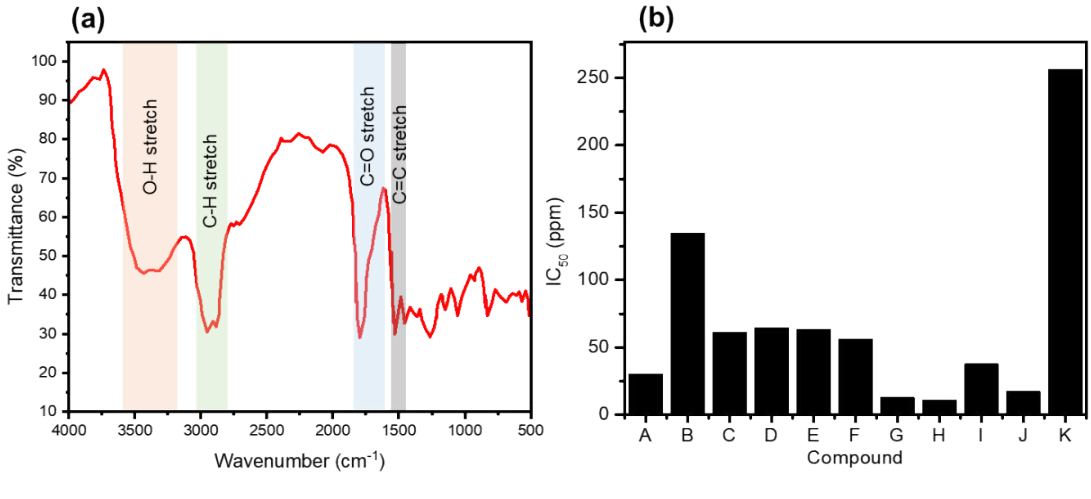


**Figure 1:** (a) FTIR spectrum of MFHB extract and (b) anticancer activity of MFHB compound fraction from A to K.

**Table 1: Antioxidant activity result of pure compound.**

| Compound | %Inhibition (ppm) | | | $IC_{50}$ (ppm) |
|---|---|---|---|---|
| | 25 | 50 | 100 | |
| MFHB (pure) | 28.73 | 35.63 | 50.11 | 99.76 |

**Table 2: Summary of anticancer activity against MCF-7 cell lines from compound A to K at 25, 50 and 100 ppm.**

| Sample | Life Average cell MCF-7 (%) | | | $IC_{50}$ (ppm) |
|---|---|---|---|---|
| | 25 ppm | 50 ppm | 100 ppm | |
| MF*n*HB A | 45.17 | 62.36 | 79.71 | 30.39 |
| MF*n*HB B | 10.47 | 9.98 | 38.30 | 134.58 |
| MF*n*HB C | 14.89 | 29.13 | 96.73 | 61.06 |
| MF*n*HB D | 17.68 | 21.44 | 91.16 | 64.66 |
| MF*n*HB E | 9.66 | 33.39 | 90.67 | 63.32 |
| MF*n*HB F | 20.79 | 44.52 | 90.34 | 56.30 |
| MF*n*HB G | 51.57 | 77.99 | 95.13 | 12.79 |
| MF*n*HB H | 64.31 | 77.04 | 96.54 | 10.75 |
| MF*n*HB I | 31.60 | 72.33 | 94.18 | 37.71 |
| MF*n*HB J | 49.84 | 74.69 | 93.24 | 17.19 |
| MF*n*HB K | 36.79 | 37.42 | 33.81 | 256.31 |
| MF*n*HB extract | 40.56 | 66.03 | 93.24 | 33.90 |

**Table 3: Summary of $^{1}H$ and $^{13}C$ NMR spectral data.**

| No | $\delta_C$ (ppm) | $\delta_H$ (ppm) | $\delta_H$ (J) | HMBC (ppm) | COSY |
|---|---|---|---|---|---|
| 1 | 173.4 (s) | - | - | - | - |
| 2 | 130.1 (d) | 5.36 | 1H (d.d) | 130.8 | 5.31<br>5.38 |
| 3 | 130.8 (d) | 5.38 | 1H (d.d) | 130.1 | 5.31 |
| 4 | 121.5 (d) | 5.31 | 1H (d) | 130.8 | 5.36<br>5.38 |
| 5 | 142.3 (s) | - | - | - | - |
| 6 | 19.8 (s) | 1.02 | 3H (s) | 142.3 | - |
| 7 | 68.0 (d) | 4.03 | 2H (t) | 65.7 | 4.10<br>0.96 |
| 8 | 65.7 (d) | 4.10 | 1H (t) | 68.0<br>19.2 | 4.03 |
| 9 | 19.2 (s) | 0.96 | 3H (d) | - | 4.10 |
| 1' | 71.6 (d) | 3.39 | 1H (d) | 34.3 | 1.06 |
| 2' | 21.8 (d) | 1.04 | 1H (d.d) | 23.3 | 1.06<br>1.29 |
| 3' | 34.3 (d) | 2.33 | 2H (d) | 71.6<br>25.5<br>21.8 | 1.60<br>2.27 |
| 4' | 25.2 (d) | 1.60 | 2H (d) | 34.0 | 2.33<br>2.27 |
| 5' | 34.0 (d) | 2.27 | 1H (d) | 25.2 | 1.60<br>2.33 |
| 6' | 38.2 (d) | 1.06 | 2H (d) | 34.0 | 3.39 |
| 7' | 23.3 (d) | 1.29 | 2H (d) | 32.6 | 1.30<br>1.04 |
| 8' | 32.6 (d) | 1.30 | 2H (d.d) | 23.3<br>0.88 | 1.29<br>0.88 |
| 9' | 14.3 (s) | 0.88 | 3H (d) | 32.6 | 1.30 |

As a result, the MFHB is a compound that composed of 18 carbon atoms comprising three of $CH_3$ group, six of CH group, 6 of $CH_2$ group, one of CO group and one of C atom with a mass of 310 g/mol. Mass spectra analysis was performed with the liquid chromatography-mass spectra (LC-MS) instrument. As a result, based on LC-MS spectra data of the MFHB based sample, it was found by the presence of molecular ions at m/z 235 (M + H)+ as shown in Figure 5. It confirms that MFHB compound has a chemical formula of $C_{18}H_{30}O_4$ and identified as a propyl (2E)-5(2z.4E)-hexa-2,4,-dio-zyl)-2propylcyclohexanol.

## CONCLUSION

In summary, MFHB isolated compound demonstrated strong antioxidant and anticancer activity. Based on FTIR, LC-MS and NMR analysis, it was confirmed that isolated compound of propyl (2E)-5(2z.4E)-hexa-2,4,-dio-zyl)-2propylcyclohexanol ($C_{18}H_{30}O_4$) play an essential role as strong antioxidant and anticancer activity against MCF-7 cell lines with an $IC_{50}$ of 99.76 and 10.75 ppm, respectively.

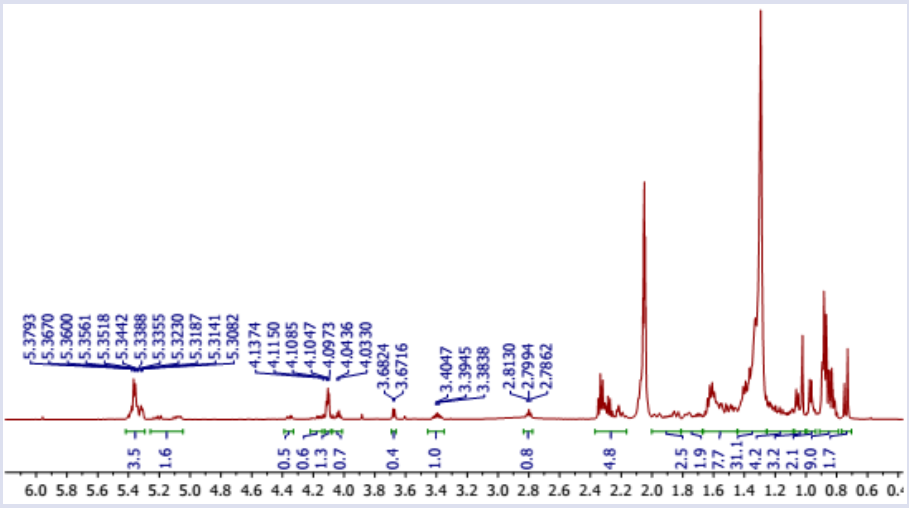


**Figure 2:** $^{1}$H-NMR spectrum of MF*n*H compound B.

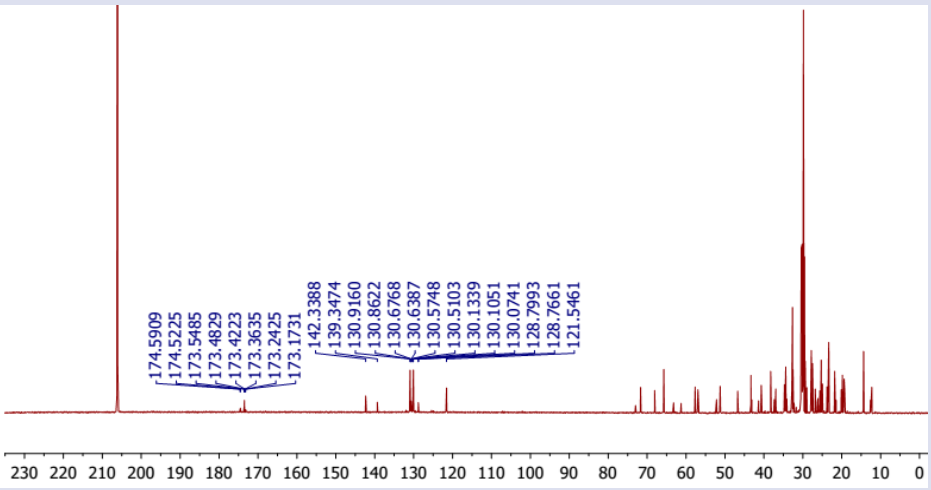


**Figure 3:** $^{13}$C-NMR spectrum of MF*n*H compound B.

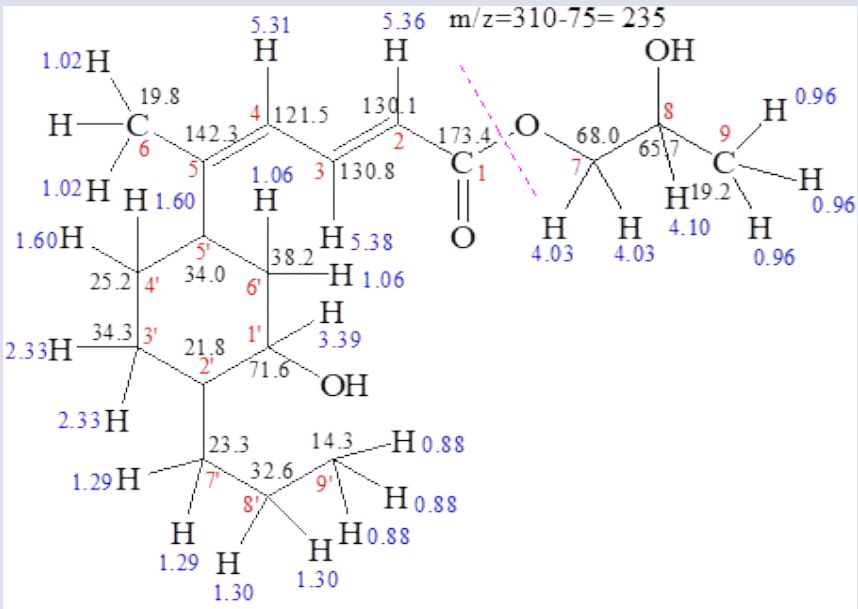


**Figure 4:** The correlation HMBC structure of MFHB compound.

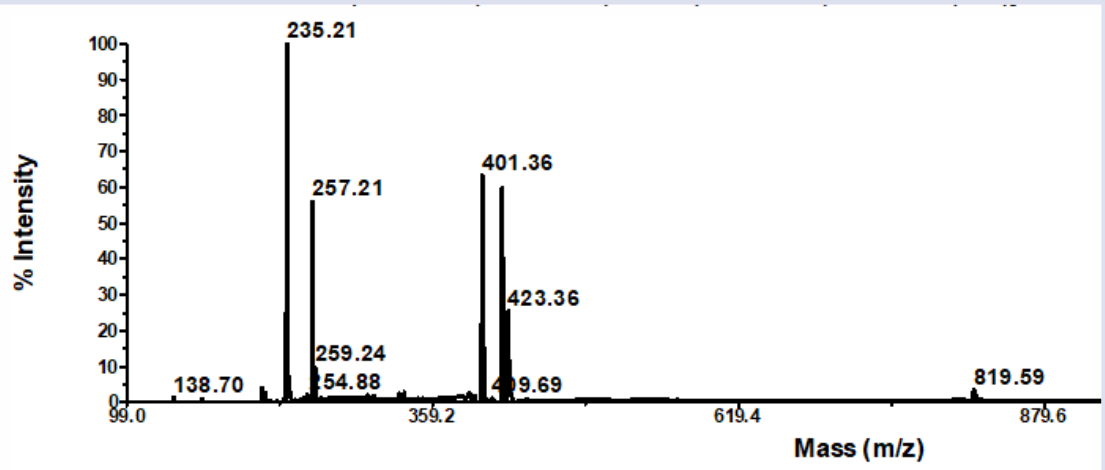


**Figure 5:** LC-MS analysis result of MF*n*H compound B.

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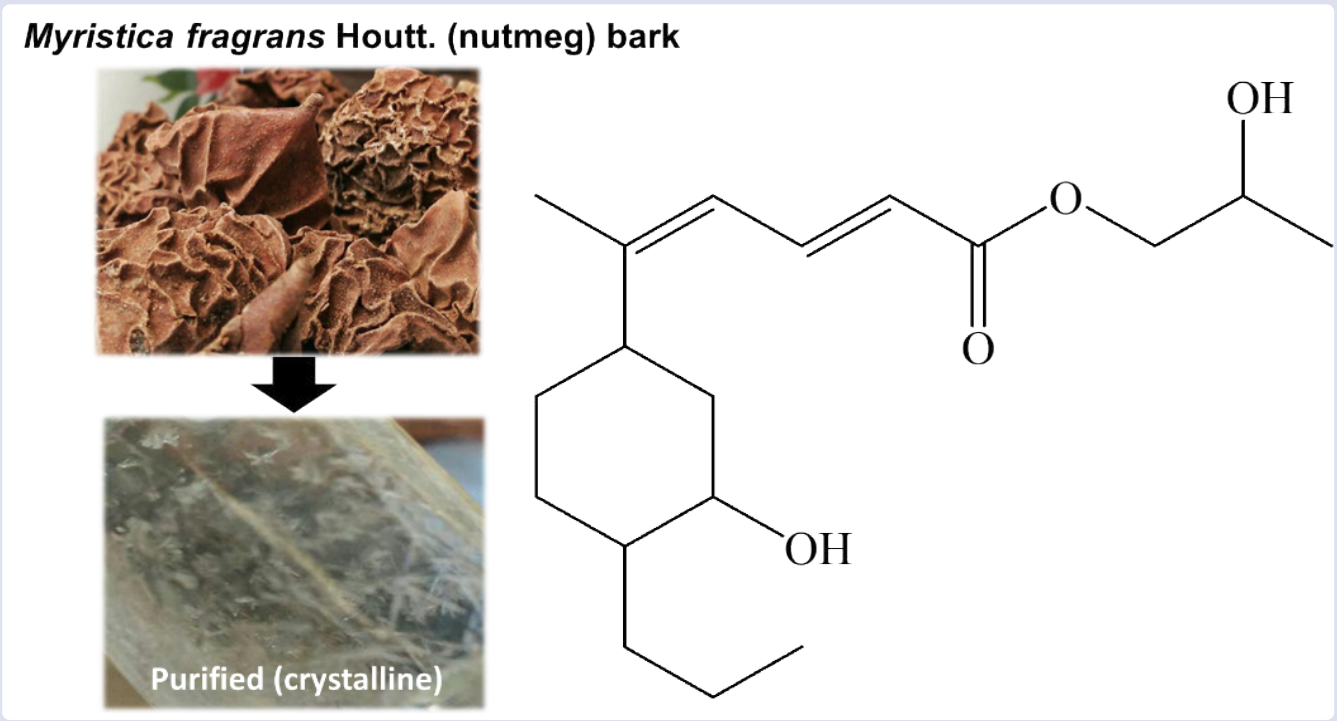
## GRAPHICAL ABSTRACT

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Murniana graduated from undergraduate degree in Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta. She completed master degree at Institut Teknologi Bandung, Indonesia majoring in bioactivities of secondary metabolite compounds from tropical natural plants. She also currently actively teaching as lecturer in Universitas Syiah Kuala.

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