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Data Article

Lignan compound isolated from n-Hexane extract myristica fragrans Houtt root as antioxidant and antitumor activities against MCF-7 cell lines data

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ABSTRACT

Nutmeg plant (Myristica fragrans Houtt) is known as one of traditional medicine. The nutmeg root has a strong potential in antioxidant and anticancer agents among other nutmeg plant parts. The n-hexane root extract has been carried out by thin-layer chromatography and obtained 8 fractions (labeled as Myristica fragrans Houtt Root: MFHR 1 – 8). Specifically, the MFHR 4 has been purified for several times to obtain a yellow-brown color. Furthermore, lignan compound 6′-methyl-(7–hydroxy-8-methylbut-9-en)-3,2′-dimethoxybiphenyl-4,5-diol) was identified with chemical formula of $C_{20}H_{24}O_5$ and analyzed using UV–vis spectroscopy, fourier-transform infrared spectroscopy (FTIR), 1D/2D nuclear magnetic resonance (NMR), and liquid chromatography–mass spectrometry (LC-MS). Based on MTT assay, MFHR demonstrated moderate anticancer activity against MCF-7 cell lines of 51.95 µM, meanwhile, DPPH activity confirmed the strong antioxidant activity with IC50 value of 12.67 ppm.

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Specifications Table

Subject	Biochemistry, Organic Chemistry, Phytochemistry
Specific subject area	Antioxidant and anticancer
Type of data	Table, Figure, Graph
How data were acquired	UV–vis spectrophotometer, FTIR, 1D/2D NMR, LC-MS, and MTT assay
Data format	Analyzed
Parameters for data collection	Thin layer chromatography (TLC) were used to isolate n-hexane extract of nutmeg root (MFHR) where 8 fractions was obtained.
Description of data collection	The antioxidant activity of the extract was carried out by the free radical DPPH method and 3–4, 5-dimethylthiazol-2-yl-2, 5-diphenyltetrazolium bromide (MTT) assay was used to evaluate the anticancer properties of MFHR against MCF-7 cell lines.
Data source location	City/Town/Region: Kampung Paya Village, North Kluet District, South Aceh Province Country: Indonesia
Data accessibility	The data are included in this article
Related research article	**none**

Value of the Data

- This data provides helpful leads for pharmacological field, n-hexane extracts of nutmeg root demonstrated great potential as antioxidant and anticancer activity.
- The data reported here provides insight to identify the bioactive molecules such as lignan compound from the n-hexane extracts that possessed antioxidant and anticancer activity.
- The dataset obtained from the UV–Vis, FTIR, LC-MS, NMR and MTT assay, will guide researchers with the utilization of lignan compound for further investigation in anticancer besides against the MCF-7 cell lines.
- The LC-MS and NMR dataset from the characterization of the obtained extract from nutmeg root will provide researchers with the biochemical constituents and other possible applications of the nutmeg root.

1. Data description

The yield of n-hexane extract of nutmeg root of 18.21% was obtained. The thin layer chromatography (TLC) has been used to identify each compound in n-hexane root extracts and 8 fractions were obtained and labeled as *Myristica fragrans Houtt Root* (MFHR) 1 – 8. To observe the antioxidant activity, the DPPH assay was employed. The DPPH assay offers a low-cost method to evaluate potential antioxidants by spectrophotometry as indicate at λ_{max} of 517 nm. The percentage of inhibition of n-hexane extract-based sample at various concentrations of 25, 50, and 100 ppm were 52.80%, 69.27%, and 75.00%, respectively. The MFHR $4-8$ exhibit a strong antioxidant activity with relatively low percentage of half maximal inhibitory concentration (IC_{50}) value implying higher percentage inhibition. Although, the MFHR 6 shows the strongest antioxidant activity with the lowest IC_{50} value of 6.00 ppm, however lower percentage inhibition with lower than 40%. Furthermore, the MFHR 1 – 3 had the weakest antioxidant activity as indicating of higher IC_{50} value of 380.02, 590.51, and 604.44 ppm, respectively. The results of antioxidant activity tests are summarized in Table 1.

The antioxidant activity is expressed by the IC_{50} value of each fraction. $^{\text{The}}$ IC_{50} $^{\text{of MFHR 1}-8}$ are higher than the result of the antioxidant activity of n-hexane extract which has an IC_{50} value of 0.216 ppm. This shows that the n-hexane extract-based sample has better inhibitory

Table 1
Summary of the antioxidant activity of n-hexane nutmeg root extracts at various concentrations.

Sample	Inhibition of concentration (%) 25 ppm	50 ppm	100 ppm	IC50 (ppm)
n-hexane extract	52.80	69.27	75.00	0.216
MFHR-1	14.55	21.33	22.55	380.02
MFHR-2	12.24	12.85	17.09	590.51
MFHR-3	19.64	20.61	23.52	604.44
MFHR-4	29.33	46.18	60.85	69.64
MFHR-5	44.61	71.27	85.21	24.54
MFHR-6	27.15	34.55	36.55	6.00
MFHR-7	57.94	72.24	84.12	39.51
MFHR-8	41.45	58.18	73.58	55.32

activity due to the presence of several active compounds, which is in good accordance with previous report that nutmeg root extracts consists of seven main compounds namely 5-octadecanoic acid, methyl ester, linalool, cyclopropanepentanoic acid-2-undercyl-methyl ester-trans-, methoxyeugenol, eugenol, hexadecenoic acid, methyl ester, and myristicin [1]. The complex fat compounds can synergize in inhibiting free radicals, thus will help to make the antioxidant activity of pristine n-hexane extract MFHR stronger than its fractions.

Although the fraction exhibit lower antioxidant activity than the pristine n-hexane extracts, the specific compound that plays a role as an antioxidant agent is still not clear. To identify it, the fractionated extract needs further analysis. Among 8 fractions, the MFHR-4 exhibited better separation, therefore the purification of MFHA-4 crystals was carried out using a column chromatography method with eluent n-hexane: ethyl acetate (9:1) gradient. The results of TLC monitor had a staining pattern and purification by washing the MFHR-4 with acetone and n-hexane for several times to obtain yellow brownish color powder of 4 mg and melting point of 132 - 136 °C. After several times of purifications, the MFHR subfraction shows better antioxidant activity with lower IC_{50} of 12.67 ppm than the first extraction process of MFHR-4.

The nutmeg root extracts are expected to have a high potential activity for the anticancer treatment. The anticancer activity of nutmeg plant methanol extract has been tested on osteosarcoma cells (MG-63) using the MTT assay and exhibited an IC_{50} value of 40.39 μg/mL (Chakraborty et al., 2015). Furthermore, the lower IC_{50} value of 24.83 μg / mL has been also reported on Vero Cell Line using the MTT assay (Piaru et al., 2012). To determine the anticancer activity level, the toxicity level was measured by analyzing the inhibitor concentration 50 (IC_{50}). The standard guideline has been established by NCI to analyze the IC_{50} value. The smaller the IC_{50} value means the stronger the anticancer activity. It has been reported that if IC_{50} below 20 ppm is classified to have strong anticancer activity for extract compounds, while below 4 ppm was for pure compound [2].

The breast cancer against MCF-7 cell lines has been examined in order to evaluate the anticancer activity of n-hexane extract at various concentrations of 25, 50, 100, and 200 ppm. Doxorubicin is also tested as a control sample. The absorbance of each sample was analyzed at a wavelength of 595 nm and relatively high IC_{50} of 51.95 µM of n-hexane extract of nutmeg root was obtained. Based on the IC_{50} value, the MFHR-4 demonstrated a moderate anticancer activity due to the higher IC_{50} value above 20 µM [3]. Although it has a weak anticancer activity, the n-hexane extract of nutmeg root has a high potential to further develop as a natural antioxidant and anticancer agent.

Ultraviolet-visible (UV–vis) absorption spectra and Fourier-transform infrared spectrum (FTIR) were performed to identify the specific compound in the MFHR. There are two peaks at 269 and 275 nm which indicate the presence of two substituted benzene rings of the compound as shown in Fig. 1a. Moreover, according to the UV absorption existence at a wavelength of 250−280 nm is a typical type of spectrum of phenolic or lignan compound, which is in good agreement with previous report (Filleur et al., 2002; Zhang et al., 2014). Fig. 1b shows the FTIR analysis results. It shows several peaks at wavenumbers of 3446−3408 cm^{-1}, 3143−3014 cm^{-1},

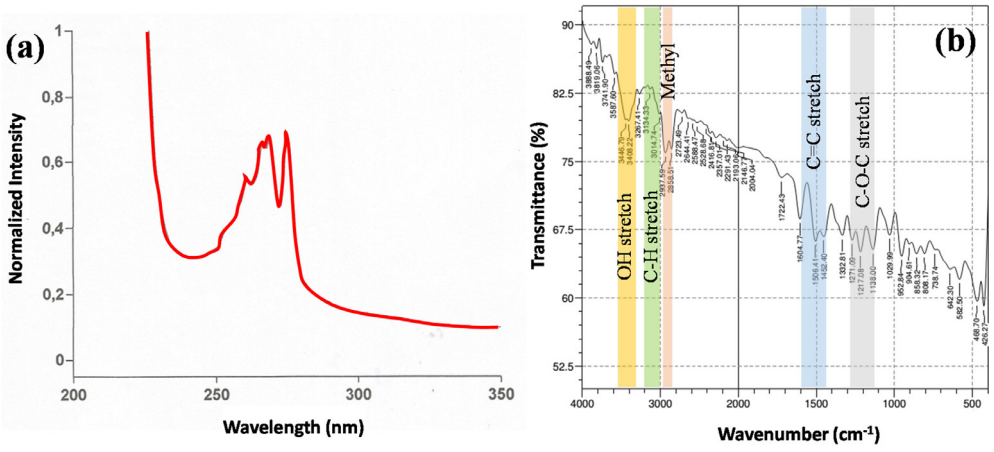

Fig. 1. (a) UV–vis absorption spectrum and (b) FT-IR analysis results of MFHR 4 according to Tables 1 and 2 data.

and 2937–2858 cm^{-1} which indicate the presence of OH stretch, C–H stretch, and the C–H stretch for methyl compound. Moreover, the $C=C$ stretch ring and C–O-C stretch are found at peaks at 1604–1452 cm^{-1} and 1271–1138 cm^{-1} which is correspond to lignan compound.

Table 1. UV–Vis spectrum data of MFHR 4 compound based on Fig. 1a.

Wavelength (nm)	Absorption (a.u.)
226.8	0.92041
228.3	0.77877
228.7	0.61789
230.9	0.50251
231.8	0.40989
233.5	0.35761
235.0	0.34595
241.0	0.31506
242.3	0.31049
246.9	0.32021
247.2	0.32186
252.5	0.34786
252.8	0.38198
252.8	0.37711
258.7	0.47465
260.9	0.56589
262.8	0.53826
264.7	0.56731
265.2	0.57239
265.6	0.62114
266.7	0.65851
268.0	0.64714
269.1	0.66989
270.1	0.64226
270.6	0.60849
271.0	0.57889
271.9	0.53826
272.9	0.47001
274.2	0.53826
274.5	0.59676

(*continued on next page*)

Wavelength (nm)	Absorption (a.u.)
275.5	0.68614
276.5	0.61364
276.8	0.60814
277.2	0.52201
277.4	0.45214
279.0	0.35111
281.7	0.23249
282.4	0.22754
288.4	0.18636
294.3	0.16062
295.9	0.15449
300.2	0.14517
306.1	0.14003
312.1	0.12973
315.8	0.12361
318.0	0.12458
323.9	0.11429
329.8	0.10914
330.9	0.11061
335.8	0.10399
341.7	0.10399
347.6	0.10399

Table 2. FTIR spectrum data of MFHR 4 compound based on Fig. 1b.

Wavenumber (cm^{-1})	Transmittance (%)
3982.58	87.91161
3937.82	86.57552
3932.85	85.93652
3893.07	86.0527
3873.17	85.41371
3858.25	86.28507
3818.47	84.13571
3828.42	85.29752
3788.63	84.60043
3748.84	84.65852
3728.95	83.72907
3728.95	83.03198
3694.14	82.79962
3664.30	80.81485
3634.46	79.47876
3594.68	77.96839
3549.92	79.42066
3525.05	80.81485
3485.27	80.58248
3425.59	81.39575
3360.94	81.1053
3326.13	79.65303
3301.26	78.43312
3291.32	76.51612
3266.45	74.94767
3226.67	75.76094
3196.83	74.94767
3191.85	76.74849
3176.93	77.96839
3162.01	79.07212
3082.44	79.07212

(*continued on next page*)

Wavenumber (cm^{-1})	Transmittance (%)
3027.74	78.02648
2947.34	78.02648
2872.74	77.38748
2827.99	76.6323
2758.36	76.39994
2693.71	75.93521
2624.09	75.4124
2509.70	75.29621
2415.21	74.54103
2335.64	73.84394
2280.94	73.08876
2231.21	72.15931
2191.42	72.50785
2111.85	71.52031
2101.91	70.00995
2057.15	67.68631
2037.26	69.89376
2007.42	71.46222
1957.69	69.54522
1952.71	67.51204
1922.87	65.17872
1882.26	66.5729
1847.45	65.87581
1807.66	67.62822
1787.77	69.25476
1733.07	67.57013
1683.33	66.22436
1648.52	67.68631
1603.76	66.75686
1608.74	65.64345
1559.01	66.34054
1544.09	65.41109
1514.25	64.59781
1474.46	66.81495
1444.62	65.52727
1419.76	64.6559
1384.95	66.68908
1350.13	68.90622
1295.43	67.39586
1255.64	66.28245
1225.81	68.15104
1195.97	66.34054
1181.05	65.23681
1166.13	63.95881
1116.40	65.58536
1091.53	65.12063
1026.88	64.24927
987.10	64.94636
957.26	64.19118
912.50	65.46918
852.82	64.6559
787.34	63.37791
717.72	62.56463
638.15	61.34473
613.28	62.50654
583.44	63.26172
538.68	61.92563
518.79	60.70573
488.95	59.25345
454.14	61.22854
424.30	59.02109
404.41	61.69327

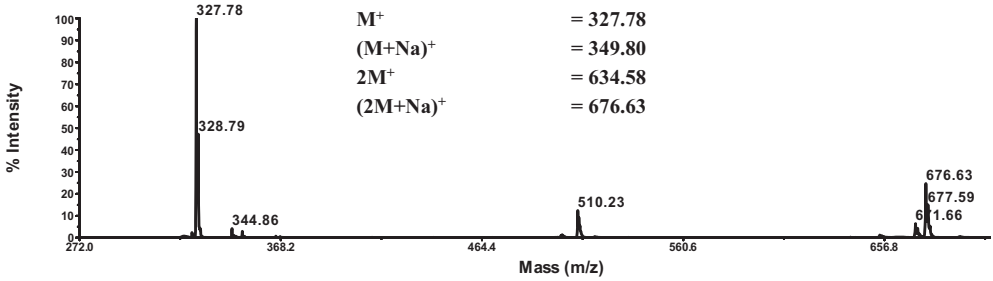

Fig. 2. LC-MS spectrum analysis of MFHR 4.

Table 2
The chemical shifts of ^{1}H and ^{13}C NMR of MFHR 4.

Number of Carbon	500 MHz; $CDCl_3$ ppm *J* in Hz $^{1}HNMR$ δ	$^{13}CNMR$ δ
1	–	132.35 (s)
2	6.97	109.00 (d)
3	6.89	114.21 (d)
4	6.78	113.45 (d)
5	−3.88	146.70 (s)
		56.14 (d)
6	–	132.22 (s)
7	5.63	93.98 (d)
8	3.45	45.78 (d)
8	1.38	17.70 (q)
9	6.35	131.07 (d)
10	6.13	123.69 (d)
11	1.86	18.58 (q)
1′	–	133.42 (s)
2′	6.97	109.16 (d)
3′	-	146.81 (s)
	3.88	56.14 (d)
4′	–	144.31 (s)
5′	–	145.92 (s)
6′	6.89	120.16 (d)

The structure of pure isolate n-hexane MFHR-4 is expected to be a group of phenolic compounds or lignans. The similar chemical structure of lignan “argenteane” from nutmeg has been reported with only different on the aliphatic chain [4, 5]. Mass spectra analysis was performed with the liquid chromatography-mass (LC-MS). As a result, it was found the present of molecular ions at *m/z* 344.86 $(M+H)^{+}$ as shown in Fig. 2. It confirms that the MFHR 4 compound has a molecular weight of 344.86 with the chemical formula of $C_{20}H_{24}O_5$ which is consistent with UV–Vis and FTIR result.

To further elucidate the lignan compound in the MFHR 4, NMR analysis was carried out. NMR spectroscopy provides comprehensive and more accurate information on both quantitative and qualitative analysis. The ^{1}H NMR and ^{13}C NMR spectra of the MFHR 4 compound were obtained by dissolving the sample in the chloroform-d ($CDCl_3$) as a solvent. The spectra recorded on the JEOL spectrophotometer (^{1}H NMR 500 MHz and ^{13}C NMR 125 MHz) are summarized in Table 2 and the raw data can be referred to Supplementary Materials.

The ^{1}H NMR spectra in Fig. 3a shows the presence of two methyl protons (CH_3) in the δ_H 1.37 (d) and δ_H 1.86 regions (d). Two methoxyl (OCH3) groups are in the δ_H 3.88 (s) area and nine methine (CH) groups in the 97H 6.97 (d) area, δ_H 6.89 (d) two protons, δ_H 6.78 (d) two protons, δ_H 6.35 (d), δ_H 6.13 (d), δ_H 3.45 (d), δ_H 5.10 (d) as shown in Fig. 3b.

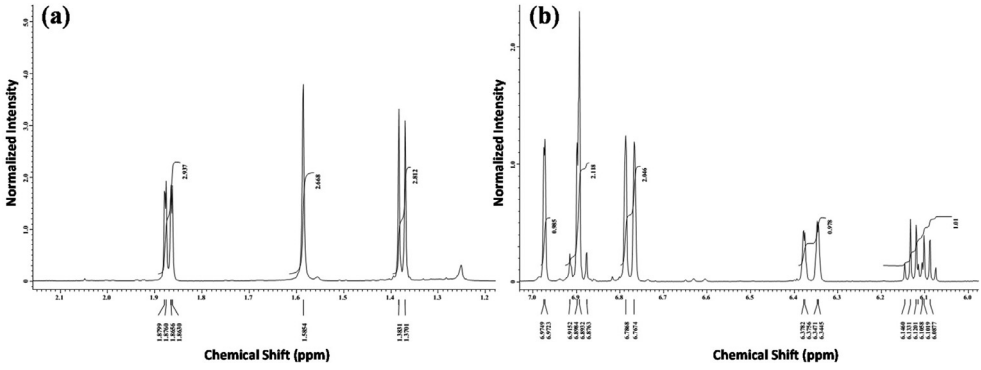

Fig. 3. 1H–NMR spectra for MFHR-4.

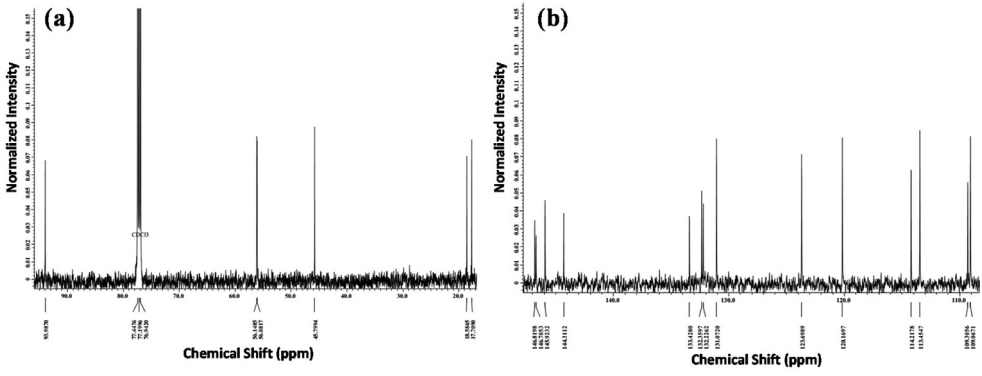

Fig. 4. 13C–NMR spectra for MFHR 4.

Fig. 4 shows the ^{13}C NMR spectra of MFHR 4. It shows the presence of 20 carbon atoms consisting of seven quaternary carbon that found in the region of δ_C 132.2 (s), δ_C 132.3 (s), δ_C 133.4 (s), δ_C 144, 3 (s), δ_C 145.9 (s), δ_C 146.7 (s) and δ_C 146.8 (s). Two methyl carbon (CH_3) are shown in the δ_C 17.7 (q) region, and δ_C 18.5 (q). Furthermore, nine carbon methins (CH) are also shown in the δ_C 45.7 (d), δ_C 93.9 (d), δ_C 109.0 (d), δ_C 109.3 (d), δ_C 113, 4 (d), δ_C 114.2 (d), δ_C 120.1 (d), δ_C 123.6 (d) and 131.0 (d). Besides, the existence of two methoxyl groups (OCH_3) in the δ_C 56.14 (d) and δ_C 56.14 (d) regions were also obtained.

The 1H–1H COSY (Correlation Spectroscopy) spectrum is shown in Fig. 5a. It shows that the proton from methyl (CH3) δ_H 1.38 ppm is associated with protons 3,H 3.45 ppm (H-8) and δ_H 6.35 ppm (H-9). The proton from methyl at 1,H 1.86 ppm (H-11) corresponds to the proton d_H 6.13 ppm (H-10) and d_H 6.35 ppm (H-9). Protons from metin δ_H 3.45 ppm (H-8) correspond to protons δ_H 5.10 ppm (H-7) as well as protons from methine (CH) 5,H 5.10 ppm (H-7) associated with proton d_H 3.45 ppm (H-8). The methane proton (CH) δ_H 6.35 ppm (H-9) corresponds to the proton δ_H 6.13 ppm (H-10) and δ_H 1.86 ppm (H-11). In the A ring, the proton of methine (CH) δ_H 6.78 ppm (H-3) corresponds to protons δ_H 6.89 (H-4) and δ_H 6.97 ppm (H-5). The proton from methine (CH) δ_H 6.89 (H-4) corresponds to the proton δ_H 6.78 ppm (H-3) and δ_H 6.97 ppm (H-5). Protons from methine (CH) δ_H 6.97 ppm (H-5) are associated with protons δ_H 6.89 ppm and δ_H 6.89 ppm (H-4).

Furthermore, hetero multiple bond connectivity (HMBC) spectra show the existence of a proton relationship with carbon more than one bond apart as shown in Fig. 5b. The ordinate axis in the HMBC spectrum was plotted with the chemical nucleus shift (13C) and the x-axis was

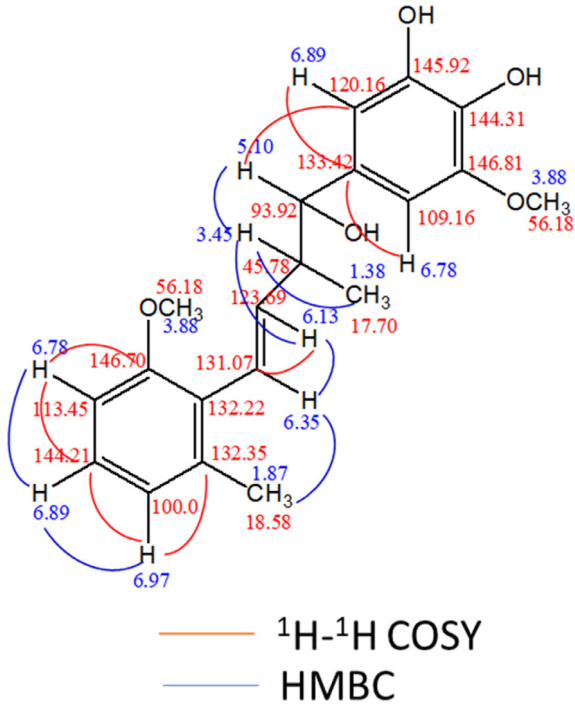

Fig. 5. Key 1H- 1H COSY and HMBC correlations of compound.

plotted with the proton-nucleus chemical shift (1H). In the HMBC spectrum of the MFHR 4 exhibit 3 bonds of the proton signal δ_H 6.97 ppm (H-2 ′) associated with δ_C 133.42 ppm (C-1 ′), δ_C 109.0 ppm (C-2), $_C$ 114.21 ppm (C-3). Moreover, the protons at δ_H 6.89 ppm (H-6 ′) give 2 bonds associated with δ_C 133.42 (C-1 ′) and 132.22 ppm (C-6). The 78H signal 6.78 ppm (H-4) corresponds to δ_C 114.21 ppm (C-3) and δ_C 146.70 ppm (C-5). The proton δ_H 6.13 ppm (H-10) corresponds to δ_C 131.07 ppm (C-9). Based on the result of 2D NMR from COSY and HMBC, the suggested chemical structure of the lignin compound is successfully demonstrated in Fig. 5. This result is in good agreement with LC-MS, FT-IR and UV–vis results.

2. Experimental design, materials and methods

2.1. Material and sample preparation

All the solvent used in this work such as methanol, petroleum ether, n-hexane, and ethyl acetate were purchased from Merck Chemicals without further purification. Furthermore, the same solvent that functions as an eluent is used as a pro analysis solvent, 60 G silica, and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

The nutmeg roots were collected from Kampung Paya Village, North Kluet District, South Aceh Province. The sample was carried out with several stages of work to separate the secondary metabolites of nutmeg root. Nutmeg root extraction was obtained by flowing distilled water to remove the dirt and followed by oven dry for overnight. Subsequently, the dried nutmeg root was blend and the powder of 483 g was macerated with n-hexane for 24 h. After that, the solution was filtered and evaporated with a rotary evaporator. As a result, the extract of n-hexane nutmeg root was obtained.

2.2. Isolation of n-hexane extract of nutmeg root

Nutmeg root n-hexane extract 4.35 g was further isolated using column chromatography, using 60 G silica gel as the stationary phase and then the eluent was determined by thin layer chromatography (TLC). The n-hexane extract was separated by its components and each fraction was collected in a test tube.

2.3. Antioxidant and anticancer activity test

The antioxidant activity of the extract was carried out by the free radical DPPH method where vitamin C (0.01–0.1 mg) was used as a reference according to previous report [6]. To investigate the anticancer activity of n-hexane extract of nutmeg root, the breast cancer (MCF-7) cells was obtained from Animal Study Center in Institut Pertanian Bogor, Indonesia according to ATCC protocols [7] has been carried out at various concentrations of 25, 50, 100, and 200 ppm. As a positive control, the doxorubicin was employed, where the cells were grown at a concentration of 5000 cells in 100 µL growth medium. The extract was added after the cells reached 50% confluence (24 h). The 3–4, 5-dimethylthiazol-2-yl-2, 5-diphenyltetrazolium bromide (MTT) assay test was carried out on day 3, by adding MTT concentration of 5 mg / mL in 10 µl per tube and incubated for 4 h at 37 °C. Then, the formazan crystals are dissolved in ethanol. The absorbance reading was carried using VersaMax Elisa Microplate Reader at a wavelength of 595 nm.

2.4. Characterizations

The structure of the isolated compound was determined by NMR (1H and 13C-on JEOL 500 spectrometer; 500 MHz for 1H NMR; 125 MHz for 13C NMR). UV–vis spectra were obtained using a Shimadzu Variant Cary 100. Fourier transform infrared spectroscopy (FTIR) analysis was perform using Perkin Elmer FTS FT-IR spectrometer. EI-MS mass spectra by LC-MS / MS (HPLC Alliance 2695, Waters Detector Photodiode Array 2996).

Ethics statement

None

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2020.105997.

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