Lignan (+)-Piperitol-γ,γ-Dimethylallylether from Stem Bark of Zanthoxylum rhetsa (Roxb.) DC (Rutaceae)

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Abstract

Lignans are a group of phenylpropanoid dimers in which the phenylpropane units are linked by their propyl side chains' central carbon (C8). Lignans have various biological activities, including antiviral, anticancer, cancer preventive, and cytotoxic. Zanthoxylum rhetsa (Roxb.) DC is a tree with prickly branchlets belonging to the Zanthoxylum genus, commonly known as panggal buaya in Indonesia. Asian tribes have used this plant as traditional medicine. In this study, (+)-piperitol-γ,γ-dimethylallylether, a furofuran lignan, was successfully isolated. The chemical structure of compound 1 was determined based on spectroscopic data, including 1D- and 2D-NMR, mass spectroscopy, and by comparing with previous spectral data. In addition, compound 1 was tested for its cytotoxic activity against MCF-7 breast cancer cell lines in vitro and showed weak activity with the IC50 value of 261.37 µg/mL.

Keywords: Furofuran lignan, MCF-7, (+) piperitol-γ,γ-dimethylallylether, Rutaceae, Zanthoxylum rhetsa

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1. INTRODUCTION

A phenylpropanoid dimer is formed by two phenylpropane units where the central carbon (C8) in the first unit links the propyl side chain in the second unit, generating a lignan compound (Sarajlija et al., 2012). The diversity of the lignan consists of eight main frameworks: furofuran, furan, dibenzylbutane, dibenzylbutirolactol, dibenzylbutirolactone, aryl tetraline, aryl naphthalene, and dibenzocyclooctadiene, as well as several unique frameworks known as neolignans (Wang et al., 2022). Lignans have various biological activities, including antiviral and anticancer (Yousefzadi et al., 2010), cancer preventive (Huang et al., 2010), and cytotoxic (Mukhija et al., 2014; Su et al., 2015).

Lignans can be found in more than 70 plant families and have been isolated from several plant parts (Pan et al., 2009), especially the Rutaceae family. One of the genera in the Rutaceae family with abundant lignan content is the Zanthoxylum genus (Wang et al., 2022). Zanthoxylum rhetsa is a big “Panggal buaya” tree in Indonesia. In Indian tribes, the plant treats many infirmities, such as diabetes, inflammation, rheumatism, toothache and diarrhea (Santanam et al., 2016). The methanol extract of the seed and root of Z. rhetsa showed antioxidant and antibacterial activity (Zohora et al., 2019; Hayat & Vandra, 2018). In addition, the Z. rhetsa stem extract gave significant anti-inflammatory activity (Parthiban et al., 2017). The previous phytochemical studies reported the presence of alkaloids, flavonoids, glycosides, saponin, tannins and terpenoids in Z. rhetsa (Kyaw et al., 2020; Mallya and Bhitre, 2020).
Furthermore, other secondary metabolites found in the *Z. rhetsa* bark are lignans which have a skeleton of furofuran, asarinin and hortsetinol and showed strong antibacterial activity against *Staphylococcus aureus* (Tantapakul *et al.*, 2012).

As part of our research on Indonesian Rutaceae plants, herein, we report the isolation, and characterization of a furofuran lignan, established as (+)-piperitol-γ,γ-dimethylallylster (1), and its cytotoxic activity assay against breast cancer MCF-7 cell lines.

2. MATERIALS AND METHODS

General

UV spectra were measured using a TECAN Infinite M200 pro with MeOH, and IR spectra were recorded on a SHIMADZU IR Prestige-21 in KBr. The mass spectra were obtained with Waters Q-TOF Xevo mass spectrometer instrument. NMR spectra were obtained on a Bruker Toppin spectrometer instrument.

METHODS

Plant Material

The stem bark of *Z. rhetsa* (Roxb) D.C. was collected in September 2018 at Bogor Botanical Garden, Bogor, West Java Province, Indonesia. The plant was identified by the staff of the Bogorienese Herbarium, Bogor, Indonesia (No. B-816).

Extraction and Isolation

The air-dried and powdered *Z. rhetsa* stem bark (5.5 kg) was extracted with methanol (36 L) at room temperature. The methanol (MeOH) extract was evaporated in a vacuum rotary evaporator to yield a concentrated MeOH extract (593.40 g). The MeOH extract was successfully dissolved in water and partitioned with *n*-hexane, methylene chloride (CH₂Cl₂), and *n*-butanol (n-BuOH). Evaporation of the solvent resulted in the concentrated extract of *n*-hexane (87.90 g), CH₂Cl₂ (73.30 g), and n-BuOH (121.50 g).

The CH₂Cl₂ soluble fraction (73.30 g) was separated by vacuum liquid chromatography (VLC) on silica gel 60 with *n*-hexane-EtOAc-MeOH as a solvent system to give five fractions (A-E). The B fraction (14.20 g) was further separated by vacuum liquid chromatography on silica gel G₆₀ with *n*-hexane-ethyl acetate 10% stepwise to give five subfractions (B1-B5). Subfraction B3 (1.17 g) was separated by vacuum liquid chromatography on silica gel G₆₀ with *n*-hexane-ethyl acetate 5% stepwise to yield four subfractions (B3a-B3d). Subfraction B3c (213.60 mg) was column chromatographed on silica gel with *n*-hexane-EtOAc (8.0:2.0) isocratic to give five subfractions (B3c1-B3c5). Subfraction B3c3 (48 mg) was separated by preparative thin layer chromatography (PTLC) on silica gel GF₂₅₄ with *n*-hexane-EtOAc (7.5:2.5) as a solvent system to give compound 1 (9.5 mg).

Spectroscopic Data of Compound 1

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viability and quantitatively proliferation of various resazurin-based cell types using live cell reduction capabilities. When cells are alive and healthy, they maintain a reduced environment in their cytosol. The reduction of resazurin (blue) works as a cell viability indicator by using absorbance or utilizing fluorescent outputs to reduce resorufin (purple). The conversion is proportional to the number of metabolically active cells. Briefly, MCF-7 cell lines were grown in 70% confluent, harvested and counted, and then diluted with complete culture RPMI medium. The cells were then transferred into 96 well plates with 170,000 cells/well. After overnight growth, the cells were treated with increasing compound 1 concentration (7.81, 15.63, 31.25, 62.50, 125, 250, 500, 1000 ppm) with co-treatment for 24 hours. After incubation, the medium was immediately replaced by 10 μL PrestoBlue reagent in 90 μL RPMI medium. The plates were incubated for 1-2 hours until resorufin was formed (colour changes from blue to purple). The absorbance was measured at 570 nm using a microplate reader. The IC50 value is the concentration for 50% growth inhibition. The percentage of cytotoxicity compared to untreated cells was calculated with the equation below. A plot of % cytotoxicity versus sample concentrations was used to calculate the concentration, which showed 50% cytotoxicity (IC50) (Camarillo et al., 2014; Machana et al., 2011). All assays and analyses were each runs in duplicate, and all were averaged.

3. RESULTS AND DISCUSSION

The concentrated MeOH extract (593.40 g) from the stem bark of Z. rhetsa was fractionated with n-hexane, methylene chloride (CH2Cl2), and n-butanol (n-BuOH) successively to yield the concentrated extract of n-hexane (87.90 g), CH2Cl2 (73.30 g), and n-BuOH (121.50 g). The CH2Cl2 fraction (73.30 g) was subjected to silica gel 60 by vacuum liquid chromatography (VLC) with n-hexane-EtOAc-MeOH as a solvent system to afford five fractions (A-E). The B fraction (14.20 g) was further separated according to the Thin Layer Chromatography (TLC) profile suggesting the presence of aromatic compounds, whose structurally gave fluorescence under UV light 254 nm. Separation with gradient technique 5% using VLC and isocratic elution of n-hexane-EtOAc (7:5:2.5) using preparative thin layer chromatography (PTLC) on silica gel GF254 were applied to give compound 1 (9.5 mg).

Compound 1, was isolated as a yellow gummy. The molecular formula was established by HR-TOF-MS as C23H25O8 from the molecular ion peak of HR-TOF-MS m/z 447.1787 [M+Na]+ (calcd. for C23H25O8Na, m/z 447.1784), indicating twelve degrees of unsaturation. The FTIR spectra indicated the presence of aliphatics (2962 and 2870 cm⁻¹), aromatic double bond (1590 cm⁻¹), gem-dimethyl (1443, 1382 cm⁻¹), and ether groups (1096 cm⁻¹). Furthermore, compound 1 showed the characteristic absorbance of phenolic compound, UV λmax nm (log ε) 209 (4.06), 232 (3.82), 285 (3.44) (Sianturi et al., 2016; Shiono et al., 2013). The 1H NMR showed an oxygenmethylene group at δ4.24 (2H, dt, J =5.6 Hz, H-9/H-9'), oxygenmethine group at δ4.73 (2H, dd, J =9.2, 4.2 Hz, H-7/H-7'), and methine group δ3.08 (2H, m, H-8/H-8'), suggesting the presence of a furofuran moiety (Arruda et al., 1994). In addition, the 1H NMR spectrum also gave five signals for six aromatic protons as two ABX system at δ8.69 (1H, s, H-2), 6.84 (1H, d, J=6.8 Hz, H-5), 6.84 (1H, dd, J=6.8 Hz, H-6), and δ8.65 (1H, d, J=2.0 Hz, H-2'), 6.78 (1H, d, J =6.7 Hz, H-5'), and 6.81 (1H, dd, J=6.7, 2.0 Hz, H-6') representing the presence of 1,3,4-trisubstituted benzene ring. These signals strengthen the existence of lignan skeleton (Arruda et al., 1994).

The 13C NMR and DEPT 135° (125 MHz, CDCl3) with the aid of an HMBC spectrum revealed 25 carbon signals, corresponding to three methyls (one oxygenated) at δC (18.2, 25.8, 55.9), three oxygenated methylenes at δC (65.8, 71.7, 71.7), one methylenedioxy at δC (101.0), 11 methines including two oxygenated at δC (85.8, 85.8), seven olefinic groups at δC (106.5, 108.2, 109.4, 112.8, 118.1, 119.3 and 119.9), two carbons at δC (54.1 and 54.3), seven olefinic quaternary carbons at δC (133.4, 135.1, 137.6, 147.1, 147.8, 147.9 and 149.6). The above data accounted for seven unsaturated degrees and required compound 1 to possess five additional rings, indicating that 1 is a tetracyclic lignan derivatives with one cyclic outside in the main
framework, namely lignan furofuran-type. The characteristic chemical shifts of lignan furofuran-type was proved by the presence of two furan rings including two oxymethylene carbon at C-9 (δC 71.7)/C-9′(δC 71.7) ppm and two oxymethine carbon at C-7 (δC 85.8)/C-7′ (δC 85.2) ppm. The HMBC spectrum also confirmed the presence of furofuran group located at C-1/C-1′ by the correlations from H-7 to C-2, C-6, C-8, H-7′ to C-2′, C-6′, and H-9′ to C-8′, as well as by the 1H-1H COSY data for the spin coupling segments of H-7/H-8/H-9 and H-7′/H-8′/H-9′ (Fig.1). Furthermore, a prenylxy group was deduced to be positioned at C-4 on the basis of HMBC correlations of H-4′ with C-5, C-3′, H-5′ with C-4′, C-3′, H-2′ with C-5′, and H-1′ with C-3′, C-4′. The long-range correlations of -O-CH2-O at δH 5.95 (2H, s) to C-3′, C-4′ supported the establishment of a methylenedioxy between C-3′ and C-4′, while the presence of methoxy group at C-3 was evidenced by the correlation of -OCH3 at δH 3.88 (3H, s) to C-3.

Subsequently, the 1H-1H COSY cross-peaks of H-5/H-6, H-5′/H-6′, and H-1′/H-2′ was also observed (Fig. 1), thereby generating the planar structure of compound 1 (Fig. 1).

Based on the analysis and the complete elucidation of 1D- and 2D-NMR. Compound 1 was established as the known compound (+)-piperitol-γ,γ-dimethylallylether along with the stereochemistry of each stereocenter carbon, owing to the chemical shift of 1H and 13C NMR of 1 which was extremely same as the literature (Vaquette et al., 1979; Arruda et al., 1994; Nissanka et al., 2001) (Fig. 3). The positive (+)-stereochernistry of compound 1 was deduced by the following optical rotation values: [α]D25+25° (Nissanka et al., 2001) and [α]D25+21° (Vaquette et al., 1979). In addition, compound 1 was reported for the first time from Z. rhesa species.

Table 1. 1H-NMR (500 MHz) and 13C-NMR (125 MHz) CDCl3 of compound 1 and related data

| No. | Compound 1       | (+)-piperitol-γ,γ-dimethylallylether |
|-----|------------------|-------------------------------------|
|     | 13C-NMR (δC/ppm) | 1H-NMR (δH (Mult, J/Hz))           | 13C-NMR (δC/ppm) literature* |
| 1   | 133.5 (s)        | -                                   | 133.4                           |
| 2   | 109.4 (d)        | 6.89 (1H, s)                        | 109.4                           |
| 3   | 147.9 (s)        | -                                   | 147.9                           |
| 4   | 149.6 (s)        | -                                   | 149.6                           |
| 5   | 112.9 (d)        | 6.84 (1H, d, 6.8)                   | 112.8                           |
| 6   | 118.1 (d)        | 6.84 (1H, d, 6.8)                   | 118.1                           |
| 7   | 85.8 (d)         | 4.73 (1H, t, 9.2)                   | 85.8                            |
| 8   | 54.2 (d)         | 1H (3.08, m)                        | 54.1                            |
| 9   | 71.7 (t)         | 4.24 (2H, dt, 5.6, 11)              | 71.7                            |
| 1′  | 135.1 (s)        | -                                   | 135.1                           |
| 2′  | 106.5 (d)        | 6.85 (1H, d, 1.2)                   | 106.5                           |
| 3′  | 147.2 (s)        | -                                   | 147.1                           |
| 4′  | 147.8 (s)        | -                                   | 147.8                           |
| 5′  | 108.2 (d)        | 6.78 (1H, d, 6.6)                   | 108.2                           |
| 6′  | 119.4 (d)        | 6.81 (1H, dd, 5.6, 1.1)             | 119.3                           |
| 7′  | 85.8 (d)         | 4.73 (1H, t, 9.2)                   | 85.8                            |
| 8′  | 54.3 (d)         | 3.08 (1H, m)                        | 54.3                            |
| 9′  | 71.7 (t)         | 4.24 (2H, dt, 5.6, 11)              | 71.7                            |
| 1″  | 65.8 (t)         | 4.57 (2H, d, 5.5)                   | 65.8                            |
| 2″  | 119.9 (d)        | 5.51 (1H, dd, 6.7, 2.7)             | 119.9                           |
| 3″  | 137.7 (s)        | -                                   | 137.6                           |
| 4″  | 18.3 (q)         | 1.72 (3H, s)                        | 18.2                            |
| 5″  | 25.8 (q)         | 1.76 (3H, s)                        | 25.8                            |
| 3-OCH3 | 55.9 (q)       | 3.88 (3H, s)                        | 55.9                            |
| O-CH2-O | 101.1 (t)     | 5.95 (2H, s)                        | 101.0                           |

*13C-NMR 50 MHz CDCl3, no 1H-NMR data (Arruda et al., 1994)
4. CONCLUSIONS

In summary, we report the known lignan furfuran-type (+)-piperitol-γ,γ-dimethylallyl ether (1). Compound 1 was isolated from the methylene chloride (CH2Cl2) extract of Z. rhetsa stem bark. The elucidation structure of compound 1 was determined based on the 1D- and 2D-NMR, as well as comparison with the previous spectral data. Furthermore, the (+)-stereochemistry of compound 1 was established based on the reported positive optical rotation values. In this study, compound 1 was evaluated for its cytotoxic activity against the MCF-7 breast cancer cell line, showing weak inhibition with an IC50 value of 261.37 μg/mL. This compound was first reported from the Zanthoxylum rhetsa species to the best of our knowledge.

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