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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 (C_8). 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 IC_{50} 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. rhetza* showed antioxidant and antibacterial activity (Zohora *et al.*, 2019; Hayat & Vandna, 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 horsfieldine 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-dimethylallylether (**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 Topspin spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C using tetramethylsilane (TMS) as an internal standard. Silica gel G_{60} (Merck) 70-230 mesh for crude separation and 200-400 mesh for purification using column chromatography. Thin Layer Chromatography (TLC) plates were precoated with silica gel GF_{254} (Merck, 0.25 mm), and detection was achieved by spraying with 10% H_2SO_4 in ethanol, followed by heating and irradiated under UV light at wavelength 254 and 365 nm.

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 Bogoriense 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 (CH_2Cl_2), and *n*-butanol (n-BuOH). Evaporation of the solvent resulted in the concentrated extract of *n*-hexane (87.90 g), CH_2Cl_2 (73.30 g), and *n*-BuOH (121.50 g). The CH_2Cl_2 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 G_{60} 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 GF_{254} with *n*-hexane-EtOAc (7.5:2.5) as a solvent system to give compound **1** (9.5 mg).

Spectroscopic Data of Compound 1

Compound **1,** yellow *gum oily*, $[\alpha]_D$ + 80.9 ($CHCl_3$, *c* 0.00037). UV λ_{max} nm (log ε) 209 (4.06), 232 (3.82), 285 (3.44). IR ν_{max} 3440, 2962, 2870, 1590, 1443, 1382, 1115 cm^{-1}. HR-TOFMS *m/z* 447.1787 $[M+Na]^+$, (calcd. $C_{25}H_{28}O_6Na$, *m/z* 447.1784). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 6.89 (1H, *s*, H-2), 6.84 (1H, *d*, *J*=6.85 Hz, H-5), 6.84 (1H, *d*, *J*=6.85 Hz, H-6), 4.73 (1H, *t*, *J*=9.2 Hz, H-7), 3.08 (1H, *m*, H-8), 4.24 (2H, *dt*, *J*=5.6, 11.0 Hz, H-9), 6.85 (1H, *d*, *J*=1.25 Hz, H-2′), 6.78 (1H, *d*, *J*=6.65 Hz, H-5′), 6.81 (1H, *dd*, *J*=5.65, 1.05 Hz, H-6′), 4.73 (1H, *t*, *J*=9.2 Hz, H-7′), 3.08 (1H, *m*, H-8′), 4.24 (2H, *dt*, *J*=5.6, 11.0 Hz, H-9′), 4.57 (2H, *d*, *J*=5.55 Hz, H-1′′), 5.51 (1H, *dd*, *J*=6.68, 2.72 Hz, H-2′′), 1.72 (3H, *s*, CH_3-4′′), 1.76 (3H, *s*, CH_3-5′′), 3.88 (3H, *s*, 3-OCH_3), 5.95 (2H, *s*, O-CH_2-O); ^{13}C-NMR ($CDCl_3$, 125 MHz): *δ*c 133.4 (C-1), 109.4 (C-2), 147.9 (C-3), 149.6 (C-4), 112.8 (C-5), 118.1 (C-6), 85.8 (C-7), 54.1 (C-8), 71.7 (C-9), 135.1 (C-1′), 106.5 (C-2′), 147.1 (C-3′), 147.8 (C-4′), 108.2 (C-5′), 119.3 (C-6′), 85.8 (C-7′), 54.3 (C-8′), 71.7 (C-9′), 65.8 (C-1′′), 119.9 (C-2′′), 137.6 (C-3′′), 18.2 (CH_3-4′′), 25.8 (CH_3-5′′), 55.9 (3-OCH_3), 101.0 (O-CH_2-O) (**Table 1**).

Cytotoxic Activity

Cell viability was assessed with Presto Blue reagent (Thermo Fisher Scientific, Uppsala, Sweden) to rapidly evaluate the

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-solvent 2% (v/v) DMSO in PBS. Cisplatin was used as the positive control. All samples were incubated at 37 °C in a 5% CO_2 incubator 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 IC_{50} value is the concentration for 50% growth inhibition. The percentage of cytotoxicity compared to untreated cells was determined with the equation below. A plot of % cytotoxicity versus sample concentrations was used to calculate the concentration, which showed 50% cytotoxicity (IC_{50}) (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 (CH_2Cl_2), and *n*-butanol (*n*-BuOH) successively to yield the concentrated extract of *n*-hexane (87.90 g), CH_2Cl_2 (73.30 g), and *n*-BuOH (121.50 g). The CH_2Cl_2 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 GF_{254} 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 $C_{25}H_{28}O_6$ from the molecular ion peak of HR-TOF-MS *m/z* 447.1787 $[M+Na]^+$ (calcd. for $C_{25}H_{28}O_6Na$, *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 λ_{max} nm (log ε) 209 (4.06), 232 (3.82), 285 (3.44) (Sianturi *et al.*, 2016; Shiono *et al.*, 2013). The ^{1}H NMR showed an oxymethylene group at δ_H 4.24 (2H, *dt* , *J*=5.6 Hz, H-9/H-9'), oxymethine group at δ_H 4.73 (2H, *dd*, *J*=9.2, 4.2 Hz, H-7/H-7'), and methine group δ_H 3,08 (2H, *m*, H-8/H-8'), suggesting the presence of a furofuran moiety (Arruda *et al.*, 1994). In addition, the ^{1}H NMR spectrum also gave five signals for six aromatic protons as two ABX system at δ_H 6.89 (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 δ_H 6.85 (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 ^{13}C NMR and DEPT 135° (125 MHz, $CDCl_3$) with the aid of an HMQC spectrum revealed 25 carbon signals, corresponding to three methyls (one oxygenated) at δ_C (18.2, 25.8, 55.9), three oxygenated methylenes at δ_C (65.8, 71.7, 71.7), one methylenedioxy at δ_C (101.0), 11 methines including two oxygenated at δ_C (85.8, 85.8), seven olefinic groups at δ_C (106.5, 108.2, 109.4, 112.8, 118.1, 119.3 and 119.9), two carbons at δ_C (54.1 and 54.3), seven olefinic quaternary carbons at δ_C (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 (δ_C 71.7)/C-9'(δ_C 71.7) ppm and two oxymethine carbon at C-7 (δ_C 85.8)/C-7' (δ_C 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 ^{1}H-^{1}H 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 prenyloxy 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-CH_2-O- at δ_H 5.95 (2H, *s*) to C-3', C-4' supported the establishment of a methylendioxy between C-3' and C-4', while the presence of methoxy group at C-3 was evidenced by the correlation of -OCH_3 at δ_H 3.88 (3H, *s*) to C-3. Subsequently, the ^{1}H-^{1}H 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 ^{1}H and ^{13}C 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 (+)-stereochemistry of compound **1** was deduced by the following optical rotation values: $[\alpha]_D^{25}$ +25° (Nissanka *et al.*, 2001) and $[\alpha]_D^{25}$ +21° (Vaquette *et al.*, 1979). In addition, compound **1** was reported for the first time from *Z. rhetsa* species.

Table 1. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) $CDCl_3$ of compound **1** and related data

No.	Compound 1		(+)-piperitol-γ,γ-dimetilalileter
	^{13}C-NMR (δ_C/ppm)	^{1}H-NMR (δ_C (Mult, *J*/Hz))	^{13}C-NMR (δ_C/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, 11.0)	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-OCH_3	55.9 (*q*)	3.88 (3H, *s*)	55.9
O-CH_2-O	101.1 (*t*)	5.95 (2H, *s*)	101.0

*^{13}C-NMR 50 MHz $CDCl_3$, no ^{1}H-NMR data (Arruda et al., 1994)

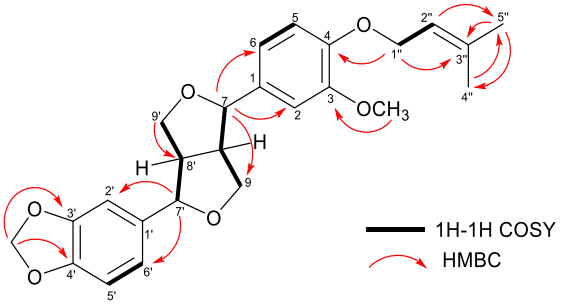

Figure 1. Key HMBC and ^{1}H-^{1}H COSY correlations of compound **1**.

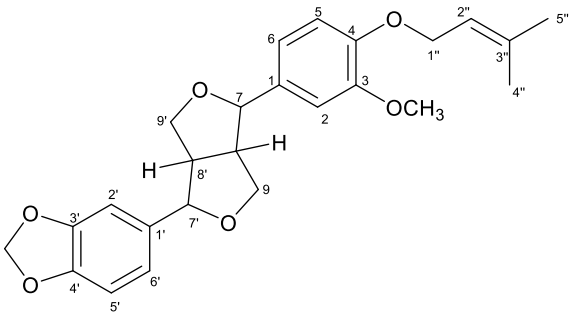

Figure 2. The planar structure of compound **1**

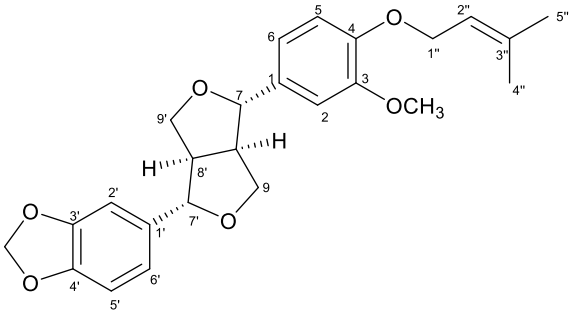

Figure 3. Structure of compound **1.**

The cytotoxic activity of the isolated compound **1** was evaluated against the MCF-7 breast cancer cell lines according to a method described (Xu *et al.*, 2015). Cisplatin (53.0 µg/mL) was used as the positive control. Compound **1** showed weak activity on MCF-7 cells with IC_{50} 261.37 µg/mL (Weerapreeyakul *et al.*, 2012).

Previously, the furofuran lignans were isolated from the roots of *Z. planispinum* showed the strongest inhibitory effect on the growth of HL-60 and PC-3 (Su *et al.*, 2015). Furofuran lignans have a variety of structures due to different substituents at aryl groups and diverse configurations at furofuran ring. They exhibit a wide range of significant biological activities, including antioxidant, anti-inflammatory, and cytotoxic (Xu *et al.*, 2018).

4. CONCLUSIONS

In summary, we report the known lignan furfuran-type (+)-piperitol-γ,γ-dimethylallylether (**1**). Compound **1** was isolated from the methylene chloride (CH_2Cl_2) extract of *Z. rhetsa* stem bark. The elucidation stucture 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 IC_{50} 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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REFERENCE

Arruda, M. S. P., Fernandes, J. B., Vieira, P. C., Da Silva, M. F., & Pirani, J. R. (1994). Protolimonoid and lignans from *Zanthoxylum petiolare***.** *Phytochemistry*. 36.(5), 1303-1306.

Camarillo, I. G., Xiao, F., Madhivanan, S., Salameh, T., Nichols, M., Reece, L. M., Leary, J. F., Otto, K. J., Natarajan, A., Ramesh, A., & Sundararajan, R. (2014). *Low and high voltage electrochemotherapy for breast cancer: An in vitro model study in Electroporation-Based Therapies for Cancer*. Eds. Sundararajan. R. Woodhead Publishing. Cambridge; United Kingdom.

Hayat, M., & Vandna, K. (2018). A review on medicinal properties of *Zanthoxylum armatum* DC. *Research Journal of Pharmacy and Technology* 11(5), 2131-2138.

Huang, W. Y., Cai, Y. Z., & Zhang, Y. (2010). Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. *Nutrition and Cancer*. 62 (1), 1-20.

Kyaw, C. M., Lae, K. Z. W., & Ngwe, D. H. (2020).

Investigation of phytochemical constituents and some biological activities of the spine of *Zanthoxylum rhetsa* (Roxb.) Dc.(Ka-Thit-Phu). *Journal of the Myanmar Academy of Arts and Science*. XVIII (1B), 103-116.

Machana, S., Weerapreeyakul, N., Barusrux, S., Nonpunya, A., Sripanidkulchai, B., & Thitimetharoch, T. (2011). Cytotoxic and apoptotic effect of six herbal plants against the human hepatocarcinoma (HepG2) cell line, *Chinese Medicine*, 6 (39), 1–8.

Mallya, R., & Bhitre, M. J. (2020). Cytotoxic activity and initiation of apoptosis via intrinsic pathway in jurkat cells by leaf extract of *Zanthoxylum rhetsa* DC. *Nutrition and Cancer*. 1-12.

Mukhija, M., Dhar, K. L., & Kalia, A. N. (2014). Bioactive lignans from *Zanthoxylum alatum* Roxb. stem bark with cytotoxic potential. *Journal of Ethnopharmacology*. 152, 106–112.

Nissanka, A. P., Karunaratne, V., Bandara, B. R., Kumar, V., Nakanishi, T., Nishi, M., Inada, A., Tillekeratne, L. M., Wijesundara, D. S., & Gunatilaka, A. L. (2001). Antimicrobial alkaloids from Zanthoxylum tetraspermum and caudatum. *Phytochemistry*. 56(8), 857-61.

Pan, J. Y., Chen, S. L., Yang, M. H., Wu, J., Sinkkonen, J., & Zou, K. (2009). An update on lignans: natural product and synthesis. *Natural Product Reports.* 26 (10), 1221-1352.

Parthiban, S., Kumar, K. G., Boopathi, T., Sangeetha, G., Kokila, T., & Devan, V. S. (2017). In vitro anti inflammatory activity of stem of *Zanthoxylum Rhetsa* (Roxb.) DC. *World Journal of Pharmaceutical Research*. 6 (7), 591-600.

Santhanam, R. K., Ahmad, S., Abas, F., Safinar, S., Ismail, I., Rukayadi, Y., Tayyab, A. M., & Shaari, K. (2016). Bioactive constituents of *Zanthoxylum rhetsa* bark and its cytotoxic potential against B16-F10 melanoma cancer and normal human dermal fibroblast (HDF) cell lines. *Molecules.* 21(6), 652.

Sarajlija, H., Čukelj, N., Novotni, D., Mršić, G., Brnćić, M., & Čurić, D. (2012). Preparation of flaxseed for lignan determination by gas chromatography-mass spectrometry method. *Czech Journal Food Scince*. 30(1), 45–52.

Shiono, Y., Sasaki, T., Shibuya, F., Yasuda, Y., Koseki, T., & Supratman, U. (2013). Isolation of a phomoxanthone A derivative, a new metabolite of tetrahydroxanthone, from a Phomopsis sp. Isolated from the mangrove, *Rhizhopora mucronata*. *Natural Product Communications*. 8(12), 1735-1737.

Sianturi, J., Harneti, D., Darwati, D., Mayanti, T., Supratman, U., & Awang, K. (2016). A new (-)-5',6-dimethoxyisolariciresinol-(3",4"-dimethoxy)-3α-*O*-β-D-glucopyranosides from the bark of *Aglaia eximia* (Meliaceae). *Natural Product Research.* 30(19): 2204–2208.

Su, G. Y., Wang, K. W., Wang, X. Y., & Wu, B. (2015). Bioactive lignans from Zanthoxylum planispinum with cytotoxic potential. *Phytochemistry Letters.* 11, 120–126.

Tantapakul, C., Phakhodee, W., Ritthiwigrom, T., Yossathera, K., Deachathai, S., & Laphookhieo, S. (2012). Antibacterial compounds from *Zanthoxylum rhetsa*. *Archives of pharmacal research.* 35(7). 1139-1142.

Vaquette, J., Cavé, A., Waterman, & P., G. (1979). Alkaloids, Triterpenes and Lignans from *Zanthoxylum sp. Sevenet* 1183[1]–a Species Inigenous to New Caledonia[2]. *Planta Medica*. 35(01), 42-7.

Wang, L. X., Wang, H. L., Huang, J., Chu, T. Z., Peng, C., Zhang, H., Chen, H. L., Xiong, Y. A., & Tan, Y. Z. (2022). Review of lignans from 2019 to 2021: Newly reported compounds, diverse activities, structure-activity relationships and clinical applications. *Phytochemistry*. 14(202):113326.

Weerapreeyakul, N., Nonpunya, A., Barusux, S., Thitimetharoch, T., & Sripanidkulchai, B. (2012). Evaluation of the anticancer potential of six herbs against a hepatoma cell line, *Chinese Medicine*, 7: 15.

Xu, M., McCanna, D. J., & Sivak, J. G. (2015). Use the viability reagent PrestoBlue in comparison with alamarBlue and MTT to assess the viability of human corneal epithelial cells, *Journal of Pharmacology and Toxicology Methods*. 71, 1–7.

Xu, W. H., Zhao, P., Wang, M., & Liang, Q. (2018). Review: Naturally occurring furofuran lignans: structural diversity and biological activities, *Natural Product*

Research. 1-17.

Yousefzadi, M., Sharifi, M., Behmanesh, M., Moyano, E., Bonfill, M., Cusido, R. M., & Palazon, J. (2010). Podophyllotoxin: Current approaches to its biotechnological production and future challenges. *Engineering in Life Sciences*. 10 (4). 281-292.

Zohora, F. T., Islam, S. N., Khan, S. A., Hasan, C. M., & Ahsan, M. (2019). Antioxidant, cytotoxic, thrombolytic and antimicrobial activity of *Zanthoxylum rhetsa* root bark with two isolated quinolone alkaloids. *Pharmacology & Pharmacy*. 10, 137-145.