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Lignans from *Phyllanthus niruri* L. and Their Antifusarium Properties

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Abstract

In this study, two lignan compounds were isolated from acetone extract of *Phyllanthus niruri* L. nirtetralin B (**1**) and phyllanthin (**2**) using several chromatographic methods followed by molecular structure elucidation mainly based on 1D and 2D of ^{1}H and ^{13}C NMR spectrum. The isolated compounds were tested for their antimicrobial properties against the plant pathogenic fungus, *Fusarium oxysporum,* using the agar plate well diffusion method. The microdilution method determined the minimum inhibitory concentration (MIC) and the minimum fungicide concentration (MFC). In addition, the microconidia germination inhibition test was carried out using the agar diffusion method. As a result, compound 1 had MIC and MFC values of 4 and 16 µg/mL, respectively. While compound **2** showed the same MIC and MFC values of 16 µg/mL. Further testing on the inhibition of germination of *F. oxysporum* microconidia showed that compound **2** inhibited microconidia germination 100% at a concentration of $2 \times$ MIC. In comparison, compound **1** at the same concentration was only able to inhibit germination by 29%. This study revealed that compound **2** is a potential new fungicide derived from local medicinal plants. However, further research is needed to identify the interaction mechanism between the test compound and the fungal pathogen *F. oxysporum* to develop new antifungal agents.

Keywords: Antifungal*, F. oxysporum,* medicinal plant, microconidia germination, *Phyllanthus niruri.*

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1. INTRODUCTION

Indonesia has a variety of plants that can be used as medicinal plants (Saifudin *et al.,* 2011; Miksusanti *et al.,* 2009). One of the medicinal plants that have the potential to be developed is green meniran. This herbaceous plant with the scientific name *Phyllanthus niruri* Linn. is a member of the Euphorbiaceae family that grows wild in damp and rocky places, such as bushes and soil among grasses. *P. niruri* has been widely used as traditional medicine (Bana *et al.,* 2016; Tjandrawinata *et al.,* 2017; Ervina & Mulyono, 2019; Destryana & Ismawati, 2019; Qasrin *et al.,* 2020). These plant species produce one or more bioactive compounds that are believed to have medicinal properties and are useful for treatment so that they are used as medicinal entities. In addition, it can contain many compounds that can serve as chemical models for new drug design, synthesis, or molecular semi-synthetics (Tjandrawinata *et al.,* 2017; Baehaki *et al.,* 2021). Bioactive compounds or secondary metabolites are standard compounds contained in a plant species (Alam *et al.,* 2016). The compounds found in *P. niruri* include flavonoids, alkaloids, triterpenes, tannins, lignans, polyphenols, and sterols (Bagalkotkar *et al.,* 2006; Paithankar *et al.,* 2011; Murugaiyah & Chan, 2006; Rivai *et al.,* 2013). These compounds play a role in various therapeutic activities, including antiviral, antimicrobial, anti-hepatic, antitumor, and antidiabetic (Paithankar *et al.,* 2011).

This plant has several names in several countries including *meniran* (Indonesia),

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dukung anak (Malay), *sampa sampalukan* (Philippines), Child pick a back (England) and *zhen chu cao* or *ye xia zhu* (China), *kilaneli* (India), and *arrebenta pedira* (Brazil). While in some areas in Indonesia, the names for this plant include *meniran* (Java), *gasau madungi* (Ternate), *memeniran* or *beunyeur* (Sunda), *baket sikolop* (Sumatra), *sidukung anak* (Sulawesi), *belalang babiji* (Maluku) and others. An ethnobotanical study on the potential of *P. niruri* L., especially in Indonesia, including as a snake-like drug or herpes zoster in the tradition of the Ngaju Dayak Tribe, Central Kalimantan (Ervina & Mulyono, 2019), facilitates the birthing process for the Kaili Rai community in Taripa Village, Sindue District, Donggala Regency, Sulawesi. Central (Bana, Khumaidi, & Pitopang, 2016), fever medicine in Banyuasin III District, South Sumatra (Awaliyah, 2018), medicine for urinary stones and diabetes in Huilelot Village and Uiasa Village, Semau District, Kupang Regency (Nomleni, Daud, & Tae, 2021), lowers blood pressure in the Dayak Tribe Pope and Malay for post-delivery maternal and child care in Pengadang Village, Sanggau Regency, West Kalimantan (Pradita *et al.*, 2021), diarrhoea and ulcers in North Sumatra (Amrul *et al.*, 2019), post-natal wound healing medicine in the Pulo Nasi Pulo Aceh settlement (Hafnidar, 2019).

The various properties of *P. niruri* cannot be separated from the content of secondary metabolites in it. Many secondary metabolites have been identified in *P. niruri*, including flavonoids, alkaloids, triterpenes, tannins, lignans, polyphenols, and sterols (Bagalkotkar *et al.,* 2006; Murugaiyah & Chan, 2006). Regarding the richness of *P. niruri* metabolites, its various therapeutic activities include antiviral, antimicrobial, anti-hepatic, antitumor, and antidiabetic (Paithankar *et al.,* 2011). Flavonoids in this plant include rutin; gallocatechin; quercetin 3-O-glucopyranoside; quercetin; kaempferol 3-O-d-glucopyranoside, and kaempferol. Ellagitannins were also identified in *P. niruri*; like geraniin; furosin; amariin; amarinic acid; geraniinic acid B; repanducinic acid A; amarulone; corilagin; elaeocarpusin; phyllanthusion A, B, C and D; isochorylamine; and melatonin (Sarin *et al.,* 2015; Mellado *et al.,* 2008; Paithankar *et al.,* 2011). The presence of these compounds in *P. niruri* plants is very interesting for researchers to find out their health benefits and study the effect of changes to these metabolites.

In addition to being used for human health, in the last few decades, there has also been a tendency to use plant extracts or secondary metabolites in handling pests or diseases in food crops and livestock (Siva *et al,* 2008; Hidanah *et al.,* 2017). One of the plant diseases that cause losses is the fungus *Fusarium oxysporum* (Matheron, n.d.) (Gordon, 2017). The fungus *F. oxysporum* is known to cause wilt disease in plants such as tomatoes, bananas, and other food crops (Ignjatov *et al.,* 2012; Steinkellner *et al*., 2008; Walduck & Daly, 2006; Sutherland *et al.,* 2013). Several plant extracts can inhibit *F. oxysporum* germination (Sutherland *et al.,* 2013). Likewise, plant extracts of *P. niruri* showed inhibitory activity against *F. oxysporum* (Siva *et al.,* 2008). However, until now, there have been no reports of testing the activity of compounds from *P. niruri* against *F. oxysporum*. Whereas compounds from the *P. myrtifolius* plant, the same genus as the secondary metabolites of the lignan group, have been reported to be able to inhibit the growth of *F. oxysporum* (Windayani *et al.,* 2014). Therefore, this study was carried out to isolate secondary metabolites from the acetone extract of *P. niruri* and in vitro test its bioactivity against the fungal pathogen *F. oxysporum*.

2. MATERIALS AND METHODS

Plant Material

The plants were collected from Bandung. The voucher specimens (*P. niruri*) were identified and deposited at the Herbarium Bandung, School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, Indonesia under specimen number HBG-12183.

Lignan Isolation

P. niruri leaf powder (1.0 kg) was macerated with acetone at room temperature for 3 × 24 hours. The solvent was evaporated at low pressure using a rotary evaporator to produce acetone extract (30.0 g). The acetone extract was fractionated using vacuum liquid chromatography (VLC) technique (*n*-hexane:EtOAc = 80:20 to 70:30, EtOAc = 100%) yielded 4 main fractions. Fraction B (2.1 g) was re-fractionated using gravity column chromatography with sephadex LH-20

stationary phase and MeOH mobile phase then followed by radial chromatography with *n*-hexane: EtOAc= 70:30 to 90:10 as mobile phase to obtain pure compounds nirtetralin (**1**) (17.4 mg) together with phyllanthin (**2**) (58.7 mg).

Antifungal Assay

Testing antifungal properties by making a suspension of fungal inoculum *F. oxysporum* refers to the CLSI M38-A2 method (CLSI, 2003). Briefly, the fungus *F. oxysporum* was grown on PDA agar plates at 35 °C for seven days (Windayani *et al.,* 2014). Seven-day fungal colonies were rinsed with 0.85% PBS (Phosphate Buffered Saline) solution to obtain a mixture of conidia, sporangiospores, and hyphae fragments. Then, the mixture was transferred into a sterile tube and left for three to five minutes until the heavier particles settle. The top (suspension) of the mixture was separated into a new tube and stirred using a vortex for fifteen seconds. Then, the density of the microconidia suspension was determined and adjusted by diluting the suspension using sterile distilled water to 1:50. Dilutions were carried out to obtain a colony density of 0.4×10^4 to 5×10^4 CFU/mL. Inoculum quantification was made by growing 0.01 mL of a suspension that had been diluted 1:100 on a PDA agar plate. Then aerobically incubated at 35 °C, the appearance of fungal colonies was observed every day. Fungal colonies after this double dilution were in the range of 5×10^4 CFU/mL.

3. RESULTS AND DISCUSSION

Phytochemical studies showed that two compounds (nirtetralin B (**1**) and phyllanthin (**2**)) of the lignan group were isolated from the acetone extract of the plant *P. niruri* (**Figure 1**). This study's lignans of *P. niruri* had an aryltetralin and dibenzylbutane framework (**Figure 1**). However, Windayani *et al.,* (2015) reported that *P. myrtifolius* plants only produced a type of arylnaphthalene lignan skeleton. At the same time, *P. niruri* plants produced several lignan skeleton such as dibenzylbutane, dibenzylbutyrolactone, aryltetralin, and seco-aryltetraline (Qi *et al.,* 2014). By comparing the type of framework and the oxygenation pattern of lignans obtained in previous work from *P. myrtifolius* with lignans from *P. niruri*, it appears that arylnaphthalene lignans are products of other reactions of lignans dibenzylbutane and aryltetralin as stated in lignan biosynthesis (Suzuki & Umezawa, 2007). The arylnaphthalene and aryltetralin of *Phyllanthus* plants are lignans with oxygen-bound at C-9 and C-9'. Their biogenesis is assumed to be formed by enantioselective dimerization of two coniferyl alcohol units to produce pinoresinol (furofuran), then undergoes reduction to secoisolarisyresinol (dibenzylbutane), which further oxidized to matairesinol (dibenzylbutyrolactone). At the same time, the aromatic substituent is a further modification of the lignan. Here it appears that the formation of the lactone ring occurs earlier than the formation of the aromatic naphthalene ring. Thus, chemotaxonomically, *P. myrtifolius* plants are similar to *P. niruri* plants but with a higher oxidation level of lignans from *P. myrtifolius* than lignans produced from *P. niruri*.

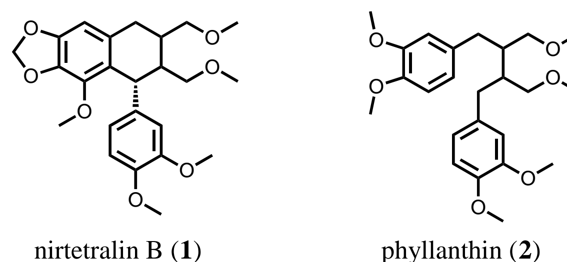

Figure 1. Molecular structures of lignan isolated from *P. niruri*

In this section, the determination of the molecular structure of each of these compounds will be discussed. Determination of the molecular structure has been carried out based on the analysis of spectroscopic data for each isolated compound, which includes infrared (IR) spectra data, one- and two-dimensional (1D and 2D) nuclear magnetic resonance (NMR) spectra (1D and 2D). The following data shows the results of the NMR spectroscopy of the two compounds that have been isolated from the acetone extract of the *P. niruri* plant:

Nirtetralin B (**1**) was obtained as colourless needle crystals. ^{1}H-NMR Spectrum (500 MHz, $CDCl_3$), δ_H (ppm): 4.33 (1H, *d, J*=6.2 Hz, H-7); 2.03(1H, *qi*, H-8); 1.75 (1H, *m*, H-8'); 2,53 (1H, *d, J*=11.7 Hz, H-7'a); 2.57 (1H, *d, J*=11.2 Hz, H-7'b); 6.45(1H, *s*, H-5); 3.27 (2H, *s*, H-2a); 3.30 (2H, *q*, H-3a); 33.33 (3H, *s*, H-2b); 3.27(3H, *s,* H-3b); 3.49 (3H, *s,* H-8-OCH_3); 5.90; 5.93 (2H, *d, J*=14 Hz, H-15

-OCH_2-); 6.79(1H, *d, J*=8,3 Hz, H-10); 3.75 (3H, *s*, H-11-OCH_3); 3.75 (3H, *s*, H-12-OCH_3); 6.76 (1H, *d, J*=1,8 Hz, H-13); 6.54(1H, *dd*, *J*=1,8; 8.2 Hz, H-14). ^{13}C NMR spectrum (125 MHz, $CDCl_3$) δ_C (ppm): 41.2 (C-1); 45.2 (C-2); 37.2 (C-3); 33.2 (C-4a); 33.2 (C-4b); 102.6 (C-5); 135.2 (C-6); 147.5 (C-7); 141.9 (C-8); 73.0 (C-2a); 75.5 (C-3a); 58.0 (C-2b); 58.0 (C-3b); 132.2 (C-4A); 124.9 (C-8A); 58.3 (C-8-OCH_3);100.7 (C-15); 139.9 (C-9); 111.7 (C-10); 149.0 (C-11); 55.3 (C-11a); 55.3 (C-12); 147.5 (C-12a); 112.9 (C-13); 119.8 (C-14).

Phyllanthin (**2**) was obtained as a white powder. ^{1}H-NMR spectrum (500 MHz, acetone-d_6), δ_H (ppm): 6.64; 6.64 (1H, *d*, *J*=1,46 Hz; H-2); 3.30 (3H, *s*, H-3a); 3.86 (3H, *s*, H-4a); 6.77;6,78 (1H, *d*, *J*= 7,92 Hz, H-5); 6.66; 6.66; 6.67;6.67 (1H, *dd*, *J*=1,43; 8.06 Hz, H-6); 2.65(2H, *m*, H-7); 2.05(1H, *t*, H-8); 3.32 (2H, *m*, H-9); 3.81(3H, *s,* 9a -OCH_3). ^{13}C NMR spectrum (125 MHz, acetone-d_6) δ_C (ppm): 133.7 (C-1); 112.3 (C-2); 147.2 (C-3); 58.8 (C-3a); 148.8 (C-4); 56.0 (C-4a); 111.0 (C-5); 121.2 (C-6); 35.0 (C-7); 40.8 (C-8); 72.7 (C-9); 55.8 (C-9a).

Compound **1** was obtained as colourless needle crystals with a melting point of 55-56 °C. The ^{13}C NMR spectrum data for compound **1** showed 24 carbon signals. In DEPT and HSQC experiments on compound **1**, five carbon methoxy signals were obtained (δ_C 58.0 (C-2b), 58.0 (C-3b), 58.3 (C-8-OCH_3), 55.3 (C-OCH_3). 11a), 55.3 (C-12a)) and one methylenedioxy signal (δ_C 100.7 (C-15)), while the other 18 carbon signals showed signals for the lignan framework, consist of twelve carbons. The aromatic *sp*^{2} carbon signal consisted of four carbon signals methyne (102.59 (C-5), 111.7 (C-10), 112.8 (C-1), 119.9 (C-14) ppm) and eight quaternary carbon signals (δ_C 124.9 (C-4A), 132.2 (C-8A), 135.2 (C-6), 147.5 (C-7), 141.9 (C-8), 140.9 (C-9), 149.1 (C-11), 147.5 (C-12). In comparison, the other six carbon signals are three carbon signals of methylene (δ_C 33.2, 73.0, 75.6) and aliphatic methyne (δ_C 41.2, 45.2, 37.2), respectively. Furthermore, the COSY spectrum data for compound **1** showed a correlation between H-1↔H-2, H-2↔H-3, H-2↔H-2a, H-4 H-5, and H-13↔H-14. The positions of the methoxy and methylenedioxy substituents were determined based on the long-range correlation data ^{1}H↔^{13}C in the HMBC spectrum of compound **1**, shown in **Fig. 2**. The information obtained from the 1D and 2D NMR experiments suggests that compound **1** has a 1-aryltetrahydronaphthalene or tetralin skeleton (Anjaneyulu *et al.,* 1973; Chang *et al.,* 2003). Comparing with previously reported data presented in **Table 1**, showing compound **1** has high compatibility with one of the anomers of nirtetralin (Wei *et al.,* 2012). The coupling constant H-1 confirms it with H-2, *J* = 6.25 Hz, which means both protons are axially-axially oriented. In contrast, the other coupling constants that determine the structure of compound **1**, namely H-2 and H-3 with *J* = 4.6 Hz, indicate both protons are axially-equatorially oriented, thus compound **1** is suggested as nirtetralin B (Wei *et al.,* 2012).

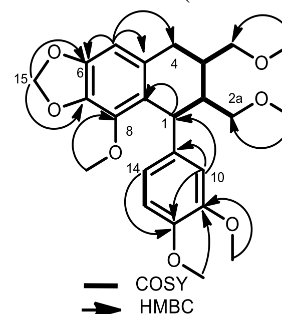

Figure 2. Important correlation HMBC (C→H) and COSY spectral on nirtetralin B (**1**)

Compound **2** was obtained as white crystals with a melting point of 96-97 °C. In the ^{13}C NMR spectrum data for this compound, 12 carbon signals appeared with the integration of each signal for two carbon atoms, so the total number of carbon atoms was 24. Based on the HSQC and DEPT experiments, of the twelve signals, three of them were methoxy (δ_C 58.8; 56.0; 55.8), while the other nine carbon signals were six aromatic *sp*^{2} carbon signals (δ_C 133.7; 112.3; 147.2; 148.8; 111.1; 121.2, two methylene (δ_C 35.0; 72.7), and one aliphatic methyne (δ_C 40.8). These data point to the skeleton of the lignan compound dibenzylbutane. Further evidence of the molecular structure of compound **2** was obtained based on long-range correlation data on the HMBC spectrum and literature search. Further, it was shown that compound two had high similarity in its NMR parameters with the phyllanthin compound, the skeleton lignan dibenzylbutane. In addition, the data on the physical properties of compound **2** also showed similarities to the previously reported data (Krithika *et al.,* 2009; Hanh *et al.,* 2013). A comparison of the ^{1}H NMR chemical shift of compound **2** with the previously reported data is presented in **Table 2**.

Table 1. Chemical shift ^{1}H NMR and ^{13}C NMR of nirtetralin B (**1**) dan (**1***) in acetone-d_6 , 500 MHz

No. C	δ_H [multiplicity, *J* (Hz)]		δ_C	
	1	**1***	**1**	**1***
1	4.33 (*d*; 6.4)	4.30(*d*; 6.2)	41.2	41.5
2	2.03 (*m*)	2.01 (*m*)	45.2	45.4
3	1.75 (*m*)	1.82(*m*)	37.2	37.1
4	2.53; 2.57 (*d*; 11.7) 2.57; 2.60 (*d*; 11.6)	2.73(*dd*; 4.2; 3.6; 11.3) 2.58(*dd*, 11.4; 12)	33.2	33.5
5	6.45(*s*)	6.42 (*s*)	102.6	103.0
6	-	-	135.6	135.7
7	-	-	147.6	147.6
8	-	-	141.9	142.0
2a	3.27 (*s*)	3.28 (*s*)	73.0	73.8
3a	3.30 (*s*)	3.35 (*s*)	75.6	76.3
2a-OMe	3.33 (*s*)	3.30 (*s*)	57.9	58.9
3a-OMe	3.40 (*s*)	3.39 (*s*)	58.0	59.0
4A	-	-	124.9	124.9
8A	-	-	132.2	132.1
8-O*Me*	3.49 (*s*)	3.49 (*s*)	60.3	59.2
15	5.90;5.93 (*d*; 13)	5.86 (*d*; 5.8)	100.6	100.8
9	-	-	139.9	139.9
10	6.79,6.81 (*d*; 8.5)	6.71(*d;* 1.5)	112.9	112.2
11	-	-	149.1	148.7
11-OMe	3.75 (*s*)	3.83 (*s*)	55.3	56.0
12-OMe	3.75 (*s*)	3.83 (*s*)	55.3	56.1
12	-	-	147.5	147.6
13	6.75;6.76 (*d*; 1.8)	6.72 (*dd;* 8.2; 1.5)	111.7	111.0
14	6.53;6.54 (*dd*; 2, 1,8)	6.56 (*m*, 1.5;2.4; 8.2)	119.9	120.0

*600 MHz (Wei *et al.*, 2012)

Table 2. ^{1}H-NMR data of phyllanthin (**2**)

No. C	δ_H [multiplicity, *J* (Hz)]	
	2	**2***
1,1’	-	-
2,2’	6.64 (2H, *d*, 1,5)	6.60 (2H, *d*, 1,9)
3,3’	-	-
4,4’	-	-
5,5’	6.77; 6,78 (2H, *d*; 8.0)	6,.75 (2H, *d*, 8.,0)
6,6’	6.65 (2H, *dd*; 1.5; 8.0)	6,.65 (2H, *dd*, 1.9;8.0)
7,7’	2.65 (4H, *m*)	2.65 (4H, *m*)
8,8’	2.03 (2H, *m*)	2.05 (2H, *m*)
9,9’	3.28 (4H, *m*)	3.25 (4H, *m*)
3(3’)-OMe	3.84 (6H, *s*)	3.83 (6H, *s*)
4(4’)-OMe	3.80 (6H, *s*)	3.78 (6H, *s*)
9(9’)-OMe	3.29 (6H, *s*)	3.30 (6H, *s*)

*Krithika *et al.,* (2009)

Furthermore, this section also presents the results of the antimicrobial activity of the two pure compounds isolated against fungal pathogens and suggestions for the relationship between structure and bioactivity. The isolated compounds from *P. niruri*, compounds **1-2,** were tested as an antifungal against *F. oxysporum*, including sensitivity tests expressed by % inhibition values, MIC, and MFC, also microconidia germination inhibition tests. Both tests were carried out based on the standard CLSI M38-A2 protocol (CLSI, 2003). For the sensitivity test, the MIC and MFC values of each pure compound at a

concentration of 1000 μg/mL are presented in **Table 3**.

The nirtetralin compound B (**1**) is a lignan of the aryltetralin type, showing a more active inhibition of the growth of *F. oxysporum* (MIC = 4.0 μg/mL) compared to the lignan dibenzylbutane, phyllanthin (**2**) (MIC = 16.0 μg/mL). Antifungal activity data on the two compounds suggested that the presence of a cyclohexane ring in compound **1** had a role in the activity of lignan compounds in inhibiting the growth of the fungus *F. oxysporum*. The effect of each compound on conidia germination of *F. oxysporum* was tested at concentrations of 0 × MIC to 4 × MIC for 72 hours at 35 ºC. The results of the *F. oxysporum* micronidia germination inhibition test are presented in **Table 4**.

In general, all tested pure compounds inhibited concentration-dependent *F. oxysporum* germination, where a sharp decrease began to occur at 0.5 × MIC. Compound **2** (phyllanthin) completely inhibited the germination of *F. oxysporum* at a concentration of 2 × MIC. These results are in agreement with the MFC values of the compounds (**Table 3**). Meanwhile compound **1** (nirtetralin B), at a concentration of 4 × MIC did not completely inhibit *F. oxysporum* germination. This result can be understood for example compound **1** has an MIC value of 4.0 μg/mL while the MFC value of compound is 16.0 μg/mL (4 × MIC). In general, these results indicate that the isolated compounds from *P. niruri* plant can inhibit the germination of *F. oxysporum* conidia since the concentration of 0.5 × MIC.

Several other lignans have been evaluated as an antifungal. For example, three lignan compounds, aliterinate C, (+)-pinoresinol, and (+)-medioresinol, exhibited better biologic effects on fungal organisms such as *Pythium insidiosum* than the antifungal drugs itraconazole and terbinafine (Sriphana *et al.,* 2013). Two aryltetralin lignans isolated from *Cordia exaltata* were found to have weak antifungal properties against several *Candida* species (Nogueeria *et al.,* 2013). Ecbolin A (furofuran lignan), a secondary metabolite of *Exbolium viride*, showed moderate antifungal activity against twelve fungi (Francina *et al.,* 2012). (+)-Medioresinol is antifungal against *Candida albicans* through apoptotic mechanisms, namely oxidative stress and mitochondrial dysfunction (Hwang *et al.,* 2012). In addition, justisidin B from *P. myrtifolius*, phyllanthin (**2**), a dibenzylbutane lignan isolated from *P. niruri*, also exhibited antifungal properties against *C. albicans* with an inhibitory level of 250 μg/mL (Windayani *et al.,* 2014). The antifungal properties of compounds **1** and **2** in this work are consistent with previous studies (Gertsch *et al.,* 2004). Therefore the results of this study add to the data on the beneficial effect of lignans of *Phyllanthus* species as antifungal agents. However, referring to the antifungal properties of lignans against *Fusarium* fungi, the data that have been reported are very limited. Pinoresinol (furanoid lignan) and secoisolariciresinol (dibenzylbutane lignan) have been shown to inhibit the growth and biosynthesis of trichothecene in *F. graminearum* (Kulik *et al.,* 2014). Lyonisid, a glucoside derivative of the lignan aryltetraline isolated from *Vaccinium myrtillus* L., showed inhibition of the mycelia growth of *F. oxysporum*. However, eudesmin (a furanoid lignan) did not show antifungal activity against *F. oxysporum* (Szakiel *et al.,* 2011). So the effect of lignans on *Fusarium* fungi depends on the type of lignan framework, and there is a tendency for lignans arylnaphthalene, aryltetralin, and dibenzylbutane to be potential antifungal compounds against *F. oxysporum*.

Table 3. Antifungal activity of isolated compound against *F. oxysporum*

Compound	**Group**	**Antifungal activity**		
		% IG[a] (1000 μg/mL)	**MIC (μg/mL)**	**MFC (μg/mL)**
Nirtetralin B (**1**)	Lignan	66.9	4.0	16.0
Phyllanthin (**2**)	Lignan	62.3	16.0	16.0
AMP-B	Control (+)	45.1	1.0	1.0
DMSO	Control (-)	0	-	-

[a] Germination inhibition

Tabel 4. Antifungal activity of isolated compounds on germination of Microconidia

Compound	Type of Lignan skeleton	% Germination				
		0 × MIC	0.5 × MIC	MIC	2 × MIC	4 × MIC
Nirtetralin B (**1**)	Aryltetralin	99.0	75.5	70.7	70.7	70.7
Phyllanthin (**2**)	Dibenzylbutane	99.0	75.0	70.7	0.0	0.0

The disclosure of conidia germination inhibitors is vital for overcoming infections caused by pathogenic organisms (Ebbole *et al.,* 2004; Cantrell *et al.,* 2005). Mycotic infections are difficult to treat since fungi are eukaryotic organisms with structures and metabolism similar to their hosts (Jin *et al.,* 2004). Furthermore, long-term treatment with commonly used antifungals, such as amphotericin B, is toxic, whereas azole antifungals have a limited spectrum and efficacy, and their use may result in resistance to some strains of pathogenic fungi (Denning *et al.,* 1997; Helmerhorst *et al.,* 1999; Wiederhold, 2017).

4. CONCLUSIONS

In this study, two pure compounds from the lignan group of aryltetraline (nirtetralin, **1**) and dibenzylbutane (phyllanthin, **2**) have been isolated. The results of the antifusarium bioactivity test showed that nirtetralin B (**1**) could inhibit the growth of *F. oxysporum* at a concentration of 4 µg/mL (MIC). Both isolated compounds **1** and **2** were fungicidal at a concentration of 16 µg/mL. Further testing of the inhibition of germination of *F. oxysporum* microconidia showed that compound **2** inhibited microconidia germination 100% at a concentration of 2× MIC. In comparison, compound **1** at the same concentration was only able to inhibit germination by 29%. So compound **2** was estimated to have potential as the main compound antifusarium. However, further studies are needed to determine its *in vivo* activity and cytotoxicity to host plants.

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