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Original Article

In vitro antimycotic activity of chemical constituents from *Dipterocarpus verrucosus, Dipterocarpus cornutus* and *Dipterocarpus crinitus* against opportunistic filamentous fungi

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Abstract

This paper will discuss, *in vitro* investigation of chemical constituents extracted from the stem bark of *Dipterocarpus verrucosus*, *Dipterocarpus crinitus* and *Dipterocarpus cornutus* against opportunistic filamentous fungi. In this research, 17 compounds comprised of twelve oligostilbenoids, (-)-ε-viniferin ,(-)-laevifonol, (-)-hopeaphenol , (-)-isohopeaphenol, vaticanol B, diptoindonesin E, hemsleyanol D, davidiol A, resveratrol, ampelopsin A, ampelopsin F**,** together with three other phenolic; gallic acid derivative, (-)-bergenin, scopoletin and 4-methoxygallocathecin and also two terpene; β-sitosterol and β-sitosterol glucoside have been isolated. In this study, the crude extracts and isolated compounds were evaluated regarding to their antifungal activity; in terms of MIC, MFC and germination assay against pathogenic fungi strains, namely *Aspergillus flavus* (AF), *Aspergillus oligosporus* (AO), *Rhizophus oryzae* (RO) and *Fusarium oxysporum* (FO) using Clinical and Laboratory Standard Institute (CLSI) methods. The MIC of crude extracts and isolated compounds against all fungi ranged from 3.8 - 500 µg/mL. *F. oxysporum* shows the most sensitive microorganisms on crude extract: *D. verrucosus*, *D. cornutus* and isolated compound: ε-viniferin with MIC of 3.8 µg/mL. The MIC was lower compared to amphotericin B (4 µg/ml). The strain was killed at the MFC of 31.3, 31.3 and 15.6 µg/mL respectively, as compared to amphotericin B (8 µg/mL). Compounds: resveratrol, laevifonol, ε-viniferin, ampelopsin F, vaticanol B, vaticanol A, isomer of hopeaphenol and isohopeaphenol, β-sitosterol and β-sitosterolglucoside possessed an inhibitory activity on the conidial germination of *F. oxysporum* at the concentration of 4× MIC. On top of that, *D. cornutus*, ampelopsin A and hemsleyanol D possessed a complete sterility at the concentration of 2×MIC while *D.verrucosus* achieved its inhibitory activity at 1× MIC. To the best of our study, there is no data discussing the inhibition of conidial germination of filamentous fungi using the tested compounds and crudes tested.

Keywords: Antifungal, *In vitro*, Opportunistic fungi, Phenolic compounds, Stilbenoid

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Introduction

Filamentous fungi; anatomically viewed from its shape, can possibly facilitate problematic infections, consequently causing invasive mycoses in both non-immunocompromised and immunocompromised individuals. These infections require prompt systemic antifungal therapy, where the effectiveness of which depends, among other things, on the *in vitro* susceptibility of the fungus to antifungal drugs (Meletiadis et al., 2000). Although *Candida albicans* is the species that is most often associated with serious fungal infections, other species of *Candida* as well as filamentous fungi such as *Aspergillus* and *Fusarium* are now considered as imperative pathogens in immunocompromised hosts (Morison et al., 1993). The incidence of invasive opportunistic filamentous fungal infections, particularly in immunosuppressed patients, have increased in past 25 years and is now becoming a significant cause of morbidity and mortality (Rukayadi & Hwang, 2007). This is particularly true in infected patients with hematological malignancies undergoing induction or consolidation chemotherapy (Annaise, 1992), in immunosuppressed organ transplant recipients, in patients with acquired immunodeficiency, and is secondary to the infection of human immunodeficiency viruses (Hadley & Karchmer, 1995). Amphotericin B and azole derivatives are among the primary drugs used for treatment of serious fungal infections. Nevertheless, limitations in efficacy or tolerability of these chemicals create an alarming call for further research to discover new drugs with a wide spectrum of effectiveness in treating said diseases caused by filamentous fungi pathogens (Rukayadi & Hwang, 2007)

The past 25 years is a mark of the advent of plants resource advancement as an effective alternative source, replacing antibiotics for clinical applications (Fenner et al., 2005). Malaysia is known as one of the 12th mega biodiversity countries that offer a plethora of natural resources to facilitate numerous researches in the quest of a novel compound discovery. Malaysia's rich rainforest and a wide array of biodiversity should be supported by the collective and continuous efforts in compiling and screening those floras, to prevent the loss of valuable sources that is possible to be developed into environmentally safe antifungal agents. Hence, to achieve such attainment, these valuable compounds or known as secondary metabolites, should be harvested from the plants, which is the main source of antifungal compounds. Antifungal compounds can act as phytoalexin, a natural antibiotic in plants which acts like a toxin that attack harmful organism (Sotheeswaran & Pasupathy, 1993). Therefore, secondary metabolism is considered as a promising source of novel antifungal properties due to the fact that it has been successfully used by plant as their defense mechanism (Basri et al., 2012). In an attempt to explore new antifungal leads, the stem bark of *Dipterocarpus* from Dipterocarpaceae family which is often neglected in timber industry was utilised. Dipterocarpaceae is the large family of tropical plants, extensively distributed across Kalimantan and Malay Peninsula (Seo, 2000). Interestingly, this stem carries an ability to produce various secondary metabolites such as oligostilbennoid, triterpenoid, flavonoid, volatile oil and arylpropanoid (Sotheeswaran & Pasupathy, 1993). Resveratrol or known as 3, 5, 4-trihydroystilbene and their oligomer is a polyphenolic commonly exists in edible foods and beverages such as mulberries, peanuts, grapes and wines (Xue et al., 2014). Since the discovery of those natural compounds, scientists have found the chemical structure of the derivatives of resveratrol and its positive effects on cellular and biological activities. For that reason, there is a growing demand and interest in exploring resveratrol produced by plants. Vitaceae, Leguminosae, Gnetaceae, Dipterocarpaceae, and Cyperaceae were five plant families isolated on resveratrol oligomers (Seo, 2000, Xue et al., 2014, Zain et al., 2011, Zain et al., 2018a, Zain et al., 2018b) . However, there is a paucity of reports on antifungal properties (Lee & Lee, 2015) especially on the chemical constituents of Dipterocarpaceae against human fungal pathogens. The first study on antifungal was evaluated on 1977 (Pryce, 1977) and the full review of antimicrobial from stilbenoids was published in 1993 (Sootheeswaran & Pasupathy, 1993). However, until now, there is only one reported literature published about the use of stilbenoids from the family of Dipterocarpaceae against filamentous fungi; by discussing on the effect of stilbenoid from *Hopea exalata* against *Fusarium oxysporum* (FO). In fact, the study (Ge et al., 2006) evaluated the same compounds with this research which are α-viniferin and vaticanol A, mostly as an approach in agricultural sectors to prevent microbial pathogens and pests' attack using natural defenses. Both compounds exhibit the capabilities of inhibiting FO with the MIC values of 25.2 µg/mL and 6.22 µg/mL, respectively,

and the compounds performed closely to the standard ketoconazole at 2.21 µg/mL. Another finding evaluated on antifungal against *C. albicans* towards two stilbenoids; 3, 4', 5-trihydroxystilbene and 3, 5-dihydroxy-4-isopropylstilbene, which demonstrated that the use of stilbene were more effective than AMP B (Kumar et al., 2012). Recently, novel mechanism on resveratrol towards apoptosis inducer in *Candida albicans* have been discovered (Lee & Lee, 2015). The study revealed the potential use of resveratrol as an apoptosis inducers in the human pathogens fungus. Surprisingly, the result indicated resveratrol induces fungal apoptosis through a caspase-dependent mitochondrial pathway (Lee & Lee, 2015).

Most studies proposed that stilbenoids possess significantly moderate potential in combating bacterial pathogens such as *E.coli*, *Staphylococcus* spp, *Bacillus* spp, MRSA (Methicillin Resistant *Staphylococcus aureus*), *Pseudomonas aeruginosa* and *Mycobacterium magmatism*· (Soothesswaran & Pasupathy, 1993, Ainaa et al., 2012, Basri et al., 2012, Wibowo et al., 2012). Some of them showed significant activity in reducing the viable cell number of MRSA. The active compounds were all identified as stilbene derivatives. Hemsleyanol D, a stilbene tetramer, isolated from *Shorea hemsleyana* (Nitta et al., 2002) revealed to be the most effective compound with an MIC value of 2µg/mL as compared to 1µg/mL of vancomycin.

Until recently, the evaluation of combining effect of stilbenoid from *Shorea gibbosa* (Dipterocarpaceae family) and antibiotics, vancomycin against MRSA was discovered as (Basri et al., 2012) suggesting that stilbenoids encompass an anti-MRSA activity thus creating a big potential as an alternative phytotherapy in combating MRSA infections. These plant-based metabolites can enhance the *in vitro* activity of some cell-wall inhibiting antibiotics by encountering the same target site such as peptidoglycans (Basri et al., 2012).

In relation to this, the need to develop alternative plant-based antibiotics to assess and solve the problem of microorganism resistance from stilbenoid is exactly auspicious. To the best of our knowledge, no prior literature has reported on stilbenoids activity against filamentous fungi and its germination assay.

Material and Methods

Plant extract preparation

Dried *Dipterocarpus verrucous* plant (5kg) were ground and extracted with methanol. Further fractionation using various chromatography techniques were carried out consecutively (Wibowo et al., 2014, Wibowo et al., 2012, Zain et al., 2011). 17 compounds were identified and analysed by ^{1}H NMR, ^{13}C NMR, LCMS, UV and IR also compared with previous data in literature. All compounds consists of 12 stilbenoid which were resveratrol (Atun et al., 2008), ε-viniferin(Li et al., 1996), laevifonol(Tanaka et al., 2000), ampelopsin F (Luo et al. 2001) , α-viniferin (Kitanaka et al., 1990), diptoindonesin E (Muhtadi et al., 2006) , hopeaphenol (Kawabata, 1992) , isohopeaphenol (Ito et al., 2008), vaticanol B (Tanaka et al., 2000), hemsleyanol D (Tanaka et al., 2001), davidiol A (Tanaka et al., 2000), ampelopsin A (Abe et al., 2011), two terpene which were β-sitosterol (Chaturvedula & Prakash 2012) and β-sitosterol glucoside (Moghaddam et al.2006) and three phenolic which were bergenin (Ito et al., 2012), scopoletin (Rohaiza et al., 2011) and 4'-*O*-methylepigallocatechin (Garcia et al.,1993). The extracts and compounds were dissolved in 10% dimethyl sulfoxide (DMSO) following the protocol of Clinical and Laboratory Standards Institute (CLSI, 2012). The final concentrations of extracts were standardized at 10 mg/mL or 1% whiles the compound at 1 mg/mL or 0.1%. DMSO at 10% was unable to kill all fungi tested in this study.

Filamentous fungi strains and growth conditions

*Aspergillus flavus (*ATCC 22546), *Aspergillus oligosporus (*ATCC 22959*), Rhizophus oryzae (*ATCC 22580) and *Fusarium oxysporum (*ATCC 44187) used in this study are all human pathogens that were purchased from the American Type Culture Collection (Rockville, MD, USA). Fungal strains were cultured and maintained on potatoes dextrose agar (PDA) (Difco, Spark, MD, USA) medium at various optimal temperatures depending on the strain.

Preparation of conidia

The method was referred according to with slight modification (Rukayadi & Hwang, 2007, Rukayadi & Hwang, 2006). Firstly, all fungi were grown on PDA at 35 ºC for 7 days (Santos et al., 2006). The conidia suspensions for all fungi were prepared according to the method described in CLSI M38-A2. Briefly, seven days colonies of *A. flavus* was covered with 1 mL of sterile phosphate buffer saline (0.85%) medium and the suspensions were made by slowly probing the colonies with the tip of a Pasteur pipette. The resulting

mixture of conidia and hyphal fragments were withdrawn and transferred into a sterile tube. Conidia quantification was made by plating 0.01 mL of 1:100 diluted of the conidia suspension on Potatoes dextrose agar (PDA) (Difco) at 35 °C for 48 h to quantify the viable number of CFU/mL. After the incubation process, the numbers of viable colonies were counted and the conidia suspensions were adjusted to approximately 5×10^4 CFU/mL. The same procedures were performed for other fungi; *A. oligosporus, F. oxysporum and R. oryzae*.

Antifungal bioassay

Susceptibility test

The well diffusion test was performed to determine the susceptibility of all antifungal agents on *A. flavus, A. oligosporus, F. oxysporum* and *R. oryzae*. Briefly, the 5 mm wells were made by cutting out the agar (PDA) using a cork borer. 30 µL of seven days old suspension of *A. flavus, A. oligosporus, F. oxysporum* and *R. oryzae* were dropped into the wells, separately. A 30 µL of antifungal agents were placed into the cultured well accordingly and were incubated at 35 °C for 72 h. The growth of mycelia was observed daily and the diameter of mycelia growth was measured. A 10% DMSO was served as negative control while amphotericin B acts as positive control. The percentage of mycelia growth inhibition was estimated by using the formula:

$$\% \text{ of inhibiton} = 1 - \frac{\text{Diameter of treated control}}{\text{Diameter of negative control colany}} \times 100\%$$

This experiment was repeated two times independently, each with a duplicate (n=2 × 2).

***In vitro* susceptibility test**

Minimum inhibitory concentrations (MIC)

MIC is the lowest concentration of antifungal agent which is obtained from the complete inhibition of visible growth. In order to determine the MIC of the crudes and isolated compounds on the fungal tested, the standard microtiter broth dilution method was applied. This was performed according to a method described by Clinical Laboratory Standard Institute, CLSI M7-A6. Briefly, a 100 µL of conidial inoculums of approximately 5.0×10^3 CFU/mL was transferred into each wells of a sterile disposable 96-well round bottom microtiter plate. Then, a two-fold dilution of antifungal agent (using 100 µL of 0.1% solution) was performed starting from well 12 (concentration of 500 µg/mL until well 3 (concentration of approximately 1 µg/mL and the remaining 100µL from well 3 was discarded. Well 1 served as a negative control (only medium) and well 2 served as a growth control (medium containing conidial inoculums). A sterile cover lid of the 96-well plate and sealed with parafilm was incubated at 37 ºC for 48 - 72 h.

Minimum fungicidal concentration (MFC)

MFC was studied by sub culturing 10 µL suspension from each well of microtiter plates on PDA agar including both positive and negative controls. The plates were incubated at 35 º C for 48 h or until the growth control was seen.

Conidial germination inhibition assay

The conidial germination inhibition assay was performed in standard MOPS-buffered RPMI 1640 according to the method of CLSI (2002). The adjusted inoculums suspension of 5×10^4 CFU/mL was diluted in 1:10 using PBS to a final concentration of 5×10^3 CFU/mL. Each concentration of crude extracts and isolated compounds were diluted 1:10 in PBS medium containing 5×10^3 CFU/mL. Each tested fungus; *A. flavus, A. oligosporus, F. oxysporum* and *R. oryzae* were finalised at the concentration of 0× MIC, 0.5× MIC,1× MIC and 2× MIC, 1 mL of final volume from each fungal culture was incubated at 35 ºC with 200 rpm agitation for 12 h, separately. An appropriate volume of 50 µL, depending on the dilution and the concentration of compounds and extracts, were spread onto PDA plates and incubated at 35 ºC for 48 h or until the growth was seen in the negative control (0 × MIC) and the percent inhibition of germination (% Gi) was calculated using the formula given below:

$$\% \text{ Gi} = \frac{\text{Average conidial germination (\%) of control} - \text{Average conidial germination (\%) of treatment}}{\text{Average conidial germination (\%) of control}} \text{ X } 100\%$$

Results and Discussion

Screening bioassay

In this study, 17 compounds including three crude extracts from the stem bark of *D. verrucosus, D. crinitus* and *D. cornutus* were evaluated against *A. flavus, A. oligosporus, R. oryzae* and *F. oxysporum*. Screening activity indicated that all samples showed potential antifungal activity against *F. oxysporum* and

A. oligosporus rather than *A. flavus* and *R. oryzae*. It can be interestingly observed in Table 1, all the compounds and crudes extracts exhibited promising potential antifungal towards *F. oxysporum* as it was comparable with AMP B. Resveratrol, ε-viniferin, ampelopsin F, catechin, bergenin, and β-sitosterol glucoside with 0.1 % concentration inhibited more than 50% the growth of fungal during 3 days of the test. The fungal inhibitions of the isolated compounds were higher than that of amphotericin B, a commercial antifungal agent, at the same concentration. The compounds have been used to treat the opportunistic filamentous fungi in order to reduce the total number of mold. The inhibition growth showed that there was no significant result on *A. flavus* and *R. oryzae*. Thus it was further determined that the Minimum inhibitory concentration (MIC), Minimum fungicidal concentration (MFC) and the germination inhibition of *F. oxysporum* and *A. oligosporus* from the observations were the potential fungi to be treated with crude extracts and isolated compounds.

The MIC and MFC of compounds and crudes against *F. oxysporum* and *A. oryzae* are shown in Table 2. The results indicated that all compounds with their concentration ranging from 3.8 - 62.5 (µg/mL) exhibited antifungal activity against *F. oxysporum*. On the other hand, the strains were germinated by the compounds at MFC ranges of 31.3-250 µg/mL. In this report, the MICs of *D. verrucosus*, *D. cornutus* and and ε-viniferin against *F. oxysporum* concentration were 3.8 µg/mL. It is important to state that this MIC value was lower in comparison to amphotericin B (4 µg/mL) and the strains were killed at MFC, between the range of 15.6 - 31.3 µg/mL. Recently, *Fusarium* species has emerged as an opportunistic pathogen in immunocompromised as well as in immunocompetent individuals. *Fusarium* is a species that contains important mycotoxin producing capabilities, possessing an ability to affect human's health. The example of *Fusarium* species such as *F. oxysporum* has become an increasingly common due to the breakthrough infections in immunosuppressed patients (Rukayadi & Hwang, 2007, Fleming et al., 2002) Hence, this compound and crude extract has the potential to be developed and used as antimycotic against *F. oxysporum*.

Meanwhile, *Aspergilli* produced a wide variety of diseases with more than 100 species of *Aspergilli*. In parallel, the MICs of all compounds against *A. oryzae* ranged at the concentrations between 15.6 - 125 µg/mL. The strains were germinated by compounds at MFC ranges of 125 - 500 µg/ml. Ampelopsin F and hemsleyanol D results were slightly comparable to AMP B with value of 15.6 µg/mL and 4 µg/mL, respectively. The results indicated that the compounds and crudes demonstrated a moderate activity against *A. oryzae* and was not fully comparable with AMP B. The MFC values for both strains indicated that the strains have higher value than their MIC values. It can therefore be interpreted that they acted against the fungal strains by fungicidal action. Considering its zones of inhibition, the MIC and MFC values, it can be concluded that the compounds and crudes were more potent against *F. oxysporum*. Further study on germination inhibition assay has been done on *A. oryzae* and *F. oxysporum* and is reported in the following section.

b) Germination inhibition assay for *F. oxysporum*

To investigate the antifungal activity in depth, inhibition conidial germination assay was performed. This test generally examines the ability of *Dipterocarpus* extract and compounds to inhibit the conidial germination based on the increasing value of predetermined MIC point. Knowledge of limit point for conidial germination is valuable to control disease caused by fungi (Jin *et al.*, 2004). In a comprehensive study on conidial germination by Osherov & May (2001) they explained that the process involved a very complex signalling process starting with conidial swelling, adhesion, nuclear decondensation and ends with hyphal formation.

Table 1 Inhibiton (100%) of antifungal on isolated compounds

	Microorganisms (Inhibition zone: 100%)											
Microorganisms	***F. oxysporum***			***A. oligosporum***			***A. flavus***			***R. oryzae***		
Sample / Day	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
D. verrucosus	46.15	41.18	25.00	14.29	12.00	2.63	31.25	21.21	14.89	11.11	4.41	5.41
D. cornutus	46.15	52.94	41.67	21.43	12.00	7.89	31.25	18.18	14.89	11.11	4.41	6.41
D. crinitus	46.15	50.00	41.67	14.29	4.00	2.63	25.00	15.15	10.64	11.11	4.41	6.41
Resveratrol	46.15	55.88	50.00	14.29	12.00	21.05	31.25	18.18	14.89	11.11	4.41	5.41
ε-viniferin	0.00	0.00	88.33	14.29	12.00	7.89	31.25	57.58	25.53	11.11	4.41	6.41
Laevifonol	46.15	41.18	25.00	14.29	12.00	7.89	25.00	12.12	14.89	11.11	4.41	6.41
Ampelopsin A	0.00	79.41	65.00	21.43	16.00	13.16	37.50	24.24	17.02	11.11	4.41	5.41
Ampelopsin F	46.15	55.88	41.67	14.29	12.00	21.05	25.00	45.45	36.17	22.22	4.41	5.43
Davidiol A	46.15	50.00	41.67	14.29	4.00	0.00	31.25	18.18	14.89	11.11	4.41	6.44
Diptoindonesin E	15.38	26.47	0.00	21.43	12.00	10.53	25.00	21.21	10.64	22.22	4.41	6.44
Hopea+ Isohopea	38.46	32.35	15.00	21.43	8.00	2.63	25.00	12.12	12.77	44.44	4.41	6.44
Vaticanol B	7.69	11.76	0.00	14.29	12.00	10.53	37.50	24.24	25.53	33.33	4.41	6.44
Hemsleyanol D	46.15	17.65	13.33	28.57	8.00	5.26	37.50	39.39	31.91	4.44	4.41	6.41
Scopoletin	46.15	41.18	33.33	14.29	0.00	7.89	25.00	9.09	19.15	11.11	4.41	6.41
4-mEpGcatechin	0.00	79.41	80.00	21.43	12.00	15.79	25.00	15.15	10.64	11.11	4.41	6.41
Bergenin	0.00	79.41	66.67	14.29	8.00	7.89	31.25	15.15	14.89	11.11	4.41	6.41
B-sitosterol	46.15	26.47	25.00	21.43	20.00	13.16	37.50	24.24	19.15	11.11	4.41	6.41
B-sitoglu	0.00	61.76	58.33	21.43	12.00	10.53	31.25	15.15	14.89	11.11	4.41	6.41
10 % DMSO	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Amphotericin B	46.15	55.88	33.33	50.00	52.00	47.37	56.25	69.70	48.94	15.56	33.82	40.82

Table 2: MIC and MFC on compounds isolated towards *A. oryzae* (*AO*) and *F. oxysporum (FO)*

	Microorganism (µg/mL)			
	AO		***FO***	
Sample	**MIC**	**MFC**	**MIC**	**MFC**
Crude extract *D. verrucosus*	125	250	3.8	31.3
D. cornutus	125	250	3.8	31.3
D. crinitus	62.5	250	15	31.3
Compounds Resveratrol	125	250	7.5	125
ε-viniferin	31.25	250	3.8	15.6
Laevifonol	62.5	125	15	62.5
Ampelopsin F	15.6	500	15	62.5
α-viniferin	31.25	250	62.5	62.5
Diptoindonesin E	31.25	125	7.5	31.3
Hopeaphenol &Isohopeaphenol	31.25	125	7.5	250
Vaticanol B	62.5	125	62.5	31.3
4-mEpGcatechin	31.25	250	7.5	31.3
Bergenin	62.5	500	30.0	31.3
Hemsleyanol D	15.6	125	62.5	125
Scopoletin	125	500	62.5	125
Davidiol A	62.5	250	7.5	31.25
Ampelopsin A	62.5	500	15	62.5
β-sitosterol	62.5	500	30	62.5
β-sitosterolglu	31.25	250	15	31.3
Amphotericin B	4	8	4	8

However, in this study, the germination was determined by calculating the percentage of colony formed on PDA plate. To the best of our knowledge, there is no report on the inhibition of conidial germination on *Dipterocarpus* extract and isolated compounds. Table 3 revealed the concentration of crude and isolated compounds in 0.5 MIC, 1 × MIC, 2 × MIC and 4 × MIC and also fungicidal points for *F.oxysporum.* A sharp reduction and complete inhibition of conidial germination for *D.crinitus* and *D.verrucosus* at 2 × MIC (30 µg/mL) and 1 × MIC (3.8 µg/mL) each was observed.

D.crinutus on the other hand, did not completely inhibit the germination conidia, however it still showed potential activity to inhibit more than 50% of conidia at 2 × MIC (30mg/mL). From the figure, dimer reseveratrol; resveratrol, ε-viniferin, ampelopsin F, laevifonol and ampelopsin A performed complete inhibition of 0% towards *F.oxysporum* at concentration of 15µg/mL (2× MIC), 15.25µg/mL (4×MIC), 30 µg/mL (2×MIC), 60 µg/mL (4×MIC) and 60 µg/mL (4×MIC), respectively.

Table 3: Concentration of crude and isolated compounds in in 0.5 MIC, 1× MIC, 2× MIC and 4× MIC and fungicidal points for *F.oxysporum*

	MIC (µg/mL)					
Sample	**0.5**	**1**	**2**	**4**	**× MIC**	**Fungicidal point Concentration (µg/mL)**
Crude extract						
D.verrucosus	1.9	3.8	7.6	15.2	1	3.8
D. cornutus	1.9	3.8	7.6	15.2	-	-
D. crinitus	7.5	15	30	60	2	30
Compounds Resveratrol	3.75	7.5	15	30	2	15
ε-viniferin	1.9	3.8	7.6	15.2	4	15.2
Ampelopsin F	7.5	15	30	60	2	30
Laevifonol	7.5	15	30	60	4	60
Ampelopsin A	7.5	15	30	60	4	60
α-viniferin	31.25	62.5	125	250	-	-
Diptoindonesin E	3.75	7.5	15	30	-	-
Hopeaphenol &Isohopeaphenol	3.75	7.5	15	30	2	15
Vaticanol B	31.25	62.5	125	250	2	125
4-mEpGcatechin	3.75	7.5	15	30	-	-
Bergenin	15	30.0	60	120	-	-
Hemsleyanol D	31.25	62.5	125	250	2	125
Scopoletin	31.25	62.5	125	250	-	-
Davidiol A	3.75	7.5	15	30	-	-
β-sitosterol	15	30	60	120	4	120
β-sitosterolglu	7.5	15	60	60	4	60

The pattern of the % germination activity were resveratrol> ε-viniferin> ampelopsin F> laevifonol, ampelopsin A. Interestingly, the structure analysis relationship showed that the presence of *trans* olefinic unit which was responsible for electron delocalization for ε-viniferin and ampelopsin F at the compounds skeleton relatively give stronger antifungal activity. These results, were in agreement with the previous study on antimicrobial (Wibowo et al., 2012) which revealed the presence of free resveratrol in upunaphenol D and flexuosol A that showed significant activity than the others. Resveratrol and ε-viniverin are the most potential compounds to inhibit the *F.oxysporum* at concentration 15µg/mL within 48 or 72 h of incubation time. However ε-viniferin required two times concentrations compared to resveratrol to inhibit the *F.oxysporum.*

Meanwhile for tetramer resveratrol; isomer of isohopeaphenol and hopeaphenol, vaticanol B and hemsleyanol D perfomed complete inhibition of 0% at concentration of 15µg/mL (2× MIC), 125µg/mL (2×MIC) and 125µg/mL (2×MIC) each. Diptoindonesin E did not reach complete inhibition however it showed good germination activity as can be seen at 0.5× MIC it inhibited 40% of *F.oxysoprum* and start at that point the graph slightly decline. This may suggest the compound required higher concentration to inhibit the *F.oxysprorum.* The figure also illustrated the trimer resveratrol, α-viniferin and davidiol, however both compounds did not achieved complete sterility of 0%. From the graph, 40% of conidia were inhibited after incubation with 0.5 × MIC. Figure 1 also portrayed the conidial germination of *F. oxysporum* under the presence of terpene (β-sitosterol and β-sitosterol-glucoside) and phenolic compounds (bergenin, catechin and scopoletin). Terpenes demonstrated better inhibition of the conidia germination as it decreased drastically at 2 MIC (60µg/mL) with 60% and lastly reached at the end point at 4 × MIC.

In comparison of dimer and tetramer, dimer resveratrol revealed the most potential in inhibiting the germination of conidia compared to tetramer except for isomer isohopeaphenol. The synergistic effect of this isomer might be contributed to the high antifungal activity.

In this study, *Dipterocarpus verrucosus* extract showed the highest inhibition activity with

concentration of 3.8µg/mL (1× MIC) activity compared to individual isolates. This is also due to the synergistic effect of the compound which contributed to the potential activity on the crude. Synergistic effect will boost significantly powerful activity from two combined elements rather than a single agent (Kumar et al., 2012). Meanwhile, the potential of tetramer vaticanol B, hemsleyanol D and isomer of hopeaphenol and isohopeaphenol as a tetramer resveratrol can be related to their structure which consists of multiple phenolic hydroxyl groups with 4-*para*hydroxyphenol group (Nitta et al., 2002). The chemical structure analysis of the complexity of phenolic content and stereoisomer, *cis* or *trans* structure, also affected the biological activity of resveratrol (Cichewicz & Kouzi, 2002). Resveratrol with more hydroxyl groups, which is known as resveratrol oligomers were recognized as fungal detoxification products or resveratrol metabolism (Cichewicz & Kouzi, 2002).

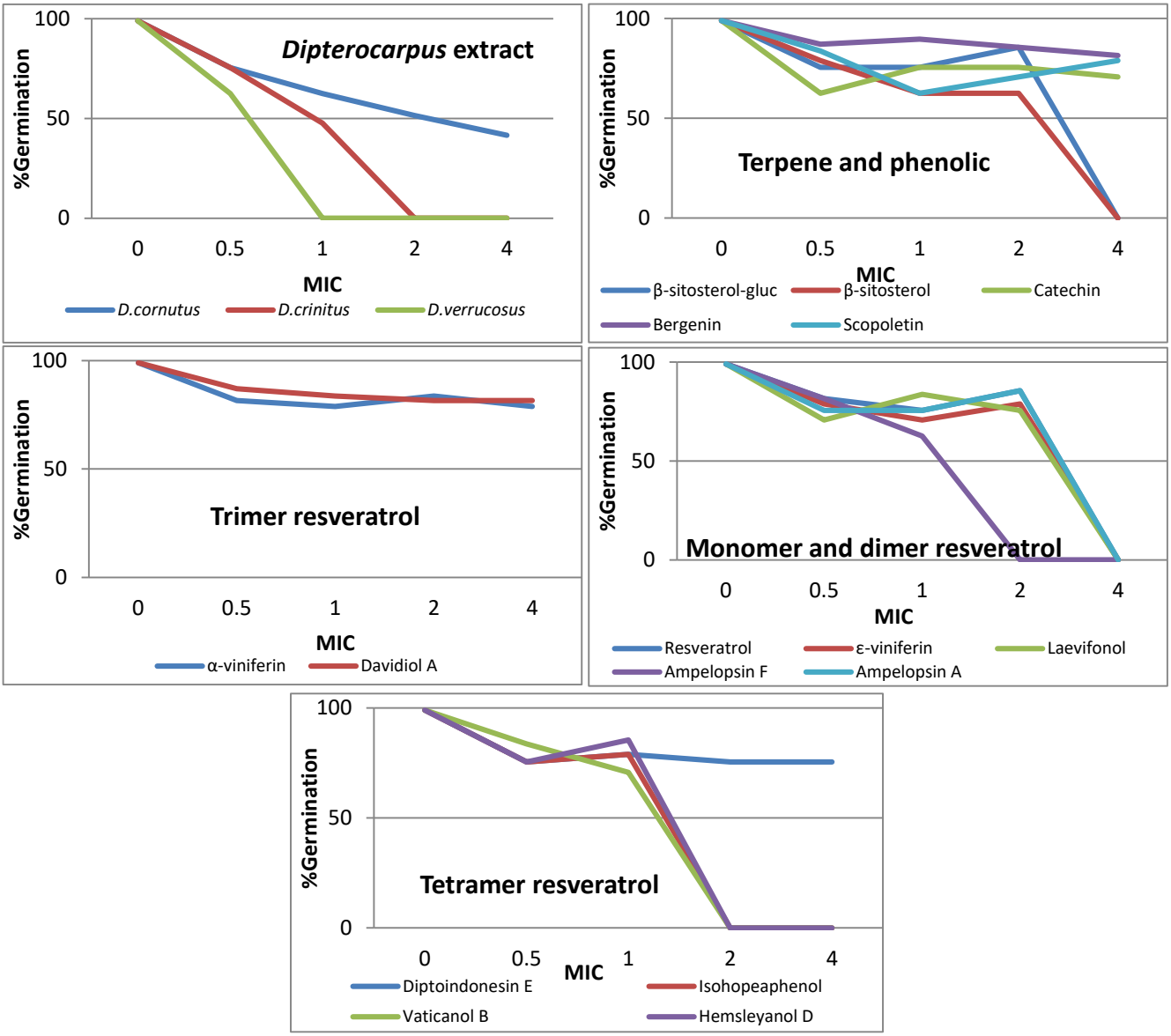

Figure 1: Effect on crudes and compounds to conidial germination of *Fusarium oxysporum*

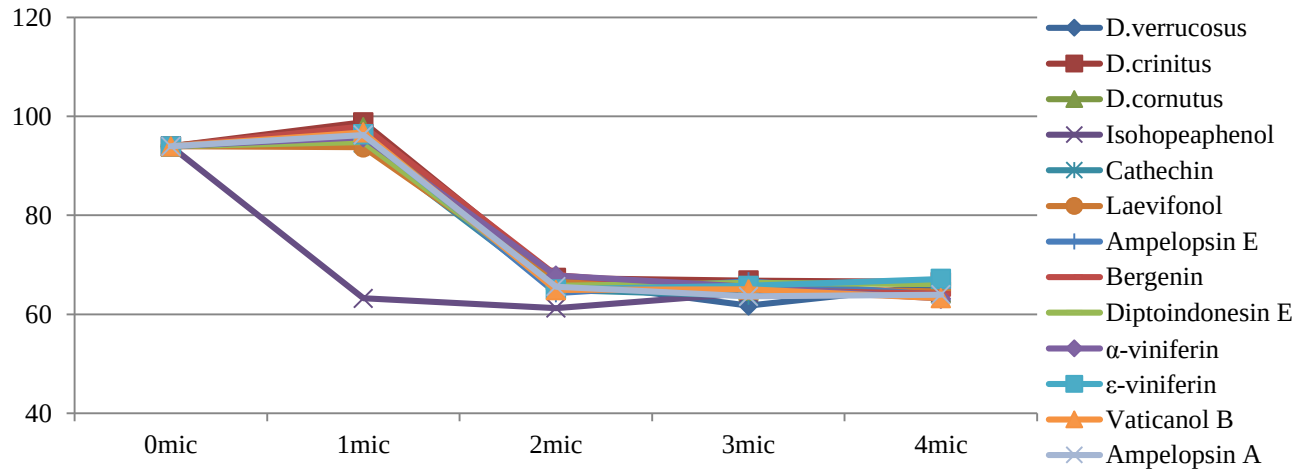

Figure 2: Effect on crudes and compounds to conidial germination of *Aspergillus oligosporus*

To the best of our knowledge, there is no reports that discuss the inhibition of conidia germination of filamentous fungi by *Dipterocarpus* crude and their chemical constituents. The discovery of new conidia germination inhibitors would be beneficial in controlling disease caused by pathogenic fungi (Jin et al., 2004). Fungi are categorised in the group of eukaryotic host organisms with a structure and metabolism attributes similar to that eukaryotic host. Therefore, adequate treatment of mycotic infections is a serious challenge in disease control. Hence, in this study, phenolics and stilbenoids compound isolated from the waste product of timber trees are potential valuable as a natural cure against mycotic or fungal infections.

(c) Germination inhibition assay for *Aspergillus oligosporus*

The germination assay of *A. oligosporus* as illustrated in Figure 2. The graph of germination indicated that all crudes and compounds have slightly similar pattern against *A. oryzae*. However, there is no compounds that achieve a complete sterility at 4 × MIC. The plotted graph shows that the average conidia germination of *F. oxysporum* was merely at 60%, describing its moderate potential against *R. oryzae*. *Oligosporus*

Conclusion

In short, the chemical constituents from the stem bark of *Dipterocarpus* against opportunistic filamentous fungi, *Fusarium oxysporum* is the most potent compound based on the fact that it competes with the standard reference of Amphotericin B. The results show the significant potential in waste product from timber to be efficiently utilized as an antifungal agent. Nevertheless, the aspects of pharmacokinetic and safety studies should be analyzed in future research to facilitates the holistic development of natural antifungals.

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Contribution of Authors

Wan ZWMZ: Data collection, conducted laboratory work, literature search, manuscript preparation
Norizan A: Design research methodology, conceived idea, advised on technical aspect
Yaya R: Design research methodology, statistical analysis, data interpretation
Che PO: Conceived idea, advised on technical aspect, manuscript preparation
Nor AHY: Statistical analysis, data interpretation, conceived idea
Neneng W: Statistical analysis, data interpretation, conceived idea

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