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Anti-candidal Activity of Crude Extracts and Compounds from *Dipterocarpus verrucosus Foxw. Ex Sloot, Dipterocarpus cornutus Dyer* and *Dipterocarpus crinitus* Dyer

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Dipterocarpus, commonly known as 'keruing,' is an important source of dammarane and contributes to a highly valuable economic plant in Southeast Asia. A preliminary study revealed that this plant is rich in phenolic compounds and potential antimicrobial properties. Thus, the stem bark of three *Dipterocarpus* species (*Dipterocarpus verrucosus*, *Dipterocarpus cornutus*, and *Dipterocarpus crinitus*) has been extensively studied chemically and biologically. The methanol extract was isolated using multiple chromatography techniques, and the structural elucidation of the compounds was characterized using UV, IR, NMR (1d &2D), HRESI-MS, and comparison with literature. The anticandidal activity of the methanolic crude extracts and compounds was determined using the disc diffusion method, Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC), and time-kill assay against pathogenic strains, namely *Candida glabrata.* In this study, three crude extracts and 12 phytochemicals of consisting of nine stilbenoids (ε-viniferin, ampelopsin A, α-viniferin, davidiol A, stenophyllol B, ampelopsin E, vaticanol B, diptoindonesin E, Hemsleyanol D, two phenolic compounds (bergenin, scopoletin) and one compound from terpene (β-sitosterol glucoside) were tested for their anticandidal activity. The disc diffusion method result showed that ε-viniferin was more susceptible to *C. glabrata* than other compounds; thus, this compound was selected for time-kill assay. The MIC and MFC ranged from 62.5 to 500 ppm. Time-kill curves demonstrated that ε-viniferin could inhibit *C. glabrata* strains at 500 ppm, after 120 min of treatment with a significant reduction of more than 3 $\log_{10}$ reduction. Results revealed the potential of ε-viniferin isolated from *Dipterocarpus* to be developed as an anticandidal agent against *C. glabrata.*

Keywords: Dipterocarpaceae; *Dipterocarpus*; *Candida glabrata*; anticandidal; time-kill assay; ε-viniferin

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Dipterocarpaceae family plants, also known as "keruing", are a significant source of dammarane, contributing to a highly valuable economic plant in Southeast Asia. It contains a variety of active compounds, including sesquiterpenes, triterpenes, flavonoids, and resveratrol oligomers [1-3]. Previously, *Dipterocarpus* extracts from the bark, stems, and fruits have been reported to have medicinal properties such as antimicrobial, antidiabetic, anti-oxidant, anti-inflammatory, anti-termite, and anticancer [4-7].

One of the active compounds found in *Dipterocarpus* plant extract is stilbenoids. Plants synthesize stilbenoids as a form of defense against pathogens, and the potential antimicrobial activity of this class of natural compounds has piqued the interest of researchers in recent years. These compounds were discovered in *Dipterocarpus* plants and were found to have antibacterial properties against methicillin-resistant *Staphylococcus aureus* (MRSA) [8]. Other studies have also reported the presence of stilbenoids in the *Dipterocarpus* [9-11]. The monomeric stilbenoids called resveratrol were reported to reversibly

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bind to ATP synthase in *Escherichia coli* at 94 µM, inhibiting bacteria growth [12]. Furthermore, resveratrol at 50-100 µg/mL exerted bacteriostatic and bactericidal activity in Gram-negative bacteria, *Arcobacter butzleri* and *Arcobacter cryaerophilus* [13].

Candida glabrata has emerged in several hospital settings, causing many systemic infections and life-threatening complications with high mortality rates [14]. Increasing hematogenic candidiasis related to *C. glabrata*, especially in high- risk cancer patients, has become a serious concern [15]. The rapid resistance of *C. glabrata* against antifungal agents was also reported by Sanguinetti *et al.* [16].

As a continuing study in resveratrol and antifungal studies [17, 18] in *Dipterocarpus*, we would like to conduct their anti-candidal activity against *C.glabrata.*

EXPERIMENTAL

Chemicals and Materials

Materials

Samples of the stem bark of *Dipterocarpus verrucosus*, *Dipterocarpus crinitus*, and *Dipterocarpus cornutus* were collected from forest reserve UiTM Jengka, Pahang, Malaysia. The plants were identified by a botanist, and a voucher specimen (SKD1, SKD2, and SKD3) was deposited in the herbarium of Universiti Teknologi MARA, Malaysia (Pahang campus).

Instrumentation

IR spectra were recorded on the Spectrum One FR-IR spectrometer (Perkin-Elmer). The UV spectra were recorded on a UV-Vis 160i (Shimadzu). The optical rotation was measured on the Autopolar VI Automatic Polari meter. The melting points (uncorrected) were determined using a micro-melting point apparatus. HRESI-MS spectra were obtained with Agilent Technologies 6224 TOF LC/MS. The 1D and 2D NMR data were obtained from FT Bruker 300 Ultra shield (300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively), JEOL UKM 500 MHz for 1H and 125 MHz for ^{13}C, respectively), JEOL Meijo Nagoya University Pharm Japan 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively) and Bruker 500 Ultra shield (500 MHz for 1H and 125 MHz for ^{13}C, respectively) (AuRIns UiTM) using various commercially available deuterated solvents such as chloroform-*d*, acetone-d_6, and methanol-d_4. Mestnova software was used to analyze the spectrum in detail. The Vacuum Liquid Chromatography (VLC) was carried out using Si-gel Merck 60 GF254 (230-400 mesh), the process of Column Chromatography was performed with Si-gel Merck 60 (200-400 mesh), Sephadex $LH_{20,}$ and TLC analysis on pre-coated Si gel plate Si- gel Merck Kieselgel 60 F254 0.25 mm, 20×20 cm.

Procedure

The stem barks of *D. verrucosus* were cut into small pieces, air-dried, and ground into fine powder. The finely grounded plant materials were weighed (6 kg) and macerated with acetone (4 × 9 L). The acetone extract was concentrated to a volume of ca. 250 mL. Diethyl ether was added to the concentrated acetone extract to obtain ether-soluble and insoluble fractions free from tannin. The soluble material was evaporated *in vacuo* at 40°C to yield 60 g crude extract. The extract was stored at room temperature. The isolation process started with 2 × 30g crude extract using VLC with a 10 cm in diameter column and silica gel weighed 250 g. Hexane chromatographed this crude: Ethyl acetate, Ethyl acetate: Methanol to methanol (100%) (Gradience of increasing methanol) to provide five fractions (DV1–DV5). The fractions were subjected to further isolation using repeated VLC. They were purified by repeated Radial Chromatography, Column Chromatography, and Preparative Thin Layer Chromatography (PTLC) on silica gel, eluted with various solvent systems such as $CHCl_3$:MeOH, Hex: $CHCl_3$:MeOH, $CHCl_3$:Hex, and $CHCl_3$:EtOAc: MeOH. The same procedure above was repeated on the samples of *D. cornutus* (5 kg) and *D. crinitus* (4 kg). [17, 18, 19, 20].

From the study, isolation using repeated VLC and then purification by repeated Radial Chromatography, Column Chromatography, and Preparative Thin Layer Chromatography (PTLC) on the stem barks of *D. verrucosus* discovered nine compounds [18]. The compound consists of eight oligo stilbenes and one phenolic compound. However, due to insufficient quantities, only six compounds will be reported in this study. Fraction 2 was fractionated using radial chromatography (eluent $CHCl_3$:EtOAc:MeOH) and yielded ε-viniferin (**1**) (6 mg) with radial chromatography (eluent $CHCl_3$: MeOH), Fraction 3 was fractionated using $CHCl_3$: MeOH) found ampelopsin E (**6**) (9 mg) and α-viniferin (**3**) (15 mg) also with radial chromatography (eluent Hex:$CHCl_3$:MeOH) yielded vaticanol B (**7**) (7 mg). In addition, Fraction 4 was refractionated using eluent $CHCl_3$:MeOH repetitive with radial chromatography found diptoindonesin E (**8**) (8 mg). Meanwhile, fraction 5 was refractionated with eluent $CHCl_3$:MeOH afforded one non-oligostilboid: bergenin (**10**) (15 mg) with eluent Hexane :$CHCl_3$: MeOH.

Meanwhile, the extraction of *D. cornutus* successfully isolated ten compounds consisting of six oligostilbenoid, three catechins, and one coumarin. In this study, only five compounds will reported. Fraction 2 was fractionated using radial chromatography eluent $CHCl_3$: MeOH found scopoletin (**11**) (17 mg), davidiol A (**4**) (15 mg), stenophyllol B (**5**) (15 mg). Additionally, Fraction 3 was fractionated with radial chromatography eluent Hex:$CHCl_3$: MeOH and PTLC (preparative thin layer chromatography) gave ε-viniferin (**1**) (8 mg). Fraction 5 was refractionated

using radial chromatography with eluent $CHCl_3$:MeOH hemsleyanol D (**9**) (15 mg).

In the meantime, eight compounds were successfully isolated from the *D. crinitus* extract, including five oligostilbenoids, two terpenoids, and one phenolic compound. In this study, only five compounds will be reported. Fraction 2 was refractionated using column chromatography with eluent Hex: $CHCl_3$) yielded β-sitosterol (**12**) (10 mg). Meanwhile, Fraction 3 was refractionated with column chromatography with eluent Hex:$CHCl_3$ yielded ε-viniverin (**1**) (9 mg). In addition, Fraction 4 successfully refractionated with column chromatography with the aid of Sephadex (eluent CHC_{l3}:MeOH) isolated ampelopsin A (**2**) (10mg), α-viniferin (**3**) (7 mg), and bergenin (**10**) (8 mg). Figure 1 shows all the isolated compounds.

ε-viniferin (**1**), obtained as brownish viscous oil, MS *m/z*: 455 $[MH]^+$. mp.: 172-176ºC. $[\alpha]_D^{20}$: -44ºC (c 0.1 MeOH). UV (MeOH) χmax (Logϵ): 203, 230, 324 nm. IR spectrum (KBr) υ max (cm^{-1}): 3383 (OH), 1640, 1514, 1440 (C=C aromatic), and 832 (*para*-disubstituent). Spectrum ^{1}H NMR (Methanol-d_4, 300 MHz) δ_H ppm: 7.18 (2H, *d*, *J* = 8.7, H-2a/6a), 6.81 (2H, *d*, *J* = 8.7, H-3a/5a), 5.39 (1H, *d*, *J* = 6.6, H-7a), 4.35 (1H, *d*, *J* = 6.6, H-8a), 6.18 (2H, *d*, *J* = 1.8, H-10a/14a), 6.20 (1H, *d*, *J* = 2.1, H-12a), 7.07 (2H, *d*, *J*=8.7, H-2b/6b), 6.68 (2H, *d*, *J* = 8.7, H-3b/5b), 6.87 (1H, *d*, *J* = 16.2, H-7b), 6.61 (1H, *d*, *J* = 16.2, H-8b), 6.27 (1H, *d*, *J* = 1.8, H-12b), 6.65 (1H, *d*, *J* = 1.8, H-14b). ^{13}C NMR (75 MHz) δ_C ppm: 132.8 (C-1a), 127.8 (C-2a/6a), 115.3 (C-3a/5a), 158.7 (C-4a), 93.0 (C-7a), 56.1 (C-8a), 146.6 (C-9a), 106.1 (C-10a), 160.0 (C-11a), 101.2 (C-12a), 160.0 (C-13a), 106.1 (C-14a), 129.1 (C-1b), 127.0 (C-2b/6b), 115.4 (C-3b/5b), 157.3 (C-4b), 122.3 (C-7b), 129.2 (C-8b), 135.5 (C-9b), 118.9 (C-10b), 161.6 (C-11b), 96.1 (C-12b), 161.6 (C-13b), 103.3 (C-14b)

Ampelopsin A (**2**) was obtained as a yellow crystal. MS *m/z*: 469 $[MH^-]$. mp.: 218-220ºC. $[\alpha]_D^{20}$: -160ºC (c 0.1 MeOH). UV (MeOH) χmax (Logϵ): 203, 226, 284 nm. IR spectrum (KBr) υ max (cm^{-1}): 3364 (OH), 2913 (C-H), 1614, 1587, 1516, 1454, 1440 (C=C aromatic) and 835 (*para*-disubstituent). Spectrum ^{1}H NMR (Acetone-d, 500 MHz) δ_H ppm: 7.11 (2H, *d*, *J* = 8.6, H-2a/6a), 6.75 (2H, *d*, *J* = 8.7, H-3a/5a), 5.75 (1H, *d*, *J* = 11.5, H-7a), 4.15 (1H, *brs*, H-8a), 6.42 (1H, *d*, *J* = 2.3, H-10a,), 6.22(1H, *d*, *J* = 2.3, H-12a), 6.89 (2H, *d*, *J* = 8.0, H-2b/6b), 6.63 (2H, *d*, *J* = 8.8, H-3b/5b), 5.44 (1H, *d*, *J* = 4.6, H-7b), 5.40 (1H, *d*, *J* = 4.6, H-8b), 6.14 (1H, *d*, *J* = 2.0, H-12b), 6.64 (1H, *d*, *J* = 2.0, H-14b). ^{13}C NMR (125 MHz) δ_C ppm: 132.7 (C-1a),129.9 (C-2a/6a), 116.0 (C-3a/5a), 158.5 (C-4a), 88.5 (C-7a), 49.6 (C-8a), 143.6 (C-9a), 118.4 (C-10a), 157.3 (C-11a), 101.6 (C-12a), 158.9 (C-13a), 105.6 (C-14a), 131.0 (C-1b), 128.8 (C-2b/6b), 115.4 (C-3b/5b), 156.1 (C-4b), 43.9 (C-7b), 71.2 (C-8b), 140.5 (C-9b), 118.9 (C-10b), 160.2 (C-11b), 97.1 (C-12b), 158.9 (C-13b), 110.5 (C-14b)

α-viniferin (**3**), obtained as pale yellow, MS *m/z*: 677 $[MH^-]$. mp.: 220-223ºC. $[\alpha]_D^{20}$: +60ºC (c 0.1 MeOH). UV (MeOH) χmax (Logϵ): 203, 226, 284 nm. IR spectrum (KBr) υ max (cm^{-1}): 3393 (OH), 1613, 1462, 1337 (C=C aromatic), and 831 (*para*-disubstituent). Spectrum ^{1}H NMR (Acetone-d_6, 300 MHz) δ_H ppm: 7.02 (2H, *d*, *J* = 8.7, H-2a/6a), 6.71 (2H, *d*, *J* = 8.7, H-3a/5a), 6.08 (1H, *s*, H-7a), 3.97(1H, *brs* , H-8a), 6.00 (1H, *d*, *J* = 2.1, H-12a), 6.23 (1H, *d* ,*J* = 2.1, H-14a), 7.22 (2H, *d*, *J* = 8.7, H-2b/6b), 6.79 (2H, *d*, *J* = 8.7, H-3b/5b), 5.96 (1H, *d*, *J* = 9.9, H-7b), 4.71 (1H, *d*, *J* = 9.9, H-8b). 6.73 (1H, *d*, *J* = 2.1, H-12b), 6.25 (1H, *d*, *J* = 2.1, H-12b), 7.06 (2H, *d*, *J* = 8.7, H-2c/6c), 6.80 (2H, *d*, *J* = 8.7, H-3c/5c), 4.91 (1H, *d*, *J* = 6.3, H-7c), 4.61 (1H, *d*, *J* = 6.3, H-8c), 6.60 (1H, *d*, *J* = 1.8, H-12c), 6.22 (1H, *d*, *J* = 2.1, H-14a), ^{13}C NMR (75 MHz) δ_C ppm: 132.0 (C-1a), 128.1 (C-2a/6a), 115.7 (C-3a/5a), 157.8 (C-4a), 86.4 (C-7a), 46.4 (C-8a), 118.8 (C-9a), 141.2 (C-10a), 159.3 (C-11a), 108.5 (C-12a), 161.5 (C-13a), 98.0 (C-14a), 132.2 (C-1b), 128.6 (C-2b/6b), 116.1 (C-3b/5b), 158.2 (C-4b), 89.9 (C-7b), 52.8 (C-8b), 120.9 (C-9b), 139.7 (C-10b), 159.34 (C-11b), 106.2 (C-12b), 158.4 (C-13b), 96.8 (C-14b), 132.4 (C-1c), 128.6 (C-2c/6c), 116.0 (C-3c/5c), 158.2 (C-4c), 95.5 (C-7c), 55.6 (C-8c), 119.6 (C-9c), 138.6 (C-10c), 160.9 (C-11c), 105.7 (C-12c), 161.7 (C-13c), 96.8 (C-14c)

Davidiol A (**4**), obtained as a brown amorphous powder. MS *m/z*: 679 $[MH^-]$. mp.: 255-257ºC. $[\alpha]_D^{20}$: -275ºC (c 0.1 MeOH). UV (MeOH) χmax (Logϵ): 203, 226, 284 nm. IR spectrum (KBr) υ max (cm^{-1}): 3418 (OH), 1614, 1515, 1455 (C=C aromatic), and 833 (*para*-disubstituent). Spectrum ^{1}H NMR (acetone-d_6, 300 MHz) δ_H ppm: 7.21 (2H, *d*, *J* = 8.7, H-2a/6a), 6.80 (2H, *d*, *J* = 8.7, H-3a/5a), 6.09 (1H, *d*, *J* = 3.0, H-7a), 4.42 (1H, *d*, *J* = 9.6, H-8a), 6.44 (1H, *d*, *J* = 2.1, H-12a), 6.57 (1H, *d*, *J* = 2.4, H-14a), 7.02 (2H, *d*, *J* = 8.7, H-2b/6b), 6.60 (2H, *d*, *J* = 8.7, H-3b/5b), 5.28 (1H, *br s*, H-7b), 4.27 (1H, *d*, *J* = 11.4, H-8b). 6.04 (1H, *s*, H-12b), 6.74 (2H, *d*, *J* = 8.7, H-2c/6c), 6.61 (2H, *d*, *J* = 8.7, H-3c/5c), 4.39 (1H, *d*, *J* = 9.3 H-7c), 2.93 (1H, *dd*, *J* = 11.7, 9.9, H-8c), 6.43 (1H, *d*, *J* = 2.4, H-10c), 6.19 (1H, *t*, *J* = 2.1, H-12c), 6.43 (1H, *d*, *J* = 2.4, H-14c). ^{13}C NMR (75 MHz) δ_C ppm: 133.4 (C-1a), 127.2 (C-2a/6a), 115.1 (C-3a/5a), 155.0 (C-4a), 85.0 (C-7a), 49.6 (C-8a), 146.2 (C-9a), 117.0 (C-10a), 158.0 (C-11a), 100.0 (C-12a), 157.2 (C-13a), 103.0 (C-14a), 136.6 (C-1b), 128.7 (C-2b/6b), 114.4 (C-3b/5b), 157.3 (C-4b), 35.7 (C-7b), 50.9 (C-8b), 142.4 (C-9b), 118.2 (C-10b), 158.6 (C-11b), 95.0 (C-12b), 153.8 (C-13b), 121.5 (C-14b), 133.5 (C-1c), 129.0 (C-2c/6c), 114.6 (C-3c/5c), 157.2 (C-4c), 55.3 (C-7c), 66.6 (C-8c), 143.2 (C-9c), 107.5 (C-10c), 158.5 (C-11c), 100.1 (C-12c), 158.7 (C-13c), 107.3 (C-14c)

Stenophyllol B (**5**) was obtained as a brown amorphous powder. MS *m/z*: 679 $[MH^-]$, mp.: 255-257ºC. $[\alpha]_D^{20}$: -20ºC (c 0.1 MeOH). UV (MeOH) χmax (Logϵ): 205, 228, 287 nm. IR spectrum (KBr) υ max (cm^{-1}): 3418 (OH), 1616, 1544, 1455 (C=C aromatic), and 831 (*para*-disubstituent). Spectrum ^{1}H NMR

(acetone-d_6, 300 MHz) δ_H ppm: 6.88 (2H, *d*, *J* = 8.7, H-2a/6a), 6.77 (2H, *d*, *J* = 8.7, H-3a/5a), 5.84 (1H, *d*, *J* = 3.3, H-7a), 5.07 (1H, *d*, *J* = 3.3, H-8a), 6.31 (1H, *d*, *J* = 2.1, H-12a), 6.25 (1H, *d*, *J* = 2.1, H-14a), 7.20 (2H, *d*, *J* = 8.4, H-2b/6b), 6.66 (2H, *d*, *J* = 8.4, H-3b/5b), 4.73 (1H, *d*, *J* = 6.3, H-7b), 4.73 (1H, *d*, *J* = 6.3, H-8b), 6.79 (1H, *s*, H-14b), 7.29 (2H, *d*, *J* = 8.1, H-2c/6c), 6.68 (2H,*d*, *J* = 8.1, H-3c/5c), 5.35 (1H, *d*, *J* = 9.6 H-7c), 4.30 (1H, *dd*, *J* = 10.5, 8.4, H-8c), 6.07 (1H, *m*, H-12c), 6.07 (1H, *m*, H-14c). ^{13}C NMR (75 MHz) δ_C ppm: 135.5 (C-1a), 128.2 (C-2a/6a), 116.9 (C-3a/5a), 158.7 (C-4a), 89.0 (C-7a), 53.5 (C-8a), 142.2 (C-9a), 124.5 (C-10a), 157.5 (C-11a), 102.4 (C-12a), 159.8 (C-13a), 107.8 (C-14a), 137.8 (C-1b), 130.9 (C-2b/6b), 116.8 (C-3b/5b), 157.1 (C-4b), 52.8 (C-7b), 57.4 (C-8b), 145.2 (C-9b), 121.4 (C-10b), 161.4 (C-11b), 96.8 (C-12b), 160.1 (C-13b), 109.3 (C-14b), 140.6 (C-1c), 130.8 (C-2c/6c), 116.8 (C-3c/5c), 157.2 (C-4c), 48.2 (C-7c), 54.5 (C-8c), 151.8 (C-9c), 124.4 (C-10c), 155.7 (C-11c), 100.1 (C-12c), 158.7 (C-13c), 107.3 (C-14c)

Ampelopsin E (**6**), obtained as a reddish yellow, MS *m/z*: 679 [M^+]. Mp.: 180-182ºC. $[\alpha]_D^{20}$: -94ºC (c 0.1 MeOH). UV (MeOH) χmax (Logϵ): 203, 230, 325 nm. IR spectrum (KBr) υ max (cm^{-1}): 3367 (OH), 2947 (C-H aliphatic), 1655, 1452 (C=C aromatic). Spectrum ^{1}H NMR (Methanol-d_4, 300 MHz) δ_H ppm: 7.27 (2H, *d*, *J* = 8.4, H-2a/6a), 6.84 (2H, *d*, *J* = 8.4, H-3a/5a), 5.45 (1H, *d*, *J* = 4.8, H-7a), 4.53 (1H, *d*,*J*=4.8 H-8a), 6.26 (1H, *d*, *J* = 2.4, H-10a), 6.26 (1H, *d*, *J* = 2.4, H-10a), 6.23 (1H, *t*, *J* = 2.0, H-12a), 6.26 (1H, *d*, *J* = 2.4, H-14a), 6.62 (2H, *d*, *J* = 8.0, H-2b/6b), 6.59 (2H, *d*, *J* = 8.4, H-3b/5b), 6.63 (1H, *d*, *J* = 16.5, H-7b), 6.59 (1H, *d*, *J* = 16.5, H-8b), 6.44 (1H, *s*, H-12b), 7.28 (2H, *d*, *J* = 8.5, H-2c/6c), 6.87 (2H, *d*, *J* = 8.5, H-3c/5c), 5.45 (1H, *d*, *J* = 5.4, H-7c), 4.56 (1H, *d*, *J* = 4.8, H-8c), 6.23 (1H, *d*, *J* = 2.4, H-10c), 6.23 (1H, *t*, *J* = 2.0, H-12c), 6.26 (1H, *d*, *J* = 2.0, H-14c). ^{13}C NMR (75 MHz) δ_C ppm: 134.0 (C-1a), 128.6 (C-2a/6a), 116.5 (C-3a/5a), 158.4 (C-4a), 94.1 (C-7a), 55.6 (C-8a), 147.3 (C-9a), 107.0 (C-10a), 160.0 (C-11a), 102.14 (C-12a), 160.0 (C-13a), 102.14 (C-14a), 133.7 (C-1b), 127.9 (C-2b/6b), 115.9 (C-3b/5b), 158.3 (C-4b), 124.6 (C-7b), 131.8 (C-8b), 130.2 (C-9b), 120.1 (C-10b), 162.5 (C-11b), 91.3 (C-12b), 162.5 (C-13b), 120.1 (C-14b), 134.0 (C-1c), 128.6 (C-2c/6c), 116.5 (C-3c/5c), 158.4 (C-4c), 94.1 (C-7c), 55.6 (C-8c), 147.3 (C-9c), 107.0 (C-10c), 160.0 (C-11c), 102.1 (C-12c), 160.0 (C-13c), 107.0 (C-14c)

Vaticanol B (**7**) was obtained as a brown amorphous powder. MS *m/z*: 905 [MH^-]. Mp.: 205-207ºC. $[\alpha]_D^{20}$: -40ºC (c 0.1 MeOH). UV (MeOH) χmax (Logϵ): 203, 230, 284 nm. IR spectrum (KBr) υ max (cm^{-1}): 3367 (OH), 2947 (C-H aliphatic), 1655, 1452 (C=C aromatic). Spectrum ^{1}H NMR (Methanol-d_4, 500 MHz) δ_H ppm: 7.18 (2H, *d*, *J* = 8.5, H-2a/6a), 6.78 (2H, *d*, *J* = 8.5, H-3a/5a), 5.72 (1H, *d*, *J* = 12.0, H-7a), 4.33 (1H, *d*,*J*= 12.0, H-8a), 6.18 (1H, *d*, *J* = 2.0, H-12a), 6.05 (1H, *s*, H-10a), 7.13 (2H, *d*, *J* = 8.5, H-2b/6b), 6.68 (2H, *d*, *J* = 8.5, H-3b/5b), 5.28 (1H, *d*, *J* = 5.5, H-7b), 3.15 (1H, *d*, *J* = 12.5, H-8b), 5.98 (1H, *s*, H-12b), 6.45 (2H, *d*, *J* = 8.5, H-2c/6c), 6.49 (2H, *d*, *J* = 8.5, H-3c/5c), 4.08 (1H, *t*, *J* = 11.5, H-7c), 4.42 (1H, *d*, *J* = 10.5, H-8c), 6.19 (1H, *s*, H-12c), 6.44 (1H, *d*, *J* = 1.5, H-14c), 7.14 (2H, *d*, *J* = 8.5, H-2d/6d), 6.75 (2H, *d*, *J* = 8.5, H-3d/5d), 5.28 (1H, *d*, *J* = 5.5, H-7d), 5.99 (2H, *d*, *J* = 2.5, H-10d/14d), 6.20 (1H, *d*, *J*=2.0,H-12d).^{13}C NMR (125 MHz) δ_C ppm: 129.7 (C-1a), 130.9 (C-2a/6a), 114.9 (C-3a/5a), 157.9 (C-4a), 89.6 (C-7a), 49.3 (C-8a), 141.3 (C-9a), 124.3 (C-10a), 154.4 (C-11a), 100.5 (C-12a), 156.9 (C-13a), 100.9 (C-14a), 132.9 (C-1b), 129.4 (C-2b/6b), 113.8 (C-3b/5b), 154.7 (C-4b), 35.8 (C-7b), 51.9 (C-8b), 147.2 (C-10b), 113.8 (C-11b), 154.7 (C-12b), 35.8 (C-13b), 51.9 (C-8b), 147.2 (C-9b), 113.8 (C-10b), 158.3 (C-11b), 94.3 (C-12b), 154.0 (C-13b), 121.6 (C-14b), 130.9 (C-1c), 129.0 (C-2c/6c), 114.2 (C-3c/5c), 155.3 (C-4c), 57.4 (C-7c), 49.3 (C-8c), 140.7 (C-9c), 122.5 (C-10c), 160.8 (C-11c), 94.2 (C-12c), 159.6 (C-13c), 104.4 (C-14c), 133.6 (C-1d), 127.3 (C-2d/6d), 114.9 (C-3d/5d), 157.4 (C-4d), 89.6 (C-7d), 56.5 (C-8d), 142.5 (C-9d), 106.1 (C-10d/14d), 160.4 (C-11d/13d), 100.5 (C-12d)

Diptoindonesin E (**8**) was obtained as a white amorphous powder. MS *m/z*: 903 [MH^-], Mp.: 233-235ºC. $[\alpha]_D^{20}$: -95ºC (c 0.1 MeOH). UV (MeOH) χmax (Logϵ): 205, 228, 325 nm. IR spectrum (KBr) υ max (cm^{-1}): 3401 (OH), 2922 (C-H aliphatic), 1655, 1452 (C=C aromatic). Spectrum ^{1}H NMR (Methanol-d_4, 300 MHz) δ_H ppm: 7.27 (2H, *d*, *J* = 8.7, H-2a/6a), 6.88 (2H, *d*, *J* = 8.7, H-3a/5a), 5.47 (1H, *d*, *J* = 12.0, H-7a), 4.69 (1H, *d*, *J*=3.9,H-8a), 6.26 (2H, *d*, *J* = 2.1, H-10a/14a), 6.19 (1H, *t*, *J* = 2.1, H-12a), 7.70 (2H, *d*, *J* = 2.1, H-2b/6b), 6.74 (2H, *d*, *J* = 8.5, H-3b/5b), 6.78 (1H, *d*, *J* = 5.5, H-7b), 6.70 (1H, *d*, *J* = 16.5, H-8b), 6.42 (1H, *s*, H-12b), 6.46 (2H, *d*, *J* = 8.7, H-2c/6c), 6.52 (2H, *d*, *J* = 8.7, H-3c/5c), 5.04 (1H, *d*, *J* = 1.9, H-7c), 4.76 (1H, *d*, *J* = 1.9, H-8c), 6.23 (1H, *d*, *J* = 2.2, H-12c), 5.99 (1H, *d*, *J* = 2.1, H-14c), 7.50 (1H, *d*, *J* = 2.4, H-2d), 6.89 (1H, *d*, *J* = 8.7, H-5d), 7.23 (*dd*, *J* = 9.0,2.1,H-6d), 5.18 (1H, *d*, *J* = 1.5, H-7d), 4.79 (1H, *d*, *J* = 1.6, H-8d), 5.95 (2H, *brd*, *J* = 2.1, H-10d/14d), 6.29 (1H, *t*, *J* = 2.1, H-12d). ^{13}C NMR (75 MHz) δ_C ppm: 133.3 (C-1a), 126.8 (C-2a/6a), 116.2 (C-3a/5a), 158.9 (C-4a), 93.6 (C-7a), 57.1 (C-8a), 141.7 (C-9a), 106.0 (C-10a/14a), 159.4 (C-11a/13a), 101.4 (C-12a), 131.5 (C-1b), 130.8 (C-2b/6b), 126.2 (C-3b), 153.8 (C-4b), 116.9 (C-5b), 128.8 (C-6b), 131.1 (C-7b), 126.9 (C-8b), 131.1 (C-9b), 115.9 (C-10b), 162.5 (C-11b), 91.6 (C-12b), 161.9 (C-13b), 122.0 (C-14b), 132.9 (C-1c), 126.9 (C-2c/6c), 115.6 (C-3c/5c), 157.4 (C-4c), 90.6 (C-7c), 51.4 (C-8c), 145.6 (C-9c), 118.9 (C-10c), 162.7 (C-11c), 95.9 (C-12c), 161.8 (C-13c), 107.2 (C-14c), 135.6 (C-1d), 131.9 (C-2d/6d), 128.4 (C-3d), 156.8 (C-4d), 115.5 (C-5d), 91.5 (C-7d), 55.2 (C-8d), 147.2 (C-9d), 106.1 (C-10d/14d), 161.8 (C-11d/13d), 102.1 (C-12d)

Hemsleyanol D (**9**) was obtained as a brownish-yellow solid. MS *m/z*: 905 [MH^-]. Mp.: 280-282ºC. $[\alpha]_D^{20}$: +29ºC(c 0.1 MeOH). UV (MeOH) χmax (Logϵ): 203, 230, 284 nm. IR spectrum (KBr) υ max (cm^{-1}):

3400 (OH), 2927 (C-H aliphatic), 1614, 1512 (C=C aromatic). Spectrum ^{1}H NMR (acetone-d_6, 300 MHz) δ_H ppm: 7.22 (2H, *d*, *J* = 8.7, H-2a/6a), 6.78 (2H, *d*, *J* = 8.5, H-3a/5a), 5.77 (1H, *d*, *J* = 11.7, H-7a), 4.41 (1H, *d*, *J* = 11.7, H-8a), 6.36 (1H, *d*, *J* = 2.4, H-12a), 6.12 (1H, *d*, *J* = H-10a), 6.94 (2H, *d*, *J* = 8.7, H-2b/6b), 6.48 (2H, *d*, *J* = 8.7, H-3b/5b), 5.29 (1H, *d*, *J* = 3.4, H-7b), 3.38 (1H, *d*, *J* = 10.9, H-8b), 6.02 (1H, *s*, H-12b), 6.72 (2H, *d*, *J* = 8.7, H-2c/6c), 6.52 (2H, *d*, *J* = 8.7, H-3c/5c), 4.55 (1H, *d*, *J* = 10.2, H-7c), 3.89 (1H, *dd*, *J*=11.7, 10.8, H-8c), 6.23 (1H, *d*, *J* = 2.0, H-12c), 6.79 (1H, *s*, H-14c), 7.06 (2H, *d*, *J* = 8.4, H-2d/6d), 6.82 (2H, *d*, *J* = 8.4, H-3d/5d), 4.92 (1H, *d*, *J* = 1.5, H-7d), 3.50 (1H, *brs*, H-8d), 5.34 (2H, *brs*, H-10d/14d), 6.07 (1H, *t*, *J* = 2.1 H-12d). ^{13}C NMR (75 MHz) δ_C ppm: 132.5 (C-1a), 129.9 (C-2a/6a), 115.3 (C-3a/5a), 157.7 (C-4a), 89.6 (C-7a), 48.0 (C-8a), 140.7 (C-9a), 124.0 (C-10a), 154.9 (C-11a), 100.6 (C-12a), 155.9 (C-13a), 104.9 (C-14a), 133.9 (C-1b), 129.3 (C-2b/6b), 115.3 (C-3b/5b), 157.1 (C-4b), 36.2 (C-7b), 56.5 (C-8b), 142.1 (C-10b), 114.9 (C-11b), 158.4 (C-12b), 153.8 (C-13b), 120.4 (C-14b), 132.5 (C-1c), 128.4 (C-2c/6c), 114.7 (C-3c/5c), 155.8 (C-4c), 53.1 (C-7c), 57.4 (C-8c), 140.1 (C-9c), 94.8 (C-10c), 162.2 (C-11c), 116.3 (C-12c), 159.5 (C-13c), 104.9 (C-14c), 136.4 (C-1d), 127.1 (C-2d/6d), 115.3 (C-3d/5d), 154.8 (C-4d), 93.1 (C-7d), 60.1 (C-8d), 147.1 (C-9d), 105.5 (C-10d/14d), 158.1 (C-11d/13d), 101.2 (C-12d)

Bergenin (**10**) was obtained as a white crystal. MS *m/z*: 327 [MH$^-$], Mp.: 244-246^{o}C. $[\alpha]_D^{20}$: -30^{o}C (c 0.1 MeOH). UV (MeOH) χmax (Logϵ): 307, 274 nm. IR spectrum (KBr) υ max (cm^{-1}): 3420 (OH), 2927 (C-H aliphatic), 1614, 1512 (C=C aromatic), 1703 (C=O), 2949 (C-H). Spectrum ^{1}H NMR (Acetone-d_6, 500 MHz) δ_H ppm: 4.00 (1H, *dd*, *J* = 10.0, 4.0, H-2), 3.45 (1H, *t*, *J* = 9.0, H-3), 3.80 (1H, *t*, *J* = 9.0, H-4), 3.70 (1H, *dd*, *J* =9.5, 7.0, H-4A), 7.08 (1H, *s*, H=7), 4.94 (1H, *d*, *J* = 10.8, H-10B), 3.65 (1H, *m*, H-11A), 4.10 (1H, *dd*, *J* = 9.5,4.0, H-11B), 3.89 (1H,*s*, OMe).^{13}C NMR (125MHz) δ_C ppm: 81.5 (C-2), 71.9 (C-3), 75.7 (C-4), 83.0 (C-4A), 165.8 (C-6), 119.526A), 111.1(C-7), 152.42(C-8), 142.3(C-9), 149.5(C-10), 117.3(C-10A), 74.32(C-10B), 62.72(C-11), 60.92(C-Ome)

Scopoletin (**11**) was obtained as a white powder. Mp.: 171-175^{o}C. UV (MeOH) χmax (Logϵ): 256, 342 nm. IR spectrum (KBr) υ max (cm^{-1}): 3536 (OH), 2927 (C-H aliphatic), 1700, 1635(C=O conjugated), 1616, 1562, 1461 (C=C aromatic), 1288, 1140 (C-O oxyaryl). Spectrum ^{1}H NMR (methanol-d_4, 300 MHz) δ_H ppm: 6.20 (1H, *d*, *J* = 9.3, H-3), 7.84 (1H, *d*, *J* = 9.3, H-4), 7.12 (1H, *s*, H-5), 6.78 (1H, *s*, H-8), 3.92 (3H, *s*, OCH_3). ^{13}C NMR (75 MHz) δ_C ppm: 161.4 (C-2), 113.3 (C-3), 144.7 (C-4), 112.1 (C-4a), 109.9 (C-5), 146.0 (C-6), 151.9 (C-7), 103.8 (C-8), 151.2 (C-8a), 56.7 (C-OMe)

β-sitosterol (12) was obtained as a whitish solid. Mp: 287-295^{o}C. The IR spectrum (KBr) υ max (cm^{-1}): 3423 (OH), 2935 (C-H), 1054 (C-O). Spectrum ^{1}H NMR (methanol-d_4, 300 MHz) δ_H ppm: 3.53 (1H, *tdd*, *J* =4.5,4.2,3.8,H-2), 5.36 (1H, *t*, *J* =6.4,H-5), 0.93 (1H, *d*, *J* =6.5, H-19), 0.84 (1H, *t*, *J* = 7.2, H-24), 0.83 (1H, *d*, *J* = 6.4, H-26), 0.81 (1H, *d*, *J* = 6.4, H-27), 0.68 (1H, *s*, H-28), 1.01 (1H, *s*, H-29). ^{13}C NMR (75 MHz) δ_C ppm: 37.2 (C1), 31.6 (C2), 71.8 (C3), 42.3 (C4), 140.8 (C5), 121.7 (C6), 31.9 (C7), 31.9 (C-8), 50.1 (C-9), 36.5 (C-10), 21.7 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 28.2 (C-16), 56.0 (C-17), 11.9 (C-18), 19.4 (C-19), 36.1 (C-20), 19.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.8 (C-24), 29.1 (C-25), 19.8 (C-26), 19.0 (C-27), 23.1 (C-28), 12.0 (C-29).

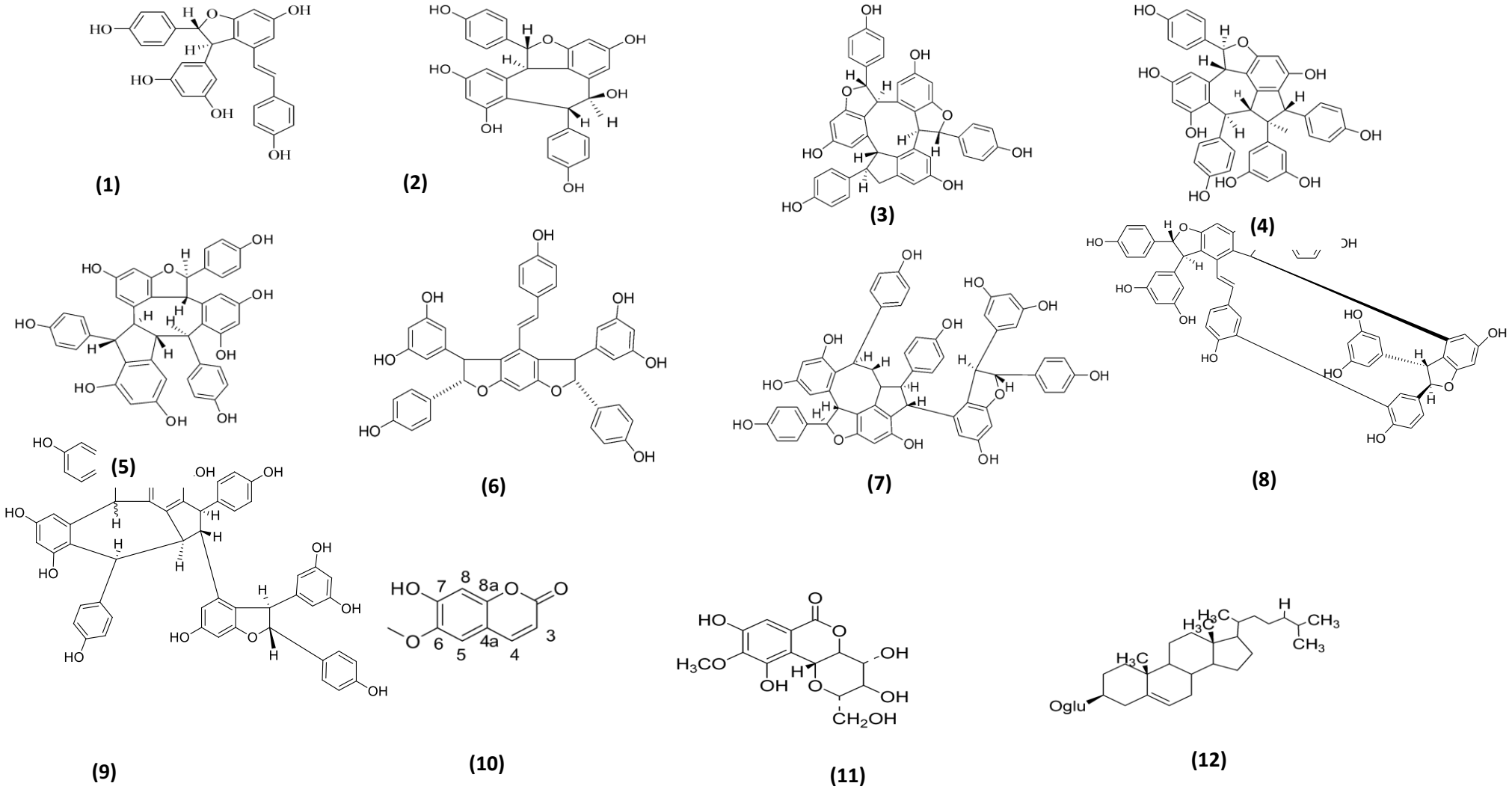

Figure 1. Isolated compounds from *D.verrucosus, D.crinitus* and *D. cornutus.*

Preparation of Antimicrobial Agents

The methanolic crude extracts and isolated compounds were dissolved in 100% dimethylsulfoxide (DMSO) (Sigma-Aldrich, Saint Louis, MO, USA) following the protocol of the Clinical Laboratory Standards Institute (CLSI) [21]. The final concentration of the methanolic extracts was standardized at 1% (10000 ppm), while the final concentration of the compounds was 0.1% (1000 ppm). However, DMSO at 10% served as a control.

***Candida* Strain and Inoculum Preparation**

Candida glabrata (ATCC 20021) used in this study was purchased from the American Type Culture Collection (Rockville, MD, USA). The *Candida* strain was cultured and maintained on Sabouraud dextrose broth (SDB) or Sabouraud dextrose agar (SDA) (Difco, Spark, MD, USA) medium at various optimal temperatures depending on the strain.

Preparation of *Candida glabrata*

The *Candida* was prepared as recommended by CLSI standards: M02-A11 and M27-A3. In brief, the stock culture of *Candida* was cultured on SDA at 37°C for 48-72 h [22]. Three to five yeast colonies were transferred into 1 mL of SDB using a sterile cotton swab and mixed with a vortex for 15 min. After the vortex, the yeast suspension was grown for 48-72 h with 200 rpm agitation. Then, 10 µL of candida suspension was transferred into 10 mL of SDB. The turbidity of inoculums was standardized to 10^6-10^8 CFU/mL before the test by using the standard broth microdilution method and inoculum quantification. Inoculum quantification was performed by plating 25 µL of candida suspension on SDA and counting the visible colonies after incubation at 37°C for 48-72 h.

Disc Diffusion Method

Using the disc diffusion method, methanolic *Dipterocarpus* crude extracts and isolated compounds were tested for anticandidal activity against *C. glabrata* strains. An overnight culture of each strain was spread on SDA using a sterile cotton swab. A sterile paper disc (6 mm) was impregnated with 10 µL of 1% extract and 0.1% compound. Amphotericin B (1%) was included as a positive control. The plates were incubated at 37°C for 48-72 h and observed for any clear zone surrounding the paper disc (including the disc diameter). The test was performed in duplicate to verify the results.

Minimum Inhibitory Concentration (MIC)

In order to determine the MIC of the methanolic crude extracts and isolated compounds on fungal tests, the standard microtitre broth dilution method was applied. This was guided according to a method described in the guidelines of CLSI, M7-A6. A 100 µL of conidial inoculum of approximately 10^6-10^8 CFU/mL of *C. glabrata* was prepared. Then, a two-fold dilution of the extract or compound agent (using 100 µL of 0.1% solution) was performed starting from well number 12 (concentration of 500 µg/mL) till well number 3 (concentration of approximately 1 µg/mL), and the remaining 100 µL from well number 3 was discarded. Well number 1 served as a negative control (only medium), and well number 2 as a growth control (medium containing conidial inoculums). The 96-well plate was then covered by a sterile cover lid and sealed with parafilm before it was incubated at 37ºC for 48-72 h.

Minimum Fungicidal Concentration (MFC)

The MFC was determined by sub-culturing 10 µL of each of the suspensions from the microtiter plates (including positive and negative growth control) onto the SDA and incubated for 48-72 h at 37°C. The least concentration that showed no visible growth was considered the MFC value [23].

Time-Kill Assay

A time-kill curve assay was carried out with the MIC values found previously in the microplate bioassay by referring to [26]. ε-viniferin was diluted with the SDB medium containing an inoculum of approximately 10^6-10^8 CFU/mL to obtain final concentrations of 0×, 0.5×, 1×, 2×, 4×, and 8×MIC for the *C. glabrata*. At 0, 15, 30, 60, 120, and 240 min, each mixture (extract with inoculum suspension) was serially diluted with 1.0% sterile phosphate buffer saline and spread separately to the SDA plate with replications, The plates were incubated at 37°C for 48-72 h, and colonies were counted after that time. All tests were done three times with three replications each (n=3×3).

RESULTS AND DISCUSSION

From the isolation, 12 compounds were identified in this study (Figure 1), analyzed with 1H NMR, 13C NMR, LCMS, UV, and IR, and compared with previous literature data. All compounds consist of 9 stilbenoids which were ε-viniferin [27], ampelopsin A [28], α-viniferin [29], davidiol A [30], stenophyllol B [31], ampelopsin E [32], vaticanol B [30], diptoindonesin E [33], hemsleyanol D [34], two phenolics which were bergenin [35] and scopoletin [36] and one terpene which was β-sitosterol glucoside [37].

The anticandidal activities of the methanolic extracts of *D. verrucosus*, *D. cornutus*, *D. crinitus* with a concentration of 1% and 12 isolated compounds with 0.1% against *C. glabrata* strains are summarized in Table 1. The principle of the disc diffusion method states that a larger inhibition zone will have more significant activity. Thus, the data indicates that all the crude extracts and compounds exhibited an anticandidal activity but had a lower inhibition

zone when compared to standard Amphotericin B, except for ε-viniferin, stenophyllol B, and β-sitosterol glucoside. ε-viniferin exhibited significantly higher anticandidal activity than amphotericin B. Meanwhile, stenophyllol B and β-sitosterol glucoside showed no significant difference with amphotericin B. The disc diffusion method is only used to screen for anticandidal activity in the plant. This test sometimes gives inaccurate results due to some limitations, such as the ability of extracts to pass through the pore discs and the inability of hydrophobic compounds to diffuse into the media agar [25]. Therefore, the MIC and MFC have to be determined. DMSO, which served as a control, was found not to kill the tested *C. glabrata* in this study.

The MIC and MFC of crude extracts and compounds against *C. glabrata* are shown in Table 1. MIC was defined as the lowest concentration of antimicrobial agents that inhibit the microorganism population. However, MFC was defined as the minimum concentration of plant extracts required to kill at least 99 % of the fungi [22]. The MFC was performed after the determination of the MIC value. The results generally show that the MIC and MFC of amphotericin B against *C. glabrata* coincide with previous reports [26]. The results indicated that all crude extracts and compounds had concentrations ranging from 62.5-500 ppm. The value of MFC might be the same or higher than the MIC value, but it is impossible to be lower than the MIC value. The MFC values for the strain in this study indicated higher values than its MIC values. It can, therefore, be interpreted that they acted against the fungal strains by fungicidal action.

Stilbenoids exist as monomers or oligomers. In this study, dimer, trimer, and tetramer oligostilbeoids have been isolated (Table 1). From this finding, it shows that molecular structure is not the main factor that influences anticandidal activity. This is supported by the data provided because diptoindonesin E, a tetramer structure, was shown to be more active with a MIC of 62.5 ppm than ampleopsin A, a dimer with a MIC of 125 ppm.

In contrast, ε-viniferin dimer has been shown to be more active than hemslyenol D and vaticanol B. This finding has also been supported by Mattio *et al.* [37] in an overview of recent achievements in the study of stilbenoids as antimicrobial agents, with particular emphasis on the sources, chemical structures, and the mechanism of action of the most promising natural compounds. However, the structure analysis relationship in this study observed that the presence of *trans* olefinic units in ε-viniferin and diptoindesin E were responsible for electron delocalization in the compounds' skeletons, which relatively gave more potent antifungal activity. These results agreed with the previous study on anti-microbials [19], which revealed the presence of free resveratrol in upunaphenol D and flexuosol A, which showed significant activity compared with the others. Our previous work on anti-fungals [17] also showed that resveratrol and ε-viniverin performed the best activities, which gave complete inhibition of 0% towards *Fusarium oxysporum* at a concentration of 15 µg/mL (2×MIC). A study done by Sahidin *et al.* [39] revealed that ε-viniferin and balanocarpol, a dimer stilbenoid, were found to be the most effective compounds against *Escherichia coli* and *Staphylococcus aureus* compared to trimer and tetramer stilbenoid. Therefore, in this case, the molecular size seemed to cause a different penetration into the microorganism, affecting the anti-bacterial activity. The molecular size of the compound significantly influences bacterial membrane permeability [40].

The MIC is the parameter commonly used to guide the selection of the antimicrobial agents used in treatment by predicting their efficacy at a standard inoculum of approximately 10^6-10^8 CFU/mL after an incubation period of 48-72 h. However, MIC only provides limited information on the kinetics of the antimicrobial action. Therefore, a time-killing assay was performed to find the correlation between the rate of bactericidal activity with the incubation time and concentration of antimicrobial agents [41]. Knowledge of the *in vitro* pharmacodynamics of amphotericin B is still limited. Therefore, the study on time-kill was done on ε-viniferin. Furthermore, no previous report for ε-viniferin against pathogenic *C. glabrata,* especially on time-kill. *C. glabrata* infection is second or third in frequency after *C. albicans* and is associated with a high mortality rate in at-risk hospitalized patients. ε-viniferin was chosen since this compound was shown to be the most susceptible to anti-candida, with a 12 mm inhibition zone (Table 1). Generally, the time-killed decreased when the incubation time was increased. Table 2 indicates the effect of ε-viniferin on the time-kill of *C. glabrata* at 0× (control), 0.5×, 1×, 2×, 4× and 8×MIC after the endpoint (240 min).

Figure 2 illustrates the time-killing curves for *C. glabrata* when exposed to ε-viniferin. At 8×MIC (500 ppm), there was a slight decline in the killing time. The time-kill shows a sharp decrease after 30 min, followed by a gradient until it completely inhibits at 120 min. The reduction in CFU/mL was ≥ 3 log units (99.9 %). However, at 4×MIC, 2×MIC, 1×MIC and 0.5×MIC of ε-viniferin, the compound did not completely inhibit the *C. glabrata*. Although the respective points did not suppress the fungus, it still showed the potential for time-killing activity. Figure 2 and Table 1 showed that the time kill gradually declined as the concentration of samples inclined. This result might suggest that the compound will completely inhibit *C. glabrata* at an incubation time of more than 240 min or 4 h.

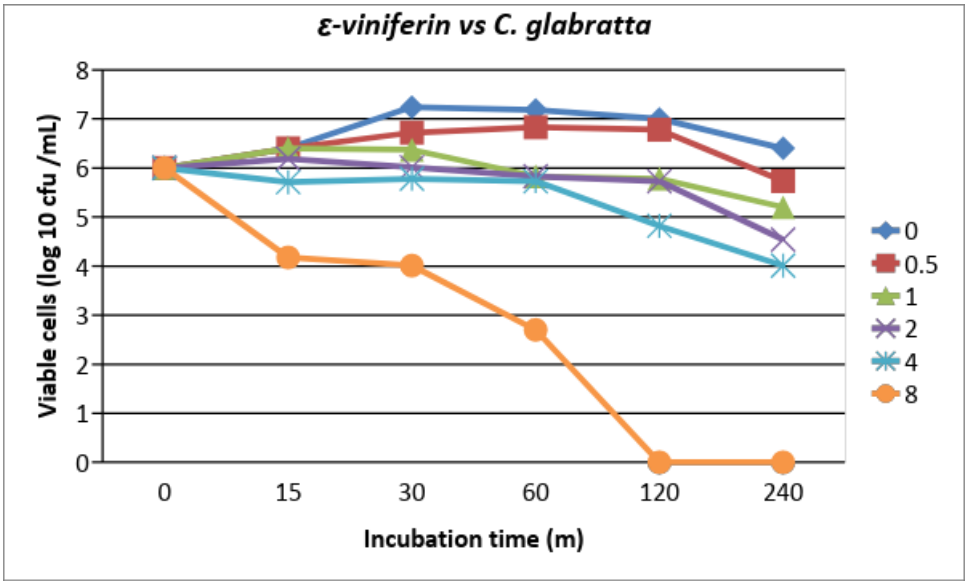

Figure 2. Time-kill curve plots for *C. glabrata* following exposure to ε-viniferin.

Table 1. Inhibition zone, MIC and MFC of crude extracts and compounds isolated from *D. verrucosus*, *D. crinitus*, and *D. cornutus* on *C. glabrata.*

Standard/ Sample	**Inhibition zone (mm)**	**MIC (ppm)**	**MFC (ppm)**
Amphotericin B	10.0 ± 0.6	2.0 ± 0.2	4.0 ± 0.2
Methanolic crude extracts			
D. verrucosus	7.0 ± 0.3	500.0 ± 1.2	500.0 ± 1.2
D. cornutus	7.0 ± 0.3	62.5 ± 0.8	250.0 ± 1.2
D. crinitus	7.0 ± 0.3	62.5 ± 0.5	125.0 ± 1.4
Dimer stilbenoid			
ε-viniferin (**1**)	12.0 ± 0.2	62.5 ± 0.8	125.0 ± 0.2
Ampelopsin A (**2**)	9.0 ± 0.2	125.0 ± 0.8	500.0 ± 1.2
Trimer stilbenoid			
α-viniferin (**3**)	8.0 ± 0.2	62.5 ± 0.8	250.0 ± 0.8
Davidiol A (**4**)	7.0 ± 0.3	62.5 ± 0.8	125.0 ± 0.8
Stenophyllol B **(5)**	10.0 ± 0.2	125.0 ± 0.7	500.0 ± 1.2
Ampelopsin E (**6**)	7.0 ± 0.2	62.5 ± 1.5	125.0 ± 1.4
Tetramer stilbenoid			
Vaticanol B (**7**)	8.0 ± 0.2	125.0 ± 0.0	250.0 ± 1.2
Diptoindonesin E (**8**)	7.0 ± 0.2	62.5 ± 1.2	250.0 ± 1.3
Hemsleyanol D (**9**)	8.0 ± 0.2	125.0 ± 0.0	500.0 ± 1.5.
Others			
*Bergenin **(10)***	7.0 ± 0.2	62.5.0 ± 0.0	125.0 ± 0.8
*Scopoletin **(11)***	8.0 ± 0.2	125.0 ± 0.0	250.0 ± 1.4
*β-sitosterol glucoside **(12)***	10.0 ± 0.2	62.5 ± 0.2	125.0 ± 0.9

Table 2. Time-kill point of ε-viniferin on *C. glabrata* at 0×MIC (control), 0.5×MIC, 1×MIC, 2×MIC, 4×MIC, and 8×MIC after endpoint (240 min).

Time (min)	Viable cell (Log 10 CFU/mL)					
	0×MIC (control)	**0.5×MIC (31.25 ppm)**	**1×MIC (62.5 ppm)**	**2×MIC (125 ppm)**	**4×MIC (250 ppm)**	**8×MIC (500 ppm)**
0	6.00 ± 0.3	6.00 ± 0.1	6.00 ± 0.1	6.00 ± 0.3	6.00 ± 0.4	6.00 ± 0.0
15	6.40 ± 0.3	6.40 ± 0.2	6.40 ± 0.1	6.19 ± 0.2	5.71 ± 0.1	4.18 ± 0.0
30	7.24 ± 0.1	6.72 ± 0.3	6.37 ± 0.1	6.01 ± 0.2	5.78 ± 0.2	4.01 ± 0.0
60	7.18 ± 0.1	6.83 ± 0.2	5.83 ± 0.2	5.73 ± 0.3	5.73 ± 0.2	2.70 ± 0.1
120	7.00 ± 0.1	6.78 ± 0.2	5.78 ± 0.2	5.73 ± 0.3	4.82 ± 0.0	0.00 ± 0.4
240	6.40 ± 0.1	5.73 ± 0.2	5.20 ± 0.2	4.01 ± 0.3	4.01 ± 0.0	0.00 ± 0.4

CONCLUSION

This anticandidal study showed that double olefinic units in the compound skeleton were important for antifungal activity. The results strongly suggest that stilbenoid has the potential to be developed as a natural antifungal agent to combat *C. glabrata*. However, a detailed study of the mechanism of actions involved during the inhibition process might be an advantage in determining their chemical-cell interactions.

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CONFLICT OF INTEREST

Author declares that no conflict of interest is present in this study.

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