Oligostilbenoids from *Vatica pauciflora* and the Oxidative Effect on Chang Cells

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ABSTRACT

Phytochemical studies on the woods and twigs of *Vatica pauciflora* (Dipterocarpaceae) have been conducted. The woods and twigs of *V. pauciflora* were extracted in methanol and ethanol respectively with Soxhlet apparatus. The crude methanol extracts from the woods were semi-purified by vacuum liquid chromatography to give 14 fractions (VMB01-VMB14). The VMB10 was further purified by radial chromatography to give pure dimmer stilbenoid. The same technique has been employed on the crude ethanol extracts from the twigs in which one pure fraction, VER612 was obtained. Based on the UV, IR, NMR and mass spectral data, the pure compounds were characterized as ε-viniferin and vaticanol G respectively. An oxidative damage test which was done on Chang liver cells has shown that ε-viniferin has a potential to be a chemopreventive agent to protect liver cells from oxidative damage.

Keywords: *Vatica pauciflora*, ε-viniferin, vaticanol G and chemopreventive.

1.0 Introduction

*Vatica pauciflora* which is known as *resak paya* or *resak laru* belongs to the family of Dipterocarpaceae [2,12]. This species has been recorded from the Malay Peninsula and the south of Sumatra [1,12]. Economically, *V. pauciflora* is one of the most species used as *laru* to prevent fermentation of toddy and its woods are used for temporary construction [2,12].
The previous study on the stem bark of this species has successfully isolated five new oligostilbenoids which are pauciflorol A, B and C, isovaticanol B and C; three new oligostilbene glucosides which are paucifloroside A, B and C; together with 17 known oligostilbenoids and bergenin [6]. Subsequent work done by the same researchers found other oligostilbenoids namely pauciflorol D, E and F [8].

Apart from that, oligostilbenoids that have been isolated from *V. pauciflora* showed biological activities against murine leukemia P-388 cells [4,11], colon cancer cell line SW480 and leukemia HL60 cells [7].

Studies had shown that resveratrol, which is the basic skeleton of oligostilbenoids exhibited chemoprevention effect and cancer therapeutics on cultured cells systems. Resveratrol acted as an antioxidant, antimutagen and inducing drug metabolism enzyme phase II (anti-initiation activity) and detoxification carcinogen, anti-inflammatory effect as well as stopping the function of cyclooxygenase and hydroperoxide [3,9].

Our work on the woods and the twigs of *V. pauciflora* has successfully isolated two pure compounds which were characterized as $\varepsilon$-viniferin and vaticanol G. As part of our continuing study on the chemical and biological activities of *V.pauciflora*, we investigated the effect of oligostilbenoids in protecting Chang liver cells from oxidative damage which has been induced by hydrogen peroxide.

### 2.0 Experimental

#### 2.1 Plant Material

*V. pauciflora* was collected from Gunung Panti, Pontian, Johor (voucher no. WYP 15, Herbarium, UKM Bangi). Dried and ground woods (400 g) and twigs (800 g) of *V. pauciflora* were extracted with methanol and ethanol respectively by using Soxhlet apparatus.

#### 2.2 Extraction and Isolation

The crude methanol extract (16.73 g) was fractionated by Vacuum Liquid Chromatography (VLC) with a mixture of hexane-ethyl acetate in increasing polarity to give 14 fractions (VMB01-14). Fraction VMB10, which was a polar fraction was further purified by radial chromatography to give a pure compound labeled as VMB1004 (210 mg).

The crude ethanol extract (24.4 g) from the twigs of *V. pauciflora* was semi-purified by VLC with a mixture of hexane-ethyl acetate in increasing polarity to give 7 fractions (VER1-VER7). Further purification on VER6 by radial chromatography had given 12 combined fractions (VER601-VER612) which one of the fractions is a pure compound labeled as VER612 (20 mg).

The structure of VMB1004 and VER612 were elucidated by means of infrared (IR), ultraviolet-visible (UV-VIS), mass (MS) and one and two dimensioned nuclear magnetic resonance spectroscopy (NMR), optical rotation analysis and direct comparison with literature data [5,10]. Chemical shift, $\delta$, ppm recorded in CD$_3$COCD$_3$.

#### 2.3 Bioassay Evaluation

Hydrogen peroxide (H$_2$O$_2$) is introduced into Chang cells for about 30 minutes to induce oxidative damage to the cell. The media contain of H$_2$O$_2$ was discarded and replaced by the media containing treatment from pure compounds obtained from the isolation. The concentration of the treatment is at 0
μM, 50 μM and 100 μM. Then, the cells were incubated for 24 hours. MTT assay was done on the cells and the viability was obtained by comparing the optical density (OD) of the treated cell and treatment with media only.

3.0 Results and Discussions

ε-viniferin (1) was obtained as brownish viscous oil. HREIMS spectrum recorded the molecular ion [M]+ peak at \( m/z \) 454 indicated a molecular formula of C\(_{28}\)H\(_{22}\)O\(_{6}\), corresponding to a dimmer stilbenoid. The UV spectrum (\( \lambda_{\text{max}} \) 206, 248, 343 nm in MeOH) showed a characteristic of an oligostilbene.

The \(^1\)H NMR spectrum of 1 showed the presence of four pairs of ortho-coupled aromatic protons at \( \delta_H \) 6.73, 6.83, 7.18, 7.20 and two sets of meta-coupled aromatic protons at \( \delta_H \) 6.23, 6.32, 6.72. There were doublet signals at \( \delta_H \) 4.47, 5.42 which represents the protons at the dihydrobenzofuran ring. A pair of doublet signals at \( \delta_H \) 6.71, 6.92 indicated the presence of olefinic protons.

The \(^{13}\)C-APT NMR spectrum exhibited twenty-eight signals which consists of six oxyaryl (\( \delta_c \) 158.3, 158.3, 159.6, 159.9, 159.9, 162.4), two aliphatic (\( \delta_c \) 57.1, 93.9), eighteen aromatic (\( \delta_c \) 96.8, 102.1, 104.1, 106.9, 106.9, 116.1, 116.1, 116.3, 119.8, 127.9, 127.9, 128.7, 128.7, 129.8, 133.7, 136.3, 147.4) and two olefinic carbons (\( \delta_c \) 123.4, 130.0).

Vaticanol G (2) obtained as brownish viscous oil. Negative ion FAB-MS: [M-H]− at \( m/z \) 679 indicated a molecular formula of C\(_{42}\)H\(_{32}\)O\(_{9}\), corresponding to a trimer stilbenoid. The UV spectrum (\( \lambda_{\text{max}} \) 204, 282 nm in MeOH) showed the presence of aromatic and phenolic system. When the UV spectrum was recorded in the presence of NaOH, there was no bathochromic shift occur at \( \lambda_{\text{max}} \) 282 which indicated that this molecule did not contain stilbene skeleton.
The $^1$H NMR spectrum of 2 showed three pairs of broad singlet at $\delta_H$ 7.13, 6.66, 6.45, 6.42, 5.97 and 5.92 which represent two 1,4-disubstituted aromatic ring. The presence of 1,2,4-trisubstituted, 1,3,5-trisubstituted, 1,2,3,5-tetrasubstituted and 1,2,3,5,6-pentasubstituted aromatic rings were shown by the signals at $\delta_H$ 6.45, 6.17, 6.08, 6.05, 5.99, 5.93, 5.74 and 5.64. Other signals shown in the spectrum are the signals of methyne protons which are a broad singlet at $\delta_H$ 4.07, two pairs of doublet signals at $\delta_H$ 4.86, 4.60, 4.52 and 3.49 and a double doublet signal at $\delta_H$ 3.83.

The $^{13}$C-APT NMR spectrum exhibited thirty – seven signals which represent fourty – two carbon atoms. There are nine oxyaryl ($\delta_c$ 159.2, 159.2, 158.6, 156.6, 156.0, 155.1, 154.9, 153.3, 153.1), ten quaternary ($\delta_c$ 147.8, 147.1, 142.0, 141.9, 140.0, 137.2, 129.3, 126.1, 121.9, 117.6), seventeen aromatic ($\delta_c$ 135.1, 130.7, 130.3, 130.3, 127.6, 119.5, 116.2, 114.8, 114.8, 114.3, 112.9, 111.5, 106.5, 106.5, 102.1, 101.7, 101.1) and six aliphatic ($\delta_c$ 63.1, 57.2, 57.1, 53.9, 50.4, 42.8) carbons.

![Figure 2. Structure of vaticanol G (2)](image)

An oxidative damage test on compounds 1-2 was performed to determine the ability of these compounds in protecting Chang cells from oxidative damage induced by hydrogen peroxide ($H_2O_2$). The percentage of cell viability before the treatment with $\varepsilon$-viniferin (1) is 78.3% while the viability after the treatment at 50 $\mu$M and 100 $\mu$M is 106.9% and 111.0% respectively. Meanwhile, the percentage of cells treated with vaticanol G (2) at 50 $\mu$M and 100 $\mu$M is 85.4% and 84.4% respectively. Before the treatment, the cell viability is 76.6%. The proliferation of Chang cells had shown a significant increase after the treatment with $\varepsilon$-viniferin (1) while the treatment with vaticanol G (2) did not show any significant increase. This significant increase in Chang cells treated $\varepsilon$-viniferin (1) shown that the dimmer stilbenoid has the ability to protect the cells from oxidative damage. High antioxidant capacity in $\varepsilon$-viniferin (1) is one of the factors that contribute to the significant protection of oxidative damage in Chang cells.
4. Conclusions
The wood of *V. pauciflora* found to contain a dimmer stilbenoid, $\varepsilon$-viniferin which is the precursor of oligostilbenoids while the isolation from the twigs of *V. pauciflora* has successfully obtained vaticanol G which is a trimer stilbenoid. The major compound $\varepsilon$-viniferin has the potential to be applied as chemopreventive agent due to its significant ability to protect liver cells from oxidative damage.

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