Australian Journal of Basic and Applied Sciences, 5(5): 926-929, 2011
ISSN 1991-8178

The Evaluation of Antioxidant, Antibacterial and Structural Identification Activity of Trimer Resveratrol from Malaysia's Dipterocarpaceae

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Abstract: *Dipterocarpus verrucosus* is known as "keruing merah" by the local is a species of tree in the family Dipterocarpaceae, endemic to Brunei, Indonesia (Kalimantan, Sumatra), Malaysia (peninsular Malaysia, Sabah, Sarawak). In this study we wish to report on characterization of trimer resveratrol from the *Dipterocarpus verrocosus* as well as the antioxidant and antibacterial activity. The structure of the compound was confirmed by 1H and 13C NMR analyses and comparison with previous data. The antioxidant activity was evaluated by DPPH, TPC, FTC and TBA. Meanwhile for antibacterial it was screened again *E.coli, Klebsiella pneunomonia, Bacillus subtilis, Staphylococcus aureus, Salmonella paratyphi* and *Pseudomonas aeruginosa.* The testing was carried out by the disc diffusion method. The result indicated that α-viniferin, resveratrol trimer from *Dipterocarpus verrucosus* gave moderate activity towards antibacterial and antioxidant values. This report is very important for the chemotaxonomy of Dipterocarpaceae family since from the literature review this is first work on this species which no chemical and biological report was evaluated.

Key words: *Dipterocarpus verrucosus,* Dipterocarpaceae, trimer resveratrol, antioxidant, antibacterial

INTRODUCTION

Dipterocarpaceae is the most important family of economic trees in Southeast Asia. The species of this family is one of major importance in the timber trade. The Dipterocarpaceae is a very large family comprising of 16 genera and about 600 species (Cronquist, 1981; Heyne, 1987). This family produce variety of compounds such as volatile oil, triterpenoid, flavonoid, arylpropanoid, and oligomeric resveratrol (Heyne, 1987; Sotheeswaran and Pasupathy, 1993). The phytochemical part of these plants have been studied before (Dai *et al*., 1998; Ito *et al*., 2000, Muhtadi 2008; Muhtadi *et al*., 2006; Zheng *et al*., 1994), but for such a broad family plants, the chemistry of Dipterocarpaceae is relatively less known. This is because the initial research on Dipterocarpaceae was mainly focused on resins such as terpenoid, sesquiterpene and triterpene.

Since the finding of hopeaphenol, a polyphenol of oligomeric resveratrol, from two species of Dipterocarpaceae, *i.e. Hopea odorata* and *Balanocarpus heimii* (Coggon *et al*., 1965) in the last two decades, the phytochemical research on Dipterocarpaceae has shifted rapidly. This is due to the fact that oligomeric resveratrol compounds are the main polyphenol which show the variety of important bioactivities, such as anti-inflammation (Bertelli *et al*., 1999), antibacterial (Geewananda *et al*.,1986), antifungal , anticancer (Lee *et al*., 2009; Aggrawal *et al*., 2004) chemo preventive, hepatoprotectiveand inhibition of topoisomerase II, gastric ATPase and 5α-reduktasereactions(Hakim, 2002), antioxidant (Hirano *et al*., 2001). Therefore, phytochemical study of resveratrol oligomer of Dipterocarpaceae family remains to be further explored, especially on genus or species that has not been studied.

MATERIALS AND METHODS

General Experimental Procedures:

UV spectra were measured with a Varian Conc. 100 instrument. IR spectra were determined with a Perkin Elmer FTIR Spectrum One spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded with a JEOL ECP400 operating at 400 (^{1}H) and 100 (^{13}C) MHz using residual and deuterated solvent peaks as reference standards. Vacuum liquid (VLC) and column chromatography were carried out using Merck silica

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gel 60 GF_{254} and silica gel G60 35-70 mesh. For TLC analysis, precoated silica gel plates (Merck Kieselgel 60 GF_{254}, 0.25 mm) were used.

Plant Materials:

Samples of the stem barks of *D.verrucosus* collected from Forest Reserve UiTM Pahang. The plant was identified by wood lecturer, UiTM Pahang; Tuan Sheikh Abdul Karim bin Tuan Yamani.

Extraction and Isolation:

The dried powdered of stem bark (6 kg) of *D.verrucosus* was macerated with acetone (3 x 5L) followed by methanol (3 x 5L) succcessively and each extract was evaporated under reduced pressure to give dark brown residues (60 g). The MeOH extract was subjected to fractionation using VLC (silica gel, *n*-hexane-EtOAc) into six major fractions DV1-6. Purification of subfraction DV3 by radial chromatography (silica gel, $CHCl_3$-MeOH) yielded α-viniferin (91mg). (Figure 1).

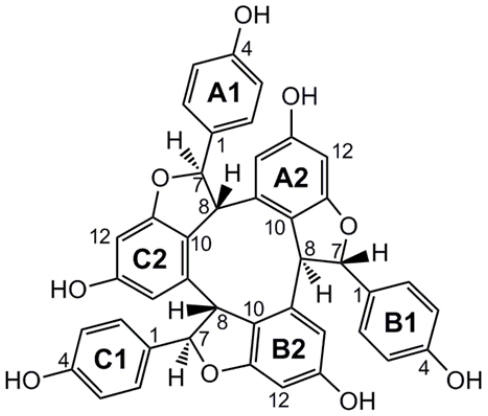

Fig. 1: (α-Viniferin).

Antibacterial Assay:

The bacteria used for the tests were obtained from Institute Medical Research (IMR), Malaysia which included both gram–positive (*Bacillus subtilis* and *Staphylococcus aeureus*) and gram-negative bacteria (*Escherechia coli, Klebsiella pneunomonia, Salmonella paratyphi* and *Pseudomonas aeruginosa*). A 100 µL of bacterial suspension was spread on nutrien agar (NA) plate. α-Viniferin (50 mg/mL) as dissolved in methanol on 6 mm sterile filter paper disc and placed on inculated agar. Paper disc with methanol solvent was used as negative control meanwhile for positive control Streptomycin was used. The plates were incubated at 37ºC for 18-24h. After incubation time, zone of inhibition was measured. This method was referred to Barry *et al*., 1994.

Antioxidant Activity:

***Total Phenolic Content (TPC)*:**

This assay was conducted as described by Velioglu *et al*., (1999). This assay was carried out using Folin-Ciocalteu agent. 0.01gram of α-Viniferin was appropriately diluted with 10 ml of methanol. 0.1 ml of the diluted α-Viniferin was added with 0.5 ml Folin-Ciocalteu phenol reagent. After 20 min in dark place, 1.5 ml of 20% Na_2CO_3 solution were added to the α-Viniferin and shaken vigorously. Then the mixture was diluted to 10 ml with distilled water and mixed thoroughly. Finally, the absorbance was measured at 760 nm with spectrophotometer after incubation for 2 h in dark at room temperature. A calibration curve was prepared, using the regression equation of the calibration curve of gallic acid ($y = 0.013x$, $r^2 = 0.998$), and contents were expressed as mg gallic acid equivalent (GAE)/g of sample.

Radical Scavenging Assay (DPPH):

This test was measured following the method of Blois (1958). 0.6 ml of the fraction solutions (50 – 250 mg/ml) of α-Viniferin was added to 4.5 ml of 0.1M methanolic DPPH solution. The mixture was incubated for 20 min of reaction at room temperature; the absorbance of the solution was measured at 517 nm while the methanol was used to zero the spectrophotometer. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a control solution (no sample). Radical scavenging activity

is expressed as percent inhibition and is calculated as follows:

% scavenging activity = 1 - (Abs sample / Abs control) x 100

where Abs control is the absorbance of DPPH solution without extracts.

Ferric Thiocyanate Method (FTC):

The antioxidant activity analysis using ferric thiocyanate was performed based on Osawa and Namiki (1981) method. 4.0 mg of the crude extracts were dissolved in 4.0 ml of ethanol absolute, followed by the addition of 4.1 ml of a 2.51% linoleic acid solution in EtOH and 8 ml of a 0.05M phosphate buffer (pH 7.0). Then the mixture was incubated at 40ºC in a screw-cap vial. During the incubation, a 1.0 ml aliquot was taken from the mixture was diluted with 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate was added. Precisely 3 min after the addition of 0.1 ml of 0.02M ferrous chloride in 3.5% hydrochloric acid, the absorbance for the red color was measured at 500 nm in a spectrophotometer.

Thiobarbituric Acid Method (TBA):

This assay was performed followed the method by Kikuzaki and Nakatani (1993). 2.0 ml of the mixture solution from the FTC method was added with 2.0 ml of 0.67% thiobarbituric acid and 1.0 ml of 20% trichloroacetic. The mixture was then heated in a boiling water bath for 10 min. After the mixture was cooled, the mixture was centrifuged at 3000 rpm for 20 min, and the absorbance was measured at 532 nm.

RESULTS AND DISCUSSION

^{1}H NMR (acetone-$_{d}$, 400 MHz) ppm: δ_H 7.02 (2H, d, J= 8.2 Hz, H-2a/6a), 6.71 (2H, d, J= 8.6 Hz, H-3a/5a), 6.05 (1H, br s, H-7a), 3.95 (1H, br s, H-8a), 6.23 (1H, d, J= 1.8 Hz, H-12a), 6.71 (1H, d, J= 1.8 Hz, H-14a), 7.04 (2H, d, J= 7.7 Hz, H-2b/6b), 6.78 (2H, d, J=8.4 Hz, H-3b/5b), 4.90 (1H, d, J=6.2 Hz, H-8b), 6.22 (1H, d, J=2.0 Hz, H-12b), 5.98 (1H, d, J=2.0 Hz, H-14b), 7.22 (2H, d, J=8.6 Hz, H-2c/6c), 6.71 (2H, d, J=8.6 Hz, H-3c/5c), 5.95 (1H, d, J=10.0 Hz, H-7c), 4.67 (1H, 10.0 Hz, H-8c), 6.20 (1H, d, J=2.0 Hz, H-12c), 6.58 (1H, d, J=1.8 Hz, H-14c).

^{13}C-APT NMR (acetone-$_{d}$, 100 MHz) ppm: δ_C 132 (C-1a), 128.3 (C-2a/6a), 115.8 (C-3a/5a), 158.3 (C-4a), 86.4 (C-7a), 46.4 (C-8a), 141.3 (C-9a), 120.9 (C-10a), 161.6 (C-11a), 98.1 (C-12a), 159.5 (C-13a), 106.3 (C-14a), 132.6 (C-1b), 128.8 (C-2b/6b), 116.2 (C-3b/5b), 158.5 (C-4b), 95.7 (C-7b), 55.8 (C-8b), 139.8 (C-9b), 118.9 (C-10b), 161.0 (C-11b), 97.0 (C-12b), 159.5 (C-13b), 108.6 (C-14b), 132.0 (C-1c), 128.2 (C-2c/6c), 116.2 (C-3c/5c), 157.9 (C-4c), 90.1 (C-7c), 52.9 (C-8c), 138.7 (C-9c), 119.7 (C-10c), 161.9 (C-11c), 96.6 (C-12c), 160.7 (C-13c), 105.9 (C-14c).

Antibacterial Activity:

As can be seen in Table 1, α-Viniferin showed strongly inhibited against *Staphylococcus aeureus* and *Escherichia coli* as compared with positive control; Streptomycin as standard. Meanwhile for *Salmonella paratyphi* it showed moderate activity as compared with Streptomycin. The three remaining bacteria and negative control (methanol) did not inhibit any bacterial growth.

Antioxidant Activity:

From this study, we have evaluated that α-Viniferin has a high total phenolic content of 340 mg/g mg/g of GAEs). However in the radical scavenging activities, α-Viniferin displayed lower scavenging activity with no IC50 which percent scavenging activity of 15.79%. While in the next two methods, a-veniferin has indicated high antioxidant for the FTC and TBA methods with percent inhibition 77.77% and 86.47% respectively. A good correlation was found among the results of TPC, FTC and TBA methods where exhibited the moderate antioxidants potential due to the high percent inhibition in those methods.

ACKNOWLEDGMENT

We would like to exprees our deep appreciation to Tuan Sheikh Abdul Karim Yamani for identification of the plant speciemen, En Sardey Idris for collection of the plant and MOHE for financial support for phd study of Wan Zuraida and also for UiTM Malaysia for all the support

Table 1: Inhibition zone of bacteria treated with α-Viniferin, Streptomycin and methanol.

Bacteria	α-Viniferin (50mg/ml)	Inhibition zone (mm) Streptomycin (Positive control)	Methanol (Negative control)
Gram positive			
Bacillus subtilis	-	1.17±1.00	-
Staphylococcus aeureus	8.8±1.061	5.83±0.29	-
Gram negative			
Escherichia coli	17±0	9.67±0.58	-
Klebsiella pneunomonia	-	10.67±0.58	-
Salmonella paratyphi	8.5	11.30±1.15	-
Pseudomonas aeruginosa	-	8.50±0.30	-

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