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Original Article

CYTOTOXICITY STUDIES OF TETRAPRELYLTOLUQUINONE, A PRENILATED HYDROQUINONE FROM *GARCINA COWA ROXB ON* H-460, MCF-7 AND DU-145

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ABSTRACT

Objective: The aim of the present study was to examine the cytotoxicity of a new ring-reduced tetra prenyltoluquinone (TPTQ), [2*E*,6*E*,10*E*]-(+)-4β-hydroxy-3-methyl-5β-(3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl-2-cyclohexen-1-one against H-460, MCF-7 and DU-145 cell lines.

Methods: Different concentrations of TPTQ were subjected to cytotoxicity study by using MTT method and calculate the percentage of cell viability.

Results: The results of this study showed that this compound has IC_{50} value 16.3 ± 3.0 µM in H-460 cancer cell lines without any activities towards another two type of cell lines.

Conclusion: TPTQ had selective activity against H-460 cancer cell lines.

Keywords: *Garcinia cowa*, TPTQ, H-460, MCF-7, DU-145, Cell viability, Cytotoxicity.

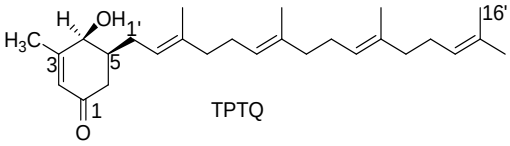
INTRODUCTION

Cancer can be defined as a disease in which the disorder occurs in the normal processes of cell division, which are controlled by the genetic material (DNA) of the cell [1]. Several plant-derived compounds are currently successfully employed in cancer treatment such as vincristine (leukemia, lymphoma, breast, lung, solid cancers and others), vinblastine (breast, lymphoma, germ-cell and renal cancer), paclitaxel (ovary, breast, lung, bladder, head and neck cancer), docetaxel (breast and lung cancer) [2].

Anticancer drugs are not selective, because they can damage normal cells that have rapid growth. It encourages researchers to conduct research in order to find an anticancer drug that has selective activity against cancer cells without damaging the normal cells.

Plants are the potential source for discovering biologically active compounds preferably those having unique and new mechanism of therapeutic action. Among of these plants, special attention is paid to genus Garcinia (Guttiferae). This genus has been extensively investigated from phyto chemical and biological point of view. Prenylated xanthones, triterpenes and bio flavonoids have been isolated from this genus. The alcoholic extract of Garcinia species were reported to have the wide range of biological activities such as anti-inflammatory, antioxidant, antibacterial and cytotoxicity [3-6].

Garcinia cowa Roxb. (Guttiferae), known as kandis in West Sumatra, is a medium-sized tree which attains a height of *ca.* 30 m and it is widely distributed throughout Indonesia and the Malay peninsula. Traditionally, kandis have been used for many purposes. The bark of kandis is used as pesticide, larva side [7] and antipyretic [8] while the sun dried fruit is used to treat dysentery [9]. Dried leaves of this species are used as a tonic [10]. Biological activity studies on this species showed that the leaves had strong antitumor promoting activity on cells-Raji [11], while the dried steam bark had antimalarial activity [12]. Previous workers have isolated a number of prenylated xanthones from the stem bark [3, 6, 8, 12]. Previous study showed that the hexane fraction of the steam bark of *G. cowa* was active against small lung cell cancer H-460 and not active against MCF-7 and DU-145 cell lines [13]. A new ring-reduced tetraprenyltoluquinone (TPTQ), [2*E*,6*E*,10*E*]-(+)-4β-hydroxy-3-methyl-5β-(3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl-2-cyclohexen-1-onewas isolated from this species[14]. Based on these data, further cytotoxicity activity studies of TPTQ on three cancer cell lines, H-460, MCF-7 and DU-145 cell lines were carried out on this compound.



TPTQ

MATERIALS AND METHODS

Instruments

UV (in absolute ethanol) and IR (KBr) spectra were recorded on a J ASCO V-560 spectrophotometer and a Perkin-Elmer 1650 FTIR spectrophotometer, respectively. High resolution fast-atom bombardment mass spectrometry (HRFAB-MS) was obtained on a J EOL J MS HX-110A spectrometer. ^{1}H and ^{13}C NMR spectra ($CDCl_3$) were were recorded on a Varian 500 MHz NMR Spectrometerat 500 MHz (^{1}H)and 125 MHz (^{13}C), respectively. The spectra was interpreted with the aid of the1H-1H COSY, HMBC, and HMQC techniques.

Holten Lamin Air microbiological safety cabinet class II was obtained from Heto-Holten (Allerød, Denmark), and Galaxy CO_2 incubator was purchased from RS Biotech (Ayrshire, Scotland). A micro plate reader equipped SOFTmax® Prosoftware (Versamax, Molecular Devices, California, USA) was used to measure of the formazan solution.

Plant material

Garcinia cowa Roxb (stem bark) was collected at Sarasah Bonta, Harau Valley, and West Sumatra at an altitude of 500 m. Plant materials were cut into small pieces (3-5 mm thickness) and air-dried under shade. The dried plant materials were ground to powder before extraction.

Cell lines

The cell lines, NCI-H460 were purchased from the American Type Culture Collection (Manassas, VA, USA). The cancer cells were cultured in RPMI 1640 medium (Life Technologies, Paisly, UK) with 10%v/v fetal calf serum (PAA Laboratories, Linz, Austria), 100 IU/mlpenicillin and 100 µg/ml streptomycin (Life Technologies,

Paisly, UK) whereas the solution trypsin-EDTA were purchased from GIBCO (Auckland, New Zealand). Dimethylsulfoxide (DMSO) was purchased from BDH Laboratory (England). Culture flask (25 cm^2 and 75 cm^2) and 10 ml serological pipettes were purchased from Becton Dickson (New Jersey, USA).

Isolation of TPTQ from the stem bark of *G. cowa*

The ground air-dried stem bark (2.2 Kg) was percolated three times with methanol (10 l). The extract was evaporated to dryness under reduced pressure to give a dark mass (113.54 g). The methanol extract was partitioned between water 200 ml and hexane (4 x 100 ml) and the combined hexane extracts was evaporated to yield a gum (5.7 g). The aqueous layer was further partitioned successively with ethyl acetate and followed by butanol, and upon evaporation 43.0 g and 45.0 g of gums were obtained from the respective solvents.

A portion (4.0 g) of the hexane extract was subjected to CC (silica gel, Merck 7734) with hexane-ethyl acetate mixture as eluent in increasing polarity manner, followed by methanol to give three sub-fractions HxA-HxC. Sub-fraction HxB (0.896 g) was further purified by silica gel CC (Merck 9385) eluting with a mixture of hexane-ethyl acetate mixture in increasing polarity mode to give four sub-fractions (Habra-HxBd). Further purification of HxBb (0.550 mg) was achieved using radial chromatography using the same eluent to yield 310 mg of[2*E*,6*E*,10*E*]-(+)-*4β-hydroxy-3-methyl-5β-(3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl-2-cyclohexen-1-one* as a yellowish oil. High-resolution FABMS gave $[M+H]^{+\bullet}$ at: 399.3263 $C_{27}H_{43}O_2$, requires *m/z* 399.3359, $[\alpha]_D^{26}$ +50.0°. UV (MeOH) λ_{max} (logε) nm: 230 (4.04); IR ν_{max}(nujol) cm^{-1}:3391, 2982, 2909, 2834, 1652, 1436, 1375, 1330, 1289, 1186, 1144, 1110, 1055, 1021, 1005,3435, 3310, 2980, 2925, 2860, 1653, 1437, 1377, 1111, 1008, 886, 667. ^{1}H NMR(500 MHz, $CDCl_3$) δ_H2.03 (3H, *s,* 3-CH_3), 5.80 (1H, *s,* H-2), 4.09(1H, *d*, *J*= 6.0 Hz, H-4), 5.2 (1H,*t*, *J*= 6.0 Hz, H-2'), 5.09 (3H, *d*, *J*= 6.0 Hz, H-6', H-10', H-14'), 2.1 (1H, *d*, *J*= 8 Hz.0, H-1'), 2.35 (1H, *d*, *J*= 8.0 Hz, H-1'),2.10 (1H, *m,* H-5), 2.51 (1H, *s*, H-6), 1.96 (3H, *m,* H-4', H-8', H-12'), 2.14 (3H, *m,* H-5', H-9', H-13').^{13}C NMR (125 MHz,$CDCl_3$) δ_{CB}199.9 (C-1); 126.4 (C-2), 164.1 (C-3), 20.4 (C-3Me), 73.1 (C-4), 43.5 (C-5), 41.1 (C-6), 30.5 (C-1'), 120.5 (C-2'), 138.2 (C-3'), 39.6 (C-5', C-8'), 39.8 (C-4'), 26.4 (C-13'), 26.5 (C-12'), 26.6 (C-9), 123.8 (C-14'), 124.1 (C-10'), 124.3 (C-6').

Culture of cells

The lung cancer (H-460), breast cancer (MCF-7), and prostate cancer (DU-145) cell lines had been used in this study. All cell lines were maintained in RPMI-1640 medium with 10% v/v foetal calf serum, 100 IU/ml penicillin and 100 g/ml streptomycin as a complete medium. Cells were grown in 25 cm^3 flask with 10 ml of complete media at 37°C with 5% CO_2. Every 3 days the cells were subcultured by splitting the culture with fresh complete media. Cells were attached by incubating suspension cells (180 ul in each well except blank) for overnight.

MTT assay [15]

Varying concentrations of TPTQ were prepared from the stock solutions by serial dilution (100 µM, 10 µM, 1 µM, 0.1 µM) in RPMI-1640 to give a volume of 200 µl in each well of the microtiter plate (96-well). The assay for each concentration was performed in quadruplicate and the culture plate was incubated at 37 ^{0}C with 5 % (v/v) CO_2 for 96 hours. After of incubation, 50 µL of 2 mg/ml MTT solution was added to each well and allowed to incubate for a further 4 hours, after which all supernatant were discarded. Subsequently, 100 µL DMSO was added to each well and vigorously mixed to dissolve the formazan crystals. Absorbance values at 550 nm were measured with a microplate reader. Cytotoxicity was expressed as IC_{50}, i. e. The concentration of the test sample to reduce the absorbance of treated cells by 50% with reference to the control (untreated cells). To determine the IC_{50} value, viability vs. dose response curve is plotted. The viability was calculated according to this formula:

% Viability = (Optical density of treated cells/ Optical density of control) X 100%

The relation, between the concentrations of the test solution with viability cell was displayed in graphical form. Of the graph was a determined price IC_{50} (concentrations that inhibit 50% living cells) test solution.

The data of relation between the concentration of the test preparations and the absorbance were statistically analyzed using a one-way variance analysis (ANOVA) followed by of Duncan's multiple regions (Duncan's Multiple Range Test).

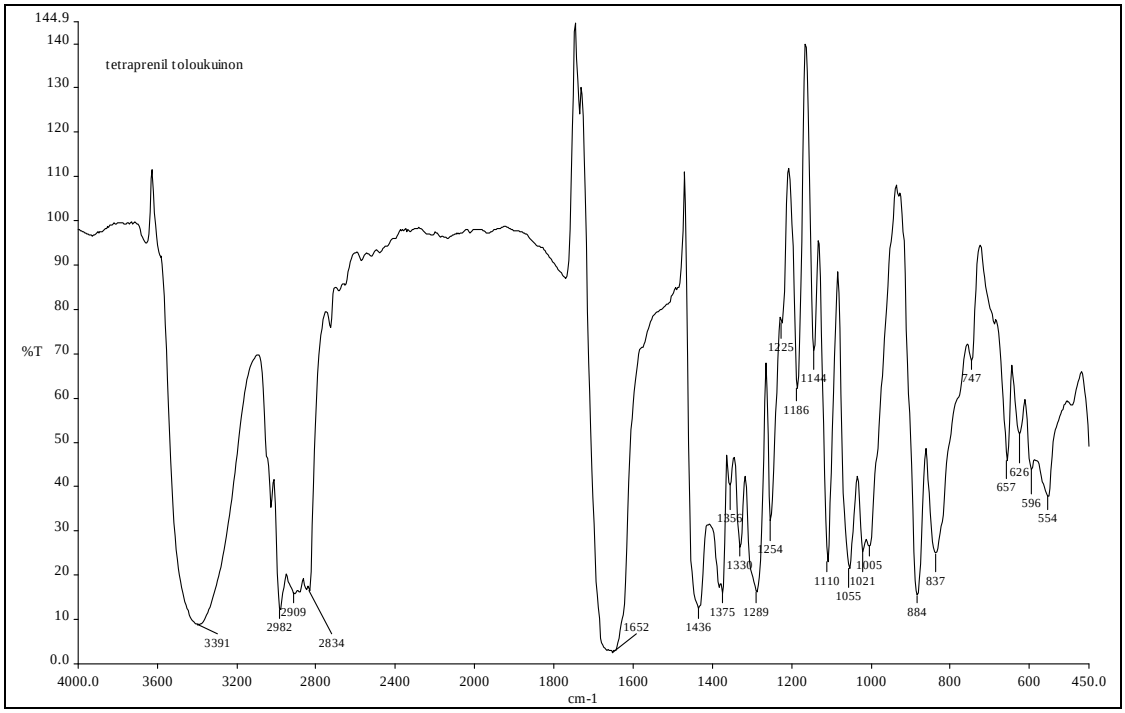
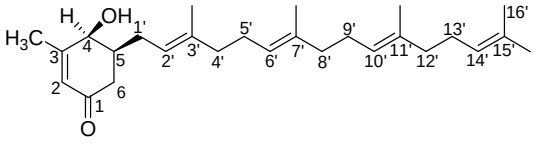


Fig. 1: FTIR spectrum of TPTQ in nujol

RESULT AND DISCUSSION

TPTQ

Chromatography of the hexane soluble portion of the methanol extract yielded yellowish oil, which was assigned structure **TPTQ** on the following grounds. The high-resolution mass spectrum (HRMS) indicated the molecular formula $C_{27}H_{42}O_2$, implying seven unsaturation.



The infrared spectrum (Fig. 1) of compound TPTQ indicated the presence of a hydroxyl function (ν_{max} 3435 cm^{-1}) and an α,β-unsaturated ketone (ν_{max} 1653 cm^{-1}) in keeping with the ^{13}C NMR spectrum (Fig. 2) at δ_C199.0 for ketone, a tertiary vanillic carbon (δ_C 126.4) and a quaternary vanillic carbon (δ_C 161.1).

The ^1H NMR (fig. 3) spectrum of side ring contain six allylic methylenes as complex multiplets between δ_H 1.96 – 2.14, three vinyl protons as multiplet at δ_H5.09 and five vinyl methyl's at δ_H1.59, 1.60, 1.60, 1.64, 1.67. The present of the geranyl-geranyl side chain was further confirmed by ^{13}C NMR, HSQC and HMBC data. Confirmation of the structure was carried out by comparing the spectroscopic data (^1H and ^{13}C NMR) of isolate with spectroscopic data of TPTQ [14].

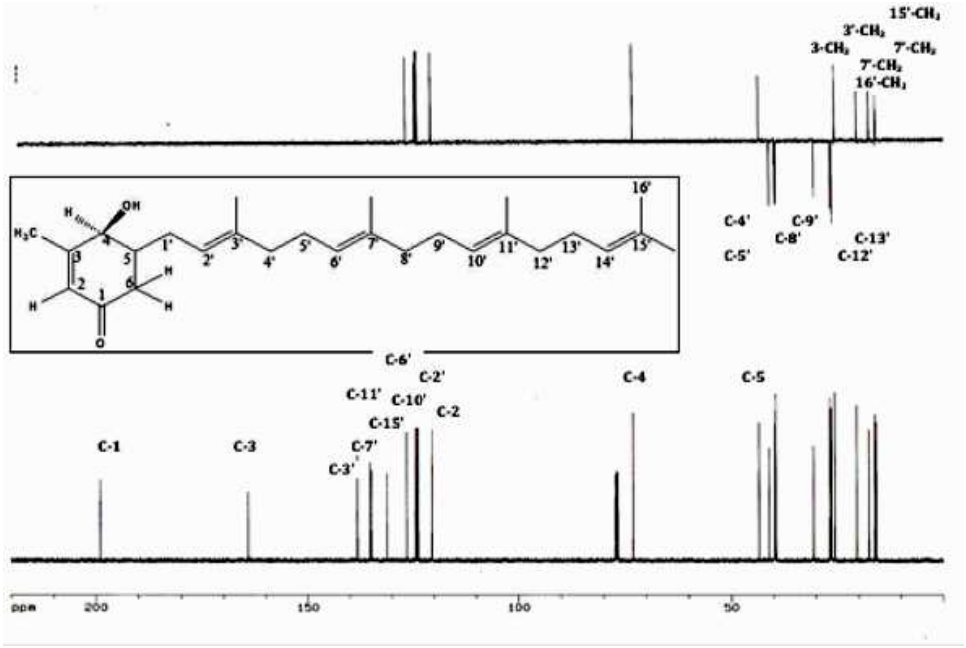


Fig. 2: [13]C-NMR and DEPT spectrums of TPTQ in $CDCl_3$

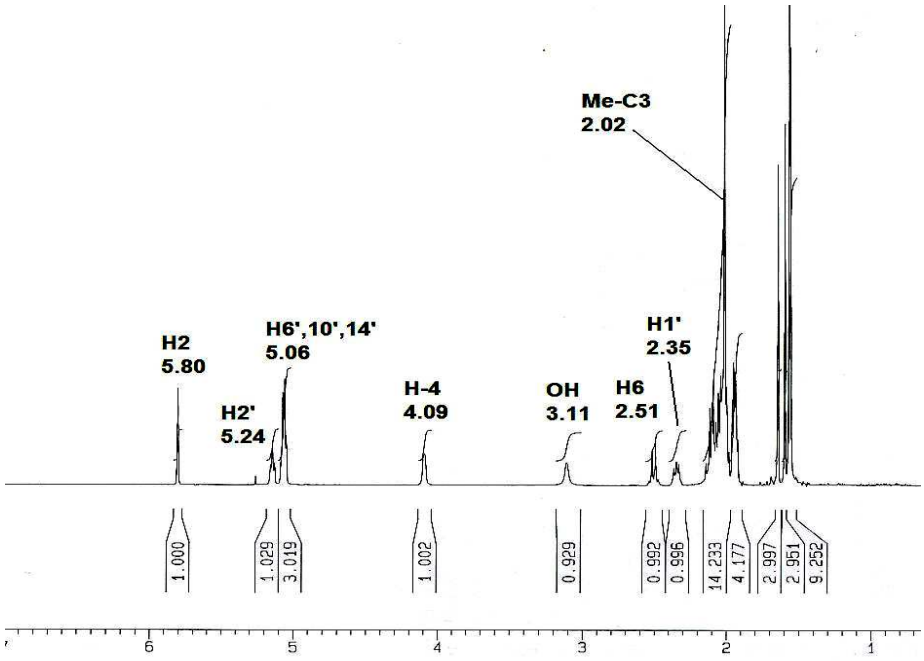


Fig. 3: ^1H-NMR spectrum of TPTQ in $CDCl_3$

Table 1: Cytotoxic activity of TPTQ towards cancer cell lines

Cell lines	IC_{50}
MCF-7	>100
H-460	16.3 ± 3.0
DU-145	>100

MCF-7= Breast cancer cells, H-460= Lung cancer cell cells, DU-145= Prostate cancer cells

TPTQ showed good and selective activity towards H-460 cell lines and not active against MCF-7 and DU-145 (Table 1). Similar results were also observed on the crude extract [13].

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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