Cytotoxic activity of *Combretum quadrangulare* leaf extracts on HepG2 cancer cell line

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Abstract

Studies on anticancer using plant extracts/compounds are promising. However, information regarding in vitro cytotoxicity is incompletely. Herein, this study aims to evaluate the cytotoxicity potentials of hexan:ethyl acetate (50:50, v:v) and ethanol extracts of *Combretum quadrangulare* leaves on HepG2 cancer cell line. Using cell viability MTT assay, hexan:ethyl acetate and ethanol extracts were defined as potential cytotoxicity with IC50 at 38 and 47 µg/mL respectively. In addition, by microscopy observation, we found that morphology of the cells apparently change in a dose and time dependent manner. The data showed in this study shed new light for further investigation of the anticancer effect of C. quadrangulare extracts and its compounds

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1. Introduction

Cancer is a class of diseases characterized by out-of-control cell growth. Nowadays, there are over 100 different types of cancer. Lung, prostate, colorectal, stomach, and liver cancer are the most common types of cancer in men, while breast, colorectal, lung, uterine cervix, and stomach cancer are the most common among women (WCRF). Studies on anticancer using plant extracts and compounds are promising. In eastern Asia, plants are widely being used for traditional medicine. *Combretum* species are widely used as medicine for the treatment of some diseases such as hepatitis, malaria, respiratory infections, and even cancer in eastern Asia [3]. *Combretum quadrangulare* is an indigenous tree in eastern Asia. In Vietnam it is commonly known as “Tram Bau”, widely contributed and used as traditional medicine as an antipyretic, antisympathetic, anthelmintic agent [4]. Several Cycloaactane Triterpenes have been isolated and shown to have biological activity on liver protection and cytotoxicity of some cancer cell lines [1, 2, 3, 4, 7, 8]. However, the activity of *C. quadrangulare* on HepG2 cells, a liver cancer cell line, is not fully understood. In this study, hexan:ethyl acetate (50:50) and ethanol extracts of *Combretum quadrangulare* leaves were tested on HepG2 cells for their cytotoxic possibilities.

2. Materials and methods

**Cell line.** HepG2 cell line was a gift of Dr. Pham Van Phuc, Lab of Stem Cells (National University in Ho Chi Minh city).

**Complete growth medium.** High glucose Dulbecco’s Modified Eagle’s Medium (DMEM, GIBCO) supplemented with 10% Fetal Bovin Serum (FBS, GIBCO) and 100 U/mL penicillin, 100 µg/mL streptomycin (Mekophar). Complete growth medium should be pre-warmed before use by placing into a water bath (Membert) set at 37°C ± 1°C for 30 minutes.

**Combretum quadrangulare leaves extracts and standards.** Hexan:ethyl acetate (H:Ea, 50:50) and ethanol (EtOH) extracts were prepared from *C. quadrangulare* leaves. Doxorubicin (Fresenius Kabi) and dimethyl sulfoxide (DMSO, Fisher chemical) was used as positive control and negative control respectively.

**Chemicals.** 0.25% (w/v) trypsin (Sigma) - 1mM Na₂-Ethylenediaminetetraacetic acid (EDTA, Thermo) was used for cell detaching and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) for cell viability assay. Detergent Reagent contained 10% (w/v) sodium dodecyl sulfate (SDS, Merck) and 10mM HCl (Merck). 0.4% (w/v) trypan-blue for cell staining was
3.1. Growth curve of HepG2 cell line using GraphPad Prism version 5.0 software. Statistical analysis applied.

Cell culture. HepG2 cell line was seeded in complete growth medium at 1 x 10^4 cells/cm^2 density and incubate at 37°C and 5% CO_2.

HepG2 growth curve. In order to assess growing possibility, HepG2 cells were seeded to 96-wells plate at 5x10^3 cells/cm^2 density and incubate at 37°C and 5% CO_2. Count the cells with a hemacytometer everyday up to 7 days of culture. The experiment was done in triplicate.

Cell viability assay. Dissolve Combretum quadrangulare extracts in DMSO at 10 mg/mL concentration. The extracts were diluted in culture medium at 100, 50, 25, 12.5, 6.25, 3.125, 0 µg/mL concentration. Stock of positive control containing 2mg/mL Doxorubicin in 20% DMSO were diluted in culture medium at corresponding concentration to dilluted C. quadrangulare extracts. DMSO was used as negative control. In addition, culture medium without cells was used as Blank. Preparation and usage of the standards were done according to ISO 10993-5 and 12 [5, 6]. Seed the extracts in DMSO at 10 mg/mL concentration. The extracts of Combretum quadrangulare leaves treatment, morphology of HepG2 cell line changed in a dose and time dependent manner. At concentration of 100 µg/mL of H:Ea extract, all cells changed from adhesion-shape to round-shape after 24 hours of treatment, but at concentration of 100 µg/mL of EtOH extract, there was quite rare round-shaped cells. At concentration of 50 µg/mL of H:Ea extract, density of round-shaped cells increased according to treatment time. At concentration of 50 µg/mL of EtOH extract, HepG2 morphology was likely unchanged.

Cytotoxic activity evaluation using MTT assay. In contrast to EtOH extract, H:Ea extract were expressed higher cytotoxic activity against HepG2 cell line. At 3.125–25 µg/mL concentration, H:Ea and EtOH extracts caused cytotoxicity to less than 15% of the cells. At 50 µg/mL concentration, EtOH extract caused 54% cells dead, H:Ea extract up to 74%. At concentration of 100µg/mL extracts, EtOH caused 60% cell dead, H:Ea extract up to 96% and this value was higher than inhibition percentage value of Doxorubicin (87%) at equivalent concentration. According to ISO 10993-5, IC_{50} value of >27 µg/mL and >31 µg/mL concentration of H:Ea and EtOH extract, respectively, was considered as potential cytotoxicity.

3.2. Cytotoxic activity

Morphological assessment. As showed in table 2, with the extracts of C. quadrangulare leaves treatment, morphology of HepG2 cell line changed in a dose and time dependent manner. At concentration of 100 µg/mL of H:Ea extract, all cells changed from adhesion-shape to round-shape after 24 hours of treatment, but at concentration of 100 µg/mL of EtOH extract, there was quite rare round-shaped cells. At concentration of 50 µg/mL of H:Ea extract, density of round-shaped cells increased according to treatment time. At concentration of 50 µg/mL of EtOH extract, HepG2 morphology was likely unchanged.
Table 1. IC50 value of *C. quadrangulare* extracts and Standard using graphic method.

<table>
<thead>
<tr>
<th>Extracts and Standard</th>
<th>IC50 (µg/mL)</th>
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<tbody>
<tr>
<td>Hexan:ethyl acetate</td>
<td>38</td>
</tr>
<tr>
<td>Ethanol</td>
<td>47</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>2</td>
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</tbody>
</table>

Table 2. Figures of HepG2 morphology at different concentrations of H:Ea and EtOH extracts and Doxorubicin after 24, 48, 72 hours treatment (100X). Scale bar, 100µm.

4. Conclusions

*C. quadrangulare* H:Ea and EtOH extracts possessed an inhibiting effect on the growth of HepG2 cell line. The extracts induce cell morphology from adhesion-shape to round-shape in a dose and time manner. Our preliminary data show that *C. quadrangulare* extracts can be consider for further investigation on its anticancer treatment.

Today, researching on plant extracts and compounds are promising way in anticancer treatment. The present study was an initial step of identifying the cytotoxicity effect of Combretum quadrangulare extracts on HepG2 cell line and in furtherance of its compounds in various in vitro studies for anticancer treatment.

References