Antiproliferative effect on cancer cells and mutagenic activity of *Pseuderanthemum palatiferum* (Nees) Radlk.

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**ABSTRACT**

*Pseuderanthemum palatiferum* (Nees) Radlk. is one of the most commonly used medicinal plants in Thailand. It has been reported to have antioxidant, anti-inflammatory, antidiabetic, and antimicrobial activities. This study aimed to investigate the *in vitro* antiproliferation of 95% ethanol extract of *P. palatiferum* (EEP) against different human cancer cell lines (Jurkat, HepG2, MCF-7, and PC-3) using the MTT assay. The mutagenic effect of EEP as a possible health risk from its long term use was also evaluated by the Ames test. The results showed that various types of cancer cells exhibited different susceptibilities to EEP in a dose dependent manner. Jurkat cells was the most sensitive to the lethal effect of EEP. The LC\(_{50}\) of EEP in Jurkat and HepG2 were 476.35 ± 31.51 and 927.01 ± 90.84 µg/ml, respectively, and the LC\(_{50}\) in MCF-7 was higher than 1,500 µg/ml. No growth inhibition of EEP on PC-3 cells was observed. The cytotoxicity of EEP was mediated through apoptotic mechanism as evidenced by the nuclear condensation and DNA laddering fragmentation profile of Jurkat cells exposed to 300 µg/ml EEP for 12 and 24 h and 600 µg/ml for 24 h. Apoptosis induction in Jurkat cells was further confirmed by Hoechst 33258 and Annexine-V/PI staining using flow cytometry. There was no mutagenic effect of EEP on the *Salmonella typhimurium* strains TA98 and TA100, regardless of the absence or presence of S9 mix. Overall, this study suggested that EEP exhibits antiproliferative effect on Jurkat cells by apoptosis induction, and the extract possesses no mutagenic activity.

**Keywords:** Antiproliferation activity, Mutagenic activity, Apoptosis induction, *Pseuderanthemum palatiferum* (Nees) Radlk.
1. INTRODUCTION

*Pseuderanthemum palatiferum* (Nees) Radlk. or Hoan-ngoc has long been used as medicinal plant by Vietnamese people. A few years ago, it has become popular among Thai people for alleviating or curing various diseases, including cancer. Hoan-ngoc leaves have been reported to have antioxidant and antidiabetic [1], anti-inflammatory [2], and antimicrobial activities [3]. Moreover, the major bioactive compounds in Hoan-ngoc leaves are revealed to be flavonoid, stigmasterol, β-sitosterol, and apigenin-7-O-β-glucoside [4]. Flavonoid and β-sitosterol were reported to induce apoptosis in cancer cells [5, 6]. However, up to present, the pharmacologic studies of Hoan-ngoc’s claimed properties are still very limited, and the long-term effects of its use are still largely unknown. Therefore, the aim of this study was to investigate the *in vitro* antiproliferative activity against different human cancer cell lines (Jurkat, HepG2, MCF-7, and PC-3) and evaluated apoptosis induction on Jurkat cells of 95% ethanol extract of *P. palatiferum* (EEP). Besides, the mutagenic effect activity of the extract was assessed.

2. MATERIALS AND METHODS

**Preparation of Pseuderanthemum palatiferum (Nees) Radlk leaves extracts**

Fresh leaves of Hoan-ngoc were blended in 95% ethanol and filtered through gauze. The extract was centrifuged and then the supernatant was filtered through a Whatman No.1 filter paper. After that, the ethanolic filtrate was concentrated using a vacuum rotary evaporator and lyophilized into powder of ethanol crude extract.

**Cell culture**

HepG2 human hepatocyte carcinoma cell line, MCF-7 human breast adenocarcinoma cell line, and PC-3 human prostate adenocarcinoma cell line were obtained from American Type Culture Collection (ATCC). Jurkat leukemic cell line was obtained from Cell Line Services (CLS), Germany. HepG2 and MCF-7 cells were cultured in DMEM with high glucose supplemented with 10% FBS and 100 U/ml penicillin and 100 µg/ml streptomycin. Jurkat cells and PC-3 cells were cultured in RPMI-1640 supplemented with 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin. All cell lines were maintained at 37°C in 5% CO₂ and 95% humidity.

**Cytotoxic assay**

The cytotoxic effect of EEP on cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described [7].

**DNA fragmentation**

Jurkat cells were treated with 100, 300, and 600 µg/ml of EEP for 24 h and 300 µg/ml for 6, 12, and 24 h. After treatment, cells were collected, and then DNA was extracted using QIAamp® DNA Mini Kit (QIAGEN, Germany). Five micrograms of DNA sample were loaded on a 1.5% agarose gel. The gel was run at 70 volts for 1.50 h and then stained with 0.5 µg/ml ethidium bromide. The DNA fragment was visualized under ultraviolet light.

**Hoechst 33258 staining**

After Jurkat cells were treated with EEP, the cells were fixed with p-formaldehyde (4%, v/v) for 20 min and further stained with 10 µg/ml of Hoechst 33258 for 30 min at room temperature in the dark. The stained cells were washed with PBS and visualized under the inverted fluorescence microscope.

**Annexin V-PI staining**

After EEP treatment, the Jurkat cells were collected and stained for 15 min with Annexin V-FITC and PI using the Annexin V-FITC Apoptosis Detection Kit (EXBIO, Czech Republic). The stained cells were analyzed by flow cytometry.

**Ames test**

The mutagenicity of EEP was evaluated by the Ames test using *Salmonella typhimurium* strains TA98 and TA100. The assay was performed by pre-incubation method [8] and conducted under both absence and presence of S9 mix. The extract was considered as mutagenic if the number of revertant per plate was at least double over the spontaneous revertant frequency.
3. RESULTS

The \textit{in vitro} cytotoxic effect of EEP against various human cancer cell lines, namely Jurkat, HepG2, MCF-7 and PC-3 after 24 h of exposure are shown in Figure 1A. The results showed that various types of cancer cells exhibited different susceptibilities to EEP in a dose dependent manner. The LC\textsubscript{50} was calculated from a dose response curve using linear regression analysis. EEP exerted antiproliferation only in Jurkat and HepG2 cell lines with the LC\textsubscript{50} values of 476.35±31.51 and 927.01 ± 90.84 µg/ml, respectively. EEP at the concentration up to 1,500 µg/ml had no cytotoxicity towards PC-3. The breast cancer MCF-7 cells showed less susceptible to EEP treatment (LC\textsubscript{50} > 1,500 µg/ml). Being the most sensitive target of EEP, the Jurkat cells were selected for further investigation whether the cytotoxic effect of EEP was mediated through the apoptotic mechanism.

![Figure 1A](image1.png)  

![Figure 1B](image2.png)

Figure 1. (A) Cytotoxic effect of EEP against different human cancer cell lines, PC-3, MCF-7, HepG2 and Jurkat cells. The cells were exposed to various concentrations of EEP for 24 h and were assessed for cell viability by MTT assay. Reported means ± SD values (n=4) are from a representative of three independent experiments. (B) DNA fragmentation in Jurkat cells exposed to EEP for 24h. Lane M, 1 kb DNA marker; lane P, 40 µg/ml of etoposide (positive control); lane C, media alone (negative control); lane VH, 0.1% DMSO (vehicle control).

The DNA fragmentation analysis (Figure 1B) showed that EEP induced the apoptotic cell death in both concentration- and time-dependent manners. The fragmented DNA was clearly observed in Jurkat cells after exposure to EEP at 300 and 600 µg/ml for 24 h and at 300 µg/ml for 12 and 24 h, respectively. No DNA fragmentation was observed in both negative and vehicle control cells, whereas the DNA ladder formation was clearly observed in the positive control group (40 µg/ml etoposide) at 24 h of exposure.

The nuclear morphological changes of Jurkat cells after EEP treatment were observed by Hoechst 33258 staining. The extent of apoptotic cell death induced by EEP was dose- and time- dependent (Figure 2A). At 300 and 600 µg/ml, EEP induced apoptotic cell death in Jurkat cells, but had no effect at 100 µg/ml. The time course study revealed that Jurkat cells exposed to 300 µg/ml of EEP showed nuclear condensation and DNA fragmentation at 12 and 24 h post exposure, but no alteration of nuclear morphological changes was observed at the earlier time point (6 h). As expected, non-treated cells showed normal nuclear morphology.

The Annexin V-PI assay was further performed to confirm the apoptotic cell death induced by EEP. AnnexinV-PI staining was used to evaluate early and late apoptotic cell death. The percentage of EEP-induced apoptosis in Jurkat cells were increased in both dose- and time-dependent manners (Figure 2B). The percentages of early apoptotic in EEP-treated Jurkat cells were 5.21%, 7.93%, and 14.24% upon treatment with 100, 300, and 600 µg/ml of EEP for 24 h, respectively. Likewise, the percentages of early apoptotic in Jurkat cells after treatment with 300µg/ml of EEP for 6, 12, and 24h were 6.93%, 8.44%, and 11.42%, respectively.

The mutagenicity of EEP was evaluated by the Ames test using \textit{S. typhimurium} strains TA98 and TA100. The assays were performed in both absence and presence of S9 mix. A compound tested with a mutagenic index of 2.0 or more is regarded as a potent mutagen. EEP ranging from 150 µg/plates up to 600 µg/plates had the mutagenic index of less than 2.0 on both tested strains, regardless the presence or absence of S9 mix (Table 1). Therefore, EEP in the range of 150 - 600 µg/plates had no mutagenic activity, whereas all positive controls always induced a clear mutagenic response with high values of mutagenic indexes (13.6-50.6).
Figure 2. (A) Effect of EEP on nuclear morphological changes in Jurkat cells. The nuclear morphological changes were evaluated by staining with Hoechst 33258 and visualized under fluorescence microscopy at 400 × magnification. The fragmented or condensed nuclei are indicated as white arrows. (B) Flow cytometric analysis of apoptosis in Jurkat cells exposed to various concentrations of EEP for 24 h and kinetics of apoptosis induction in Jurkat cells exposed to 300 µg/ml of EEP. The apoptosis of Jurkat cells was detected by flow cytometry using AnnexinV-PI staining method. Data are a representative of two independent experiments.
Table 1. Mutagenic effect of EEP on the <i>Salmonella typhimurium</i> strains TA98 and TA100 in the absence and presence of S9 mix

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of revertants/plate (Mean±SD), (Mutagenic Index)</th>
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<tbody>
<tr>
<td></td>
<td>TA98</td>
</tr>
<tr>
<td>Control</td>
<td>22±2</td>
</tr>
<tr>
<td>Vehicle control (1.4% DMSO)</td>
<td>20±5</td>
</tr>
<tr>
<td>2-NF (10µg/plate)&lt;sup&gt;PC&lt;/sup&gt;</td>
<td>1,012±225,(50.6)</td>
</tr>
<tr>
<td>Sodium azide (10µg/plate)&lt;sup&gt;PC&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>2-AA (2.5µg/plate)&lt;sup&gt;PC&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>EEP (150µg/plate)</td>
<td>24±2, (1.20)</td>
</tr>
<tr>
<td>EEP (300µg/plate)</td>
<td>20±4, (1.00)</td>
</tr>
<tr>
<td>EEP (600µg/plate)</td>
<td>20±3, (1.00)</td>
</tr>
</tbody>
</table>

PC, positive control. Data were expressed as means ± SD of two independent experiments (n=3). Mutagenic Index (MI) = Number of revertant colonies of the extract/Number of revertant colonies of the vehicle control (spontaneous revertant). Values in brackets (MI) ≥2 indicate mutagenicity.

4. CONCLUSIONS

The current study demonstrated that the ethanolic extract of <i>Pseuderanthemum palatiferum</i> exerts the most potent antiproliferative effect on human T cell leukemia Jurkat cells, and its cytotoxicity is mediated through apoptosis pathway. The extract in the range of 150 to 600 µg/plate has no mutagenicity in the Ames assay.

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REFERENCES