ANTIPROLIFERATIVE EFFECT ON COLON CANCER CELL LINES BY AQUEOUS EXTRACT FROM THE BARK OF MILLINGTONIA HORTENSIS

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Abstract Millingtonia hortensis is a traditional medicinal plant widely used in South-East Asia. This study focused on the antiproliferative effect when comparing between aqueous extract and ethanol extract from the bark of M. hortensis using human colon cancer cell lines, DLD-1, HCT15, SW48 and SW480. In in vitro experiments, the MTT method was used to determine the antiproliferative effect of M. hortensis extract on colon cancer cells. An aqueous extract of M. hortensis significantly reduced the proliferation rate of colon cancer cell lines in a dose-dependent manner. However, no antiproliferative effect was observed by ethanol extract of M. hortensis. This finding indicated that only aqueous extract from the bark of M. hortensis is able to inhibit colon cancer cell proliferation, and its mechanism is now under investigation. Chiang Mai Medical Journal 2007;46(2):61-66.

Keywords: Millingtonia hortensis, colon cancer cell lines, antiproliferative effect, MTT method

Cancer is a genetic disease caused by the accumulation of numerous mutations that render the tumor cell insensitive to control by the local cellular environment.1 Colon cancer is one of the most common malignancies worldwide,2-4 and is increasing in Asian countries.5 Although many anticancer drugs have been developed and applied, resistance to anticancer drugs was found. Therefore, the research and development of new and safe drugs have become necessary.6 Chemoprevention refers to the use of natural or synthetic compounds to prevent, reverse, or delay the development of cancer. Plants have been a source of medicinal substances for a long time, and phytoproducts play an essential role in medicine.7 Millingtonia hortensis belongs to the Family Bignoniaceae. It is an important plant in Asia and South-East Asia, ranging from...
India, Burma, Thailand, Vietnam, Southern China and Indonesia. In Thailand, this plant is called ‘Peep’ and is used for the treatment of asthma and sinusitis, and as a tonic.\(^{6-9}\)

In our laboratory, we found by using Ames test that \textit{M. hortensis} has an antimutagenic activity against Glu-P-1, Glu-P-2, Trp-P-1 and Trp-P-2. However, there was no evidence as to whether \textit{M. hortensis} effects tumor cell growth. These data led us to investigate the effect of aqueous and ethanol extract from the bark of \textit{M. hortensis} on the proliferation of colon cancer cell lines.

In this study, we used four types of colon cancer cell lines; DLD-1, HCT15, SW48 and SW480. DLD-1 and HCT15 cell lines, which were established separately from a colon carcinoma, have totally different chromosome changes, but the same genetic origin.\(^{10}\) They are a poorly differentiated cell. DLD-1 carries a mutation of \textit{p53}\(^{11}\) and adenomatous polyposis coli (\textit{APC}).\(^{12}\) SW48 was derived from poorly differentiated colon adenocarcinoma. This cell line contains wild type \textit{p53}\(^{13}\) and \textit{APC},\(^{14}\) whereas, SW480 was derived from a primary colon adenocarcinoma. SW480 is a poorly differentiated cell that exhibits mutation of the tumor suppressor genes, \textit{APC} and \textit{p53}.\(^{15}\)

**Preparation of \textit{Millingtonia hortensis} extracts**

\textit{Millingtonia hortensis} Linn.f. (Bignoniaceae) was authenticated by Dr. Chusie Trisonthi, Department of Biology, Chiang Mai University. The bark of \textit{M. hortensis} was dried in a hot air oven and ground to a power. One hundred grams of the powder was extracted with 1,000 mL of distilled water or 80% ethanol by stirring for 4 hours. Then, the supernatants from the ethanol extraction were directly filtered through Whatman filter paper number 1, while the supernatants from the water extraction were centrifuged at 3,500 rpm for 15 min at 4 °C, before filtering. The filtrates were evaporated using a rotating evaporator (60 °C) followed by lyophilization until dry. The residues were dissolved in cultured medium to get a stock solution of 20 mg/mL. The stock extracts were sterilized by Millipore filter membrane 0.22 μm.

**MTT assay**

Cells (1x10^4 per well) were plated in triplicate in a 96-well culture plate followed by cell adherence overnight. They were treated with different concentrations of the extract (50, 100, 200, 400, 800 μg/mL) for 24, 48, and 72 hours, respectively. After incubation, culture media were discarded and new culture media containing 10% of 5 mg/mL of MTT ([3,4,5-dimethyl thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide], Sigma Chemical Co., Dorset, UK) were added and incubated further at 37 °C for 4 hours. After incubation, culture media were discarded before adding 0.1 mL of dimethyl sulfoxide (DMSO, Merck, Hohenbrunn, Germany) to each well to solubilize the formed formazine crystals. The absorbance (OD) was measured at 540 nm using a microplate reader spectrophotometer. The results were
expressed as percent of cell viability.

**Statistical analysis**

Significance of difference between values were assessed using the Mann-Whitney U test and all values were expressed as mean ± SD. A p value of <0.05 was considered significant.

**Result**

**Effect of M. hortensis extract on the inhibition of tumor cell growth**

The effect of *M. hortensis* extract on induced colon cancer cell death was evaluated by MTT assay. The rate of cell survival was significantly decreased when treated with different concentrations (50 µg/mL to 800 µg/mL) of aqueous extract of *M. hortensis* (Fig. 1). At 72h, aqueous extract of *M. hortensis* showed the highest cytotoxic activity against HCT15 colon cancer cells and less cytotoxic activity against DLD-1 colon cancer cells. In line with these profiles, the IC$_{50}$ values (i.e. inhibitory concentration at 50% effective level) are shown in Table 1. The cell growth and proliferation were inhibited in a dose-dependent manner and seemed to decreased slightly after increase incubation times (24 to 72 hours). However, an ethanol extract did not affect the rate of cell survival on colon cancer cell lines (Fig. 2). This result indicated that only aqueous extract of *M. hortensis* had an antiproliferative effect on colon cancer cell lines.

![Figure 1](image1.png)

**Figure 1.** Effect of aqueous extract of *M. hortensis* treatment on the viability of colon cancer cell lines. DLD-1 (A), HCT15 (B), SW48 (C) and SW480 (D) were seeded on a 96-well plate (1 x 10$^4$ cells/well). Aqueous extract of *M. hortensis* was added to the cultures at concentrations of 0, 50, 100, 200, 400, and 800 µg/mL, respectively, before incubation. The cultures were maintained at 37°C for 24, 48, and 72 h, respectively. The percent viability was determined by the MTT dye reduction assay. Values are the mean ± S.D. of three independent experiments. (*, p < 0.05 was significantly different from the control).
Table 1. Cytotoxic activity (IC\textsubscript{50} µg/mL) of aqueous extract of \textit{M. hortensis} against colon cancer cells (HCT15, DLD-1, SW48, SW480) with an exposure time of 72 hours and \textit{n} = 3

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC\textsubscript{50} (µg/mL)</th>
</tr>
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<tbody>
<tr>
<td>HCT15</td>
<td>290</td>
</tr>
<tr>
<td>DLD-1</td>
<td>420</td>
</tr>
<tr>
<td>SW48</td>
<td>385</td>
</tr>
<tr>
<td>SW480</td>
<td>330</td>
</tr>
</tbody>
</table>

Discussion and conclusion

In Asian countries, a wide variety of traditional plants have been used as food and medicine. A large number of candidate compounds, which may be practically useful for human cancer prevention, have been discovered. \textit{Millingtonia hortensis} is one important traditional plant widely used as medicine in Southeast Asia.

In this study, we purposed to compare the antiproliferative effect between aqueous and ethanol extract from the bark of \textit{M. hortensis} on human colon cancer cell lines. An MTT based cytotoxic assay was carried out using four types of these cell lines, in which their

![Figure 2. Effect of ethanol extract of \textit{M. hortensis} treatment on the viability of colon cancer cell lines. DLD-1 (A), HCT15 (B), SW48 (C) and SW480 (D) were seeded on a 96-well plate (1 x 10\textsuperscript{4} cells/well). Ethanol extract of \textit{M. hortensis} was added to the cultures at concentrations of 0, 50, 100, 200, 400, and 800 µg/mL, respectively, before incubation. The cultures were maintained at 37°C for 24, 48, and 72 h, respectively. The percentage viability was determined by the MTT dye reduction assay. Values are the mean ± S.D. of three independent experiments. (*, \textit{p} < 0.05 was significantly different from the control).]
growth and proliferation in this study were significantly inhibited by an aqueous extract of *M. hortensis*. Among the four types of colon cancer cell lines, HCT15 and DLD-1 were the most and less sensitive aqueous extract of *M. hortensis*. Although HCT15 is a microsatellite-unstable cell line, which derived from the same tumor as 1 and shared various mutations with DLD-1, it has a different karyotype. Whether HCT15 is highly sensitive to the extract is unclear. However, the ethanol extract of *M. hortensis* has no ability to inhibit the growth and proliferation of colon cancer cell lines.

The more pronounced concentration- and time-dependent antiproliferative activity for aqueous extract of *M. hortensis* may be due to its different phytochemical profiles, as compared to the ethanol extract of *M. hortensis*.

Uncontrolled growth and proliferation are a universal property of cancer cells. This study presents the probable activity of an aqueous extract of *M. hortensis* as an antitumor agent for the treatment of colon cancer. Although the IC$_{50}$ value tends to be high, it is an aqueous crude extract. Further studies should involve partial purification for increasing the effectiveness of this extract and investigating its apoptosis effect on the same cell lines. We suppose that this extract can be used as a therapeutic agent in colon cancer.

**Acknowledgement**

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**References**

การยับยั้งการแบ่งตัวของเซลล์มะเร็งลำไส้ใหญ่โดยสารสกัดด้วยน้ำจากเปลือกของต้นปีบ

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บทคัดย่อ ปีบเป็นพืชสมุนไพรที่ใช้ทางการแพทย์แผนโบราณอย่างกว้างขวางทางแถบเอเชียตะวันออกเฉียงใต้ การศึกษาครั้งนี้มุ่งเน้นไปที่การเปรียบเทียบฤทธิ์ยับยั้งการแบ่งตัวของเซลล์มะเร็งลำไส้ใหญ่ระหว่างสารสกัดจากเปลือกต้นปีบโดยใช้หลอดทดลอง สารสกัดด้วยน้ำโดยใช้วิธี MTT เพื่อตรวจสอบฤทธิ์ยับยั้งการแบ่งตัวของเซลล์มะเร็งลำไส้ใหญ่ ผลการศึกษาพบว่าสารสกัดด้วยน้ำมีฤทธิ์ยับยั้งการแบ่งตัวของเซลล์มะเร็งลำไส้ใหญ่โดยมีการเปลี่ยนแปลงของความเข้มข้นของสารสกัดด้วยน้ำ อย่างมีนัยสำคัญ อย่างไรก็ตามไม่พบสารสกัดด้วยเอทานอล สารสกัดด้วยเอทานอลให้ผลการยับยั้งการแบ่งตัวของเซลล์มะเร็งลำไส้ใหญ่ ผลการศึกษาเป็นผลต่อการแบ่งตัวโดยสารสกัดด้วยน้ำอย่างมีนัยสำคัญ คุณค่าเรื่องราว

คำสำคัญ: ปีบ เซลล์มะเร็งลำไส้ใหญ่ ฤทธิ์ยับยั้งการแบ่งตัว วิธี MTT