Cytotoxicity effects of leaf extracts of Ciplukan (*Physalis angulata*; Solanaceae) on human blood and ovary cancer cell lines

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**Abstract.** *Physalis angulata* has identified by molecular phylogenetic analysis and the results showed genetically close related with an anticancer plant, *Withania somnifera*. The aims of this preliminary study was to evaluate cytotoxicity effects of leaf extract of *P. angulata* (LEP) either in powder or pasta form on cells viability, cells proliferation, and inhibition on human ovary cancer cell lines (SKOV3) and human blood cancer cell lines (HL60). Cytotoxicity effects was analysed by MTS Cell Proliferation Assay Kit. Doxorubicin was used as positive control in this experiment. Surprisingly, two treatments (powder and pasta) have different toxic effects. We found that IC50 and viability (LC50) for SKOV3 cell lines for powder was between 93 ug/ml and 187 ug/ml, and 187 ug/ml and 375 ug/ml for pasta. IC50 for HL60 cell lines was 23 ug/ml for powder and around 18 ug/ml for pasta. Cell viability (LC50) of HL60 treated with LEP showed that 23 ug/ml for powder or 46 ug/ml for pasta. These results suggested that LEP have antiproliferative and inhibition activities both on SKOV3 or HL60.

1. Introduction

All part of the plant contain many potential chemical substances especially for therapy purposes and treat some diseases. Ciplukan (*Physalis angulata*) is a species of seed plant that have been frequently used by local peoples in Indonesia as medicinal plant since long time ago. As traditional medicine, this plant is readily available in rural areas and of course cheaper than any kind of modern drugs. Recent molecular phylogenetic study reported that Ciplukan is relative to that of endemic plant to South Asia *Withania somnifera* (Ashwaganda) [1], a plant that has been proved efficacious as an anticancer, antioxidant and anti-ageing activities [2,3].

*P. angulata* also used as traditional medicines preparation for diabetes, hepatitis, ashma and malaria in Taiwan [4]. In Africa, *P. angulata* is used as traditional medicines for cancer treatment [5], cytotoxic and antitumor, anticancer activity [6-8]. Other compound called physalin, induces cell apoptosis in human renal carcinoma cells and generating reactive oxigen species (ROS) [9]. Antiproliferative and anti inflammatory activities is also showed by withanolides from leaf extracts of *P. angulata* [10-12]. Leaf extract of *P. angulata* has antimitotic activities through inducing G2/M phase arrest in human breast cancer cells [4]. Other experiment also showed that *P. angulata* induces in vitro differentiation of murine bone marrow cells into macrophages [13]. Ethanol and methanol leaf extract
of *P. angulata* can inhibit α-glucosidase enzyme activity, indicating that this plant has a potential compound for diabetes treatment [14].

However, in Indonesia, *P. angulata* is just a plant that used as traditional cure and information of scientific evidences about its effect to cancer cell is not sufficient for the society. Therefore, we conducted this preliminary research to evaluate cytotoxicity effects of leaf extract of *P. angulata* (LEP) to the cancer cell.

2. Methods

2.1. Plant materials

The fresh leaves of *P. angulata* were collected from Lembang and Bandung region, West Java Indonesia. The plant was authenticated at Laboratory of Plant Structure, Department of Biology Education, Universitas Pendidikan Indonesia (UPI), Indonesia.

2.2. Leaf extraction

The leaves of were firstly washed and sun dried 3 days and were further dried in an oven at 40-50°C for 48 h. The dried leaves were coarsely powdered using mechanical grinder. The powdered leaves were extracted by 70% ethanol (EtOH) distilation using maceration to produce ethanol extracts for 24 h [15]. Rotary evaporimeter was used to make high concentration of the leaves extract.

2.3. Cell culture and treatment

SKOV3 cell lines were seeded into 96-well plate with 5x10³ per well. Growth medium consist DMEM (Gibco, 11995-065) and RPMI-1640 medium (Sigma Aldrich, USA) for HL60 cell lines + 10% FBS (Gibco, 10270), and 1% Abam (Gibco, 15240-062) in a final volume of 100 μL/well culture medium. The cells were incubated in a humidified atmosphere with 37°C and 5% CO₂. After 24 h incubation with 50% confluency, the cells were treated with 1500, 750, 375, 187.5, 93.8, 46.9, 23.4, or and 11.7 μg/ml or ppm of LEP either in powder or pasta forms. Subsequently, those cells were incubated for 24 h and 20 μL MTS reagent were added and incubated for 3 h followed by spectrophotometry measurement at 490 nm wavelength. Doxorubicin was used as positive control in this experiment.

2.4. MTS-Viability and Proliferation Assay

MTS [3-(4,5-dimetiltiazol-2-il)-5-(3-karboksimetoksifenil)-2-(4-sulfofenil)-2H-tetrozolium is tetrazolium component in salt form. MTS assay was used for evaluate cell proliferation by cell viability according to metabolic activities of the cells. MTS Cell Proliferation Assay Kit (Abcam #ab197010) was used in this experiment. Interaction between reagents with viable cells produce color solution called formazen. Color solution (formazen) equivalent indicates number of life cells in the cultur, both in treatment group or control. Formazen measured by spectrophotometer with 490 nm. In vitro cytotoxicity was assessed as percentage of growth inhibition adopted from Fanfan et al. [7], which was calculated as 100% - [(OD490 of drug treated cells/OD490 of untreated cells) x 100%].

2.5. Statistical analysis

Data analysis was performed using SPSS version 16 for Windows and comparisons between the group concentrations and the control groups in cell viability were performed by one-way ANOVA.

3. Results and Discussion

Cytotoxicity of the phytochemical compounds on the cancer cells can induce cell proliferation inhibition that indicated by cells viability in vitro. According to our previous research suggested that LEP contains several substances categorized in flavonoid, such as quercetin-3-O-rutinoside, 3-O-metilquercetin-7-O-rutinoside, physalin D, physalin G, and physalin F [16]. Interestingly, as shown in Figure 1, the cell-treated powder and pasta have different effects in concentration both on cell viability or inhibition activities on cells proliferation of SKOV3 or HL60 cell lines.
Figure 1. Cell viability of SKOV3 cell-lines treated with LEP

Figure 1 shows that LEP tends to decrease cells viability corresponding to the increase of concentration. Powder concentration in range between 187 and 375 µg/mL induced 50% cell viable (LC50) or between 93 and 187 µg/mL 93 and 187 µg/mL of cell proliferation inhibition (IC50) for SKOV3 cell lines. Both of the form of leaf extracts suggested that pasta formulation was stronger than powder to induce cell viability or inhibition of cells proliferation on SKOV3 cell lines. Other research showed that several compound of P. angulata, such as physalin, withangulatin, whitaferin, glycosides, and myrcetin induce cytotoxic effects [17]. Citotoxicity of this plant on breast cancer cells have been identified and indicated by proliferation inhibition [18].

Antiproliferative activities of Physalis compounds, such as withanolides have potent cytotoxic effects on cells in vitro and withanolide variety demonstrated that the Physalis genus was a good source of diverse withanolides and clearly suggested that other Solanaceae species were worthy of further exploration [19]. Cell viability of SKOV3 cell lines treated by LEP for 24 hours was suppressed. Viability of SKOV3 cell lines indicated by MTS assay results includes inhibition of cells proliferation.

Figure 2. Cell inhibition of SKOV3 cell-lines treated with LEP

According to Figure 2, inhibition activity was increase according to increasing concentration in powder or paste form. Pasta form formulation showed stronger than powder one in inhibition of proliferation SKOV3 cells.

On the other hand, we found that HL60 cell lines showed different sensitivities on LEP both powder or pasta. LC50 of powder or pasta was between 187 and 375 µg/mL or 93 µg/mL, respectively and IC50 of powder or pasta was 187 µg/mL or 93 µg/mL, respectively. We found that HL60 cell lines more sensitive to pasta formulation than powder to induce cell viability. Wu et al. [20] suggested that ethanol extracts of P. angulata clearly have high toxic effects on hepatoma cancer cells (HepG2 cell
lines), therefore we found that both SKOV3 or HL-60 cell lines showed toxic response, especially on pasta formulation that extracted by ethanol.

Figure 3. Cell viability of HL-60 cell-lines treated with LEP

![Figure 3](image)

Figure 4. Cell inhibition of HL-60 cell-lines treated with LEP

![Figure 4](image)

Similarly in SKOV3 cell-lines, HL-60 cell is more sensitive on LEP in pasta formulation rather than powder. Sensitivity of these cells is close related with the cells viability, both on SKOV3 or HL60 cell-lines. Qing et al. [21] also suggested that methanol extracts of *P. angulata* exhibited the strong antiproliferative activities towards human colorectal carcinoma (HCT116 cell lines) and human non small cell lung cancer (NCI-H460 cell lines) in vitro. Pasta was extracted by ethanol and similarly showed the high cytotoxic effects than powder one.

Figure 3 and Figure 4 above show that increasing concentration causes the number of the cells viable was decreased and inhibition activity of this extracts on HL60 cell lines depend on concentration. Cell viability and inhibition of cell proliferation was concentration dependen maner. Physalin F, Physalin B and Quercertin are strongly having growth inhibition on cancer cells by apoptotic cascade [22]. Physagulides from *P. angulata* can exhibit cytotoxic effects on cancer cells [23]. Zhang et al. [24] demonstrated that fourteen withanolides from genus *Physalis* at aerial part showed potent cytotoxicity against human head and neck squamous cell carcinoma (JMAR and MDA1986), melanoma (B16F10 and SKME128), and normal fetal fibroblast (MRC5) cells.

Kheng et al. [25] reported that cytotoxicity of physalin F to induce cell death by apoptosis pathway that involved caspase activation on human breast T-47D carcinoma. The other compound of Physalin genus such as Physalin A, is bioactive withanolides of Physalis showed have potent cytotoxicity with similar mechanism with Physalin F. Hao et al. [26] found that Physalin A induces cell death or apoptosis via p53 activation and ROS generation on human melanoma A375-S2 cells in vitro.
Cells death is the common phenomenon of cytotoxic effects of bioactive chemicals of Physalis. Cytotoxic affects that proved by apoptosis can be via several mechanism including p53 activation, ROS generation and trigger caspase [27]. Cell cycle arrest that caused by physalin treatment also have demonstrated. Physalin A induces cell cycle arrest in G2/M phase on human non-small cell lung cancer cells. The cell cycle arrest involved ROS generation and p38 MAPK activation [27].

Physalin B one of the dominant steroidal active compound of the genus Physalis also induce cytotoxicity on human breast cancer cells. Recent study from Wang et al. [28] suggested that phsylalin B causes cell cycle arrest in G2/M phase and induces apoptosis by modulating p53, activation of caspase 3, caspase 7 and caspase 9 on human breast cancer cells (MCF7, MDAMB231 and T47D cell lines) in vitro.

4. Conclusion
We concluded that ethanol extracts of P. angulata, either in the powder or pasta formulation have cytotoxicity effects indicated by cells viability and inhibition of proliferative activity on SKOV3 and HL60 cell lines.

5. References
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