Nanoparticles of *Selaginella doederleinii* leaf extract inhibit human lung cancer cells A549

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Abstract. The aim of the present study is to evaluate cytotoxicity effect of nanoparticles of *Selaginella doederleinii* (*S. doederleinii*) leaves extract. *S. doederleinii* was extracted by maceration method using 70%(v/v) ethanol as solvent. Phytochemical content was analyzed qualitatively by using Harborne and Thin Layer Chromatography (TLC) methods. Nanoparticle extract was prepared by ionic gelation using chitosan as encapsulant agent. Anticancer activity was performed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The results showed that *S. doederleinii* contains of flavonoids. Nanoparticle of *S. doederleinii* leaves extract greatly inhibited A549 cells growth (cancer cells), with IC_{50} of 3% or 1020 µg/ml. These nanoparticles extract also inhibited the growth of Chang cells (normal cells), with IC_{50} of 4% or 1442 µg/ml. The effective concentration of nanoparticles extract which inhibits cancer cells without harming the normal cells is 0.5% or 167 µg/ml. Further studies are needed to obtain the concentration of nanoparticles extract which can selectively suppress cancer cells.

1. Introduction

Lung cancer contributed 13% of the total number of new cancer cases diagnosed in the world [1]. Torre et al. [2] reported that number of death caused by lung cancer are greater than breast cancer. Currently, herbal medicine is one of the alternative drugs for curing the cancer, in addition to chemotherapy. One of the plants used as anticancer herbal medicine is *S. doederleinii*. Phytochemical screening of *S. doederleinii* discovers abundant of bioflavonoids. These compounds possess strong bioactivities, such as anti-inflammatory, antiviral, antioxidant, anticancer, and antitumor [3]. Anticancer research showed that essential oil from *S. doederleinii* could inhibit A549 and 7721 cell line [4].

Despite *S. doederleinii* has been widely used as anticancer agent, in-depth investigations are required to gain the information on anticancer ability of S. *doederleinii*, especially in its nanoparticle extract. Nanoparticle form of bioactive compounds expected to improve the substances distribution ability in human body and reach the targeted cells [5]. In this study, we evaluated the cytotoxicity effect of nanoparticle extract from *S. doederleinii* leaves on lung cancer cells A549.

2. Materials and methods

2.1. Plant collection and identification

S. doederleinii leaves were collected, identified, and authenticated by Biopharmaca Research Center, Bogor Agricultural University, Indonesia. The collected materials were washed in running tap water to eliminate clay and grimes followed by drying in the oven at 50°C, cut into small pieces and then powdered using mechanical blender and sieved with 40 mesh.

2.2. Moisture content determination
Determination of moisture content was done according to AOAC protocol [6].

2.3. Plant extraction
The powder of *S. doederleinii* were extracted using maceration method as described by Koirewoa [7]. Twenty grams of *S. doederleinii* powder was extracted in 200 ml of 70% ethanol for 24 h. The obtained extracts were subjected to vacuum evaporator (N-1100, Shanghai Eyela Co. Ltd., China) for excess solvent evaporation.

2.4. Phytochemical identification
The flavonoids, phytochemical compounds, were screened qualitatively using method described by Harborne [8]. *S. doederleinii* extract as much as the tip of a spatula is mixed with distilled water 10 ml. Drops of amyl alcohol, 5 drops of 1%(v/v) HCl and 70%(v/v) ethanol was added to this mixture. Afterwards, the solution was affixed with a little powder of magnesium (tip of a spatula). Yellow-orange-red appeared indicating the presence of flavonoids.

2.5. Synthesis of nanoparticles
S. doederleinii's nanoparticles were obtained by ionic gelation method. *S. doederleinii* dry extract was dissolved using a solvent and then stirred using the magnetic stirrer for 1 h. Then, the dissolved extracts were coated using chitosan with a ratio of 3.75:1 (v/v). A total of 12 ml of *S. doederleinii* extract was added to chitosan solution (45 ml). Magnetic stirring was conducted for 1 h for to ensure that crosslinking process was completed [9].

2.6. Particle size analysis
Particle size analysis of *S. doederleinii* nanoparticles was conducted by Particle Size Analyzer (PSA) using Beckman Coulter DelsaTM Nanoparticle Analyzer (USA) at 25°C [9].

2.7. Zeta potential measurements
Zeta potential measurements were conducted by micro-electrophoresis using a Malvern Zetasizer Nanoseries Nano ZS (Malvern Instruments, Herrenberg, Germany) [10].

2.8. Cell culture
Human lung cancer cell line A549 and normal cells (Chang cells) were obtained from ATCC (American Type Culture Collection). Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) medium supplemented with 10% Fetal Bovine Serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin at 37°C in an incubator containing 5%(v/v) CO_2. All materials were purchased from Sigma-Aldrich, Germany.

2.9. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay
MTT assay was performed as described by Wang et al. [11] with slight modification. Briefly, cells were seeded at a concentration of 5000 cells/100 µl DMSO (dimethyl sulfoxide) in a 96-well plate. After 24 h of incubation and cells' confluence has reached 50%, serial concentrations of nanoparticle extracts (0.06, 0.12, 0.25, 0.5, 1, 2, 4, 8, 12, and 16 %(w/v)), were added. Since *Curcuma zedoaria* Rosc. extracts (75 µg/ml) was added to drug treatment group, control group was added with DMSO. The use of *Curcuma zedoria* Rosc. as control is based on previous studies showing inhibition to various cancer cells [12, 13, 14]. Each concentration was repeated three times. These cells were incubated in a

humidified atmosphere with 5% CO_2 for 3 days. Then, 10 µl MTT (Sigma-Aldrich, Germany) solution (5 mg/ml) was added to each well and incubated at 37°C for 4 hours. In cells, dehydrogenase and reductase would reduce this solution into formazan, a purple color of artificial chromogenic products. The medium was removed and formazan was dissolved in 96%(v/v) ethanol 100 µl. The OD (optical density) was measured at 595 nm using a Bio-assay reader (Bio-Rad, USA). The growth inhibition was determined using following formula:

$$\%\ growth\ inhibition\ = \frac{OD\ control - OD\ sample}{OD\ control} \times 100\%$$

2.10. Statistical analysis

Cytotoxicity analysis was expressed as the mean ± SEM of pooled results obtained from at least three independent experiments. Statistical analysis was performed by one-way ANOVA and followed by Duncan test. Significance level was considered as $P < 0.05$.

3. Result and discussion

3.1. Extract and phytochemical content of S. doederleinii

The moisture content of *S. doederleinii* leaves powder was 7.62%(w/w). Moisture content described the content of organic compounds that can be isolated. Isolation method using ethanol as solvent yielded 10.87% (w/w) of extracts. Phytochemical screening showed that those extracts possessed flavonoids. It was proven with a yellow-orange appearance by Harborne' method. Previous phytochemical studies on the Selaginella species of Java led to discover many compounds, such as alkaloid, phenolic (flavonoid, tannin, saponin), and terpenoid (triterpene, steroid) [15].

3.2. Nanoparticles extract

Particle size analyzer resulted that the average nanoparticle of *S. doederleinii* were obtained 62.5 nm. This value was strengthened by previous research reported that nanoparticle encapsulated by chitosan resulted nanoparticle measurement at 40–100 nm [16]. *S. doederleinii* nanoparticle's extract was synthesized by physical modification with ionic gelation method. Crosslinking was made by adding sodium tripolyphosphate to extract. Toxicity effect after physical electrostatic interaction obtained by ionic gelation method less than that chemical process [17]. Nanoparticles from red betle extract were made by ionic gelation with chitosan and maltodextrin as chelating agent [18]. By controlling critical parameter in nanoparticle synthesis, such as chitosan concentration and ratio between chitosan and tripolyphosphate (TPP), there is spontaneous production of nanoparticle when TPP's were added at room temperature [19].

3.3. In vitro cytotoxicity using A549 and Chang cells

Figure 1 depicts the cytotoxicity activity of *S. doederleinii* nanoparticle extract. The results showed that this extract, at concentration of 0.6 to 1%, did not inhibit the growth of normal cells (Chang cells). In contrast, it could inhibit the growth of normal cells at concentration > 1%. Cytotoxicity analysis proved that this extract could inhibit cancer cells (A549 cells) at concentration > 0.25%. At concentration ≤ 0.25%, this extract has not been able to suppress the growth of cancer cells. This finding also indicated that *S. doederleinii* nanoparticle extract was toxic to cancer cells, but not to normal cells in particular concentrations, i.e. 0.5% and 1%.

The effective concentration of *S. doederleinii* nanoparticles extract required for 50% inhibition of the cell viability is expressed by IC_{50} [20]. Statistical analysis performed to determine the IC_{50} value. Anticancer assay towards normal cells showed that IC_{50} value was 4.33% or 1442 µg/ml, while IC_{50} for cancer cells was 3.06% or 1020 µg/ml. Wang et al. [21] reported that ethyl acetate extract from this plant had significantly exhibited anticancer activity with a low IC_{50} value. Other research stated that *S. doederleinii* had cytotoxic effect on murine cells [22].

Anticancer ability of this extract might be caused by its phytochemical compounds, especially flavonoids. Li et al. [23] reported that some of flavonoids, i.e. 2'',3''-dihydro-3',3'''-biapigenin and 3', 3'''-binaringenin from *S. doederleinii*, could inhibit human cancer cell. A combination of chromatographic techniques and cytotoxic assay also revealed that some natural secondary metabolites from the ethanolic extract of Selaginellaceae had ability to against cancer cells [22].

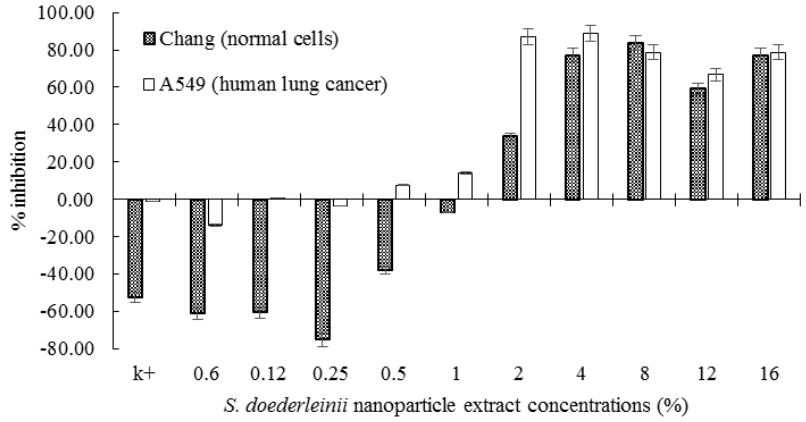

Figure 1. Cytotoxic activity of *S. doederleinii* nanoparticle extract.

4. Conclusion

We conclude that *S. doederleinii* extract contains flavonoids compounds. Its nanoparticle extract greatly inhibited A549 cell growth, with IC_{50} of 3% or 1020 μg/ml. At 0.5% or 167 μg/ml, the nanoparticles extract could inhibit lung cancer cells but not to normal cells. It suggests that this value indicated the effective concentration of nanoparticles extract suppressed cancer cells.

Acknowledgement

This study was supported by Indonesian Directorate General of Higher Education, Ministry of Research, Technology and Higher Education, Indonesia through the Program Kreativitas Mahasiswa 2015. The authors also appreciate Nanotech Herbal Indonesia, Indonesia, for its support in nanoparticle production.

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