



Antioxidant Activity and Anticarcinogenic Properties of "Sisik Naga" (*Drymoglossum piloselloides* Presl.)

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KEYWORDS MTT assay; "sisik naga"; MCF-7; Antioxidant activity; cytotoxic properties

ABSTRACT The research was conducted to determine the anticarcinogenic properties of "sisik naga" (*Drymoglossum piloselloides* Presl.), by the microculture tetrazolium salt (MTT) assay on the human breast carcinoma dependent-hormone (MCF-7) cell lines. The preliminary results showed that the "sisik naga" extract displayed the cytotoxic effects against MCF-7 with IC_{50} -value of $83.63 \mu\text{g/ml}$. The antioxidative activity of the extracts which could contribute to their cytotoxic properties was also studied. The "sisik naga" extract was found to have high antioxidant activity with IC_{50} -value of 4.229 ppm. The strong cytotoxic properties of the "sisik naga" extract could be due to its high antioxidant activity.

Indonesia has a population of more than 200 million people and data collected from hospitals in several regions was shown that cancer incidence increased by 2-8% per year during the last decade (Tjindarbumi and Mangunkusumo, 2002). Breast cancer is one of the most prevalent cancers in Indonesian female. Indonesian people have known a lot of traditional medicinal plants. Among them is "sisik naga".

Sisik naga (*Drymoglossum piloselloides* Presl.) has synonym names, i.e. sakat ribu-ribu (Sumatera), pakis duwitan (Java), *duitvaeren* (Dutch), *bao shu lian* (China) (Hariana, 2006). Sisik naga is a plant epiphyte (family: Polypodiaceae) but not the parasite because it can make its own food. It can be found throughout tropical Asia region and it has long been used traditionally as an anti cancer especially on breast cancer (Wijayakusuma, 2004). This plant can also treat various diseases, such as gingivitis, rheumatism, and tuberculosis (Kusuma and Zaki, 2005). According to Hariana (2006) this plant contained saponins, polyphenols, tannins and flavonoids. However, the scientific study on this plant is still lacking.

The research was conducted to determine the anticarcinogenic properties of "sisik naga" by the microculture tetrazolium salt (MTT) assay on the human breast carcinoma dependent-hormone (MCF-7) cell lines. The antioxidative activity of the extract which could contribute to its cytotoxic properties was also studied.

MATERIALS AND METHODS

Plant Materials and Extractions

The whole plant of "sisik naga" was extracted with 95% methanol at room temperature. Then the extracts were filtered through a Whatman No. 1 filter paper. The collected filtrate was evaporated to dryness under vacuum at 40°C using a rotary evaporator (N-1000V-W, Eyela, Japan). After evaporation, the yield of dried methanol extract was about 10% of the original plant sample. The methanol extract of plant was used for measuring DPPH radical scavenging activity and MTT assay.

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Determination of the scavenging activity

1,1-Diphenyl-2-picrylhydrazyl free radical scavenging assay (DPPH) was carried out according to the following procedure. Each methanol extract at various concentrations (10, 50, 100, and 200 ppm) was added to a 1.5×10^{-4} M solution of DPPH in methanol and the reaction mixture was shaken vigorously. The amount of DPPH remaining was determined at 520 nm, and the radical scavenging activity was obtained from the following equation: Radical scavenging activity (%) = $\{(OD \text{ control} - OD \text{ sample}) / OD \text{ control}\} \times 100$. The antioxidant activity of plant extract was partially expressed as IC_{50} , which was defined as the concentration (in ppm) of extract required to inhibit the formation of DPPH radicals by 50%.

Culturing of Cells

MCF-7 cell lines was obtained from American Type Culture Collection (ATCC, USA). The cells were grown in Dulbecco's Modified Eagle medium (Gibco, USA) supplemented with 10% of fetal calf serum, 100 IU/ml penicillin and 100 μ g/ml of streptomycin (Gibco, USA) using 25-cm² flasks (Nunc, Denmark), in a CO₂ incubator (Sanyo, Japan) at 37°C.

MTT Assay

The viability of cells was determined with trypan blue. Exponentially growing cells were harvested, counted by using hemocytometer, and diluted with medium, yielding a concentration of 1×10^5 cells ml⁻¹. From this cell suspension, 100 μ l were pipetted into 96-well microtiter plates (Nunc, Denmark) and these wells were

incubated for 24 hours in 5% CO₂ incubator (Sanyo, Japan) at 37°C. The diluted range of test extracts being 10, 20, 30, 40, 60, 80 and 100 μ g ml⁻¹. After adding the extract samples, new medium were added to make up the final volume of 200 μ l each well. The plate was incubated in 5% CO₂ incubator (Sanyo, Japan) at 37°C for 96 hours. Then, 20 μ l of MTT reagent (Roche, USA) was added into each well. This plate was incubated again for 4 hours in CO₂ incubator (Sanyo, Japan) at 37°C. After incubation, 200 μ l solubilization solution (Roche, USA) was added into each well. The cell was then left overnight at 37°C, 5% CO₂ incubator. Finally, the absorbance was read with the ELISA reader (LX-800).

RESULTS

DPPH radical scavenging activity

The methanol extracts of "sisik naga" had high DPPH radical scavenging activity, with an IC_{50} -value of 4.229 ppm. The result is categorized as a very active antioxidant and the value almost reached to the antioxidant activity of the standard vitamin C (Ascorbic acid) with IC_{50} values of 2.701 ppm. The sample demonstrated a dose-dependent DPPH radical scavenging activity.

Anticarcinogenic properties

As was mentioned, the methanol extracts of the studied plant was tested for its anticancer activities on MCF-7 cell lines by the MTT assay. The result was shown in Figure 1. As can be seen, the "sisik naga" exhibited the anticancer activity on MCF-7 cell lines with the IC_{50} value of 83.63 μ g/ml.

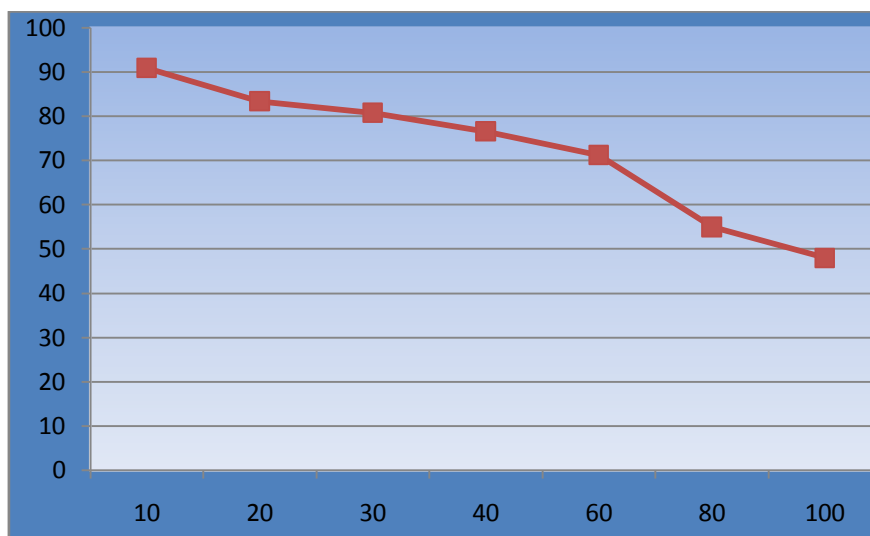


Figure 1. Cytotoxicity effect of "sisik naga" on human breast carcinoma cell lines, MCF-7

DISCUSSION

Free radical scavenging is generally the accepted mechanism for antioxidants to inhibit lipid oxidation. Antioxidants, inhibitors of lipid peroxidation, are important not only for food protection but also for the defense of living cells against oxidative damage (Barbaste *et al.*, 2002). The preferable method for evaluation of the scavenging free radicals activities is 1,1-diphenyl-2-picrylhydrazyl test - DPPH (Brand-Williams *et al.*, 1995). DPPH in comparison with other methods is able in a relatively short time evaluate the scavenging free radicals activities. Therefore, in this study the DPPH test was used.

It was found that the methanol extract of "sisik naga" had the high DPPH radical scavenging activity. The "sisik naga" also exhibited the high anticancer activity on MCF-7 cell lines with the IC₅₀-value of 83,63µg/ml. The anticancer activities result was consistent with the findings of DPPH radical scavenging activity. These results are in accordance with the results of other author (Heti, 2008). Heti (2008) reported that "sisik naga" extract had a significant inhibition of cell growth towards T47D cell

lines. Susilowati (1988) has also reported the antibacterial activities of "sisik naga" on *Escherichia coli* and *Streptococcus aureus*.

The "sisik naga" extract was reported to have a number of common bioactive constituents, including flavonoid, tannin, polyphenol, essential oil, and triterpenoid (Hariana, 2006). The existences of flavonoid and phenolic compound in the "sisik naga" extracts correlates with its high antioxidant activities. The strong cytotoxic properties of the extract could be due to its high antioxidant activities.

CONCLUSION

The "sisik naga" has high anti-oxidant activity and this plant has also potent cytotoxic properties. The strong cytotoxic properties of the "sisik naga" extract could be due to its high antioxidant activity. Further studies are required to find out whether this cytotoxic property would be beneficial for future anti-carcinogenic purpose in clinical practice.

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