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Full Length Research Paper

Antioxidant activity and anticarcinogenic properties of "rumput mutiara" {*Hedyotis corymbosa* (L.) Lam.} and "pohpohan" {*Pilea trinervia* (Roxb.) Wight}

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The research was conducted to determine the anticarcinogenic properties of "rumput mutiara" (*Hedyotis corymbosa* (L.) Lam) and "pohpohan" (*Pilea trinervia* (Roxb.) Wight), by the microculture tetrazolium salt (MTT) assay on the human breast carcinoma dependent-hormone (MCF-7) cell lines. The preliminary results showed that the "rumput mutiara" extract displayed the cytotoxic effects against MCF-7 with IC_{50}-value of 22,67 µg/ml. However, the "pohpohan" extract did not show the IC_{50}-value against MCF-7 cell lines. The antioxidative activities of the extracts which could contribute to their cytotoxic properties were also studied. The "rumput mutiara" extract was found to have higher antioxidant activity compared with "pohpohan" extract. The strong cytotoxic properties of the "rumput mutiara" extract could be due to its high antioxidant activity.

Key words: Microculture tetrazolium salt assay, *Hedyotis corymbosa, Pilea trinervia*, MCF-7, antioxidant activity, cytotoxic properties.

INTRODUCTION

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

Rumput mutiara (*Hedyotis corymbosa* (L.) Lam.) has the synonym name, *Oldenlandia corymbosa*, Linn. According to Kusuma and Zaky (2005), the whole plant of "rumput mutiara" can be used as medicine. It has long been used traditionally as an anti-inflammatory diuretics, antipyretic, and antitoxin and enhances phagocytosis white blood cell and hormonal immunity (Dalimarta, 2005). This grass can also treat various diseases, such as hepatitis, gallbladder inflammation, hypertension, tonsilis, bronchitis, mumps, pneumonia, colitis appendicitis, urinary tract infections, pelvic inflammatory, boils and ulcers (Permadi, 2006). On the other hand, "Pohpohan" is a herbaceous plant with more than 5 m. The leaves are soft aromatic and are commonly used as an upset for stomache. "pohpohan" belongs to family Urticaceae. Indonesian people commonly use "pohpohan" leaves as a salad in their consumption. However, the scientific study on this plant is still lacking (Mishra et al., 2009). The purpose of this research was to determine the anticarcinogenic properties of "rumput mutiara" and "pohpohan" by the microculture tetrazolium salt (MTT) assay on the human breast carcinoma dependent-hormone (MCF-7) cell lines. A further investigation was made to determine antioxidative activities of both extracts which could contribute to their cytotoxic properties were also studied.

MATERIALS AND METHODS

Plant materials and extractions

The whole plant of "rumput mutiara" and "pohpohan" leaves were extracted with 95% methanol at room temperature. The extracts were then filtered through a Whatman No. 1 filter paper. The collected filtrates were evaporated to dryness under vacuum at 40°C using a rotary evaporator (N-1000V-W, Eyela, Japan). The extraction methods were obtained from Ali et al. (1996) with slight modification. After evaporation, the yield of dried methanol extracts were about 10% of the original plant samples. The methanol

Table 1. DPPH radical-scavenging activity in methanol extracts of "rumput mutiara", "pohpohan", and ascorbic acid.

Extracts/compounds	IC_{50} (ppm)
Rumput Mutiara (*Hedyotis corymbosa* (L.) Lam)	270.529
Pohpohan (*Pilea trinervia* Wight)	1876.221
Ascorbic acid	2.701

extracts of plants were used for measuring DPPH radical scavenging activity and MTT assay.

DPPH assay

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 x 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 control –OD sample) / OD control} x 100. The antioxidant activity of plants extracts 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 µg/ml of streptomycin (Gibco, USA) using 25 cm^2 flasks (Nunc, Denmark), in a CO_2 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 x 10^5 cells ml^{-1}. From this cell suspension, 100 µl were pipetted into 96 well microtiter plates (Nunc, Denmark) and these wells were incubated for 24 h in 5% CO_2 incubator (Sanyo, Japan) at 37°C. The diluted range of test extracts being 0.468, 0.937, 1.875, 3.750, 7.5, 15 and 30 µg ml^{-1}. After adding the extract samples, new medium were added to make up the final volume of 200 µl each well. The plate was incubated in 5% CO_2 incubator (Sanyo, Japan) at 37°C for 96 h. Then, 20 µl of MTT reagent (Roche, USA) was added into each well. This plate was incubated again for 4 h in CO_2 incubator (Sanyo, Japan) at 37°C. After incubation, 200 µl solubilization solution (Roche, USA) was added into each well. The cell was then left overnight at 37°C, 5% CO_2 incubator. Finally, the absorbance was read with the ELISA reader (LX-800).

RESULTS

DPPH radical scavenging activity

The results of the determination of DPPH radical scavenging activity of the studied plants are summarized in the Table 1. As can be seen, the methanol extracts of "rumput mutiara" had the highest DPPH radical scavenging activity, with an IC_{50}-value of 270.529 ppm and followed by "pohpohan" (1876.221 ppm). The results of the determination of the radical scavenging activity of the studied plants were lower than of synthetic antioxidant vitamin C (Ascorbic acid) with IC_{50} values of 2.701 ppm. All samples of the studied plants demonstrated a dose-dependent DPPH radical scavenging activity.

Anticarcinogenic properties

As was mentioned, the methanol extracts of the studied plants were tested for their anticancer activities on MCF-7 cell lines by the MTT assay. The results of the determination of the anticarcinogenic properties of the studied plants are shown in Figure 1. As can be seen, the "rumput mutiara" exhibited the highest anticancer activity on MCF-7 cell lines with the IC_{50} value of 22.67 µg/ml. However, the "pohpohan" extract did not show the IC_{50}-value against MCF-7 cell lines.

DISCUSSION

Free radical scavenging is generally the accepted mechanism for antioxidants to inhibit lipid oxidation (Barbaste et al., 2002). 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 out that the methanol extracts of "rumput mutiara" had the highest DPPH radical scavenging activity compared with "pohpohan". The "rumput mutiara" also exhibited the highest anticancer activity on MCF-7 cell lines with the IC_{50} value of 22.67 µg/ml, whereas the "pohpohan" extract did not show the IC_{50}-value against MCF-7 cell lines. The anticancer activities results were consistent with the findings of DPPH radical scavenging activity. These results are in accordance with the results of other authors (Sasikumar et al., 2010). According to Sasikumar et al. (2010), the "rumput mutiara" extract exhibited high antiradical activity against ABTS, nitric oxide and hydroxyl radicals with EC_{50} value of 150, 130, and 170 µg/ml, respectively. Lee et al. (2011) was reported that "rumput mutiara" extract had a significant inhibition of cell growth and induction of cell apoptosis in COLO 205 (colon cancer), Hep3B (hepatocellular carcinoma) and H460 (lung cancer) cell lines. "Rumput mutiara" extract also enhanced the immunocompetence

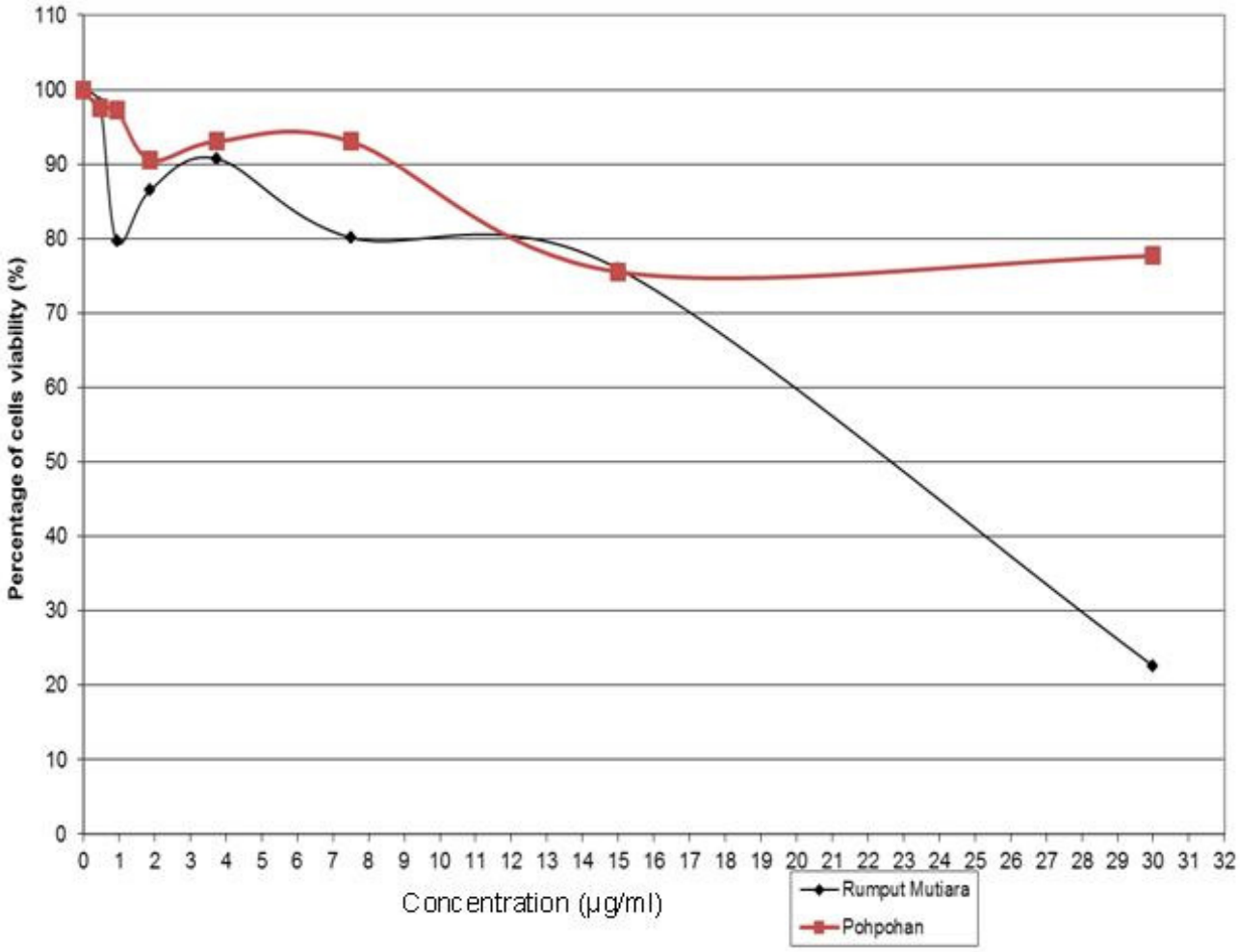

Figure 1. Cytotoxic effect of "rumput mutiara" and "pohpohan" on human breast carcinoma cell lines, MCF-7.

of mice after sub-lethal irradiation (Yang et al., 1997).

The "rumput mutiara" extract were reported to have a number of common bioactive constituents, including geniposidic acid, geniposide, oleanolic acid and ursolic acid (Wijayakusuma, 2004; Soenanto and Kuncoro, 2005). Oleanolic acid and ursolic acid exhibited strong inhibition on tumor growth and accelerated the recovery from radiation injuries in mice (Hsu et al., 1997a). Geniposidic acid and geniposide possessed antitumor and radioprotective activities (Hsu et al., 1997b). Geniposidic acid was also demonstrated to facilitate the conjugation and biliary excretion of alpha-naphthylisocyanate and/or its toxic metabolites. An acylated flavonol glycoside isolated from "rumput mutiara" exhibited antioxidative effects on xanthine oxidase inhibition, xanthine-xanthine oxidase cytochrome c and TBAMDA systems (Lu et al., 2000). Other phytochemicals found in *Hedyotis* species are n-bezoyl-L-phenylalanylphenylalaninol acetate, asperuloside acid, deacetyl asperulosidic acid and scandoside (Lin et al., 2004). In a recent study, a number of pure constituents isolated from "rumput mutiara" were reported to possess neuroprotective activity (Kim et al., 2001). The existences of flavonoid and phenolic compound in the "rumput mutiara" extracts correlates with its high antioxidant activities. The strong cytotoxic properties of the extract could be due to its high antioxidant activities. Even though "pohpohan" was also reported to have a number of flavonoid and phenolic compounds (Amalia et al., 2006), the antioxidant activity of this plant is much lesser than "rumput mutiara". The mechanisms of the cytotoxic effects of these plants are being studied.

Conclusion

The "rumput mutiara" has higher antioxidant activity compared with "pohpohan". This plant has also potent cytotoxicity activity. The strong cytotoxic properties of the "rumput mutiara" extract could be due to its high

antioxidant activities. Further study is needed on their mechanism of action since these plants have pronounced the anticancer effects towards breast cancer cell lines.

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