Cytotoxic effect of four Makassarese medicinal plants on human cervical cell lines and its selectivity

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ABSTRACT

The study was aimed to evaluate the anticancer activity of four Makassarese medicinal plants: Boehmeria virgata (Forst), Acanthus ilicifolius Linn, Eupatorium odoratum and Acalypha indica L., on human cervical cell lines (HeLa) and macrophage cells and its selectivities. The cytotoxic activity was evaluated by MTT method. IC50 on HeLa cell line were Boehmeria virgata (Forst) > Acanthus ilicifolius Linn > Eupatorium odoratum > Acalypha indica L. > Acanthus ilicifolius Linn (9.40, 32.81, 179.02 and 223.64 µg/mL) respectively, while Doxorubicin was 1.053 µg/mL. But only Boehmeria virgata (Forst) and Acanthus ilicifolius Linn selective as anticancer (SI were 3.10 and 3.14) and have potential to be developed as an anticancer agent.

Keywords: Cytotoxic, Makassarese Medicinal Plant, HeLa cell, macrophage, and selectivity

INTRODUCTION

In Indonesia, cancer is a major public health problem, becoming the 7th largest cause of death based on a national survey in 2007 (accounting for 5.7% of all mortality) [1, 2]. The most frequent and primary cancers identified in Indonesia are cervix, breast, lymph node, skin and nasopharynx [3].

The Indonesian archipelago consists of over 17,000 islands and five large islands: Sumatra, Java, Borneo, Sulawesi, and Papua. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries [4]. On the Sulawesi Island, South Sulawesi precisely, the research institute discovered 449 species of medicinal plants are still used today by dozens of herbs are still used to this day by local residents as a traditional medicine. Ethnic communities Bugis Makassar know how to treatment for illness and health care in the form of a historical manuscript evidence Lontara Pabbura was have inherited by their ancestors [5, 6].

Based on ethnopharmacological data especially in Makassar, we were evaluated the in vitro cytotoxic effect of four Makassarese medicinal plant are Parang Romang (Boehmeria virgata (Forst) Guillem), Daruju (Acanthus ilicifolius Linn), Laruna (Eupatorium odoratum) and Kucing-kucingan (Acalypha indica L.) on human cervical cell lines (HeLa) and their selectivities.

EXPERIMENTAL SECTION

Reagents

RPMI 1640 medium, M199 medium, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide (DMSO), doxorubicin were obtained from Sigma-Aldrich Company, UK. Fetal bovine serum
(FBS), penicillin, L-glutamine, streptomycin, thioglycollate broth and amphotericin B were from Gibco, USA. Dimethyl sulfoxide (DMSO), Sodium dodecyl sulfate (SDS) from Sigma-Aldrich, St. Louis-USA

Plant materials and preparation of crude extracts
The plants were collected from Makassar, South Sulawesi, Indonesia in June 2013 and were identified in Herbarium Bogoriensi (BO) (Bogor, West Java, Indonesia). The plant materials were rinsed thoroughly with tap water to remove extraneous contaminants and cut into small pieces, oven-dried at 50°C and then ground into powder with an electric-grinder. Extraction was carried out by macerating the powdered plant materials (100 g) in stoppered flasks containing 500 ml of 96% ethanol at room temperature (25-30°C) for 3 days. The extracts were evaporated under reduced pressure by rotary evaporation.

Culturing HeLa cell
HeLa cells line were grown in RPMI-1640 [each 100 mL of RPMI-1640 was supplemented with 10% (FBS), 1 mL of penicillin/streptomycin (50 IU/mL and 50 µg/mL respectively), fungizone 0.5 mL, NaHCO₃ (0.2 g) and 1 mL of L-glutamine (2 mM)]. The final medium was then sterilized using 0.22 µ microfilters and stored at 4 ºC before use. The cells were cultured in a 5% CO₂ incubator (Thermo Scientific) at 37°C in a humidified atmosphere. The culture was subcultured when cells are 70-80% confluent and routinely checked under an inverted microscope [7, 8].

Culturing Macrophage Cell
Macrophage cells were isolated from the mouse peritoneal. Mouse was injected with 2 ml of 4% thioglycollate broth. After 4 days, peritoneal exudate macrophages were isolated from peritoneal lavage of mice. Macrophage cells were cultured with M199 [each 100 mL of M199 was supplemented with 10% (FBS), 1 mL of penicillin/streptomycin (50 IU/mL and 50 µg/mL respectively), fungizone 0.5 mL, NaHCO₃ (0.2 g) and 1 mL of L-glutamine (2 mM)]. The final medium was then sterilized using 0.22 µ microfilters and stored at 4 ºC before use. The cells were cultured in a 5% CO₂ incubator (Thermo Scientific) at 37°C in a humidified atmosphere. The culture was subculture when cells are 70-80% confluent and routinely checked under an inverted microscope [9-11].

Cytotoxicity assay: MTT Assay
The MTT assay is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity [12, 13]. Briefly, the cells were seeded in 96-well plates (HeLa and macrophage at a density of 1x10⁴ cells/well and 1x10³ cells/well, respectively) following 24h incubation and attachment, the cells were treated with different concentrations of plant extract for 24 h. Following washing and incubation with MTT solution (0.5 mg/mL) for 4 h, cells were lysed with DMSO. The absorbance was measured after 24 h using a Microplate Reader (Thermo) at a wavelength of 595 nm [14]. The percentage of cytotoxicity compared to the untreated cells was determined with the equation:

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\text{Cell mortality (\%)} = \frac{\text{OD of treated cells}}{\text{OD of control cells}} \times 100
\]

Inhibition Concentration (IC50)
The results were generated from three independent experiments; each experiment was performed in triplicate. The IC50 values were calculated using software.

Selectivity Index (SI)
The selectivity index (SI) was also calculated from the IC50 ratio of macrophage and HeLa cells cancer.

RESULTS AND DISCUSSION
The Indonesian archipelago consists of over 17 000 islands and five large islands: Sumatra, Java, Borneo, Sulawesi, and Papua. On the Sulawesi Island, SouthSulawesi precisely, ethnic communities Bugis Makassar know how to treatment for illness and health care in the form of a historical manuscript evidence Lontara Pabbura was have inherited by their ancestors [5, 6].

Based on ethnopharmacological data especially in Makassar, we were evaluated the in vitro cytotoxic effect of four Makassarese medicinal plant are ParangRomang (Boehmeriavirgata (Forst) Guillem), Daruju (Acanthus ilicifolius Linn), Laruna (Eupatorium odoratum) and Kucing-kucingan (Acalyphaindica L.) on human cervical cell lines (HeLa) and its selectivity by MTT method.

Viable cells with active metabolism convert MTT into a purple colored formazan product with an absorbance maximum near 595 nm [14]. When cells die, they lose the ability to convert MTT into formazan, thus color
Cytotoxic activity against HeLa cell lines
Cytotoxic activities of the plant extract against HeLa cell lines were compared with the doxorubicin as standard by measuring the viability of cells (%). Percentage cells viability of cells line was carried out by MTT method. The cytotoxic effects of extract are presented in Figure 1.

Cytotoxic activities of four plants extracts were carried out against HeLa cell line at different concentrations to determine the IC50 (50% growth inhibition) by MTT assay. Results of different concentrations including 7.813, 15.625, 31.25, 62.5, 125.0, 250 and 500 µg/mL. Extracts showed that the percentage of growth inhibition to be increasing with increasing concentration of test compounds. The Boehmeria virgata (Forst) was showed highest cytotoxicity against HeLa cell. The IC50 value of all extracts showed in Table 1.

Cytotoxic activity against macrophage cell
Cytotoxic activities of the four plant extract against macrophage cells was measured by the viability of cells (%). Percentage viability of cells was carried out by MTT method. The cytotoxic effect of extracts are presented in Figure 2.

Cytotoxic activity of four plants extracts were carried out against Macrophage cells at different concentrations to determine the IC50 (50% growth inhibition) by MTT assay. Results of different concentrations including 7.813, 15.625, 31.25, 62.5, 125.0, 250 and 500 µg/mL. The all extracts was showed that the percentage of growth inhibition to be increasing with increasing concentration of test compounds. The Boehmeria virgata (Forst) and Acalypha indica L. was showed highest cytotoxicity against macrophage cells compared with AncantusiliciliusLinn. and Eupatorium odoratum L. The IC50 value of all extracts showed in Table 1.

IC50 and selectivity
The IC50 is a measure of how effective a drug is. It indicates how much of a drug is needed to inhibit a given biological process by half. In other words, it is the half minimal (50%) inhibitory concentration (IC) of a substance (50% IC, or IC50).
In this study, Prevention as anticancer and have potential to be developed as an anticancer agent. The selectivity index of the sample to the cell lines tested. Samples with SI value greater than 3 were considered to have high selectivity [18]. The selectivity index \textit{Boehmeria virgata} (Forst), \textit{Acanthus ilicifolius} Linn, \textit{Eupatorium odoratum} and \textit{Acalypha indica} L. were 3.10, 3.14, 0.29 and 0.05, respectively. So, only \textit{Boehmeria virgata} (Forst) and \textit{Acanthus ilicifolius} Linn have most selective cytotoxic activity.

**CONCLUSION**

In this study, \textit{Boehmeria virgata} (Forst), \textit{Acanthus ilicifolius} Linn, \textit{Eupatorium odoratum} and \textit{Acalypha indica} L. were shown cytotoxic effect on HeLa cell cancer but only \textit{Boehmeria virgata} (Forst) and \textit{Acanthus ilicifolius} Linn selective as anticancer and have potential to be developed as an anticancer agent.

**REFERENCES**