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Research Article

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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 plant: Boehmeriavirgata(Forst), Acanthus ilicifoliusLinn, Eupatorium odoratum and AcalyphaindicaL, 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 wereBoehmeriavirgata (Forst) >Acanthus ilicifolius Linn >Acalyphaindica L. >Eupatorium odoratum and on macrophage cells are Acalyphaindica L. >Boehmeriavirgata (Forst) >Eupatorium odoratum>Acanthus ilicifolius Linn (9.40, 32.81, 179.02 and 223.64 µg/mL) respectively, while Doxorubicin was 1.053 µg/mL. But only Boehmeriavirgata(Forst) and Acanthus ilicifoliusLinn 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

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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 incident 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, SouthSulawesi 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 Makassaresemedicinal 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 theirselectivities.

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 Bogoriense (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, $NaHCO_3$ (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% CO_2 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, $NaHCO_3$ (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% CO_2 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 $1x10^4$ cells/well and $1x10^3$ 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:

Cell mortality (%) = OD of treated cells/OD of control cells×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

formation serves as a useful and convenient marker of only the viable cells. The formazan product of the MTT tetrazolium accumulates as an insoluble precipitate inside cells [15, 16].

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.

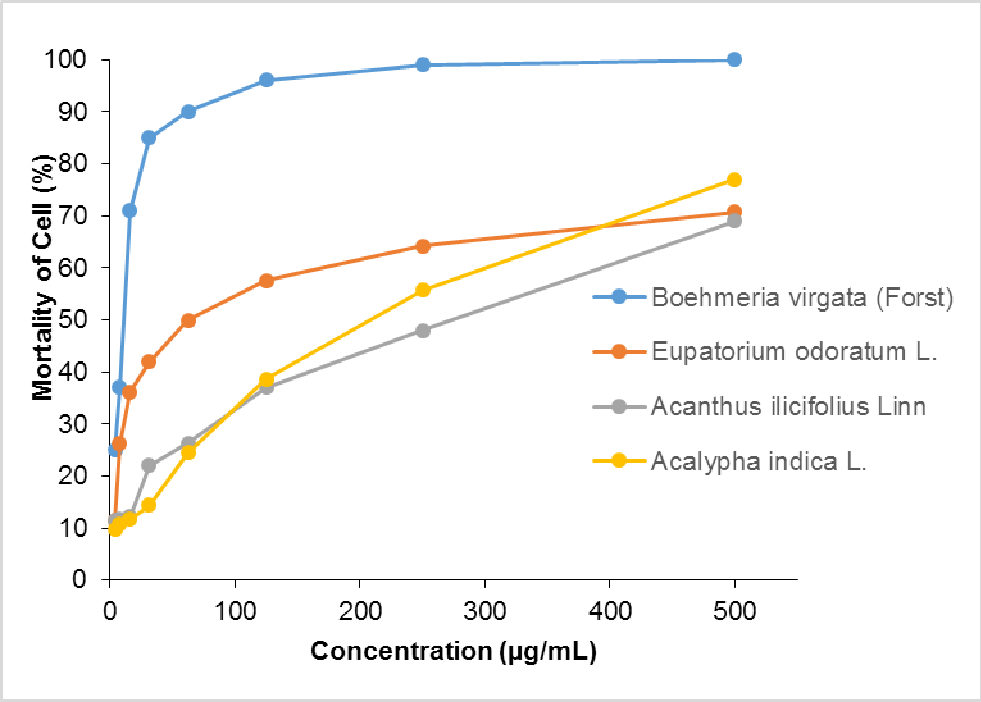

Fig 1. Cytotoxicity effect of Makassarese medicinal plant in HeLa cell cancer

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 virgate* (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.

Cytotoxicity 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 virgate* (Forst) and *Acalyphaindica*L was showed highest cytotoxicity against marcophage cells compared with *Ancantusiliciliius*Linn. 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).

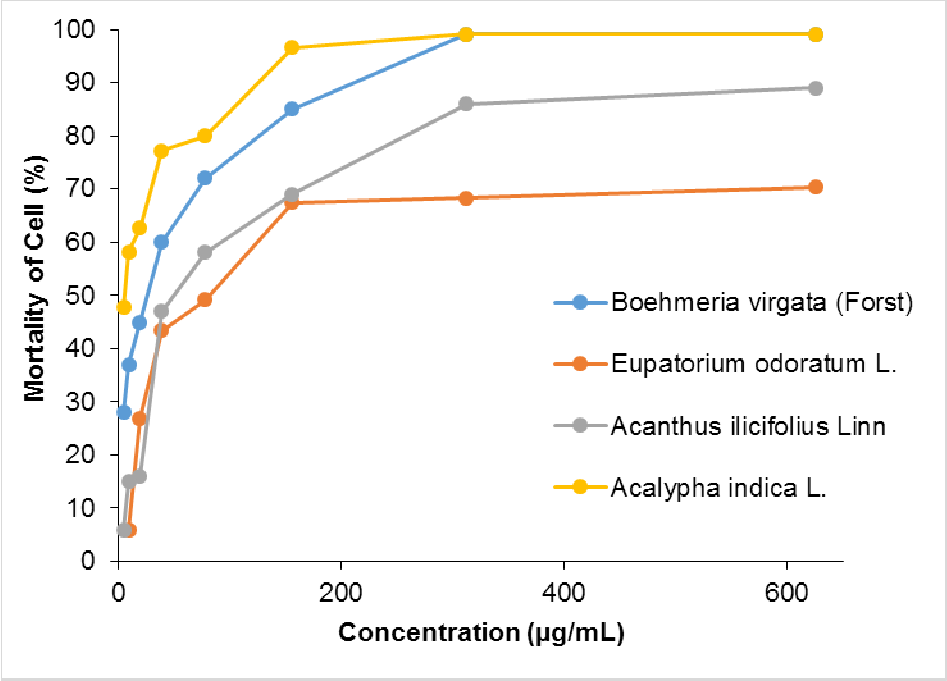

Fig 2. Cytotoxicity effect of Makassarese medicinal plant in mice macrophage

Tabel 1. IC50 and SI of Makassarese medicinal plant on HeLa cell cancer and macrophage

Sample	IC50 (µg/mL)		SI
	HeLa	Macrophage	
Boehmeriavirgata (Forst)	9.40$^{\alpha}$	29.10	3.10$^{\beta}$
*Acanthus ilicifolius*Linn	32.81$^{\alpha}$	103.04	3.14$^{\beta}$
Eupatorium odoratum	223.64	65.46	0.29
*Acalyphaindica*L.	179.02	8.70	0.05
Doxorubicin	1.052		

Note: $^{\alpha}$*significant cytotoxic effect of promising anticancer product for future,* $^{\beta}$*selective as an anticancer*

The IC50 of *Boehmeriavirgata*(Forst), *Acanthus ilicifolius*Linn, *Eupatorium odoratum* and *Acalyphaindica L.* were 9.4, 32.81, 223.64 and 179.02 µg/mL for cytotoxic against HeLa cells line and 29.10, 103.04, 65.46 and 8.70µg/mL for cytotoxic against macrophage cells, respectively. Base on The American National Cancer Institute category, only *Boehmeriavirgata (Forst)* was categorized as expertly (IC50 value <30 µg/mL), *Acanthus ilicifolius*Linn was categorized as moderately (IC50 value >30-100 µg/mL), *Acalyphaindica*L. was categorized as toxic (IC50 value 101-150 µg/mL) and *Eupatorium odoratum* was categorized as slightly cytotoxic (IC50 value 151-250 µg/mL) [17].

Selectivity of the cytotoxic activity of the four tested extracts was determined by comparing the cytotoxic activity (IC50) of each plant extract against HeLa cells line with the macrophage cell (Table 1). SI value indicates selectivity 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 *Boehmeriavirgata*(Forst), *Acanthus ilicifolius*Linn, *Eupatorium odoratum* and *Acalyphaindica*L. are 3.10, 3.14, 0.29 and 0.05, respectively. So, only *Boehmeriavirgata*(Forst) and*Acanthus ilicifolius*Linn have most selective cytotoxic activity.

CONCLUSION

In this study, *Boehmeriavirgata*(Forst), *Acanthus ilicifolius*Linn, *Eupatorium odoratum* and *Acalyphaindica*L. were shown cytotoxic effect on HeLa cell cancer but only *Boehmeriavirgata*(Forst) and *Acanthus ilicifolius*Linn selective as anticancer and have potential to be developed as an anticancer agent.

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