

Anticancer Activity of Selected Medicinal Plants Indigenous to Duri Ethnic

ERMINA PAKKI^{1*}, GEMINI ALAM¹, USMAR¹, RAHMAWATI SYUKUR¹, LUKMAN MUSLIMIN²

¹Faculty of Pharmacy, Hasanuddin University, Perintis Kemerdekaan KM 10, Makassar, Indonesia 90245

²Sekolah Tinggi Ilmu Farmasi Makassar, Perintis Kemerdekaan Street KM 13.7, Makassar, Indonesia 90241

*Corresponding Author

Email ID: er_pakki@yahoo.co.id

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ABSTRACT

Introduction: Natural products are well recognised as sources of drugs in several human ailments. Purpose: In the present work was carried out a preliminary screening of 32 medicinal plants indigenous to Duri Ethnic against HeLa cell lines.

Method: In addition, the extracts were tested for their selectivity towards cancer cells (HeLa) as compared to normal cells (Vero). The toxicity was tested by MTT assay in both cancer and normal cells at various concentrations. The extracts exhibited dose-dependent cytotoxicity in both cells with varying indices of selectivity index (SI).

Result: The results obtained indicate that only 4 plants have a tremendous anticancer activity ($IC_{50} < 50.00 \mu\text{g/mL}$): *Annona squamosa*, *Areca catechu*, *Macrosolen cochinchinensis*, and *Piper caducibracteum*. While selectivity was only found to be most favourable in *A. catechu* (SI= 1.24).

Value: The overall results of the present study provided evidence for the anticancer activity of *A. catechu* and bring supportive data for future investigations that effective and safety in cancer therapy.

Keywords: Anticancer, Medicinal plants, Selectivity index

INTRODUCTION

Approximately, the incidence of cervical cancer about 12% of all cancers in women. Cervical cancer becomes the second rank mortality in developing country before breast cancer, and also contribute 83% of all deaths due to cervical cancer are in developing, low- and middle-income countries (Arbyn et al., 2011; Denny, 2012).

The recurrence rate of cervical cancer in a developing country is still high. Surgery still the best choice to discard the tumor mass. However, chemotherapy and radioactive rays in order to kill cancer cells still as adjuvant therapy after surgery (Wang and Qiao, 2015). Beside kill the cancer cell, chemotherapy also effects on normal cells to cause adverse side effects, such as nail and hair problems, bone marrow malfunction, nausea and vomiting (Baskar et al., 2014; Huang et al., 2017; Sak, 2012; Schirrmacher, 2019). Therefore, a new design of therapy should be expanded to improve the clinical outcome of patients with cervical cancer

like complementary and alternative medicine (CAM).

Many countries rely on traditional medicine. Indonesia is rich in plant biodiversity that can be source of medicinal plants which the population has used traditionally for their problems, such as diabetes, infection, wound, pain, malaria, fractures and cancer (Kandowanko et al., 2018; Nugraha and Keller, 2011; Subeki, 2008; Syamsiah et al., 2016). Especially in Duri ethnic in Enrekang, South Sulawesi, Indonesia traditional healer are organized in public health. They are taught by their parent how to compromise disease and treat patients using local plants.

Therefore, the goals of this work are to measure the cytotoxicity against cancer cells (HeLa) and non-tumor (Vero) lines for the medical plant that used of Duri ethnic to treat the cervix cancer. The ability of Vero cell to survive due to toxicity of the extract was seen by selectivity value as an indicator of extract safety.

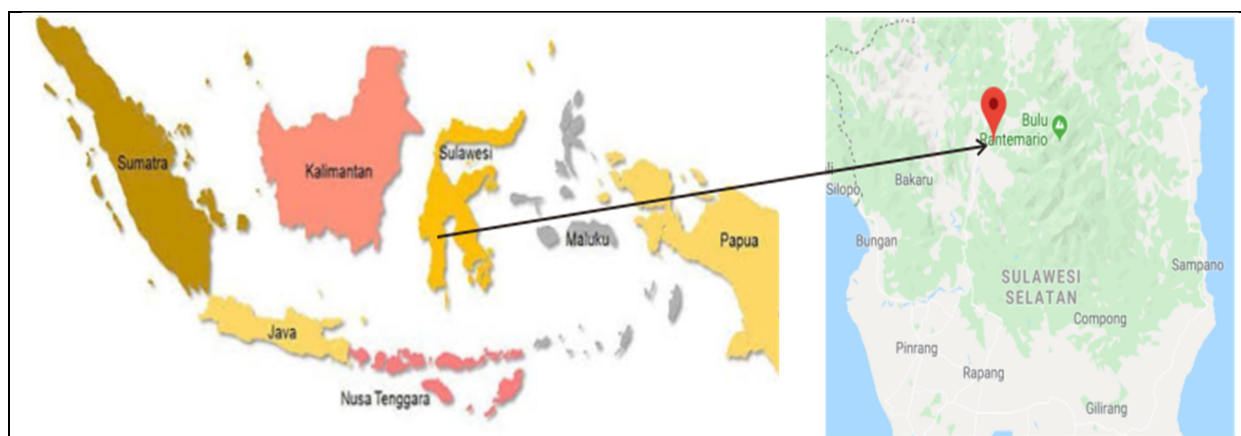


Fig.1: Malua District, Enrekang, South Sulawesi Province, Indonesia

MATERIAL AND METHOD

Material

Materials that were used in this study include Roswell Park Memorial Institute (RPMI 1640, Gibco), M 199 medium (Gibco), dimethyl sulfoxide (DMSO) (Merck), Fetal Bovine Serum 10% (FBS, Gibco), penicillin-streptomycin 1% (Gibco), trypsin (Gibco), sodium dodecyl sulfate (SDS, Gibco), 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma), and fungizone (Gibco).

Plant material

The part of the plant that collected base on traditional formula (Table 1). Specimens were collected at Malua (3°14'36" to 3°50'00" SL and 119°40'53" to 120°06'33" EL and is at an altitude of 442 m above sea level), Enrekang, South Sulawesi, Indonesia on July 2017 and identified in Laboratory of Pharmacognosy Phytochemistry, Hasanuddin University.

Table 1: Medicinal plants used by traditional healers of Duri ethnic: Information is listed in this order scientific name, local name and parts used

Scientific name	Local name	Part used
<i>Aleurites moluccanus</i>	Pelleng	Seed
<i>Allium cepa</i> var <i>ascalonicum</i> (L) Back	Lasuna cella	Bulb
<i>Allium sativum</i>	Lasuna pute	Bulb
<i>Allium tuberosum</i> Rottler ex Spreng	Gonda	Bulb
<i>Alpinia galanga</i>	Lekku	Rhizome
<i>Andrographis paniculata</i>	Daung pai-pai	Leaf
<i>Annona squamosa</i>	Sarikaja	Leaf
<i>Areca catechu</i>	Pinang	Seed
<i>Artocarpus heterophyllus</i>	Panasa	Leaf
<i>Capsicum annum</i> L.	Ladang	Fruit and leaf
<i>Carthamus tinctorius</i>	Ralle	Flower
<i>Coleus benth</i>	Miana	Leaf
<i>Curcuma caesia</i>	Onyyi lotong	Rhizome and leaf
<i>Curcuma domestica</i>	Onyyi	Rhizome
<i>Hibiscus sabdariffa</i> L.	Rosella	Flower

<i>Jatropha curcas</i> L.	Pelle kaliki	Leaf
<i>Lannea coromandelica</i> [Houtt.] Merr	Aju jawa	Cortex
<i>Macrosolen cochinchinensis</i> (Lour.) van Tiegh	Benalu	Root
<i>Mimosa pudica</i>	Tindo-tindo	Whole plant
<i>Morinda citrifolia</i> L	Bampu	Fruit
<i>Moringa oleifera</i>	Kiloro	Fruit and leaf
<i>Musa paradisiaca</i>	Otti	Fruit
<i>Orthosiphon aristatus</i>	Kumis kucing	Leaf with flower
<i>Oryza sativa</i> L	Were	Flour
<i>Piper caducibracteum</i>	Otta	Leaf
<i>Piper nigrum</i> Linn.	Borica	Seed
<i>Pogostemon cablin</i>	Laruna	Leaf
<i>Psidium guajava</i>	Jampu benni	Leaf
<i>Pluchea indica</i>	Kamboja	Cortex
<i>Solanum lycopersicum</i>	Lambace	Leaf
<i>Tamarindus indica</i>	Cemba	Leaf
<i>Zingiber cassumunar</i>	Temmu	Rhizome

Extraction procedure

All the specimens were dried at room temperature and then subjected to successive extraction based on the plant organs (leaves, flowers and bulbs by cold maceration method, while seeds and rhizomes by hot reflux method). All of the obtained extracts were concentrated in a rotary evaporator at 40 °C, and then each extract was stored at 2 - 8 °C in the dark.

Cell lines

The following established in vitro cell lines were applied in HeLa (cervix cell line) compared to Vero normal cells. HeLa cells were cultured in RPMI 1640 medium supplemented 10% FBS, 1% pen-strep, 0.5 % fungizone, while Vero cells were cultured in M 199 medium with the same supplement. All cell lines were grown at 37 °C with 5% CO₂ humidified atmosphere.

Cytotoxicity Assay In Vitro

The antiproliferation activity of all extracts against HeLa and Vero line lines was screened using MTT colorimetric assay. The harvested cells were seeded in 96-plates (Iwaki, Japan) at a density of 1 × 10⁴ cells per well in 100 μL of a culture medium. Each plant extract at a various concentration (from 3.91 to 125.0 μg/mL) was tested in a single experiment, which was repeated 3 times (n= 3). To helping the solubility of extract, DMSO was used as a solvent.

After incubation at 37 °C with 5% CO₂ humidified atmosphere for 24 h, the culture medium was removed and washed with PBS pH 7.4 to remove the extract residue which sticking on plate. The cleaning cells were exposed to 100 μL of MTT (0.5 mg/mL) and incubated for 4 h. Then, 100 μL of SDS 10% was added to stop the reaction. The absorbance of samples was recorded using a multi-plate reader (Biotek elx800) at a wavelength of 599 nm. The half-maximal inhibitory concentration (IC₅₀) was determined as a concentration of an extract that inhibited cell proliferation by 50% compared to control (untreated cells). Only extract that IC₅₀ value below 100 μg/mL continues to evaluate their toxicity in Vero cell.

Selectivity index

The selectivity of each extract in HeLa cell line was analyzed by calculating the selectivity index (SI) as IC₅₀ Vero / IC₅₀ HeLa cell line. It is categorized selective if the SI value were equal to or greater than 1 (Oliveira et al., 2015).

Statistical analysis

All results were presented as mean ± standard deviation (SD) of 3 independent experiments. All experiments are performed in triplicates. IC₅₀ values were extracted from dose-response curves for each extract on HeLa or Vero, as a concentration of a drug that inhibited cell survival by 50%.

RESULT**Cytotoxic activity**

The cytotoxic activity of all plant extracts was evaluated using a colorimetric assay, *in vitro* against HeLa cell line and one non-tumor cell line of Vero. IC₅₀ values were obtained from this assay and are summarized in Table 2. For the HeLa cancer cell lines, there were 9 plants showed toxic (< 100 µg/mL): *M. cochinchinensis* > *P. nigrum* > *A. catechu* > *A. squamosa* > *L. coromandelica* > *P. indica* > *M. citrifolia* > *A. heterophyllus* > *M. paradisiaca* with IC₅₀ value were 30.62 > 31.23 >

40.93 > 41.49 > 59.27 > 67.86 > 69.90 > 76.17 > 78.42 µg/mL, respectively and only 4 of them has a greatest activity (IC₅₀ > 50 µg/mL).

Selectivity of cytotoxic effect

The selectivity measurement was carried out using a ratio between the non-cancer cell line and the cancer cell line (IC₅₀ Vero / IC₅₀ HeLa cell line). The results of the selectivity for all tested extract are presented in Table 2. Although 4 plant extracts have a potential but only *A. catechu* selective to kill cancer cell line with SI value 1.24.

Table 2: Cytotoxic effect of all plant extracts (IC50 µg/mL) for HeLa dan Vero cell lines and SI

Scientific name	IC50		SI
	HeLa	Vero	
<i>Aleurites moluccanus</i>	> 125.00	nt	nc
<i>Allium cepa</i> var <i>ascalonicum</i> (L) Back	> 125.00	nt	nc
<i>Allium sativum</i>	> 125.00	nt	nc
<i>Allium tuberosum</i> Rottler ex Spreng	> 125.00	nt	nc
<i>Alpinia galanga</i>	> 125.00	nt	nc
<i>Andrographis paniculata</i>	> 125.00	nt	nc
<i>Annona squamosa</i>	41.49	22.31	0.54
<i>Areca catechu</i>	40.93	50.86	1.24
<i>Artocarpus heterophyllus</i>	76.17	60.25	0.79
<i>Capsicum annum</i> L.	> 125.00	nt	nc
<i>Carthamus tinctorius</i>	> 125.00	nt	nc
<i>Coleus benth</i>	> 125.00	nt	nc
<i>Curcuma caesia</i>	> 125.00	nt	nc
<i>Curcuma domestica</i>	> 125.00	nt	nc
<i>Hibiscus sabdariffa</i> L.	> 125.00	nt	nc
<i>Jatropha curcas</i> L.	> 125.00	nt	nc
<i>Lannea coromandelica</i> [Houtt.] Merr	59.27	50.33	0.85
<i>Macrosolen cochinchinensis</i> (Lour.) van Tiegh	30.62	19.32	0.63
<i>Mimosa pudica</i>	> 125.00	nt	nc
<i>Morinda citrifolia</i> L	69.90	58.95	0.84
<i>Moringa oleifera</i>	> 125.00	nt	nc
<i>Musa paradisiaca</i>	78.42	62.81	0.80
<i>Orthosiphon aristatus</i>	> 125.00	nt	nc
<i>Oryza sativa</i> L	> 125.00	nt	nc
<i>Piper caducibracteum</i>	31.23	28.64	0.92
<i>Piper nigrum</i> Linn.	> 125.00	nt	nc
<i>Pogostemon cablin</i>	> 125.00	nt	nc
<i>Psidium guajava</i>	> 125.00	nt	nc
<i>Pluchea indica</i>	67.86	42.29	0.62
<i>Solanum lycopersicum</i>	> 125.00	nt	nc
<i>Tamarindus indica</i>	> 125.00	nt	nc
<i>Zingiber cassumunar</i>	> 125.00	nt	nc

Note: no tested (nt) and nc (no calculated)

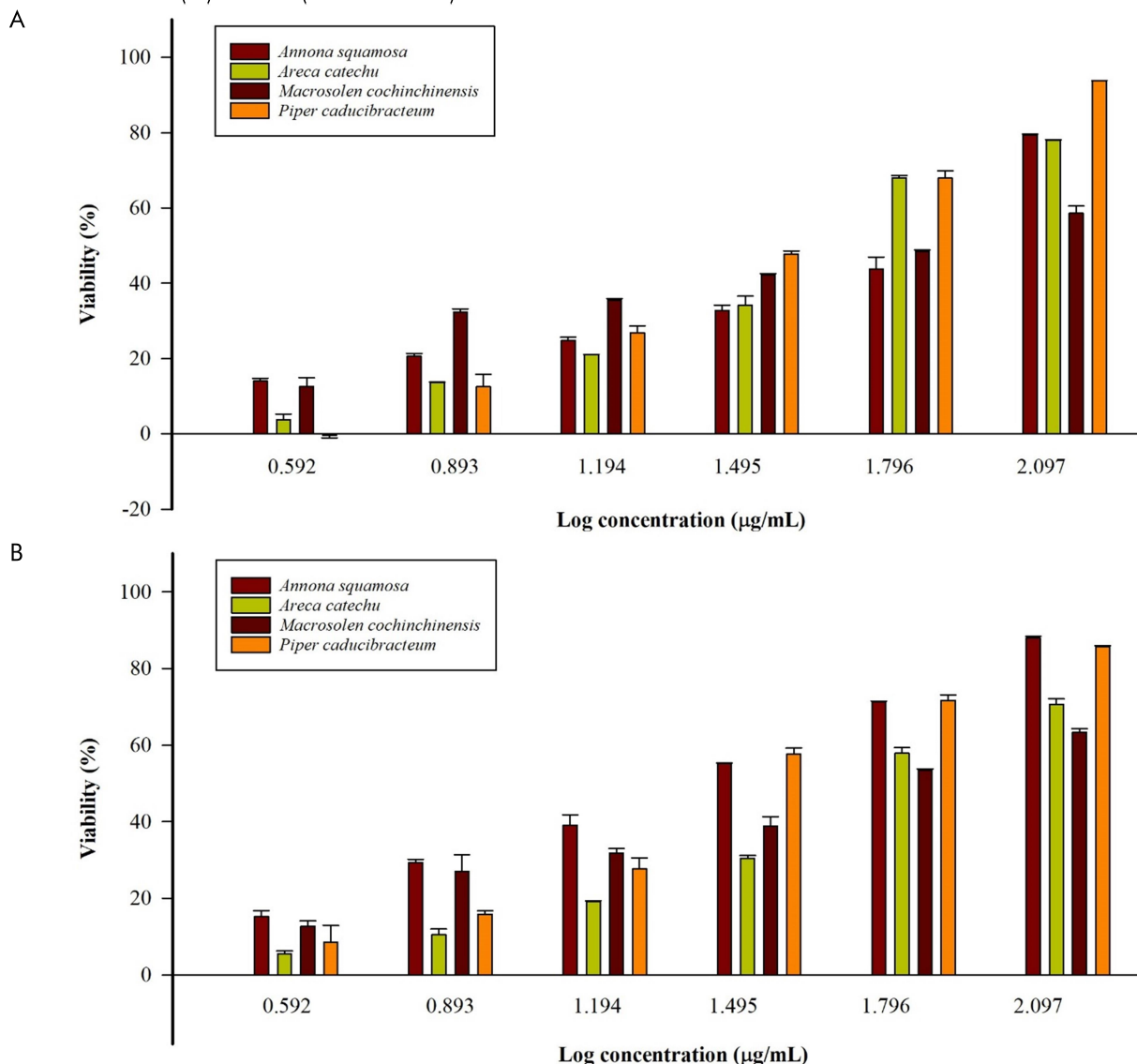


Fig.1: Cytotoxic activity of 4 selected plant extracts (plant that has a greatest activity) against HeLa cell lines (A), Vero normal cells (B) were treated with various concentration for 24 h. Cell viability was determined based on the MTT assay. Data are the means±SD of 3 independent experiments

DISCUSSION

Traditional herbal medicine knowledge not only for keeping of biodiversity but also useful for the medication and furthermore to discovery a new active drug in the future (Kipkore et al., 2014). Several researchers reported that about 80 % of rural populations in the developing countries consider herbal remedies as integral parts for treatment (Lanzotti, 2014; Li et al., 2015). The list

of plants that traditionally were used by Duri ethnic to treat cervix cancer along with their toxicity effect on Hela and Vero cell line and their SI is given Table 2.

Table 2 shows that the medicinal plants still play a role in the care of cancer patients in Duri ethnic in Enrekang. Many of these medicinal plants possess good cytotoxic properties, which may lead to anticancer activities. For 32 plant species, only 4

plant species that consider cytotoxic effect on laboratories, even though many researchers reported more of than 4. This study just reported *A. squamosa*, *A. catechu*, *M. cochinchinensis*, and *P. caducibracteum* possess as anticancer with the concentration linear to cytotoxic activity (Fig 2).

Wang et al. (2014) reported aqueous extracts of leaf of *A. squamosa* toxic to human epidermoid carcinoma cell line KB-3-1 and colon cancer cell line HCT-116 (Wang et al., 2014). Against oral squamous cell carcinoma HSC-2 and HSC-3, *A. catechu* treatment demonstrated apoptosis-inducing mechanism by increased caspase-3 activities (Sari et al., 2019). Belonging to the Loranthaceae family, *M. cochinchinensis* was selectively can inhibit the growth of breast cancer cell line MCF-7 (Lim et al., 2017). Contrary to some other researchers like *C. domestica* (Kirana et al., 2003), *Z. cassumunar* (Singh and Chanu, 2015), *C. tinctorius* (Chang et al., 2011) were reported have anticancer activity. Many of multiple ecological factors, such as temperature, humidity, and solar radiation have effect on the plant chemical constituent. Inderjit et al. (1997) reported that control and unamended control soil the significant different in 5 phenolic compounds: catechol, protocatechuic, p-coumaric, p-hydroxybenzoic, and ferulic acids (Inderjit and Mallik, 1997). Opposite, environmental factors, such as soil pH, temperature, oxygen, and substrate, can affect the degradation of phenolics (Min et al., 2015). The harvesting collection time would have influence in compound variability between the different months (Borges et al., 2013). The limitation of this study, there no literature that explain about environmental factors, such as soil composition, temperature, humidity, rainfall and ultraviolet (UV) radiation incidence on the concentrations or composition of plant in Enrekang. This investigation is very important to preserve the knowledge of anticancer medicinal plant used by the people of the Duri ethnic in Enrekang. Moreover, clinical studies were needed to improve the the efficacy, safety of treatment, especially for the most frequently reported medicinal plants.

CONCLUSION

Due to crossed by the equator become Indonesia is one of the richest in the world in natural resources. In numerous particular varieties of native plants that are very important from the viewpoint of finding new naturally compound occurring drug materials. Based on the Duri traditional healer in Enrekang, 32 plant species belonging to 22 families were used in for treatment cervix cancer. All the plants

were tested in a preliminary biological screening for cytotoxic effect against HeLa cell cancer. The remaining 4 plant extracts of the medicinal plant were a high anticancer activity: *A. squamosa*, *A. catechu*, *M. cochinchinensis*, and *P. caducibracteum*. While selectivity was only found to be most favorable in *A. catechu*. Moreover, exploring its active compound, formulation and dose determination is needed in order to achieve the best benefit from this plant and also devoid of harmful side effects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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