Cytotoxic Activity of Three South Sulawesi Medicinal Plant Extracts Used in the Treatment of HeLa Cell Line: Jati Putih (Gmelina arborea Roxb.), Jati Belanda (Guazuma ulmifolia Lamk.) and Lakka-lakka (Curculigo orchioides Gaerth)

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ABSTRACT

Gmelina arborea Roxb, Guazuma ulmifolia Lamk and Curculigo orchioides Gaerth, the three plants frequently used in South Sulawesi for the treatment of cancerous diseases, have been selected to examine their action in cervical epithelial carcinoma. These extracts were assessed using HeLa cell cancer (Human cervix cancer) and doxorubicin was used as the positive control. Data are presented as the dose that inhibited 50% control growth (IC50). Cytotoxic activity was measured using MTT colorimetric assay. Dose-dependent studies revealed IC50 of 113.61±0.12 µg/mL, 174.90±1.22 µg/mL and 126.05±2.43 µg/mL for eGA, eGU and eCO on HeLa cell cancer, respectively and correlated with treatment of cancer.

KEYWORD: Cytotoxic, HeLa, Gmelina arborea Roxb, Guazuma ulmifolia Lamk and Curculigo orchioides Gaerth

INTRODUCTION

Cancer death rates have been continuously declining for the past 2 decades. Overall, the risk of dying from cancer decreased by 20% between 1991 and 2010 (1). An estimated 12.66 million people were diagnosed with cancer was estimated to account for around 14% of all deaths (due to any cause) worldwide (2).

It is well known that the use of plants as a therapeutic material and safer or with few or no side effects (3, 4). Many of pharmaceutical agents have been discovered source from plant natural product, based on ethnopharmacological data. Especially in Makassar ethnic in South Sulawesi still using plant to treat cancer such as Jati Pute (Gmelina arborea Roxb), Jati Belanda (Guazuma ulmifolia Lamk) and Lakka-lakka (Curculigo orchioides Gaerth).

This study determined the cytotoxic activities in ethanol extracts of three plants used by traditional healers in Makassar South Sulawesi to treat HeLa cell lines using the MTT reduction test.

MATERIALS AND METHODS

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

Table 1. Ethnobotanical data and some reported pharmacological activities of plants species used in this study.

Plants species (Family)	Trivial name	Place of collection	Part plant collected	Traditional use	Pharmacological activities
Gmelina arborea Roxb (Lamiaceae)	Jati putih	Makassar	Leaves	Anticancer	1. Anti-hyperglycemic activity (5, 6) 2. Antibacterial activity (6) 3. Antioxidant activity (6) (7) 4. Anti-inflammation activity (8)
Guazuma ulmifolia Lamk (Sterculiaceae)	Jati Belanda	Makassar	Leaves	1. Anticancer 2. Anti-hyperlipidemia	1. Antiulcer activity (9) 2. Anti-hyperglycemic activity (10) 3. Anti-hyperlipidemia activity (11) 4. Hypotensive (12) 5. Vasorelaxant (12) 6. Antisecretory activity (13)
Curculigo orchioides Gaerth (Amaryllidaceae)	Lakka-lakka	Makassar	Stem bark	1. Anticancer 2. Whitening	1. Wound healing activity (14) 2. Anti-hyperglycemic activity (15) 3. Mast cell stabilization and antihistaminic (16) 4. Antiosteoporotic activity (17) 5. Immunostimulant activity (18)

Plant material

The fresh leaves of Gmelina arborea, Guazuma ulmifolia Lamk and Curculigo orchioides Gaerth were collected from Makassar, South Sulawesi, Indonesia in June 2013. The plants were identified by Herbarium Bogoriense (BO) (Bogor, West Java, Indonesia).

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Preparation of Extracts

The leaves of Gmelina arborea, Guazuma ulmifolia Lamk and Curculigo orchioides Gaerth were washed, dried (38°C) and ground to fine powder. The dried, ground leaves were extracted three times with ethanol 96% at room temperature using maceration method. Extract was filtered and then evaporated, it was dried in a vacuum desiccator. The ethanol extracts were Gmelina arborea named as eGA, ethanol extracts were Guazuma ulmifolia Lamk named as eGU and ethanol extracts were Curculigo orchioides Gaerth named as eCO. All the extracts were kept in the dark at 4°C to evaluation of effect cytotoxicity. All the extracts were dissolved in DMSO to form stock solutions of 10 mg/ml and stored at−20°C before cytotoxicity testing.

Cell line

HeLa cells line were grown in RPMI-1640 [each 100 mL of RPMI-1640 was supplemented with 10% fetal bovine serum (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.

Cytotoxicity assay: MTT Assay

Cytotoxic effect of the extracts against Hela cells was determined by a rapid colorimetric assay, using 3-(4,5-dimethyl thiazol-2-yl)-2,5diphenyl tetrazolium bromide (MTT) and compared with doxorubicin as a positive control was carried out as previously described (19). Briefly, cells at a concentration $1x10^4$ cells/mL were seeded in a 96-well plate (Iwaki) and incubated in a CO_2 incubator at 37°C to allow the cells to adhere. After 24 hours, the cells were treated with extracts at six different concentrations and incubated for 24 hours. The cytotoxic activity of each extract was expressed as IC50, which is the concentration of extract that causes 50% inhibition or cell death. DMSO was used to dilute the extracts and the final concentration of DMSO in each well was not in excess of 0.5% (v/v). No adverse effect due to presence of DMSO was observed.

MTT consists in the absorption of yellow tetrazolium salts by mitochondrial reductase of active cells, called formazan and accumulated in intacellular cell, is extracted by adding an organic solvet (20). SDS 10% was added to each of well to solubilize the MTT formazan. After incubate for 24 h at room temperature, the plate were read with an Elisa Reader (bio-Rad) at 570 nm (21).

Percentage cell viability (CV)

Percentage cell inhibition was calculated as follows:

$$CV = \frac{\text{OD of control} - \text{OD of treatment}}{\text{OD of control}} x\ 100\%$$

RESULTS

Cytotoxic effect of Gmelina arborea Roxb on HeLa cells cancer

Cytotoxic effect of eGA was tested against HeLa using colorimetric method MTT assay. All the cells were exposed to various concentration (Figure 1).

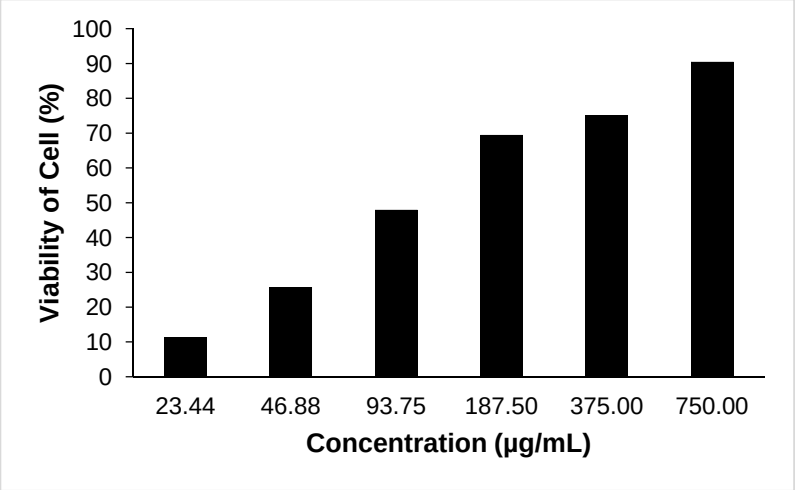

Figure 1. Anti-proliferative activity of eGA (n=3) on HeLa cells line, the cell viability was determinated by MTT assay using ELISA reader after 24 hr cultivation.

Cytotoxic effect of Guazuma ulmifolia Lamk on HeLa cells cancer

Cytotoxic effect of eGU was tested against HeLa using colorimetric method MTT assay. All the cells were exposed to various concentration (Figure 2).

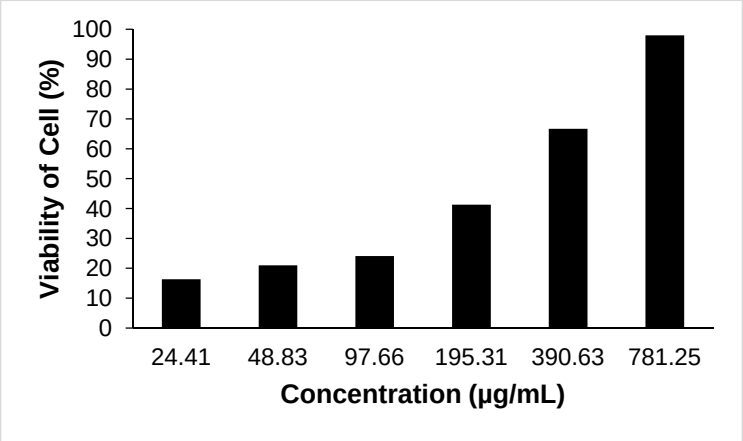

Figure 2. Anti-proliferative activity of eGU (n=3) on HeLa cells line, the cell viability was determinated by MTT assay using ELISA reader after 24 hr cultivation.

Cytotoxic effect of Curculigo orchioides Gaerth on HeLa cells cancer

Cytotoxic effect of eCU was tested against HeLa using colorimetric method MTT assay. All the cells were exposed to various concentration (Figure 3).

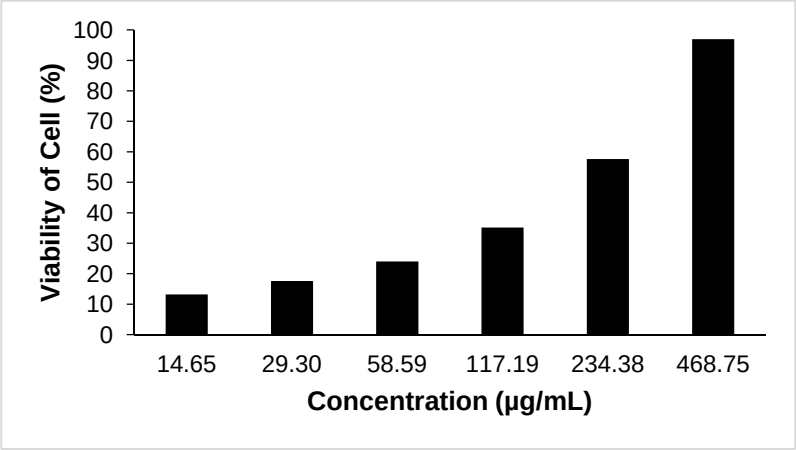

Figure 3. Anti-proliferative activity of eCU (n=3) on HeLa cells line, the cell viability was determinated by MTT assay using ELISA reader after 24 hr cultivation.

Cytotoxic effect of Doxorubicin on HeLa cells cancer

Cytotoxic effect of doxorubicin as positive control was tested against HeLa using colorimetric method MTT assay. All the cells were exposed to various concentration (Figure 4).

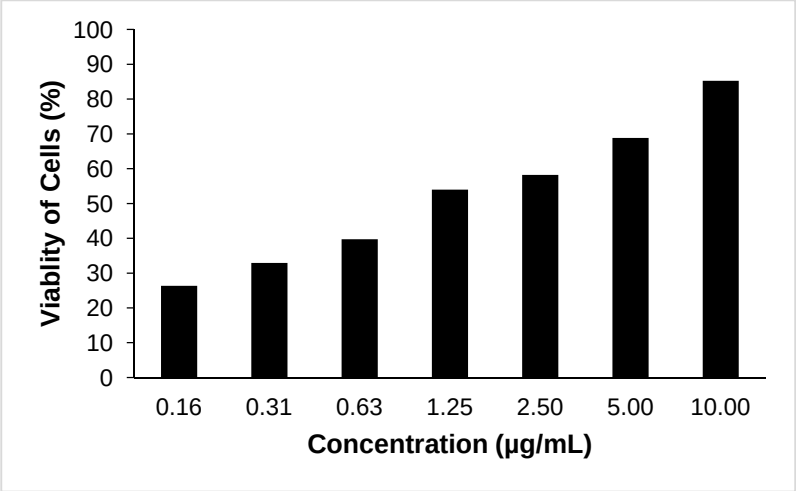

Figure 4. Anti-proliferative activity of doxorubicin (n=3) on HeLa cells line, the cell viability was determinated by MTT assay using ELISA reader after 24 hr cultivation

Inhibition concentration of three extracts (IC50)

Table 2. The comparison between IC50 of three extracts and doxorubicin

Sample	IC50 (µg/mL)
Gmelina arborea Roxb	113.608
Guazuma ulmifolia Lamk	174.899
Curculigo orchioides Gaerth	126.048
Doxorubicin	2.535

DISCUSSION

Using the ethnomedical data approach, some South Sulawesi medicinal plants that are used in the South Sulawesi traditional medicine for various diseases, including cancer, were collected and evaluated for their cytotoxic activities. The search for new anti-cancer drugs is one of the most prominent research areas of natural products.

To investigate the cytotoxic potential of 3 extracts from South Sulawesi plants used in traditional medicine for the treatment of various diseases such as cancer, we collected a selection of 3 plants in order to screen them for possible cytotoxic activity against cervical cancer cell lines (HeLa).

The cytotoxic activity was evaluated on human cervical cancer cell lines (HeLa). HeLa cell, is a cell type in an immortal cell line used in scientific research. It is the oldest and most commonly used human cell line (22).

Among the three medicinal plants tested showed significant growth inhibitory effects in HeLa cells. The IC50 of Gmelina arborea Roxb, Guazuma ulmifolia Lamk and Curculigo orchioides Gaerth are 113.608, 174.899 and 126.048 µg/mL, respectively while doxorubicin 2.535 µg/mL as positive control.

The American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer product for future bio-guided studies if it exerts an IC50 value <30 µg/mL (23). In this preliminary study, we have focused our interest on crude plant extracts, the cytotoxic activity could be due to the presence in the ethanolic extracts of active products that could probably have highly anti-growth effects.

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