

Screening of *in vitro* Cytotoxicities from methanol extract of Acehnese *Murrayakoenigii* Leaf

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Abstract. *Murrayakoenigii* (Linn.) Sprengis known as the miracle plant that are rich in medicinal properties. Aceh was used this plant as the local condiment that familiarize the specific food of Aceh. This research aims to screening the cytotoxic activities from methanol extract of *M. Koenigii* leaf from Aceh. The *invitro* cytotoxic activities were evaluated with using MTT assay method. Three cell lines were used as bioindicators including MCF-7, HeLa, and Raji. The result showed that the extract gives a very good activities against MCF-7 and HeLa cell lines with CD₅₀ value of < 1 µg/ml and 2.25 µg/ml respectively, but the cytotoxicity against Raji cell lines showed as weak with CD₅₀ value of > 1000 µg/ml. It can be concluded that the methanol extract of *M. Koenigii* leaf showed the potential activities against adherent cell type, but not potential to suspension cell type.

1. Introduction

Murrayakoenigii (Linn.) Spreng which has the local name of “Temurui” in Aceh, is known as the miracle plant that are rich in medicinal properties [1]. Majority of Acehnese people was used this plant as the local condiments that familiarize the specific food of Aceh. The present of many bioactivity of *M. Koenigii* is contribute from a very large secondary metabolites contained in this plant. The phytochemicals screening of Acehnese *M. Koenigii* has been reported in previous research that contain the group of alkaloids, terpenoids, tannins, flavonoids, steroids, and saponins [2,3]. Research on *M. Koenigii* as a bioactivity has been widely studied and reported active as cytotoxic, antitumor, antioxidant, antimutagen, anti-inflammatory, antidiabetic, anticarcinogenic, antidysenteric, stimulant, hypoglycaemic, and antimicrobial activities [1,4].

Cancer is the third leading caused of death worldwide, only preceded by cardiovascular disease, infectious, and parasitic disease[5]. Cancer may be caused by incorrect diet, genetic predisposition, and via the environment. Most of all cancers are caused by lifestyle and it may take as long as 20-30 years to develop [6]. Statistical data states that around 12 million people suffering from cancer every year and around 7 million of them are recorded dead. This number is expected to reach 27 million in 2030 and around 17 million sufferers will die. It is estimated that the development of cancer will increase in the following years due to an increasingly uncontrolled lifestyle [6,7].

The increasing cases of deaths caused by cancer encouraged researchers to conduct research to find potential anticancer drugs. Some chemotherapy prevention agents using synthetic drugs have been used to treat cancers, but it is relatively expensive and cause poisoning that limits their use. In the last few decades, the field of herbal medicine research has been gaining significant importance and the demand to use natural products in treatment of diseases is increasing worldwide [8]. The present review indicates that most of secondary metabolites isolated from large number of plant families showed specific emphases on their potential development as anticancer agents [6,9]. WHO calculated that 65-80% of diseases from the human body should treated by medicinal herbal drugs [10]. The use of medicines from natural product was increased and demanded relatively caused of cheaper compared to synthetic drugs and believed to be safer and minor side effects [11,12].

In Indonesia, especially in Aceh, research on the potential of *M. Koenigii* as anticancer agent has never been reported before. Refers to chemotaxonomy review, *M. Koenigii* can be potentially active as anticancer. This research used three types of different cancer cell lines to show the potential activity to the specific cell line including cervical cancer, breast cancer, and lymphocyte cancer cell lines. The results of this study are expected to contribute in the medical to develop *M. koenigii*(L.) Spreng as a natural source for anticancer drug and can be widely used as a safe anticancer drug.

2. Materials and Methods

2.1 Plant Material and Bioindicator

M. koenigii (L.) Spreng leaves were collected from Langsa, Aceh (Indonesia). The bioindicator used in this research is MCF-7 (Breast Cancer), HeLa (Cervical Cancer), and Raji (Burkitt's Lymphoma; B Lymphocyte Cancer) cell lines.

2.2 Extraction

The air-dried leaves (1,2 Kg) of plant materials were ground and extracted with increasing polarity of n-hexane, ethyl acetate, and methanol by maceration method for 3 x 24 hours, the maceration was repeated until the filtrate is clear. The extracts solution was filtered and evaporated by rotary evaporator to give methanol extract with yield of 4.1%.

2.3 Phytochemical Screening

Phytochemical screening of *M.koenigii* was done including alkaloid, terpenoid, steroid, saponin, flavonoid, and tannin refer to previous research [3].

2.4 Cytotoxic Screening (MTT Assay)

Cytotoxic activity in this study was treated against three types of cell lines, HeLa, MCF-7, and Raji cell lines. All cells were recognized from the American Type Cell Collection (ATCC). Medium without compound was used as negative control. The cell was cultured using Roswell Park Memorial Institute Medium (RPMI) 1640, Dulbecco's Modified Eagle's Medium (D-MEM), Fetal Bovine Serum (FBS) 5% Penicillin 100 U/mL, and Streptomycin 100 U/mL, maintained at 37°C in 5% CO₂ atmosphere and counted using hemocytometer. The MTT assay was carried out in the 96-wells plate. Briefly, a volume of 100.0 µL of complete growth medium was added into each well of 96-wells flat bottom microtiter plate (Nunc, USA). Extracts were varied with concentration of 1000, 500, 100, 50, 20, 10, 5, dan 1 µg/ml, aliquoted into wells in triplicate and serially diluted. A volume of 100.0 µL of

1×10^5 cells/mL cells were seeded into 96-wells flat microtiter plates and incubated for 24 hours in CO₂ incubator. After 24 hours incubation, a volume of 100.0 μ L of MTT solution was added into each well and incubated for 4 hours. The culture medium was removed and the SDS 10% in 0.1 N HCl solution was added to each well to solubilise the formazan formed. The plate was read using the plate reader at 595nm wavelength (Infinite M200, Tecan, Switzerland).

3. Results and Discussion

Methanol extract was investigated for cytotoxic activity with using MTT assay according to the method carried out by Tajuddin et al. [13]. Three cell lines were used as the bioindicator, HeLa, MCF-7, and Raji. The cytotoxicity of the extract was assayed at various concentrations and treated to three types of cell lines. Result showed as CD₅₀ which summarized in Table 1. The CD₅₀ value was obtained from the plot of the concentrations of extract versus percent of cell viability. The value was used to describe the degree of cytotoxicity of the extract towards cell lines¹². Figure 1 showed the plotted of % viability with concentration doses to determine CD₅₀.

Table 1. Cytotoxic activity of methanol extract of *M.koenigii* Leaf against Three Cell Lines

Cancer Cell Line	CD ₅₀ (μ g/ml)
HeLa	2.25
MCF-7	< 1
Raji	>1000

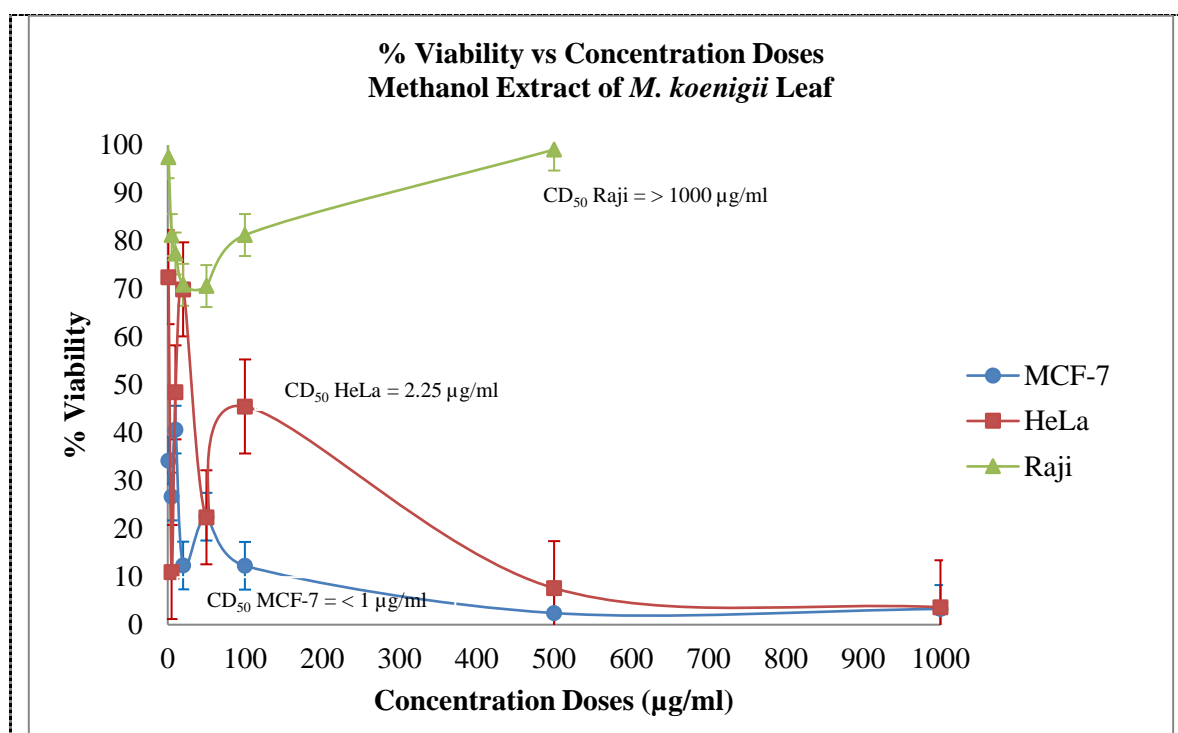


Figure 1. Viability (%) vs Concentration Doses ($\mu\text{g/ml}$) of Methanol Extract from *M.koenigii* Leaf against Three Cell Lines

Table 1 and Figure 1 showed the CD_{50} of methanol extract of *M. koenigii* against three cell lines, MCF-7, HeLa, and Raji. Cytotoxic activities represent as CD_{50} . Based on result of cytotoxicity, the extract indicated as a very good activity against MCF-7 and HeLa cell lines with CD_{50} value of $< 1 \mu\text{g/ml}$ and $2.25 \mu\text{g/ml}$, respectively, but the cytotoxicity against Raji cell lines showed as weak with CD_{50} value of $> 1000 \mu\text{g/ml}$. Refers to Tajuddin et al, 2012, value of CD_{50} demonstrated the potentiality of cytotoxic, compound with CD_{50} value of less than $5.0 \mu\text{g/mL}$ were considered as very active, while compounds with the CD_{50} value between 5.0 and $10.0 \mu\text{g/mL}$ were classified as moderately active. Those compounds that have CD_{50} value of $10\text{--}25 \mu\text{g/mL}$ were considered to be weak in cytotoxicity [13].

The cytotoxicity of the extract was treated to three types of cell lines, which one of them is the suspension cell line, Raji, and other are adherent cell lines, HeLa and MCF-7. The different types of cell lines were used to determine the effect of the extract to specific cells. The result of cytotoxicity screening showed the different potentiality against suspension and adherent cell lines, where the extract is very active against adherent cells but weak to suspension cell. Kill or Inhibit the suspension cell is harder than adherent cell, as in general, suspension cell lines are spread more widely than adherent ones.

This cytotoxic activity of methanol extract from *M.koenigii* leaves is contributed by secondary metabolites contained in the plant that can kill or inhibit cancer cell growth. Previous research has been reported that methanol extract from *M.koenigii* leaves contain the group of secondary metabolites, flavonoids, tannins and saponins [3]. This result showed a potential natural product of *M.koenigii* and could be developed as anticancer agent specific to adherent cell lines including MCF-7 and HeLa.

4. Conclusion

Methanol extract of *M.koenigii* Leaf provided a very good activities against MCF-7 and HeLa cell lines with CD_{50} value of $< 1 \mu\text{g/ml}$ and $2.25 \mu\text{g/ml}$, respectively, but the cytotoxicity against Raji cell lines showed as weak with CD_{50} value of $> 1000 \mu\text{g/ml}$. This result showed that the methanol extract of *M. Koenigii* leaf give the potential activities against adherent cell type, but not potential to suspension cell type.

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