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Phytochemical screening and *In vitro* Cytotoxic activity of Hexane extract of Temurui (*Murraya koenigii* (Linn.) Spreng) leaves against Human Cervical Cancer (HeLa) cell line

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Abstract. Cancer is a chronic disease caused by the growth of abnormal cells in body tissues and includes the second deadly disease in the world where the number of sufferers increases every year. Some chemotherapy prevention agents using synthetic drugs have been used to treat cancer, but it is relatively expensive and cause poisoning that limits their use. The aim of this study was to evaluate the phytochemical and develop natural anticancer drugs from hexane extract of Temurui (*M. koenigii* (Linn.) Spreng) leaves. The phytochemical analysis showed the presence of terpenoids and steroids. Then, the hexane extract of Temurui leaves was screened for *in vitro* cytotoxic activity against human cervical cancer (HeLa) cell line by using the MTT assay. The result showed a very strong cytotoxic activity effect with CD₅₀ values less than 1 µg/ml. This indicated as a potent cytotoxic activity agent for HeLa cancer cells. Therefore, it is expected to conduct further research for cytotoxic test of other cancer cell lines so that it could be developed as raw materials for the manufacture of new drugs.

1. Introduction

Cancer is a chronic disease caused by the growth of abnormal cells in the body's tissues that destroy the normal cells. Cervical cancer is the most common genital cancer and one of the leading causes of death among female population. It accounts for approximately 12% of all cancers in women and it is the second most common cancers in women worldwide most especially in developing country, including Indonesia [1, 2, 3]. WHO reports that cervical cancer is the second most dominant cancer after breast cancer in Indonesian women, with prevalence in woman aged 15 to 44 years [1]. Among the gynaecological cancers, cervix cancer is the most common cancer, followed by cancer of the ovary, the uterus, the vulva, the vagina, and fallopian tube cancer. Indonesia is the 4th ranking country in South-East Asia, with the highest cervical cancer incidence after Cambodia, Myanmar, and Thailand. A comprehensive statistic estimated in 2012, reports that about 20,928 new cervical cancer cases are diagnosed annually in Indonesia. The incidence rate of cervical cancer is 17 per 100,000 women per year [3].

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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. Nowadays, research about finding anticancer agent from plants is widely developing. The present review presents that most of secondary metabolites isolated from large number of plant families showed specific emphases on their potential development as anticancer agents [4, 5]. WHO noted that 65-80% of diseases from the human body can be treated with drugs from nature [6]. 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 [7, 8].

Research on medicinal plants has been based on the development of ethnobotany and the chemotaxonomy of plants and also emphasized by the researchers on the availability of local natural products. This research focused on the development of anticancer agent from Aceh local plant, temurui. Temurui is a local name of *M. koenigii* (L.) Spreng and widely spread in the province of Aceh. The majority of Acehnese people use this plant as spices. Traditionally this plant has also been used as herbs, condiments and also used to treat various types of ailments in Indian traditional system [9]. Research on *M. koenigii* (L.) Spreng as a bioactivity has been widely studied and reportedly active as antitumor, antioxidant, antimutagen, anti-inflammatory, antidiabetic, stimulant and antibacterial [9]. Research of this plant as anticancer has also been widely reported in several countries, including HT-29 intestinal cancer [10], HL-60 blood cancer and HeLa cervix cancer [11], HTB-37 colon cancer, liver HB-8065 [12] and breast cancer MBA-NB-231 [13].

In Indonesia, especially in Aceh, research on the potential of *M. koenigii* (L.) as anticancer agent has never been reported before. Refers to chemotaxonomy review, *M. koenigii* (L.) Spreng can be potentially active as anticancer. Based on the increasing the number of cervical cancer patients in Indonesia, the researchers focus to develop of *M. koenigii* (L.) Spreng leaves as a natural product for cervical cancer drugs. 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. Methodology

2.1 Plant Material and Bioindicator

M. koenigii leaves were collected from Langsa, Aceh (Indonesia) in February 2018. The bioindicator used in this research is human cervical cancer (HeLa) cell line.

2.2 Extraction

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

2.3 Phytochemical Screening

2.3.1 Alkaloid.

About 2 g of plant materials were crushed then added 1 mL of ammonia. Furthermore, 10 mL of chloroform was added, then crushed and filtered. The filtrate was added 10 mL of sulfuric acid 2N, shaken vigorously, left for a minute until the sulfuric acid solution and chloroform separated. The sulfuric acid layer is taken and divided into three test tubes and each test tube is tested by Meyer, Dragendorff, and Wagner reagents to determine the presence of alkaloids. The addition of Meyer reagent established white precipitate, Dragendorff reagent caused reddish precipitate, and Wagner reagent raised yellow precipitate. Those results indicate the presence of alkaloids.

2.3.2 Terpenoid, Steroid, and Saponin.

Ten grams of plant materials were finely ground, then extracted with hot methanol. The obtained filtrate was concentrated with rotary evaporator to yield methanol extract. The methanol extract was partitioned

with hexane. The soluble extract in hexane was tested with the Liebermann-Burchard reagent. The blue or green color exhibits the presence of steroids and red color for terpenoids. The insoluble residue in hexane is added water and shaken vigorously. The presence of the stable foam for 30 minutes indicates the existence of saponins, if positive for saponins.

2.3.3 Flavonoid.

Plant materials (10 g) was extracted with methanol and concentrated. The concentrated methanol extract was partitioned with hexane. The residue was extracted with 10 mL of 80% ethanol, subsequently added 0.5 mg of magnesium and HCl 0.5 M. The pink or purple color shows the presence of flavonoids.

2.3.4 Phenol.

Plant materials was tested by Ferric Chloride. Add 3 – 4 drops of FeCl₃ solution into extract, the formation of bluish black color exhibits the phenol compound.

2.3.5 Tannin.

About 0.5 g of plant materials were boiled in 10 ml of water in the test tube and then filtered. Add a few drops of FeCl₃ 0.1%. Forming of a brownish green or bluish black colour indicates tannins.

2.4 MTT Assay

Cytotoxic activity in this study was treated against breast cancer (MCF-7) cell line. The cell was 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% and Penicillin 100 U/mL, 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). The hexane extract was varied with concentration of 1000, 500, 100, 50, 20, 10, 5, and 1 µg/ml, aliquoted into wells in triplicate and serially diluted. A volume of 100.0 µl of 1x10⁵ cells/mL MCF-7 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 µl 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) [14].

3. Result and Discussion

3.1 Phytochemical Screening

Phytochemical screening aim to identify the compound groups contained in the sample. The phytochemical screening was carried out on leaves and hexane extract of *M. koenigii* (Linn.) Spreng using various phytochemical reagents. Examination on leaves showed the active phytochemical classes as alkaloids, terpenoids, flavonoids, phenols, and tannins, while hexane extract just presence the terpenoids as showed in Table 1.

Phytochemical screening of Hexane Extract of *M. koenigii* (Linn.) Spreng Leaves showed the presence of terpenoids, a nonpolar secondary metabolite. Hexane is a nonpolar solvent that caused the secondary metabolites extracted by hexane should be a nonpolar. Alkaloids, flavonoids, Saponins, Phenols and Tannins are polar secondary metabolite. So, they should not presence in the extract of hexane. Major classes of anticancer compounds include alkaloids, terpenoids, flavonoids and lignans [15]. The presence of terpenoid from *M. koenigii* (Linn.) Spreng leaves also reported from previous studies [16, 17, 18].

Table 1. Phytochemical Screening of *M. koenigii* (Linn.) Spreng

Secondary Metabolites	Leaves of <i>Murayya koenigii</i> (L.) Spreng	Hexane Extract of <i>Murayya koenigii</i> (L.) Spreng Leaves
Alkaloid	+	-
Terpenoid	+	+
Steroid	-	-
Saponin	-	-
Flavonoid	+	-
Phenol	+	-
Tannin	+	-

Terpenoids composed of “isoprenoid” units constitute one of the largest groups of natural products accounting for more than 40 000 individual compounds, with several new compounds being discovered every year. Terpenoids are synthesized from two five-carbon building blocks. Based on the number of building blocks, terpenoids are classified into several classes, such as monoterpenes, diterpenes, triterpenes and tetraterpenes. The diverse array of terpenoid structures and functions has provoked increased interest in their commercial use. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer, and also use as antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti inflammatory, and immunomodulatory properties [19].

3.2 Cytotoxic Activity

In this study, the hexane extract from *M. koenigii* (Linn.) Spreng leaves was evaluated for cytotoxic activity against HeLa cell line. The cytotoxicity of the extract was assayed at various concentrations of 1000, 500, 100, 50, 20, 10, 5, and 1 $\mu\text{g/ml}$ under continuous exposure for 72 h, are expressed in CD_{50} values and are summarized in Table 2. Then, viability data is plotted with concentration doses to determine CD_{50} (Figure 1). Results showed as CD_{50} represent the extract concentration doses that reduced the mean absorbance at 595 nm to 50% of those in the untreated control wells. The CD_{50} 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 lines [14].

Table 2. Cytotoxic Activity of Hexane Extract of *M. koenigii* (Linn.) Spreng Against Servical Cancer (HeLa) Cell Line

Hexane Extract ($\mu\text{g/ml}$)	I	II	III	Average	%Viability
1000	0.016	0.029	0.031	0.025	2.484
500	0.016	0.028	0.033	0.026	2.517
100	0.414	0.337	0.119	0.290	28.441
50	0.034	0.365	0.083	0.161	15.757
20	0.149	0.095	0.050	0.098	9.611
10	0.162	0.202	0.047	0.137	13.436
5	0.054	0.068	0.076	0.066	6.473
1	0.058	0.064	0.081	0.068	6.636
Cell control	1.004	1.012	1.043	1.020	100.000

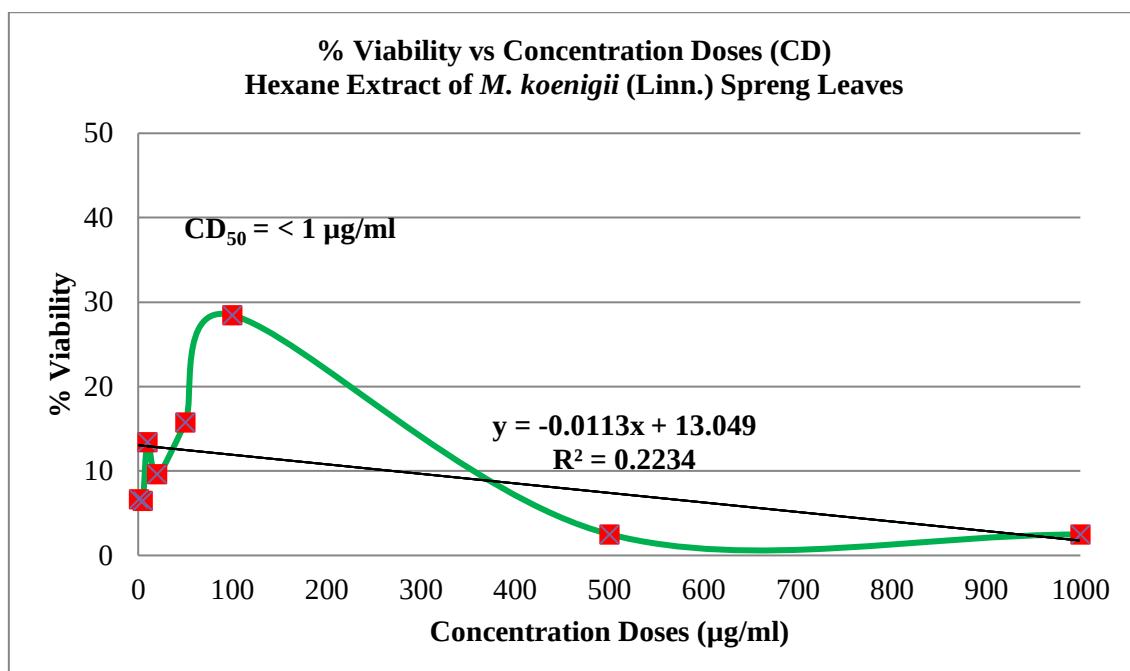


Figure 1. Viability (%) vs Concentration Doses (µg/ml) of Hexane Extract from *M.koenigii* (L) Spreng Leaves

Based on the result of cytotoxicity against HeLa cell line, hexane extract of *M.koenigii* leaves showed cytotoxic activity with CD_{50} value less than $<1 \mu\text{g/ml}$. Previous studies, vincristine, a conventional drug of cancer, was used as positive control against HeLa cell line and showed CD_{50} value of $0.4 \mu\text{g/ml}$ [20]. Compare with the positive control of vincristine, hexane extract of *M.koenigii* leaves gave a very good cytotoxic activity against HeLa cell line. Plant extract with CD_{50} less than $1000 \mu\text{g/ml}$ identified as active against cancer cell line. This cytotoxic activity of hexane extract from *M.koenigii* leaves is contributed by secondary metabolites contained in the plant that can kill or inhibit cancer cell growth. This result showed a potential natural product of *M. koenigii* and could be developed as anticancer agent.

4. Conclusion

The phytochemical screening performed on the hexane extract of *M. koenigii* (Linn.) Spreng showed the presence of terpenoids. This extract showed a very strong cytotoxic activity effect against HeLa cell line with CD_{50} values less than $1 \mu\text{g/mL}$. It indicated as a potent cytotoxic activity agent for HeLa cancer cell. Therefore, it is expected to conduct further research for cytotoxic test of other cancer cell lines so that it could be developed as raw materials for the manufacture of new drugs.

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