Research Article

In Vitro Cytotoxic Activity against MCF-7 Cell Lines from Methanol Extract of Temurui (*Murraya koenigii)* Leaves

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Received: 29-01-2019; **Revised:** 25-02-2019; **Accepted:** 04-03-2019.

ABSTRACT

This research aims to evaluate the screening of phytochemical from methanol extract of *Murraya koenigii* (L.) Spreng leaves and tested for *in vitro* cytotoxic activity against MCF-7 (Breast Cancer) cell line to provide the potent activity from the plant. Phytochemical studies involved extraction by using methanol as the solvents. The screening of phytochemicals include test of secondary metabolites which contained in the sample. The extract was then assayed for cytotoxicity against MCF-7 cell lines by using the MTT assay. The result of phytochemical studies showed that the methanol extract of *Murayya koenigii* (L.) Spreng leaves contain tannins, saponins and flavonoids. The cytotoxic activity effect against MCF-7 cell line showed the CD_{50} value less than 1 µg/ml, that present as very good activity. The conclusion of this research is the *Murayya koenigii* (L.) Spreng leaves potent to develop as anticancer agent for breast cancer.

Keywords: Phytochemical; Cytotoxic; Temurui; *Murraya koenigii*; MCF-7, Cancer.

INTRODUCTION

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. Nowadays, research about finding anticancer agent from plant 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[1,2].

Temurui is a local name from Aceh that refer to *Murayya koenigii* (L.) Spreng, or commonly called as curry leaves in other places. This plant is widely found in the province of Aceh. The majority of Acehnese people use this plant as spices. Traditionally this plant has also been used as a treatment of rheumatic diseases, wound drugs, dysentery, diarrhea and snake bites. Research on *Murayya koenigii* (L.) Spreng as a bioactivity has been widely studied and reportedly active as antitumor, antioxidant, antimutagen, anti-inflammatory, antidiabetic, antidisentri, stimulant and antibacterial[3,4]. Research of this plant as anticancer has also been widely reported in several countries, including HT-29 intestinal cancer[5], HL-60 blood cancer and HeLa cervix[6], HTB-37 colon cancer and liver HB-8065[7].

Based on chemotaxonomy review, *Murayya koenigii* (L.) Spreng can be potentially active as anticancer. Based on the increasing the number of breast cancer patients in Aceh, the researchers focus to develop of *Murayya koenigii* (L.) Spreng leaves as a natural product for breast cancer drugs. The results of this study are expected to contribute in the medical to develop *Murayya koenigii* (L.) Spreng as a natural source for anticancer drug and can be widely used as a safe anticancer drug.

MATERIALS AND METHODS

Plant Material and Bioindicator

M. koenigii (L.) Spreng leaves were collected from Langsa, Aceh (Indonesia) in February 2018. The bioindicator used ini this research is MCF-7 (Breast Cancer) cell line.

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 were filtered and evaporated by rotary evaporator to give methanol extract with yield of 4.1%.

Phytochemical Screening

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.

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 Libermann-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, the solution was hydrolyzed with HCl and tested with the Libermann-Burchard reagent. The green or blue color indicates the presence of steroidal saponins and the purple or red color shows the existence of terpenoid saponins.

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.

Phenolic. The extract was tested by Ferric Chloride. Add 3 – 4 drops of $FeCl_3$ solution into extract, the formation of bluish black color exhibits the phenol compound.

Tannin. About 0.5 g of extract was boiled in 10 ml of water in the test tube and then filtered. Add a few drops of $FeCl_3$ 0.1%. Forming of a brownish green or bluish black colour indicates tannins.

Cytotoxic Evaluation (MTT Assay)

Cytotoxic activity in this study was treated against servical cancer (HeLa) 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% CO_2 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 (Nunclon, 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 $1x10^5$ cells/mL MCF-7 cells were seeded into 96-wells flat microtiter plates and incubated for 24 hours in CO_2 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 red using the plate reader at 595nm wavelength (Infinite M200, Tecan, Switzerland).

RESULTS AND DISCUSSIONS

Phytochemical Screening

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

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

Secondary Metabolites	Leaves of *Murayya koenigii* (L.) Spreng	Methanol Extract of *Murayya koenigii* (L.) Spreng Leaves
Alkaloid	+	-
Terpenoid	+	-
Steroid	-	-
Saponin	-	+
Flavonoid	+	+
Phenol	+	-
Tannin	+	+

Phytochemical screening of Methanol Extract of *M. koenigii* Leaves showed the presence of flavonoids, tannins and saponins. All of them is polar secondary metabolite. Methanol is a polar solvent that caused the secondary metabolites extracted by methanol should be a polar. Major classes of anticancer compounds include alkaloids, terpenoids, flavonoids and lignans[8]. Previous chemotaxonomy review of *M.koenigii* showed the presence of very large phytoconstituent from different chemical groups including alkaloids, terpenoids, phenolics, flavonoids, minerals, protein, carbohydrate, and fat[9,10]. The secondary metabolites could serve many biological activities including anticancer[3,4,11]. Some difference chemical compounds that presence in previous research should be caused by the different places and climates.

In- vitro cytotoxic

In this study, methanol extracts from *M. koenigii* (L) Spreng leaves were evaluated for cytotoxic activity against MCF-7 (Breast Cancer) cell line. The cytotoxicity of the extracts was assayed at various concentrations of 1000, 500, 100, 50, 20, 10, 5, and 1 µg/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[12].

Table 2: Cytotoxic Activity of Hexane Extract of *M. koenigii* (Linn.) Spreng Against MCF-7 (Breast Cancer) Cell Line

Methanol Extract (µg/ml)	I	II	III	Average	%Viability
1000	0.025	0.033	0.003	0.020	3.287
500	0.023	0.007	0.015	0.015	2.425
100	0.062	0.072	0.094	0.076	12.284
50	0.125	0.063	0.230	0.139	22.522
20	0.036	0.050	0.143	0.076	12.338
10	0.057	0.658	0.040	0.252	40.679
5	0.059	0.392	0.045	0.165	26.724
1	0.050	0.547	0.038	0.212	34.213
Cell control	0.523	0.624	0.709	0.619	100.000

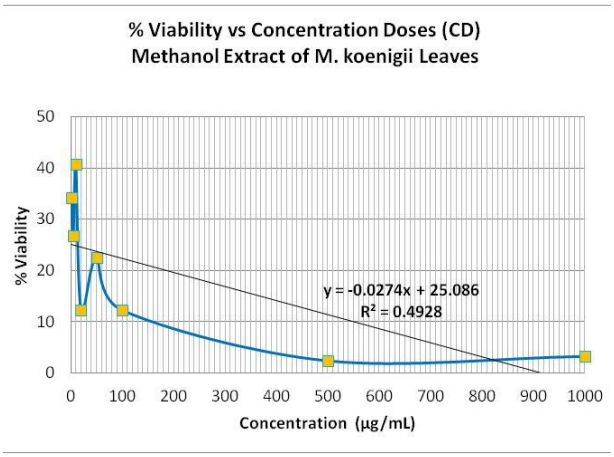

Figure 1: Viability (%) vs Concentration Doses (µg/ml) of Methanol Extract from *M.koenigii* (L) Spreng Leaves

Figure 1 represent the CD50 methanol extract *M.koenigii* leaves against MCF-7 cell line. The result showed cytotoxic activity with CD_{50} value less than <1 µg/ml. Compounds which demonstrated the CD50 value less than 5.0 µg/mL were considered very active, while compounds with the CD_{50} value between 5.0 and 10.0 µg/mL were classified as moderately active. Those compounds that have CD_{50} value of 10–25 µg/mL were considered to be weak in cytotoxicity[13,14].

Based on the result of cytotoxicity, methanol extract could be classified as very active. 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. This result showed a potential natural product of *M. koenigii* and could be developed as anticancer agent for breast cancer.

CONCLUSIONS

The phytochemical screening performed on the methanol extract of *M. koenigii* (Linn.) Spreng showed the presence of flavonnoids, tannins, and saponnins. This extract showed a very strong cytotoxic activity effect against HeLa cell line with CD_{50} values less than 1 µg/mL. It indicated as a potent cytotoxic activity agent for MCF-7 (Breast Cancer) cell line. 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.

Acknowledgements: The authors would like to thanks to The Ministry of Research, Technology and Higher Education of Republic of Indonesia who have fully funded this research through the Grant of Partnership Research of Higher Education. Acknowledgments also addressed to Samudra University and Syiah Kuala University for all technical supports.

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Source of Support: Nil, **Conflict of Interest:** None.