

Evaluation of cytotoxic activity from Temurui (*Murraya koenigii* [Linn.] Spreng) leaf extracts against HeLa cell line using MTT assay

Ulil Amna, Halimatussakdiah,
Puji Wahyuningsih, Nurdin Saidi¹,
Rosnani Nasution¹

Department of Chemistry, Faculty of Engineering, Universitas Samudra, Langsa, ¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia

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ABSTRACT

Temurui (*Murraya koenigii* [Linn.] Spreng) is a local plant of Aceh. The leaves of *M. koenigii* are used in most of the local foods as spices. Nowadays, cancer is claimed as the second deadly disease in the world where the number of sufferers increases every year. Cervical cancer (HeLa) is one of the most dominant cancers that happen in developing country, including Indonesia. Some chemotherapeutic agents using synthetic drugs have been used to treat cancer, but they are relatively expensive and cause poisoning that limits their use. Based on literatures, *M. koenigii* serves the potential secondary metabolites that could be developed as anticancer agent. This study aims to evaluate the cytotoxic activity from three extracts of *M. koenigii* leaves against HeLa cell line, including hexane, ethyl acetate, and methanol. Methodology used included extraction process, phytochemical screening, and cytotoxicity evaluation. The result showed that all the three extracts demonstrated a potent cytotoxic activity for HeLa cancer cells. Hexane and ethyl acetate showed a very strong cytotoxic effect with CD_{50} values $< 1 \mu\text{g/mL}$, whereas methanol extract showed cytotoxic effect with CD_{50} value of $2.25 \mu\text{g/mL}$. These results prove the potential of *M. koenigii* as an anticancer agent.

Key words: Anticancer, cytotoxic, HeLa, *Murraya koenigii*, Temurui

INTRODUCTION

Murraya koenigii (Linn.) Spreng is an evergreen plant, which belongs to *Murraya* genus of the Rutaceae family.^[1,2] Commonly, this plant is known as curry leaf or Temurui as a local name in Aceh, Indonesia. It is mostly distributed in tropical and subtropical regions, including Indonesia, and widely cultivated for its aromatic leaves.^[3,4] The leaves are used as traditional vegetable, especially in Indian cookery for flavoring foodstuffs.^[5] The majority of Acehnese

people use this plant as spices in a local culinary, and Aceh is known as the highest producer of curry leaves in Indonesia. Conventionally, these leaves are also used as herbs and condiments and treat various types of ailments such as rheumatism, traumatic injury, dysentery, diarrhea, and snake bite. Internally, the leaves are also used to cure dysentery, diabetes mellitus, and currently used as a stimulant and antidysentric agent. An infusion of the toasted leaves is used as an antiemetic agent. The steam distillates of the leaves are used as stomachic, carminative, purgative, febrifuge, and antianemic agents. The leaves and roots, owing to their bitter and acrid properties, show cooling, antihelminthic, and analgesic actions.^[2,6-8]

Address for correspondence:

Ms. Ulil Amna,
Department of Chemistry, Faculty of Engineering,
Universitas Samudra, 24416 Langsa, Aceh, Indonesia.
E-mail: ulil_amna@unsam.ac.id

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Based on chemotaxonomy literature, *M. koenigii* plants are known to contain many metabolite compounds such as alkaloids, terpenoids, phenolics, flavonoids, carbohydrates, protein, and amino acids. The secondary metabolites could serve many biological activities. Research on *M. koenigii* as a bioactivity has been widely carried out, and the leaves have been reported to possess cytotoxic, antitumor, antioxidant, antimutagenic, anti-inflammatory, antidiabetic, anticarcinogenic, antidysenteric, stimulant, hypoglycemic, and antimicrobial activities.^[2,9]

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 cancer in women worldwide, especially in developing countries, including Indonesia.^[10-12] The increasing cases of deaths caused by cancer encouraged researchers to conduct research to find potential anticancer drugs. Some chemotherapeutic agents using synthetic drugs have been used to treat cancers, but they are relatively expensive and cause poisoning that limits their use. Nowadays, research on anticancer agents from plants is widely developing. A recent review describes that most of the secondary metabolites isolated from a large number of plant families showed special emphasis on their potential development as anticancer agents.^[13,14] The WHO noted that 65%–80% of diseases in the human body can be treated with drugs from nature.^[15] The use of medicines from natural products has been increased and even highly demanded because of affordable prices compared to synthetic drugs, beside natural drugs have few side effect.^[16,17]

In Indonesia, especially in Aceh, research on the potential of *M. koenigii* as anticancer agent has never been reported previously. Based on chemotaxonomy review, *M. koenigii* has been found to be potentially active as an anticancer agent. Based on the increasing number of cervical cancer patients in Indonesia, this research focuses to develop *M. koenigii* leaves as a natural product for cervical cancer drugs. The results of this study are expected to contribute in the medicinal field to develop *M. koenigii* as a natural source for anticancer agents and therefore can be widely used as a safe anticancer drug.

SUBJECTS AND METHODS

Plant material and bioindicator

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

Extraction

Air-dried leaves (1.2 kg) of the plant materials were ground and extracted with increasing polarity of n-hexane, ethyl

acetate, and methanol by maceration method for 3 × 24 hours; the maceration was repeated until the filtrate is clear. The extract solutions were filtered and evaporated by a rotary evaporator to obtain the extract of hexane (2.4%), ethyl acetate (13.8%), and methanol (4.1%).

Phytochemical screening

Alkaloids

About 2 g of the plant materials was crushed and then 1 mL of ammonia was added. Furthermore, 10 mL of chloroform was added, and then the materials were crushed and filtered. 10 mL of sulfuric acid 2N was added to the filtrate, shaken vigorously, and left for a minute until the sulfuric acid solution and chloroform separated. The sulfuric acid layer was taken and divided into three test tubes and each test tube was 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. These results indicate the presence of alkaloids.

Terpenoids, steroids, and saponins

Ten grams of the plant materials was finely ground, and then extracted with hot methanol. The obtained filtrate was concentrated with the 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 exhibits terpenoids. The insoluble residue in hexane was added with water and shaken vigorously. The presence of stable foam for 30 min indicates the existence of saponins; if positive for saponins, the solution was hydrolyzed with HCl and tested with the Liebermann–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.

Flavonoids

Plant materials (10 g) were extracted with methanol and concentrated. The concentrated methanol extract was partitioned with hexane. The residue was extracted with 10 mL of 80% ethanol, and 1 mg of magnesium and HCl 0.5 M were subsequently added. The pink or purple color shows the presence of flavonoids.

Phenolics

The extract was tested by ferric chloride. Three or four drops of FeCl₃ solution were to the extract, and the formation of bluish black color exhibits the phenol compound.

Tannins

About 1 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of FeCl₃ 0.1% were added. The formation of a brownish green or bluish black color indicates the presence of tannins.

Cytotoxic evaluation (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide assay)

Cytotoxic activity of the leaves was tested against cervical cancer (HeLa) cell line. The cells were recognized from the American Type Cell Collection. Medium without compound was used as negative control. The cells were cultured using Roswell Park Memorial Institute Medium 1640, Dulbecco's Modified Eagle's Medium, fetal bovine serum 5% and penicillin 100 U/mL, and streptomycin 100 U/mL, maintained at 37°C in 5% CO₂ atmosphere and counted using hemocytometer. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was carried out in a 96-well plate. Briefly, a volume of 100 µL of complete growth medium was added into the each well of 96-well flat-bottomed microtiter plate (Nunclon, USA). Extracts varied at concentrations of 1000, 500, 100, 50, 20, 10, 5, and 1 µg/ml, which were aliquoted into wells in triplicate and serially diluted. A volume of 100 µL of 1 × 10⁵ cells/mL MCF-7 cells was seeded into 96-well flat-bottomed microtiter plates and incubated for 24 h in CO₂ incubator. After 24 h incubation, a volume of 100 µL of MTT solution was added into each well and incubated for 4 h. The culture medium was removed, and sodium dodecyl sulfate 10% in 0.1 N HCl solution was added to each well to solubilize the formazan formed. The plate was read using the plate reader at 595 nm wavelength (Infinite M200, Tecan, Switzerland).

RESULTS AND DISCUSSIONS

Phytochemical screening

Phytochemical screening was performed to determine the chemical compound groups contained in the sample. Screening of extracts was performed by phytochemical tests including alkaloid, terpenoid, saponin, steroid, flavonoid, phenol, and tannin tests. The test results are shown in Table 1.

Based on Table 1, it can be concluded that different extracts of *M. koenigii* leaves contain chemical compound group of terpenoids, steroids, alkaloids, flavonoids, saponins, and tannins. The contained chemical compound groups is based on the polarity level of solvents. Hexane extract contains group of terpenoids and steroids, the nonpolar secondary metabolites, because hexane is a nonpolar solvent, so the secondary metabolites extracted by hexane should be a nonpolar compound group. The extract of ethyl acetate showed the presence of semi-polar chemical compounds including alkaloids and flavonoids. Methanol is a polar solvent, so it will extract the polar chemical compounds of tannins, saponins, and flavonoids.

Chemotaxonomy review of *M. koenigii* showed the presence of very large phytoconstituents from different chemical groups including alkaloids, terpenoids, phenolics, flavonoids, minerals, protein, carbohydrates, and fat.^[18,19] The presence of many secondary metabolites could serve good biological activity, including as an anticancer agent.

Previous research showed the potentiality of *M. koenigii* as an anticancer agent such as HT-29 intestinal cancer,^[20] HL-60 blood cancer,^[21] HTB-37 colon cancer, HB-8065TM,^[22] and breast cancer MBA-NB-231.^[23]

Cytotoxic activity

In this study, all the three extracts of *M. koenigii* (L) Spreng leaves were evaluated for cytotoxic activity against cervical cancer (HeLa) cell line. Vincristine, a conventional drug of cancer, was used as a positive control against HeLa cell line and showed CD₅₀ value of 0.4 µg/ml.^[24] 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, which are expressed in CD₅₀ values and are summarized in Table 2.

Figure 1 shows the percentage viability of cancer cell line after treatment with different concentration doses of each extract of hexane, ethyl acetate, and methanol. Results showed as CD₅₀ represent the extract concentration doses that kill 50% of cell lines. The CD₅₀ value was obtained from

Table 1: Phytochemical screening of extracts of *Murraya koenigii* leaves

Secondary metabolites	HE	EAE	ME
Alkaloids	-	+	-
Terpenoids	+	-	-
Steroids	+	-	-
Saponins	-	-	+
Flavonoids	-	+	+
Phenols	-	-	-
Tannins	-	-	+

ME: Methanol extract, EAE: Ethyl acetate extract, HE: Hexane extract

Table 2: Cytotoxic activity of extract of *Murraya koenigii* leaves against HeLa cell line

Sample	CD ₅₀ (µg/ml)
HE	<1
EAE	<1
ME	2.25
Vincristine	0.4

ME: Methanol extract, EAE: Ethyl acetate extract, HE: Hexane extract

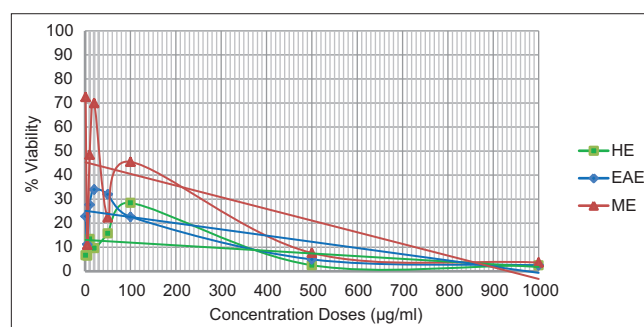


Figure 1: Viability (%) versus concentration doses (µg/ml) of extract from *Murraya koenigii* leaves

the plot of the concentrations of extract versus percentage of cell viability. The value was used to describe the degree of cytotoxicity of the extract toward cell lines. Compounds which demonstrated the CD_{50} value $<5.0 \mu\text{g/mL}$ were considered very active, whereas 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.^[25]

Based on the results of cytotoxicity, all extracts showed a very good activity. Hexane and ethyl acetate extracts showed cytotoxic activity with CD_{50} value $<1 \mu\text{g/mL}$, whereas methanol extract showed the CD_{50} value of $2.25 \mu\text{g/mL}$. All the three extracts could be classified as very active. This cytotoxic activity of 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 an anticancer agent.

CONCLUSIONS

The phytochemical screening performed on the leaf extract of *M. koenigii* (Linn.) Spreng showed the presence of terpenoids. All three extracts showed a very active with value of $<1 \mu\text{g/mL}$ to $2.25 \mu\text{g/mL}$. *M. koenigii* (Linn.) Spreng leaves proved to be a potent cytotoxic activity agent against 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 manufacturing of new drugs.

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Conflicts of interest

There are no conflicts of interest.

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