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Anticancer Activity of Turmeric Rhizome Extract (Curcuma longa Linn) In-vitro Against MCF7 Breast Cancer Line Cells

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Turmeric or Curcuma longa Linn is one of the native spices and medicinal plants from Southeast Asia. Turmeric contains a compound named curcumin. Curcumin (diferuloylmethane) is a yellow pigment in turmeric that is widely used as a spice, food coloring (curry) and preservative. Curcumin shows various pharmacological effects that have been reported scientifically in research, one of which is as an anticancer. This study aims to perform a cytotoxic test of turmeric rhizome extract (Curcuma longa Linn) in-vitro with the Microtetrazolim (MTT) assay method to grasp and determine the effect of preventing the growth of MCF_7 breast cancer cells, so that the IC_{50} value can be known. In this study, it was found that turmeric rhizome extract can be used as a natural ingredient that has the potential to inhibit the growth of MCF_7 breast cancer cells. Turmeric rhizome extract has the effectiveness to inhibit the growth of MCF_7 breast cancer cells with an IC_{50} value of 26.30 ppm. This value is categorized as moderate or moderately active cytotoxic.

INTRODUCTION

Breast cancer (BC) is the cancer that causes the highest mortality rate in women among other cancers. In Indonesia, this disease is ranked second most suffered by women. About more than 30% of people with cancer are breast cancer. Nationally, the prevalence of cancer suffered by residents of all ages in 2013 was 1.4% or estimated at around 347,792 people (Irawan, 2018). Meanwhile, based on epidemiological data survey by global con in 2018, breast cancer in Indonesia has reached 16.7% of the total cancer patients of 348,809 thousand and is the most commonly found type of cancer in Indonesia. Early screening is important in dealing with breast cancer and early management provides maximum results and provides more diverse therapeutic options (Smith & Isaacs, 2011). There are various treatments of breast cancer including chemotherapy, but this chemotherapy treatment has a lot of cost, so it is not uncommon for patients to prefer to use other alternative treatments. One of them is using natural ingredients as supporting therapy regiment to increase the immunity of breast cancer patients (Cahya & Prabowo, 2019). Turmeric or with the Latin name Curcumin Longa Linn is one of the plants that has been widely known by the people of Indonesia. This plant that lives in the lowlands has a lot of potential. Studies on the phytochemicals turmeric have revealed, it contains curcuminoids and essential oils as its main components. Curcumin and two derivatives of demethoxy, demethoxycurcumin and bisdemethoxycurcumin, is the main curcuminoids in turmeric, which has anti-cancer, anti-inflammatory, neuroprotective, anti-alzheimer's and antioxidant activity (Abdurrahman, 2019) Curcumin can affect human breast cancer cells through cell cycle induction in phase G2M and phase S end in MCF7 cells. Curcumin causes a pronounced increase in the fraction of phase G2M. Curcumin induces phase termination of cancer cells by regulating spindle-related signaling pathways. Profound effects on the mitosis spindle are exerted by curcumin directly, and monopolar spindles (Zahra et al., 2020) To determine the high anticancer potential in turmeric rhizomes can be done using the colorimetric Micro Tetrazolium (MTT) assay method, namely by reading the absorbance value of the resulting formalizan, then the results obtained are used to measure the magnitude of the IC50. This research

was conducted in vitro usingMTT assay method and the target cell is MCF7 (hormone- dependent breast carcinoma cells) (Haryanti & Widiyastuti, 2017). This study aims to determine the magnitude of the IC50. ethanol extract, turmeric rhizome extract through cytotoxic assay using MTT assay method against MCF7 breast cancer cell growth, and to measure the degree of correlation of extract concentration variations as preliminary data to provide information on the potential of turmeric rhizome plants as an alternative anti-breast cancer drug that is cheap, easy to obtain, and economical.

MATERIALS AND METHODS

Simplisia turmeric rhizomes with solvents used in this test wereEthanol 96%, HCL 2N, Lierbermen-Burhardat, Mayer, Dragendrof, MCF7(hormone- dependent breast carcinoma cells) (ATCC HTB 22), RPMI 1640,Fetal Bovine Serum (FBS) 5%, Penicillin, Streptomycin, MTT.

The method in this study was carried out with the MTT Assay method todetermine the anticancer activity of turmeric rhizome extract.

Material preparation

The material used is turmeric rhizome (*Curcuma longa Linn*) taken from the Balittro Cibinong Bogor experimental garden.

Plant Determination

Examination of materials with determination of turmeric plants at the Bogoriense Herbarium Laboratory in the Field of Botany, BRIN-Cibinong Biology Research Center, Bogor. Examination of turmeric rhizome simplisia (*Curcuma longa Linn*) is carried out before the study with the aim of ascertaining the correctness of use.

Manufacture of Simplisia turmeric (Curcuma longa Linn)

Turmeric rhizomes are harvested, sorted and washed with clean water after washing thoroughly then turmeric rhizomes are thinly sliced so that they dry quicker when drying or when drying in the sun. Drying in the sun is carried out for 3 days.

1. **Quality Inspection of Simplisia Turmeric**

1. Organoleptic Examination of Simplician Powder Organoleptic examination of simplician of turmeric rhizomes by observing the shape, color, taste, and smell.
2. Microscopic Examination of Simplician Powder Microscopic examination of turmeric rhizome simplicia powder (Curcuma Longa Linn) was carried out at the Pharmaceutical Microbiology Laboratory of Binawan University Jakarta. Microscopic examination of turmeric rhizome simplicia powder by observing starch grains, covering hairs, essential oil glands and parenchymal tissue by weighing a total of 50 mg of turmeric rhizome simplisia powder obtained from turmeric rhizome simplisia that has been finely blended then placed on the object glass then dripped with 1 drop of aquades then covered with object glass and observed turmeric rhizome fragments using an electron microscope (Cahya & Prabowo, 2019)

5. Phytochemical screening

Phytochemical screening of turmeric rhizome simplicia powder were carried out at the

Phytochemical Laboratory of the Faculty of Pharmacy, Binawan University, Jakarta. The examination of the chemical content of simplicial powder is carried out to determine the type of chemical compounds contained in the turmeric rhizome simplisia powder (Cahya & Prabowo, 2019)

6. Extraction of Turmeric Rhizome Simplicia (*Curcuma longa Linn*)

The extraction was carried out at the Bogor Spice and Medicinal Plants Research Center. Extraction process: simplisia turmeric rhizome powdered using a grinder tool with a fine sieve size of mess 60. After simplisia turmeric rhizomes are mashed then the powder is mixed with 96% ethanol solvent with a ratio of 1000 g of turmeric rhizome simplisia powder: 6 L of 96% ethanol solvent, then stirred and shaken for 2-3 hours after stirring then precipitated and macerated for 2 days. After maceration, the filtrate is filtered using filter paper, then separated between the filtrates with the dregs. After filtering, the filtrate is evaporated with a Rotavapor tool with a temperature of 40 – 50 °C for 6 hours until a thick extract is obtained from the rhizome of turmeric.

7. Cytotoxicity Test

The cytotoxicity test was carried out at the laboratory of the Bogor primate study center, using tissue culture plates of 96 wells and RPMI as test media. A total of 100 µL of cell suspension in the serum RPMI medium was inserted into each well on the tissue culture plate, then incubated in a 5% CO_2 incubator at 37^oC for 48 h to obtain good growth. After 48 hours the cells will be attached to the base of the microplate, then the medium is discarded, into each well added 200 µL of test solution (turmeric rhizome extract) in the medium RPMI 1640. Then it is incubated at a temperature of 37^oC in a 5% CO_2 incubator for 24 hours. Cells are observed with a microscope at the time of incubation of 4, 8, and 24 hours. The work in the BSC of each medium in the well is disposed of. Then 100 µL PBS is added and then shaken and discarded. A total of 100 µL of RPMI serum and 10 µL of MTT were added to each well, then incubated in a 5% CO_2 incubator at a temperature of 37^oC for 4 hours, removed from the incubator and observed purple formazan crystals formed with a microscope. Formazan crystals are dissolved in 100µ1 ethanol. The absorbance value readings were performed at a wavelength of 595 nm. IC_{50} analysis was performed using linear regression (Kurniawan et al., 2016)

RESULTS AND DISCUSSION

1. **Results of Organoleptic Examination of Turmeric Rhizomes**

Results of organoleptic examination of simplisia turmeric rhizomes;Having a round round shape, sometimes there are branches with lightweight and brittle, have a size with a diameter of 2-3 cm with athickness of 1-3 mm, are reddish orange, smell typical of aromaticturmeric, have a bitter aftertaste on the tongue and the surface on thesimplicia fracture is flat and the presence of powdery powder.

Table 1. Results of Organoleptic Examination of TurmericRhizomes

Simplician observations	Observations Result
Shape	Round round sometimes there are those that have branches, light and fragile
Size	Thickness 1-3 mm, Diameter 2-3 cm
Color	Reddish orange
Construction	Aromatic
Taste	Bitter
Surface	The fault surface is flat and has flour

Table 1.

2. Microscopic Examination Results of Turmeric RhizomeSimplisia Powder

There are fragments of turmeric rhizome specifications in the formof starch grains, covering hairs, fragments of parenchyma with secretioncells, fragments of wood vessels. Microscopic examination of turmerictissue anatomy has the characteristic that there are cell clots,parenchyma and hair cover

Figure 1. Microscopic Image of 100x Magnification `

Information:

1. Starch grains
2. Hair cover

1. Fragmen parenkim dengan sel sekresi
2. Fragmen pembuluh kayu

Organoleptic and microscopic macroscopic observations of turmericrhizome simplisia are in accordance with the organoleptic andmicroscopic characteristics of *C.longa* simplisia asstated in the Indonesian Herbal Pharmacopoeia (Ministry of Health,2017).

1. **Results of Identification of Secondary Metabolite**

Compounds of Simplisia Turmeric Rhizome Powder

Identification of secondary metabolites was carried out to determinethe compounds contained in the ethanol extract of turmeric rhizomes. Thetest was carried out qualitatively against several secondarymetabolites. Results can be seen in table 2.

Table 2. Identification of Secondary Metabolite Compounds ofSimplisia Turmeric Rhizome Powder

Secondary Metabolites	Result
Flavonoid	Positive
Alkaloid	Positive
Saponin	Positive
Tannin	Positive
Triterpenoid	Positive

Table 2.

The genus Curcuma family Zingiberaceae has also long been utilized intraditional medicine and can be developed as cancer. Turmeric has beenstudied to contain chemical compounds referred to as curcuminoids(curcumin 75%, demethoxycurcumin 15-20% and bisdemetoksikurkuminapproximately 3% (Rosita Melannisa, Muhammad Da'i, 2011). Curcumin isone of the secondary metabolite compounds of the phenolic group which isknown to have important biological activities, such as antibacterial,anticancer, antioxidant, antidiabetic and anti-inflammatory (Puteri,2020).

1. **Extraction of Turmeric Rhizome Simplisia**

The results of turmeric rhizome simplisia extraction for themanufacture of turmeric rhizome extract by maceration using solvent 96%ethanol obtained an extract yield of 79.9 g (7.99%). The yield resultsof a sample are very necessary because it is necessary to know theamount of extract obtained during the extraction process. In addition,the data from the amendment results have something to do with the activecompounds of a sample so that if the number of amendments is increasing,the number of active compounds contained in the sample is alsoincreasing (Kiswandono, 2007). Calculation of the yield of extracts fromthe maceration process could be seen in table 3.

Table 3. Turmeric Rhizome Extract Amendment Results

Weight of Simplisia (g)	Extract Weights (g)	Amendments (%)
1000	79,9	7,99

Table 3.

5. Cytotoxicity Test Results

This anticancer activity test is carried out using the MTT method todetermine cell viability so that it can be used to determine itscytotoxic effects. The principle is the breakdown of tetrazolium MTTsalts (3-(4,5-dimetiltiazol-2-il)- 2,5difenyltetrazoliumbromide) by thetetrazolium succinate reductase (or succinate dehydrogenase) enzymesystem contained in the mitochondria of living cells so that purpleformazan crystals are formed (Suyati et al., 2010).

This test uses a variation of 3 concentrations, namely 10, 20, and40. Testing was also carried out with positive control, namely the drugDoxorubicin. The largest percentage of inhibition in MCF_7line cells was found in the turmeric rhizome extract concentration of 40ppm with a percent inhibition of 88.60%.

Extract concentration (ppm)	% inhibition of MCF_7 cancer cells			Average % inhibition
	P 1	P 2	P 3	
10	3,58	5,69	6,0	5,09
20	21,51	32,8	39,89	31,40
40	91,69	91,94	82,17	88,60

Table 4.

In testing using a concentration of turmeric rhizome extract of 40ppm, it can be clearly seen that there is an inhibition of cancer cellgrowth with discoloration in the microplate, namely purple residents arefading, and if further observations are made, it can be seen that thereis an inhibition of cancer cell growth in microplate media.

- (b)

(c) (d)

Figure 2. MTT-Assay Testing

Information:

a: MCF_7 control cell

b: Turmeric Rhizome Extract concentration of 80 ppm

c: Turmeric Rhizome Extract after administration of MTT reagent

d: Observations on microplates

Based on the results above table 4. The above is then created alinear regression graph using Microsoft Excel to calculate theIC_{50} value.

[CHART]

After doing the calculation, the IC_{50} value was obtained,which was 26.30 ppm. These results suggest that turmeric rhizome extractusing ethanol solvents belongs to the moderate cytotoxic category or ismoderately active. Turmeric has the potential to be anticancer, this canbe caused by the presence of an active compound in the form of curcumin.Curcumin contained in turmeric extract has been shown to have theability to induce cell cycle arrest and induce apoptosis. The mechanismby which curcumin induces apoptosis varies widely, and is thought toinhibit some cell-signaling pathways (Zhang et al., 2015) (Annapurna,2011). Curcumin can affect human breast cancer cells through cell cycleinduction in phase G_2M and phase S end in MCF_7cells. Curcumin causes a pronounced increase in the fraction of phaseG_2M. Curcumin induces the cessation of cancer cell phases byregulating spindle-related signaling pathways (Zahra et al., 2020).Differences in sensitivity to exposure to extracts are often found instudies. This can be happened due to several factors, such as the levelsof curcumin contained in the extract, the type of extract, and thedifference in cell lines used, so that each cell can give a differentresponse to exposure to the extract (Kurniawan et al., 2016).

CONCLUSION

The results showed that turmeric rhizome extract with ethanol solvent contains secondary metabolite compounds of flavonoids, alkaloids, tannins, saponins and triterpenoid. In cytotoxic testing, turmeric rhizome extract can be used as a natural ingredient that has the potential to inhibit the growth of MCF_7 breast cancer cells. The effectiveness of turmeric rhizomes in inhibiting the growth of MCF_7 breast cancer line cells with an IC_{50} of 26.30 ppm. The value belongs to the category of moderate cytotoxic or moderately active. So it is necessary to carry out further testing using flowcytometry to determine the mechanism of turmeric rhizome extract in cell death.

Kekurangan Penelitian

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