Original Research

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

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Abstract: 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 MCF7 breast cancer cells, so that the IC50 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 MCF7 breast cancer cells. Turmeric rhizome extract has the effectiveness to inhibit the growth of MCF7 breast cancer cells with an IC50 value of 26.30 ppm. This value is categorized as moderate or moderately active cytotoxic.

Keywords: Turmeric extract, MTT assay, MCF7, Anticancer.

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 an 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 type of cancer that is commonly found 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 regimens to increase the immunity of breast cancer patients (Cahya & Prabowo, 2019).

Turmeric (*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. Research on phytochemicals turmeric has revealed, it contains curcuminoids and essential oils as its main components. Curcumin and two derivatives of dimethoxy, desmethoxycurcumin, and bisdemethoxycurcumin, are 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). Determine the high anticancer potential in turmeric rhizomes can be done using the colorimetric Micro Tetrazolium (MTT) assay method, by reading the absorbance value of the resulting formalizing, then the results obtained are used to measure the magnitude of the IC50.

This research was conducted in vitro using the MTT assay method and the target cell is MCF7 (hormone-dependent breast carcinoma cells) as previously done by 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

Turmeric rhizomes with solvents used with ethanol 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 to determine the anticancer activity of turmeric rhizome extract.

Material preparation

Turmeric rhizome (*Curcuma longa Linn*) taken from the Balittro Cibinong Bogor experimental garden. Examination of materials with determination of turmeric plants at the Bogoriense Herbarium Laboratory in the field of Botany, BRIN-Cibinong Biology Research Centre, Bogor. The phytochemical screening of turmeric rhizome powder was carried out at the Phytochemical Laboratory of the Faculty of Pharmacy, Binawan University, Jakarta. The cytotoxicity test was carried out at the laboratory of the Bogor primate study center. Examination of *Curcuma Longa Linn* is carried out before the study with the aim of ascertaining the correctness of use. 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.

Organoleptic examination by observing the shape, color, taste, and smell of the rhizome. Microscopic examination of turmeric rhizome powder by observing starch grains, covering hairs, essential oil glands, and parenchymal tissue by weighing a total of 50 mg of turmeric rhizome powder mixed with 1 drop of aquades. The chemical content of the powder is examined to determine the type of chemical compounds contained in the turmeric rhizome powder.

Extraction

Extraction process: 1) turmeric rhizome powdered using a grinder tool with a fine sieve size of a mess 60. 2) The powder is mixed with 96% ethanol solvent with a ratio of 1000 g of turmeric rhizome powder. 3) 6 L of 96% ethanol solvent, then stirred and shaken for 2-3 hours after stirring then precipitated and macerated for 2 days. 4) after maceration, the filtrate is filtered using filter paper, then separated between the filtrates with the dregs. 5) 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.

Cytotoxicity Test

Cytotoxicity test 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. incubator at 37.C 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.C in a 5% CO. 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 . incubator at a temperature of 37.C 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

Organoleptic Examination

The turmeric rhizomes simplicial have a round shape, sometimes there are branches with lightweight and brittle, have a size with a diameter of 2-3 cm with a thickness of 1-3 mm, are reddish-orange, and smell typical of aromatic turmeric, have a bitter aftertaste on the tongue and the surface on the fracture is flat and the presence of powdery powder.

Table 1
Results of Organoleptic Examination of Turmeric Rhizomes

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

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Identification of Secondary Metabolite

Identification of secondary metabolites was carried out to determine the compounds contained in the ethanol extract of turmeric rhizomes. The test was carried out qualitatively against several secondary metabolites.

Table 2
Identification of Secondary Metabolite Compounds of Turmeric Rhizome Powder

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

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The genus Curcuma family Zingiberaceae has also long been utilized in traditional medicine and can be developed as cancer. Turmeric has been studied to contain chemical compounds referred to as curcuminoids (curcumin 75%, desmethoxycurcumin 15-20%, and bisdemethoxycurcumin approximately 3% (Melannisa & Da'i, 2011). Curcumin is one of the secondary metabolite compounds of the phenolic group which is known to have important biological activities, such as antibacterial, anticancer, antioxidant, antidiabetic, and anti-inflammatory (Puteri, 2020).

Table 3
Turmeric Rhizome Extract Amendment Results

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

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The results of turmeric rhizome extraction for the manufacture of turmeric rhizome extract by maceration using solvent 96% ethanol obtained an extract yield of 79.9 g (7.99%). The yield results of a sample are essential because it is necessary to know the amount of extract obtained during the extraction process. In addition, the data from the amendment results have something to do with the active compounds of a sample so if the number of amendments is increasing, the number of active compounds contained in the sample is also increasing (Kiswandono, 2017).

Cytotoxicity Test Results

Table 4
Turmeric Rhizome Extract Cytotoxicity Test Result

Extraction Concentration (ppm)	Percentation Inhibition of MCF_7 Cancer Cells			Average Percentacion Inhibition
	P1	P2	P3	
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

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This test uses a variation of 3 concentrations, 10, 20, and 40. Testing was also carried out with positive control, namely the drug Doxorubicin. The most significant percentage of inhibition in MCF7line cells was found in the turmeric rhizome extract concentration of 40 ppm with a percent inhibition of 88.60%.

After doing the calculation, the IC50 value was obtained, which was 26.30 ppm. These results suggest that turmeric rhizome extract using ethanol solvents belongs to the moderate cytotoxic category or is moderately active. This anticancer activity test is carried out using the MTT method to determine cell viability so that it can be used to determine its cytotoxic effects. The principle is the breakdown of tetrazolium MTT salts (3-(4,5-dimetiltiazol-2-il) -2,5 difenyltetrazoliumbromide) by the tetrazolium succinate reductase (or

succinate dehydrogenase) enzyme system contained in the mitochondria of living cells so that purple formazan crystals are formed (Akula et al., 2011; Zhang et al., 2015). In testing using a concentration of turmeric rhizome extract of 40 ppm, it can be clearly seen that there is an inhibition of cancer cell growth with discoloration in the microplate, namely purple residents are fading, and if further observations are made, there is an inhibition of cancer cell growth in microplate media.

These results suggest that turmeric rhizome extract using ethanol solvents belongs to the moderate cytotoxic category or is moderately active. Turmeric has the potential to be anticancer, this can be caused by the presence of an active compound in the form of curcumin. Curcumin contained in turmeric extract has been shown to have the ability to induce cell cycle arrest and induce apoptosis. The mechanism by which curcumin induces apoptosis varies widely and is thought to inhibit some cell-signaling pathways (Akula et al., 2011; Zhang et al., 2015). 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 the cessation of cancer cell phases by regulating spindle-related signaling pathways (Zahra et al., 2020). Differences in sensitivity to exposure to extracts are often found in studies. This can be happened due to several factors, such as the levels of curcumin contained in the extract, the type of extract, and the difference in cell lines used, so that each cell can give a different response 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 triterpenoids. In cytotoxic testing, turmeric rhizome extract has the potential as a natural ingredient that has the potential to inhibit the growth of MCF. breast cancer cells. The effectiveness of turmeric rhizomes in inhibiting the growth of MCF. breast cancer line cells with an IC_{50} of 26.30 ppm. The value belongs to the category of moderate cytotoxic or moderately active. It is necessary to carry out further testing using flow cytometry to determine the mechanism of turmeric rhizome extract in cell death.

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Notes

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