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Anti-Breast Cancer Potency of Multistage Extraction from Jamur Dewa (*Agaricus blazei* Murill) Solvents on MCF-7 Cells

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Abstract

ABM (*Agaricus blazei* Murill) is a basidiomycetes fungus. ABM is used by people for the treatment of diabetes, antihypertention, anticholesterol, anticancer, and immunostimulant. ABM contains terpene, steroids, agaritine, vitamin C, vitamin E, and betaglucane. In this research, ABM extract was tested as an anti-breast cancer in vitro using MCF-7 breast cancer cells. The extract was obtained from the multistage extraction process of several solvents in turn, the solvent used, among others, n-hexane, dichloromethane (DCM), chloroform, ethyl acetate, butanol, and water. The results of the research were the obtained IC_{50} value from n-hexane extract 247.17 μg/mL; extract DCM 227μg/mL; chloroform extract 215.64 μg/mL; extract of ethyl acetate 234.9 μg/mL; butanol extract 500,78 μg/mL; while the water extract was inactive. Based on these results can be considered for further research to fractionate in order to know which class compounds have the potency as anticancer within the extracts.

Keywords : *Agaricus blazei*, multistage extraction, MCF-7 cells.

INTRODUCTION

Breast cancer is the second most common cancer in women worldwide and in Indonesia. Breast cancer is the most commonly found after cervical cancer. The latest data from the American Cancer Society estimated in 2017 showed that there are approximately 252,710 cases of breast cancer in women, while about 40,610 women died because ofbreast cancer. The number of breast cancer patients in Indonesia is second ranked after cervical cancer. The causes of breast cancer have not be known for certain, but there are several possibilities: (a) genes, *i.e.*, BRCA1, BRCA2, p53, Bcl2 genes are inherited genes that can trigger the breast cancer; (b) the use of certain drugs, such as hormonal therapy; (c) other factors, *i.e.*, not married, married but without children, giving birth to the first child after the age of 35 years, never breastfeeding the child; (d) have had an infection or trauma (Desen, 2008).

Treatments performed for breast cancer patients are chemotherapy, radiotherapy, and surgery. These treatments have negative effects, especially if it is already at metastasis stage. Based on this condition the researchers tried to find alternative treatment using natural ingredients with the aim of minimizing the side effects. One of the natural materials that can be used is *Agaricus blazei* Murill (ABM). ABM is a basidiomycetes fungus. People use ABM as a functional food. Empirically ABM is used as antidiabetic, antihypertention, anticholesterol, anticancer, and immunostimulant. ABM contains α- (1-4) -; β- (1-6) -glucan (Fujimiya, *et al*., 1998), α- (1-6) -; α- (1-4) -glucan, β- (1-6) -; β- (1-3) -glucan, β- (1-6) -; α- (1-3) -glucan (Mizuno, *et al*., 1990), lectins (Kawagishi, *et al*., 1990), riboglucan (Cho, *et al*., 1999), glucomannan (Hikichi, *et al*., 1999), ergosterol Takaku, *et al*., 2001), sodium pyroglutamate (Kimura, *et al*., 2004), RNA-protein complex (Gao, *et al.*, 2007), Agaritin (Stijve, *et al*., 2003; Nagaoka, *et al.*, 2006; Akiyama, *et al.*, 2011), blazein (Itoh and Hibasami, 2008), agariblazepirol C (Hirotani, *et al*., 2005), ascorbic acid, α- and δ-tocopherol, total phenol (Huang and Mau, 2006).

Based these contents, ABM can be used as an anticancer agent. 50% ABM ethanol extract can inhibited Hela cell growth with IC_{50} value 194.4 μg/mL (Misgiati, 2011). Agaritin isolated from ABM could inhibit the proliferation of U937, MOLT4, HL60 and K562 cells with IC_{50} values of 2,7 , 9,4 , 13, and 16 μg/mL, and in normal lymphatic cells there was no resistance to concentration 40 μg/mL (Akiyama, *et al*., 2011).

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Blazein, which also resulted from ABM isolation, could induce the death of LU99 human lung and stomach KATO III cancer cell line at a concentration of 200 µg/mL (Itoh, *et al*., 2008). Polysaccharides generated from ABM mightinhibit osteosarcoma cell proliferation (HOS cell line) at the concentration of 100 µg/mL. ABM might inhibit proliferation of prostate cancer cells DU145 and PC3 400 µg/mL and 800 µg/mL (Yu, *et al*., 2009). Previous research, had not inform the active compound or activity on breast cancer cells MCF-7. The purpose of this study was to find out the active ingredients that have cytotoxic activity against MCF-7 cancer cells.

MATERIALS AND METHODS

Simplicia Preparation

All parts of the ABM fungus were used. ABM was cleaned from soil and root, then washed with running water. ABM dried with oven at 70^{o}C temperature until obtained the dried ABM with water content of not more than 10%.

Extraction

The amount of 100 grams of dried ABM was added by n-hexane pro analysis (p.a.) for 150 ml and extracted with ultrasonic at 40^{0}C for 10 minutes. Extraction was done three times. The resulted filtrate was collected, subsequently evaporated until the solvent allows to be removed for drying. The dregs was extracted using a 150 ml Dichlormetane solvent. The same treatment was carried out in extraction with n-hexane. Other solvents are chloroform, ethyl acetate, n-butanol, and hot water (100^{o}C).

Ethanol Extract 50% Preparation

A hundred grams of dried ABM were immersed in 50% ethanol as much as 150 mL, allowed to stand for 2 days, then strained. The dregs was again immersed using 50% ethanol as much as 150 ml, strained again. Repeat again for the dregs. Filtrate was collected, evaporated, then dried in 40^{o}C oven.

Extracts For Rich Alkaloids Preparation

Amount of 50% ethanolic extracts were added by 0.5 N hydrochloric acid, then added by 2% ammonium hydroxide.

Identification of Extracts

The resulting extracts were identified by TLC (Thin Layer Chromatography). The stationary phase used silica gel GF254, while the mobile phase used two eluents. First eluen was hexane: ethylacetate (4: 1) and second eluent was ethyl acetate: methanol: water (0.5: 8: 1.5). Each extract was spotted as much as 5 µl on the TLC plate. It was eluted with the available eluent (the eluent used based on observation). Staining for mobile phase n-hexane-ethyl acetate was using Lieberman and heating 105^{0}C, then observed on UV light 254 nm and 355 nm. Staining for the ethyl acetate-methanol-water mobile phase used Dragendorf.

Cytotoxic Testing

MCF-7 cells were seeded on microplate 96 wells as much as 5000 cells/wells and incubated for 48 hours to achieve good growth. The MEM medium was replaced with a new one by adding the extract at various concentrations (0 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL, and 400 µg/mL) with DMSO as co-solvent and incubating at 37^{o}C in a 5% CO_2 incubator for 48 hours. At the end of incubation, MEM media andextracts were discarded and then the cells were washed with PBS. At each well, 10 µL of MTT reagent (5 mg/mL) was added. Cells were incubated for 4-6 hours in a 5% incubator CO_2 of 37^{o}C. The MTT reaction was discontinued with isopropanol acid reagent (HCl 4N and isopropanol ; 1:4), shaked gently over the shaker for 10 minutes. Absorption was read by ELISA reader at 550 nm wavelength. The data collection for the cytotoxic test were: a) The absorbance data obtained from the cytotoxic test that was converted to live cell percent, b) Percent of living cell was calculated using the formula:

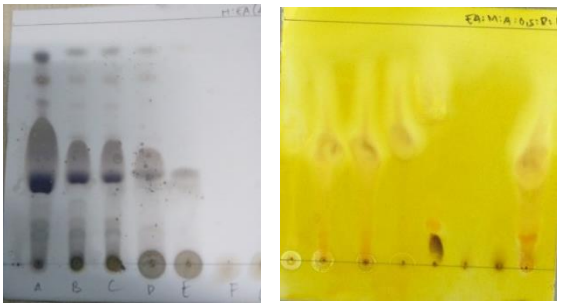
$$\text{Cell viability: } \frac{\text{absorption of cell with treatment} - \text{absorption of cell medium control}}{\text{absorption of cell medium} - \text{absorption of medium control}} \times 100\%$$

RESULT

The dried ABM had water content of not more than 10%. Extracts resulted from n-hexane solvent with rendement of 0.66%, 0.47% for chloroform solvent, 0.28% for chloroform solvent, 0.52% for ethyl acetate solvent, 0.60% for n-butanol solvent, and 46.97% for hot water solvent.

The resulted extracts were identified by TLC. Based on the eluent used n-hexane: methanol (4:1), there were several classes of terpenoid and steroids compounds in the treatment of extraction with n-hexane solvent, dichlormethane, chloroform. (Fig. 1), whereas in the eluent of ethyl acetate: methanol: water (0.5:8:1.5) there was only a class of alkaloid compound in the treatment of solvent extraction in chloride, chloroform, and n-butanol (Fig. 2).

Result of cell viability as on the diagram below (Fig. 2). Based on the cell viability percentage, resulted IC_{50} value on each extract as described in table below (Table 1).



A **B**

Figure 1. (A) Terpenoids and steroids compound, (B) Alkaloid compounds.

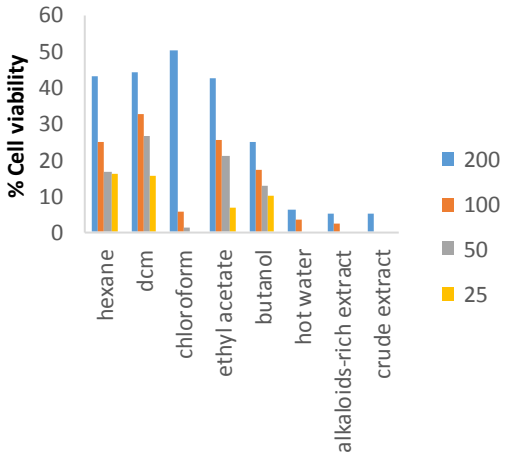


Figure 2. Cell viability of MCF-7 after treatment by extract with some solvents. Cytotoxic test was determined with MTT assay.

Table 1. IC_{50} value from some solvents on ABM (µg/mL)

Kind of extracts	IC_{50} value
n-hexane	247.17
dcm	227
chlroform	215.64
ethyl acetate	234.90
n-butanol	500.78
hot water	inactive
alkaloids extract	inactive
50% ethanolic extract	446

DISCUSSION

Extract with n-hexane solvent had IC_{50} value of 247 µg/mL. Groups of soluble compounds in n-hexane solvent were nonpolar compounds, namely terpenoids and steroids. These were confirmed by the results of identification with TLC showing a positive result. Group of steroids was capable to induce cell death and morphological changes through chromatin condensation causing apoptosis in lung cancer cells LU99 and KATO III stomach cancer cells (Itoh, *et al*., 2008).

Extract with solvent dichloromethane has had IC_{50} value of 227 µg/mL. Based on the identification with TLC there were terpenoid groups, steroids and alkaloids (Fig. 1 & 2). Likewise for extracts with chloroform solvent that had IC_{50} of 215.64 µg/mL and ethyl acetate solvent extract, also gave the same color as the solvent extract in the TLC identification. The dichlormethane and the chloroform solvent had a polarity index that was not too far away, so that the group of dissolved compounds in them were not much different from their kind. Extracts with n-butanol and hot water solvents showed no activity, nor did alkaloids-rich extracts and crude extracts.

According to Machana, *et al*., (2011), the extract having activity as an anticancer has IC_{50} value of 100-500 µg/mL. While chloroform extract of purple tapak leaves (*Catharantus ruseus* [L] G.Don var roseus) had anticancer activity with IC_{50} of 188.949 µg/mL, and white cypress leaves (*Catharantus roseus* [L] G.Don var albus) had IC_{50} of 201.371 µg/mL (Kusumastuti, 2013). Purple and white treads contain classes of alkaloids such as vincristine, vinblastine, catharantin. In the extract of petroleum ether and black cumin chloroform (*Nigella sativa*), cytotoxicity test was performed on MCF-7, HeLa, and wiDr cells with IC_{50} values of 164 µg/mL, 161 µg/mL, 402 µg/mL, 267 µg /mL, 331 µg/mL, and 280 µg/mL (Ekowati, *et al*., 2011). Other data are Aspergillus sp etil acetate extract and methanol extract: hexane (1:1) having IC_{50} values of 153.266 µg/mL and 208.305 µg/mL in T47D cells, wherein this extract contains alkaloid secondary metabolites, steroids, terpenoids, polyphenols, flavonoids, phenolic (Winarno, 2011).

CONCLUSION

Based on the above, ABM extract with n-hexane, dichloromethane, chloroform, and ethylacetate solvents have an anticancer ability in MCF-7 cells. This extract can be developed as an anticancer candidate in MCF-7 cells.

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