ANTI BREAST CANCER ACTIVITY OF ETHYL ACETATE EXTRACT OF FERMENTATION BROTH EMPLOYING ENDOPHYTIC FUNGI TAXUS SUMATRANA ISOLATES

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ABSTRACT

Fermentation broths of TSC 18 and TSC 22 fungi isolates obtained from Taxus sumatrana plant were investigated for their in vitro anti breast cancer activity against T47D cell line. The isolates were further identified by PCR and sequencing methods. In the fermentation experiments, the growth curves were constructed by plate count method and the pH change were recorded, while glucose and protein dynamics of the broth were determined by Nelson-Somogy and Lowry methods respectively. Anti-cancer activity was assessed by SRB (Sulforhodamine B) method. The investigation indicates the fermentation broth both isolates possessed the anticancer activity with IC_{50} of 42.6 ppm for TSC 18 and 55.7 ppm for TSC 22. Cisplatin which is used as a reference compound gave IC_{50} of 15.9 ppm . The PCR and tree view program proved that TSC 18 is Phomopsis sp. strain MAFF-238472 and TSC 22 is Coprinopsis cinerrea strain NBRC30628.

Keyword : Anticance, Isolates TSC 18 and TSC 22 Taxus Sumatrana

INTISARI

Telah dilakukan penelitian untuk mengetahui kemampuan aktivitas in vitro antikanker payudara pada cell line T47D dari hasil fermentasi isolat jamur endofitik Taxus sumatrana , yakni TSC 18 dan TSC 22 serta mengidentifikasi spesies jamur tersebut yang berpotensi sebagai penghasil senyawa antikanker . Tahapan penelitian mencakup Uji fermentasi dari kedua isolat tersebut diatas dengan mengukur parameter kurva pertumbuhan dengan metoda plate count, perubahan pH, kadar glukosa dengan metoda Nelson-Somogy dan kadar protein dengan metoda Lowry dari cairan fermentasi; Uji aktivitas anti kanker dengan metoda Sulforhodamin B (SRB); dan Identifikasi jamur dengan metoda Polymerase Chain Reaction (PCR) dan sequencing. Hasil penelitian menunjukkan bahwa hasil fermentasi menggunakan isolat TSC 18 dan TSC 22 memiliki aktivitas antikanker payudara pada cell line T47D dengan konsentrasi penghambatan 50 % (IC 50), untuk TSC 18 sebesar 42,6 ppm dan TSC 22 sebesar 55,7 ppm. Cisplatin yang digunakan sebagai rujukan senyawa antikanker menunjukkan IC 50 sebesar 15,9 ppm. Dengan demikian isolat TSC 18 memiliki nilai aktivitas yang lebih baik daripada TSC 22. Hasil identifikasi dengan metoda PCR dan melalui pprogram tree view, menunjukkan bahwa isolat TSC 18 adalah Phomopsis sp. strain MAFF-238472 dan isolat TSC 22 adalah Coprinopsis cinerrea strain NBRC30628.

Kata Kunci : Isolates TSC 18 dan TSC 22 Taxus Sumatrana, Anti kanker

INTRODUCTION

One may suffer from cancer irrespective of race, age and gender. It has been the second death cause after cardiovascular disease in the world and for Indonesia it is number six. It is estimated that globally 15 out of 100.000 population suffer from cancer, therefore this will be a significant health

problem in the future both for developed and less developed countries including Indonesia with estimation of 100 sufferer in 100.000 population. Cancer can appear within various cells including breast cells. Taxol which is a toxoid compound possesses anticancer activity, originated from Pacific yew tree, known as Taxus brevifolia , a slow growing tree. This compound can also be obtained in the endophytic fungi of various Taxus plants.

Active secondary metabolites produced by a plant is also a result of microbes contribution. These microbes live within the plant cells known as endophytic microbes (Strobel et al. 2004). However investigation on the anticancer compounds produced by endophytic microbes endemic in taxus genus includes Taxus sumatrana is scarce (Strobel, 2004). This plant grows in Indonesia although in a very limited area such as in Gunung Kerinci (West Sumatra) region and in highland of Sulawesi with an altitude of 1500-2000 metres above sea level.

Fungi and bacteria are often discovered as endophytic microbes. Seven bacteria isolates and 31 fungi isolates, both endophytic, have been reported to be present in Taxus sumatrana (Rumampuk , 2005). The present investigation is carried out to find some potential isolates which produce anticancer against breast cancer cell-line (T47D) and further identify the isolates.

MATERIALS AND METHODS

The endophytic isolates were obtained from Research Centre for Chemistry collection known as TSC 18 and TSC 22 isolates. Fermentation took place in PDB (Potato Dextrose Broth) media in 500 mL shake flask at 30°C , 120 rpm for 7 days. Daily observation was conducted to follow the growth by measuring its total plate counts and pH changes, while glucose and protein contents were determined by Nelson-Somogy and Lowry method respectively.

Anticancer activity was assessed by in vitro test employing breast cancer cell line T47D. This is performed by SRB (Suforhodamin B) staining to determine the percentage of survival T47D cells and the IC-50. Initially the T47D cells were trypsinised then stained with 0.4% SRB solved in 1% acetic acid and then fixed with TCA (tricholoroacetic acid) 5%. The survive T47D cells was observed in a plate reader at 515 nm (Skehan et al., 1990).

The fermentation broth was extracted with ethyl acetate and then the solvent was evaporated to dryness for in vitro testing as above. Cisplatin was used as reference. Dose-response curves were constructed by varying dose concentrations and calculating the survive cells. Inhibitory concentration at 50% of survive cells was calculated by using logarithmic regression of the dose-response curves.

The identification of potential isolates was carried out by 18S RNA with PCR (Polymerase Chain Reaction) and sequencing methods (Maniatis et al., 1989).

RESULTS AND DISCUSSION

Figures (1 and 2) and regression models in Table 1 show the dynamics of the fermentation process employing TSC 18 and TSC 22 isolate respectively. The number of fungi colonies increase during the fermentation indicates the adaptability of the fungi towards the temperature, aeration and medium compositions. Fast growth was shown during day-3 to day-5 and then followed by stationary phase in which the secondary metabolites was produced initially.

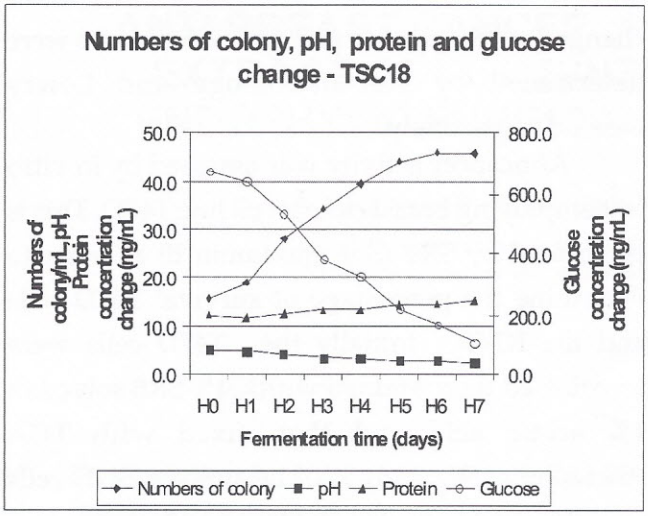

Figure 1. Fermentation with TSC 18.

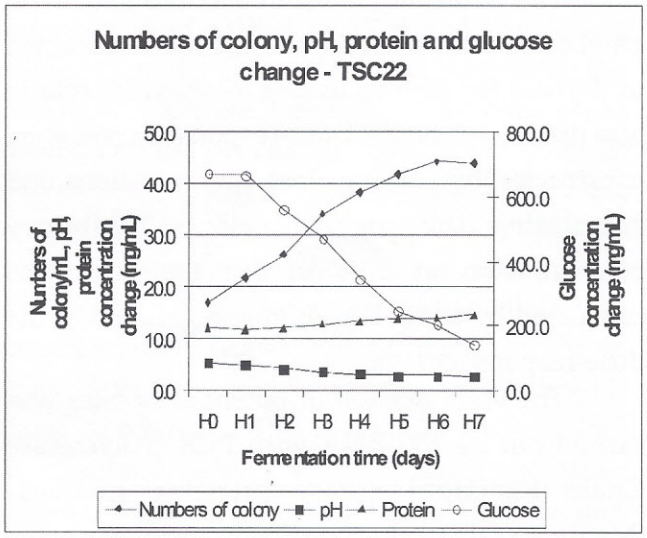

Figure 2. Fermentation with TSC 22.

The pH during fermentation decreased from 5.15 to 2.66 due to production of organic acids during growth with the presence of glucose and appropriate aeration. Different isolates will result in different metabolites, hence nutrient utilization and product formation will also differ and this affects the pH change. During the fermentation the growth of TSC 18 and its product formation is faster as compared to TSC 22.

The decrease of glucose concentration during the 7 days of fermentation is due to media degradation for growth, for formation of energy and secondary metabolites. Faster utilization of glucose appeared during day-1 to day-3 when the fungi were growing exponentially, especially the TSC 18. The increase of protein in the media have been due to excretion of various protein such as extra cellular enzymes. Again TSC 18 released more protein.

Table 1. Regression model in the fermentation process with endophytic fungi isolates

Variable (Y vs X)	TSC 18	TSC 22
Fermentation Process		
Number of colony vs day	Y= 15,897 Ln (x) + 13,428	Y= 14,66 Ln (x) + 14,067
pH vs day	Y= -0,3834x + 5,4196	Y = - 0,3814x + 5,3689
Glucose (mg/mL) vs day	Y = - 87,559x + 774,11	Y = -84,738x + 793,51
Protein (mg/mL) vs day	Y = 0,4878x + 11,289	Y= 0,3876x + 11,358

All regression equations gave $R^2 > 0.90$

Evaporation to dryness of the fermentation broths after extraction with ethyl acetate yielded 42.8 mg and 25.7 mg for respective isolate TSC 18 and TSC 22. Both were proven to be active against T47D cells as shown in Figures 3 and 4 using cisplatin as a reference for anti cancer agent (Figure 5).

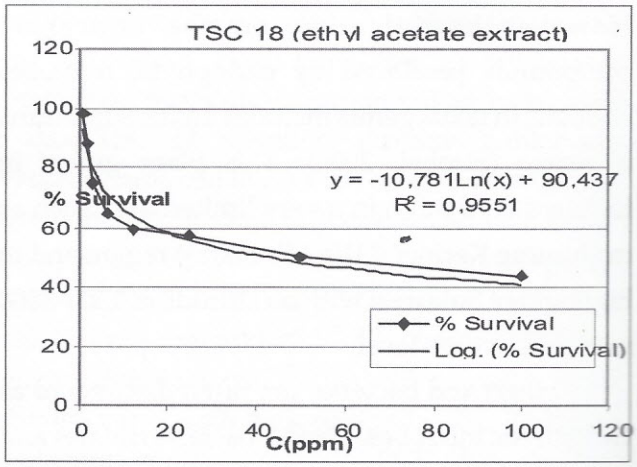

Figure 3. Logarithmic curve of cell (T47D) survival at various concentrations of TSC 18 ethyl acetate extract

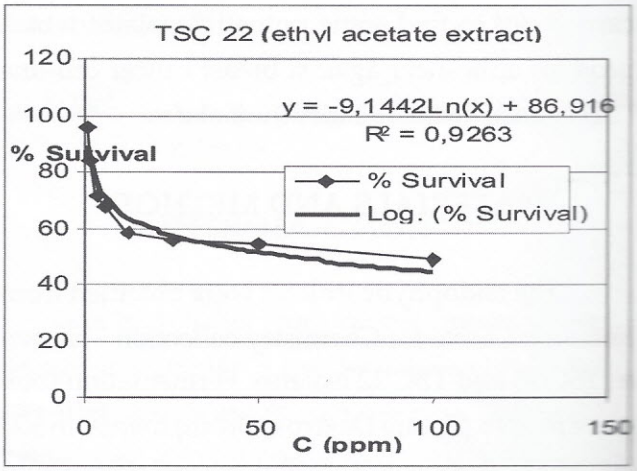

Figure 4. Logarithmic curve of cell (T47D) survival at various concentrations of TSC 22 ethyl acetate extract

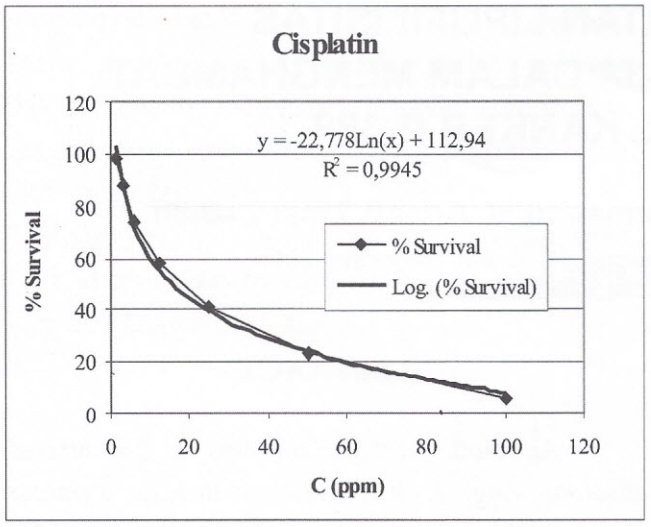

Figure 5. Logarithmic curve of cell (T47D) survival at various concentrations of cisplatin.

Based on the IC 50% obtained on the regression model curves of TSC 18 and TSC 22, the results are listed in the following Table 2.

Table 2. Ic_{50} of the ethyl acetate extracts as an anticancer activity on T47D breast cancer cell line.

Test Sample	Logartithmic Response Model	IC 50 ppm
Cisplatin*	Y=-22,78 Ln(x) + 112,94 R^2= 0,9945	15,9
TSC 18; Ethyl acetate extract	Y= -10,78 Ln(x) + 90,44 R^2= 0,9551	42,6
TSC 22; Ethyl acetate extract	Y= -9,14 Ln(x) + 86,92 R^2= 0,9263	56,7

** Reference*

Identification of TSC 18 and TSC 22 isolates

Most of the endophytic microbes belong to Ascomycetes and Deuteromycetes or imperfect fungi which in normal conditions are symbiotic with the plant. Based on the phylogenetic tree it is known that TSC 18 is Phomopsis sp. strain ZH79 and TSC 22 is Coprinopsis cinerea strain NBRC30628. Their taxonomy are as follows.

Phomopsis sp. strain Zh79

Kingdom : Fungi; Phylum : Ascomycota;
Class : Sordariomycetes
Ordo : Sordariomycetidae; Family : Diaporthales;
Genus : Phomopsis
Spesies : Phomopsis sp. Strain ZH79

Coprinopsis cinerea strain NBRC30628

Kingdom : Fungi; Phylum : Basidiomycota;
Class : Agaricomycetes
Ordo : Agaricomycetidae; Family : Agricales;
Genus : Coprinopsis
Spesies : Coprinopsis cinerea strain NBRC30628

CONCLUSION

The investigation indicates the fermentation broth both isolates possessed the anticancer activity with IC_{50} of 42.6 ppm for TSC 18 and 55.7 ppm for TSC 22. Cisplatin which is used as a reference compound gave IC_{50} of 15.9 ppm. The PCR and tree view program proved that TSC 18 is Phomopsis sp. strain MAFF-238472 and TSC 22 is Coprinopsis cinerrea strain NBRC30628.

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