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ABSTRACT

Background: In an effort to cancer treatment, failure oftenoccurs primarily through chemotherapy, due to the reduce defficacy of chemotherapeutic agents because of the multi-drug resistance mechanisms, including the doxorubicin. Addition to suppress cancer cell doxorubicines also have an effect on normal cells. Additionally, doxorubicin tends to cause resistance cell against to drug. Some natural products research begin to be directed for co-chemotherapy agent with the aims for increase sensitivity, supress resistance of cancer cells and decrease of side effect posed by chemotherapy agent.
Objective: The pasak bumi roots have great potential for anticancer and co-chemotherapy for breast cancer. Therefore, the pasak bumi roots (*Eurycoma Longifolia* Jack.) can be combined with doxorubicin for drug of choice in breast cancer.
Outcome: IC50
Methods: the method of this study will be performed by in vitro test. Amount of 500 g of pasak bumi root were macerated with ethanol 70 % during 24 hours and remacerated for 3 times. Fractination of extract was use ethyl acetate and aquadest. Cytotoxic test and combination test in T47D cells were used by MTT method.The concentrations of Ethyl acetate fraction used 2000 µg, 1000, 500, 250, 125, 62,5 and 31,125 µg/ml with incubation time 24 hours. And then, observed using *Elisa plate reader* in 595 nm. Absorbance readings were then converted into a percentage of the life of the cell to the IC_{50}values calculated.
Result: IC_{50} values from ethyl acetate fraction of the pasak bumi roots obtained was 340 µg/ml. The concentration of ethyl acetate fraction whichwas used for combination test were 340, 255, 170, 85 µg/ml. The combination index was 0,23682.
Conclussion: This result indicate that combination of pasak bumi roots and doxorubicin have strong synegristic effect with dose selection for pasak bumi roots of 255 µg/ml and doxorubicin of 0,00193 µg/ml.

Keywords : Breast Cancer T47D, Doxorubicin, Ethyl acetate fraction of pasak bumi roots, co-chemotherapy

INTRODUCTION

Breast cancer is a malignancy that is often found in women. With incidence 20% of all malignancies (Jemal *et al.*, 2008). Doxorubicin is the one of chemotherapeutic agent that

is widely used for breast cancer (Childs *et al.,* 2002). However, the use of these drugs can cause the patient to be susceptible to diseases and other infections (Patel *et.al.,* 2007). This medicine can cause side effect and No specific. It can damage normal cell of the body and cause to resistance (Smith *et al.*, 2006). Dose reductions will to reduce the side effect of doxorubicin. Therefore, need something to improve the clinical application of Chemotherapeutic agents to be more effective (Jenie *et al.*, 2009). The addition of co-chemotherapeutic is the right choice to overcome this (Diaz-Montero *et al.*, 2009) where chemoprevention compounds that are non-toxic or less toxic in combination with chemotherapeutic agents to increase efficacy by reducing their toxicity to normal tissue.

The one of chemoprevention agent that can be used is Pasak Bumi Roots (*Eurycoma longifolia* Jack.). Pasak bumi roots have bioactive compounds for anticancer, chemoprevention and immunomodulator. There are such quassinoids, flavonoids and alkaloids (Nurani *et al*, 2009). The ethanol extract have more great potential for cytotoxic and antiproliferative against T47D cell than Chloroform and water extracts (Nurani, *et al.,* 2009). The water extract, methanol-water (1 :1 v/v) a methanol shown to have antiproliferative activity against HeLa cells, A549, 26-L5, LLC, and B16-BL6 melanoma cells (Ueda *et al.*, 2002). The methanol extract, n-buthanol, chloroform, and water extract from pasak bumi roots have also been tested with MTT cytotoxicity activity on KB cells, DU-145, RD, MCF-7, CaOV-3, and MDBK. All extract except water extract have cytotoxic effects against all cancer cells but which have the greatest effect is a semipolar extract (Nurhanan *et al.*, 2005). Based of that research, Pasak bumi roots (*Eurycoma longifolia* Jack.) is possible to have great potential to be developed as a co-chemotherapeutic along chemotherapeutic agent has been widely used in cancer therapy. This study will examine whether the ethyl acetate fraction ethanolic extract of the pasak bumi roots have the ability as a co-chemotherapeutic agent in order to reduce the toxicity of chemotherapeutic agents, in this case is doxorubicin with view of the IC_{50} and CI (Combination Index).

MATERIALS AND METHODS

Instrument

Glass were, pump, chamber, stirrer electrics, pipette volume, water bath, plate 96 well, incubator, microscope, blue tip, yellow tip, laminar air flow, buchner, rotary evaporator.

Materials

Cell line T47D, fraction extract *E. Longifolia* jack, doxorubicin, ethanol 70%, aquadest, complete medium, DMEM medium, SDS, reagent MTT.

Plant material

Roots *E. Longifolia* were collected from Banjarmasin, Indonesia and identified for correctness in department of pharmacognosy /faculty of pharmacy, University of Ahmad Dahlan with hand book flora of java (Becker and Van de Brink, 1965 in Sangat, 2000).

Preparation of *E.longifolia* roots extract

The air-dried powdered roots of E.longifolia (500 mg) were extracted two times with ethanol 70% (2L), then stirred for tree hours using stirrer electrics to maximize the process of extraction after that deposit for 24 hours. Using buchner for getting the filtrats and placed in rotary evaporator until extract thickness and continued with water bath until extract more thickness.

Preparation of *E.longifolia* roots fractination

Making a fractination of ethyl acetate was done by aquadest and extract 1:1 and fractionated with ethyl acetate in tree times and obtained with rotary evaporator until thickness.

Cytotoxity studies

This studies using MTT methods adopt reductase cell system. Cell lines were seeded in 96-well culture plate for 24 hours. The addition of media complete after 24 hours. added MTT reagent, than incubated for 4 hours, given stoper reagent (SDS). The life cell with make reaction with MTT forming purple formazan crystal, can be read in ELISA reader with λ 595nm. Determination was done by IC50 counting absorbance :

% cell life = (absorbance treatment - absorbance medium control)/(absorbance control cells - absorbance medium control) x 100%

Combination index studies

Cell cancer were seeded in 96-well culture plate for 24 hours. Treatments was done by adding a few series concentration fraction ethyl acetate *E.longifolia* and 50 µl doxorubicin as and incubation for 24 hours. after adding MTT reagent then incubated for 4 hours, given reagent stopper (SDS) and can be read in ELISA reader with λ 595nm, Counting Combination index (CI) will give addictive effect as two combination compound, as a synergy, addictive or antagonist. Value CI can count with formula : CI = (D)1/(Dx)1+ (D)2/(Dx)2

Dx = one compound concentration given effect as combination

(D)1, (D)2 = two compound concentration given same effect

RESULTS AND DISCUSSION

This study explained ethyl acetate fraction from ethanolic extract of *E. longifolia* root. The result obtained from the drying shrinkage 70% ethanol extract of *E. longifolia* root was 3.2% from 500 mg of dry powder. *E.longifolia*was extracted with maceration method, because this way is more simpler, easier and relatively cheaper. Selection of 70% ethanol as a solvent is expected to attract active component contained in the *E.longifolia*.. Cytotoxicity assay begins mediapraparation, cultured T47D cells, and give treatment. Results of cells density were calculated by hemocytometer from 20 µL suspension contained 10^5 cells. So thatobtained1,05x10^6 cells/ml cell density. For cytotoxicity assay, the cells were plated at a density of 4 x 10^4 cells/ml cell/well. T47D cells used in this study, because they were one of type of breast cancer cells that are found in patients with cancer as a cause of death. These cells are often used in research because it is safe to use in the cytotoxicity assay, easy in culturing and treatment. The medium used for culturing the cells are RPMI 1640 medium,because of this medium contains complete nutrition and appropriate for the growth of T47D cells. Cytotoxicity assay was read using Elisa reader. This observation is supported by looking at the shape of T47D cell morphology, compared with cells that had been given tretment pasak bumi roots. Where the dead cells will appear dark and the shape was not round anymore, whereas living cells still looks round and lighter.

All corpuscule and dead cells were calculated, obtained percentage cell viability and linear regression equation to obtain IC_{50} withantilog value of x. The result cytotoxicity assayof doxorubicin against T47D cells are shown in Table 1 and the result cytotoxicity

assayofethyl acetate fraction ethanol extract *E.longifolia*root against T47D cells are shown in Table 2.

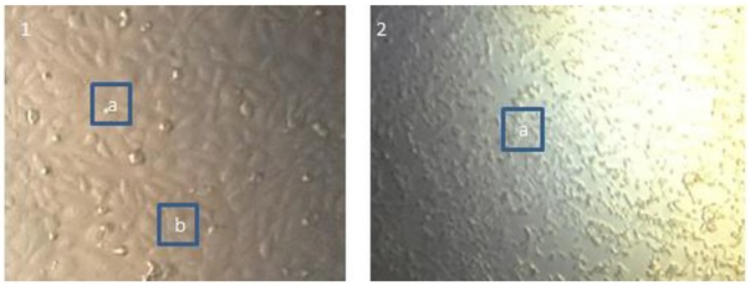

Figure 1. The morphology of T47D breast cancer cells (1) under a microscope with 100x magnification in wells with 1000 mg/ml ethyl acetate fraction of ethanol extract of the pasak bumiroottreatment and T47D cells before treatment (2). (a) Cells were living corpusculelooks spherical. (b) dead cells appeared to be a change in morphology.

Cell were treated with doxorubicin at the 7 different concentrations, cell culturedin incubation for 24 hours. Obtained the highest percentage of cells viability is 60.92% at 0.062 mg/mlconcentration and the lowest percentage is 26.76% at 0,05 µg/ml concentration. IC_{50} value of doxorubicin is 0.00385 mg/ml.

Cell were treated with ethyl acetate fraction ethanol extract *E.longifolia*root at the 7 different concentrations, cell culturedin incubation for 24 hours.Obtained the the highest percentage of cells viability is 85.48% at 31.125 µg/mlconcentration and the lowest percentage is 1.92% at 2000 µg/ml concentration. IC_{50} value of 340 µg/ml.

Table 1. The results ofdoxorubicin cytotoxicity assay against T47D cells

Concentration	%Viabilitas	log cons
4	50,26552288	0,602059991
2	44,87336601	0,301029996
1	29,59558824	0
0,5	26,75653595	-0,301029996
0,25	33,70098039	-0,602059991
0,125	43,91339869	-0,903089987
0,0625	60,92728758	-1,204119983
a=,35243877		a=40,21285598
b=1,835037271		b=-4,05402785
Equation	y=39,35243877+1,835037271x	y=40,21285598-4,05402785x
IC50	50=39,35243877+1,835037271x	50=40,21285598-4,05402785x
IC50(x)	5,802367831	-2,414177796
	Antilog (Concentration)	0,00385 micrograms

Table 2. The results of cytotoxicity assay ethyl acetate fraction ethanol extract *E.longifolia*root against T47D cells

Concentration	%Viabilitas	log Const
2000	1,92	3,30
1000	24,33	3
500	56,21	2,69
250	70,99	2,39
125	76,21	2,097
62,5	76,59	1,79
31,125	85,48	1,49
a=80,07		a=162,61
b=-0,042		b=-44,48
Equation	Y=80,069-0,04x	y=162,61-44,48x
IC50	50=80,07-0,04x	50=162,61-44,48x
IC50(x)	707,13	2,53
	Antilog (Concentration)	340 micrograms/mL

The calculation of IC_{50} can be seen that the IC_{50} of doxorubicin is much smaller than the IC_{50} value of the test sample that ethyl acetate fraction ethanol extract *E.longifolia*root which means that doxorubicin has cytotoxic activity against T47D cells were greater than the cytotoxic activity of ethyl acetate fraction ethanol extract *E.longifolia*root. This happens because of doxorubicin is a single compound that functions as an anti-cancer while ethyl acetate fraction ethanol extract *E.longifolia*root is composed of a variety of compounds that efficacy is unknown. So it can be concluded that the ethyl acetate fraction ethanol extract *E.longifolia*root has a lower cytotoxic activity than doxorubicin. In this case, the use of ethyl acetate fraction of ethanol 70%extract of the pasak bumi roots as an alternative to breast anticancer chemotherapy to try to test the co-chemotherapy. Therefore, followed by a testcombination. The combination of both is able to inhibit the growth of T47D cells. It shows that the*E. longifolia* roots able to increase the efficacy of Doxorubicin in T47D breast cancer cells. Furthermore, the result co-chemotherapy test turned out to demonstrate the efficacy of chemotherapy both strong synergistic (Table 3). This study will be of interest to be tested on breast cancer cells because each cell has a different molecular characteristics that would be comparable response to co-chemotherapy applications. The survey results revealed that the cells are so responsive to doxorubicin with results Combination Index (CI) were obtained by 0.23682. These results indicate that the combination of ethyl acetate fraction ethanol extract *E.longifolia*root with doxorubicin has a strong synergistic effect with doses of choice for earth peg root of 255 µg/ml and doxorubicin at 0.00193 µg/ml. Synergy effects of doxorubicin with ethyl acetate fraction ethanol extract *E.longifolia*root presumably through induction of apoptosis mechanisms that need to be observed induction of apoptosis by the two compounds (Maruti *et. al.*, 2011).

Table 3. Test a combination of ethyl acetate fraction ethanol extract of the *E. longifolia*root with doxorubicin

Dose APB	Dose DOX	Effect	CI
340.0	0.00385	0.588	1.09792
255.0	0.00385	0.6931	1.62145
170.0	0.00385	0.7327	1.86813
85.0	0.00385	0.7494	1.93890
340.0	0.00289	0.8691	3.86617
255.0	0.00289	0.9352	8.08517
170.0	0.00289	0.9686	17.1234
85.0	0.00289	0.9832	32.6734
340.0	0.00193	0.4377	0.39897
255.0	**0.00193**	**0.3298**	**0.23682**
170.0	0.00193	0.4787	0.36182
85.0	0.00193	0.5205	0.36896
340.0	9.63E-4	0.6158	0.51428
255.0	9.63E-4	0.7807	0.89105
170.0	9.63E-4	0.7807	0.77817
85.0	9.63E-4	0.4398	0.15628

CONCLUSION

The combination index is 0,23682. This result indicate that combination of pasak bumi roots and doxorubicin have strong synegristic effect with dose selection for pasak bumi roots was amount of 255 µg/ml and doxorubicin was 0,00193 µg/ml.

DISCLOUSURE: -

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