Conference Paper

Potential Anticancer Activity of *Caesalpinia sappan* Linn., in Silico and In Vitro Studies

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Abstract

The bark of *Caesalpinia sappan* Linn. has been used in Indonesian traditional medicine for long time. Current studies on plant chemistry reveal the compound of the bark of CS. Among other compounds, Brazillin is the major flavonoid constituent of *Caesalpinia sappan* Linn. This paper aimed to report a in silico study of Brazillin molecule for its capacity to activate Adenosine Monophosphate Activated Kinase (AMPK) and a in vitro study to examine anticancer potential of methanolic extract of the bark of *Caesalpinia sappan* Linn against breast adenocarcinoma cell line MCF-7. Molecular docking was conducted using AutoDockVina software. The antiproliferative activity was examined with MTT assay. Furthermore, the activity of MeOH extract to induce apoptosis was examined using PI-Annexin V assay. The docking result showed that ' activates the γ subunit of AMPK with the docking score on Asp^{89}, Asp^{244}, Asp^{316} were -7.5 Kal/mol, -8.3 Kal/mol, and -8.6 Kal/mol, respectively. Activation of AMPK exerts antiproliferative effect toward actively dividing cells through inhibition of mTOR complex molecule. The IC50 of the extract toward MCF-7 cells growth was 48 µg/ml. The flowcytometry analysis showed that MeOH extract of *Caesalpinia sappan* Linn induced apoptosis of MCF-7 cell. In conclusion, MeOH extract of *Caesalpinia sappan* Linn showed a potential inhibition activity toward MCF-7 proliferation. Further study to justify the anticancer mechanism of methanolic extract of *Caesalpinia sappan* Linn. is required.

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1. Introduction

Caesalpinia sappan Linn. (CS) is a tropical plant distributed widely across the Southeast Asia region. The use of the bark of CS in Indonesian traditional medicine has been documented in the Javanese literature of traditional medicine [1]. *Caesalpinia sappan* Linn. contains various compound of phenolic components including xanthone, coumarin, chalcones, flavones, homoisoflavonoids which show various therapeutic activity [2]. Investigation of the potential use of CS for treatment of infection, metabolic and degenerative diseases has been reported in the last decade. Brazilin is considered as the

major compound of CS. Nirmal *et al.* [3] reported antibacterial, antioxidant, and anti-inflamatory activity of brazilin, one of homoisoflavonoid content of CS. The molecular structure of brazilin has a similarity with Adenosine Monophosphate (AMP). Adenosine monophosphate is molecule which activates Adenosine monophosphate activated kinase (AMPK), a key player which regulates cells' bioenergetics.

The role of AMPK activity in carcinogenesis is currently identified. The decrease in AMPK activity correlates with the increase in protein synthesis which promotes cells proliferation in cancer cells [4]. The decrease of AMPK activity on breast cancer has been reported; therefore, modulation of AMPK activity by an AMPK activator could be a therapeutic target for combating breast cancer.

This research involved the use of molecular docking program to investigate the potential of brazilin to activate AMPK. Activation of AMPK exerts an antiproliferative activity against actively divided cells. Furthermore, in vitro study was conducted to examine the antiproliferative activity of CS extract toward the growth of carcinoma mammae cell line (MCF-7).

2. Material and Method

2.1. In silico study

In silico study was conducted using AutodockVina 1.1.2 version software analysis to get the drug-receptor binding energy. The structure of Adenosine Monophosphate Activated Kinase (AMPK) and Adenosine Monophosphate (AMP) molecules were retrieved from Protein Data bank (www.rscb.org). Open Babel program was used for the conversion of virtual format into formatted data compatible with AutodockVina 1.1.2 software. The structure of brazilin molecule was retrieved from pubChem NCBI.Chimera 1.9 version was used for visualisation of moleculare complex.

2.2. Sample preparation

Caesalpinia sappan Linn. heartwood was collected from herb store in Surakarta region. The extraction was conducted using maceration method to get brazilin rich extract according to the method reported by Nirmal et al [3]. *Caesalpinia sappan* Linn. heartwood was air dried and ground prior to maceration using methanol. An amount of 500 g of grounded CS heartwood was macerated in 2.5 L of methanol for 3x24 h. The extract were filtered using Whartman No.2 and the filtrate was then concentrated in vacuo at

30 °C using a rotary evaporator. The final yield was 43.0 g of darkbrown solid extract. The tested sample was prepared by dissolving the extract using DMSO. The final DMSO consentration on the tested sample was adjusted into 0.1%.

2.3. Cytotoxic assay

Breast-adenocarcinoma cell line MCF-7 were cultured according to standard protocol. The medium was prepared by mixing Dulbecco's modified Eagle's medium (DMEM) with 10% FBS and 2 mM L-glutamine, and 1% penicillin/ streptomycin (PenStrep). Exponentially growing MCF-7 cells were seeded on 96-well plates at 5×10^4 cells per well and incubated for 24 h prior to addition of the extract. Following a 24 h incubation at 37 °C, 5% CO_2, the media was replaced with 100 µL culture media containing series of concentrations of MeOH extract of *Caesalpinia sappan* (250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml, and 7.813 µg/ml). Each tested concentration was repeated in triplicate and cells were further incubated for 24 h. The MTT assay was conducted following the standard procedure to measure the inhibition activity of tested sample toward MCF-7 cells proliferation.

2.4. Apoptosis assay

Apoptotic cells population was determined using PI-Annexin V assay (Annexin V-FITC Apoptosis Detection Kit Roche). A number of 5×10^4 cells/well were seeded in 6-well plate and incubated for 24 hour at 37 °C and 5% CO_2. The IC50 concentration of tested sample were used to investigate the apoptosis activity on MCF-7. After the 24 hours incubation, cells were treated with IC50 concentrations of sample for 24 h. The apoptosis assay was conducted according to manual protocol using flowcytometry (Biorad).

3. Result and Discussion

3.1. In Silico study

A prior molecular docking study screened over 6776 active compounds derived from 3810 herbal plants from Indonesia. The results pointed out one active compound (brazilin) derived from *Caesalpinia sappan* Linn., which is expected to act as an AMPK activator. This study measured the interaction of each active compound and AMPK by comparing AMP and AMPK interaction in three binding sites which are Asp89, Asp244,

and Asp316. Before screening thousands of active compounds, it is important to validate affinity and interaction between AMP and AMPK by using AutoDock Vina. The validation plays a role as the cut-off in determining the docking score between active compounds and AMPK. The validation results showed docking score of AMP interaction with AMPK on Asp89, Asp244, Asp316 of γ subunit were: -7.5 Kal/mol, -8.3 Kal/mol, and -8.6 Kal/mol, respectively. Furthermore, the docking score of brazilin interaction with Asp89, Asp244, Asp316 of γ subunit of AMPK were: -8.5, -9, and -9 Kal/mol, respectively. The scores of brazilin-AMPK interaction confirmed that brazilin is a potential AMPK activator. The comparison between the docking score of AMP and brazilin was shown in Table 1, while the molecular interaction between brazilin and γ subunit of AMPK molecule was visualized in Figure 1.

Table 1: The docking score between brazilin-AMPK and AMP-AMPK.

Active Compunds	*PubChem* ID	Melecule Weight (g/mol)	*Docking Score* (Kal/mol)		
			Asp^{89}	Asp^{244}	Asp^{316}
AMP	6083	347.221222	-7.5	-8.3	-8.6
Brazilin	73384	286.27936	-8.5	-9	-9

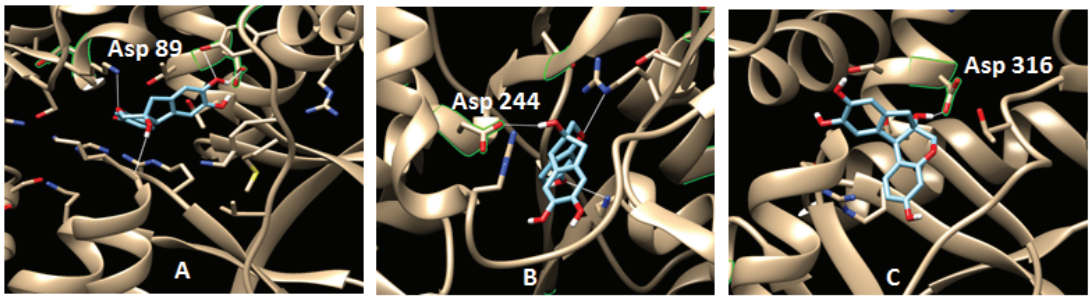

Figure 1: Interaction of brazilin with γ subunit of AMPK. A. Interaction of brazilin molecule with site 1 of AMPK (Asp^{89}). B. Interaction of brazilin molecule with site 3 of AMPK (Asp^{244}). C. Interaction of brazilin molecule with site 4 of AMPK (Asp^{316}).

Activation of AMPK regulates the use of cellular energy. Several cancer cells ignore the cellular bioenergetics signals therefore cancer cells continue to proliferate in a diminished nutrition condition [5, 6]. The increase of AMPK activation on cancer cells will drive the cells to undergo autophagy and apoptosis.

3.2. Antiproliverative assay

The effect of MeOH extract of CS heartwood on MCF-7 proliferation was examined using MTT assay method. The cells were treated with serial concentration of the extract (250

µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml, and 7.812 µg/ml). The IC50 concentration was calculated using formula:

$$IC50 = ((X2-X1) \times (50-Y1)/(Y2-Y1)) + X1,$$

where X1 and X2 are the higher and lower used concentrations bordering the 50% cell viability, while Y1 and Y2 are the mean percentages of viable cells at the X1 and X2 concentrations, respectively [7]. The dose-dependent effect of MeOH extract of *Caesalpinia sappan* Linn. on MCF-7 cell line is shown in Figure 2. The IC50 of MeOH extract of CS toward MCF-7 proliferation was 48µg/ml. This finding confirmed the anticancer potential of MeOH CS extract.

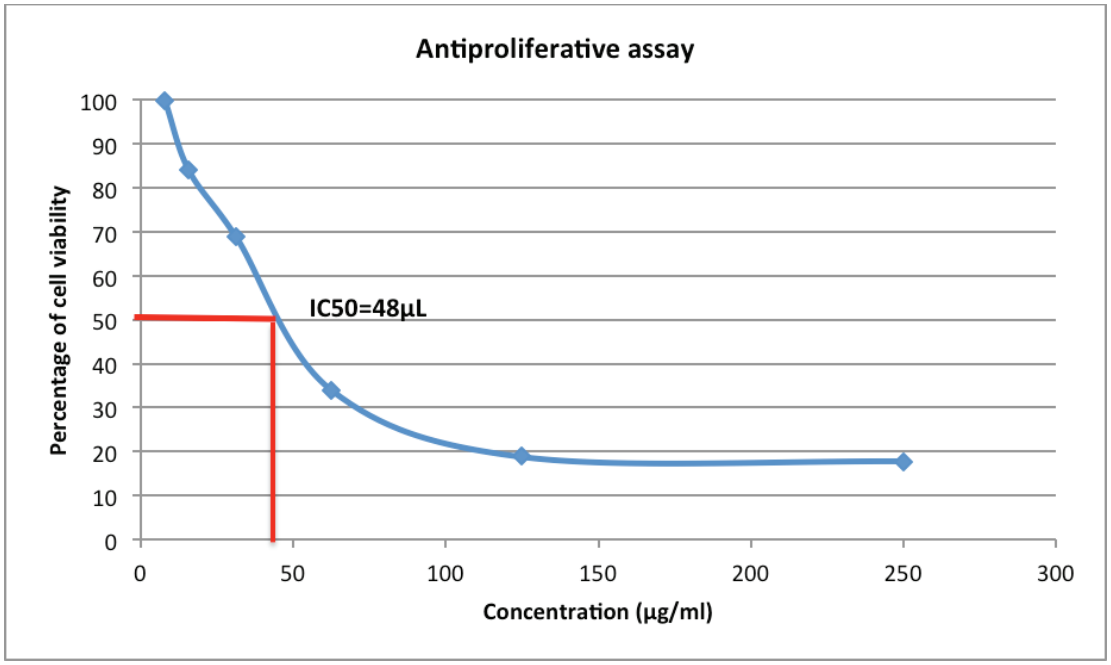

Figure 2: Antiproliferative activity of MeOH extract of *Caesalpinia sappan* Linn. heartwood toward MCF-7 cell. The dose dependent effect of MeOH extract of *Caesalpinia sappan* Linn.

3.3. Apoptosis induction of MeOH extract of Caesalpinia sappan heartwood toward MCF-7 cells

The changes in MCF-7 cell morphology on treated cells as compared to the untreated cells indicated the activity of the extract to induce cell death. The changes in morphology of MCF-7 cells treated with the IC50 dose of the extract as compared to the untreated cells was showed in Figure 3. Flowcytometry analysis confirmed this assumption, as can be seen in the Figure 4, there was an increase proportion of cells undergoing apoptosis after 24 hours incubation with IC50 dose of MeOH extract of CS. The flowcytometry analysis showed that the cell populations undergoing early and late apoptosis were

higher in the treated cell as compared to the cell population on the untreated cell. As can be seen on the flowcytometry data showed in Figure 4, the decrease of cells viability after treatment with the IC50 dose of MeOH CS extract was resulted from apoptosis. This data indicated that methanolic extract of CS induce apoptosis in MCF-7 breast adenocarcinoma cells line.

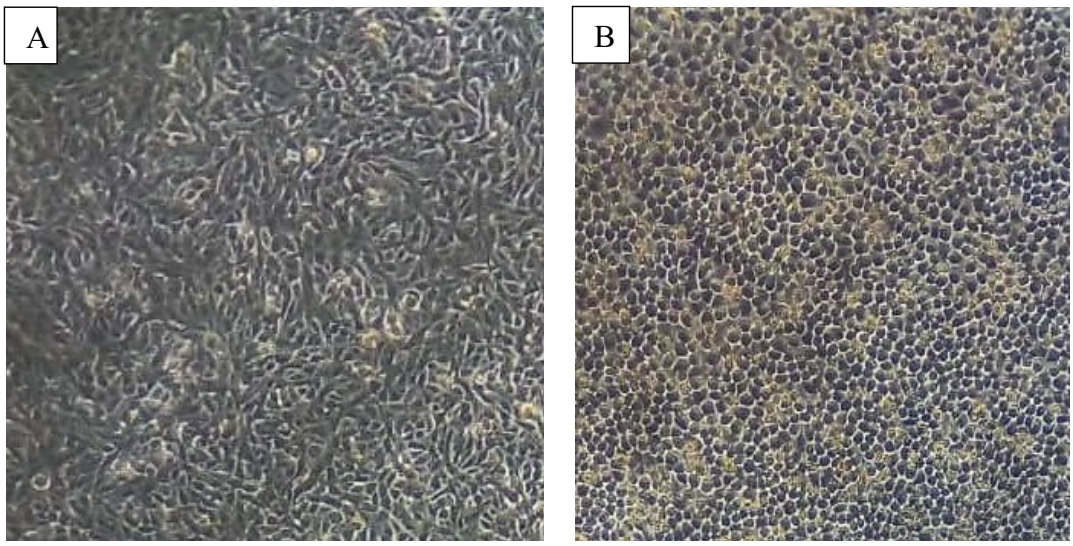

Figure 3: Morphology of cultured MCF-7 cells. A. Control cells (untreated cells). B. MCF-7 cells treated with IC50 dose (48 µg/ml) of MeOH *Caesalpinia sappan* Linn. heartwood extract. The changes in morphology of MCF-7 cell can be observed on the MCF-7 cell treated with 48 µg/ml of MeOH extract of *Caesalpinia sappan* Linn heartwood.

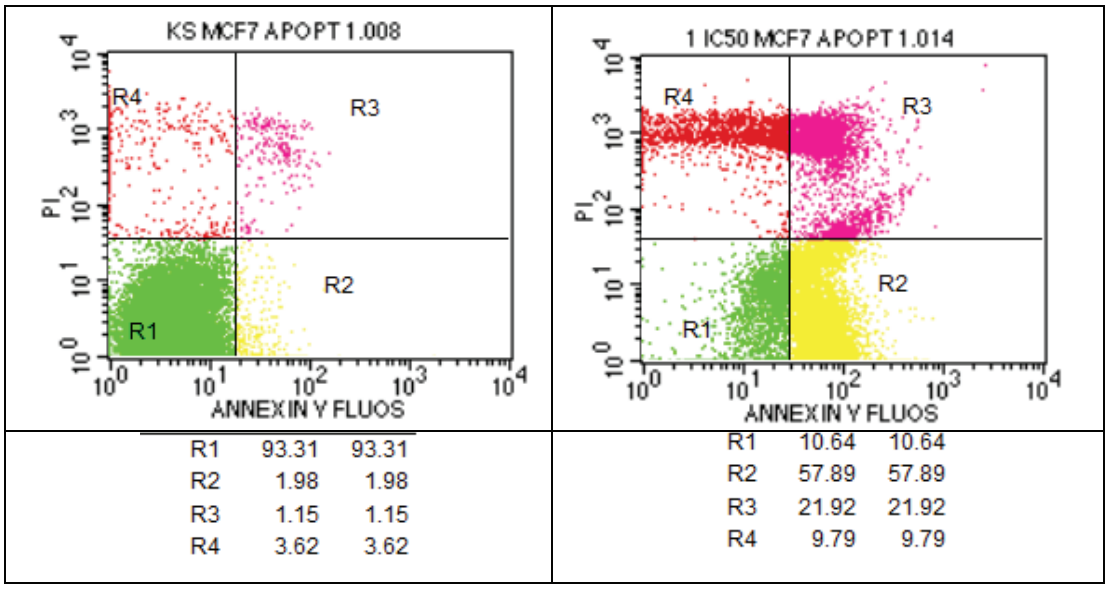

Figure 4: Flowcytometry analysis of MCF-7 cells. R1. Population of viable cells. R2. Early apoptotic cells, R3. Late apoptotic cells, R4. Necrotic cells.

4. Conclusion

The docking score of interaction between brazilin and AMPK molecules confirmed that brazilin is a potential AMPK activator candidate. The in vitro study showed the potency of MeOH extract of CS for anticancer.

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