Conference Paper

Sinensetin-Rich Fraction Solid Dispersion Inhibits Cancer Cell Cycle

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Abstract

Drug development efforts to find new selective and safe drugs for cancer from natural resources are promising ones. The natural products are obtained in the multiple or single compounds. One of them is a sinensetin found in ethanol extract of *Orthosiphon stamineus* Benth. Sinensetin could inhibit cancer cell proliferation. However, it has a poor solubility so the absorption is low and then it impacts on the low bioavailability. The solubility problem in conventional drug could be solved by pharmaceutical manipulation. In the previous research, the manipulation was tried although there was no single compound found in the material tested. We found an optimal formula of a manipulation using solid disperse system of polyethylene glycol (PEG 6000) 15 times higher than sinensetin weight. This research was focused on observing the effect of the optimal formula of solid disperse system to inhibit cancer cell cycle. The cell lines used were T47D cells. The method used was flow cytometry. The result showed that the optimum formula has a consistent effect on the concentration of 40 and 60µg/mL. The sinensetin increase cell accumulation on S phase at the percentage of 18.80% (40µg/mL) and 22.21% (60µg/mL) compared to T47D normal cells. It reflects the S phase as the longest time experienced by the cells. Inhibition on S phase (S arrest) resulted from a DNA elongation. It causes an inhibition of DNA synthesis process. It could be concluded that the solid disperse of sinensetin was active to inhibit cancer cells proliferation on phase S.

Keywords: cell cycle; sinensetin; solid disperse; poor solubility.

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1. Introduction

The efforts to treat cancer patients until today are focused on inhibiting and killing the growth of cancer cells. The ideal drug is the selective one with the minimal side effect. Drug development to obtain this ideal drug is in progress. The potential materials in Indonesia are natural resources. The bioactive compounds would be a lead compound

to find an effective drug for cancer. One of a bioactive compound found in the medicinal plant is sinensetin, which is specifically found in *Orthosiphon stamineus* Benth.

Sinensetin is one of the active markers in *Orthosiphon stamineus* Benth. It has an antibacterial and anticancer effect [1]. The previous study reported the activity of *Orthosiphon stamineus* Benth. extract inhibits oral cancer cells on human [2]. It supported by another research states that the methanol extract of this plant has a capacity to increase tamoxifen effect on breast cancer cell (MCF 7) proliferation [3]. However, the sinensetin has a limitation on the solubility which it is partially soluble in water. The data showed the Log P of sinensetin is 2.98 in n-butanol-water [4]. This poor solubility causes the low absorption and the rate of dissolution result in a bad bioavailability.

This limitation could be solved by using a solid disperse system. This system is a system of a solute dispersed in another material or dispersing agent. The optimum formula of sinensetin solid dispersion has found in the previous research. This research focused on the activity of the optimum formula in each phase of cancer cell cycle. The doses used were 40 and 60µg/mL to know whether the effect is quantal or gradual. By using this method, we observed the main mechanism of sinensetin in solid disperse form to inhibit the cancer cell growth.

2. Materials and methods

2.1. Materials

The *Orthosiphon stamineus* Benth leaves used was obtained from Balai Materia Medika, Malang, East Java.

The chemical used was n-hexane p.a., dichloromethane p.a., ethyl acetate p.a., chloroform p.a., PEG 6000 (Merck), RPMI 1640 (Gibco, Invitrogen), Fetal Bovine Serum (FBS) (Sigma), Penicillin-Streptomycin (Sigma), Amphotericin B (Sigma), Dimethyl sulfoxide (DMSO) (Sigma), PBS (Phosphate Buffer Saline) (Sigma), Annexin V, Propidium iodide (PI).

2.2. Methods

2.2.1. Sinensetin-rich fraction

The fractionation method used was remaceration by utilizing three level of polarity. The first solvent used was n-hexane to minimize the lipophilic compounds and

to separate the chlorophylls. The second maceration was using a combination of dichloromethane: ethyl acetate at a ratio of 1:1. The result was predicted as a fraction with the highest content of sinensetin. Moreover, the ethanol was used to obtain polar metabolite which did not contain sinensetin or other poly methoxy flavones. The phytochemical analysis was performed using thin layer chromatography (TLC), for observing the sinensetin profile.

2.2.2. Solid dispersion formula of sinensetin-rich fraction

The second fraction was diluted in ethanol, and then the PEG 6000 was added in the solvent at the weight of 10, 15, 20 and 25 times higher than the fraction weight. Each formula was evaporated until the solid disperse was formed. The optimum formula was known on the previous research, so the ratio of PEG 15 times higher than the fraction used as the standard formula for this research.

2.2.3. Measurement of inhibition of cycle cell proliferation by means of flow cytometry method

This observation was started by preparing cancer cells at the number of 1 x 105 cell/mL in 6 well-plate, and then it was incubated overnight. It resulted in mature cells in a lag phase that was ready for drug testing. Then, the solid disperse of sinensetin-rich fractions at both doses were tested on these cells in each well and then it was incubated overnight. After the incubation, the cells were washed by using PBS, and then the trypsin-EDTA was added. Then, it was incubated for 5-8 minutes. The cells were moved from the wells into the tubes. It was fixated using cold ethanol for 10 minutes. It was centrifuged for 3-5 minutes at 2000 rpm. The supernatants were removed, the 1 mL PBS was added. Then the samples were used for further staining and read using flow cytometer [5].

3. Results and Discussion

3.1. The phytochemical analysis

Chromatography fingerprint (profile) was performed to describe components found in a sinensetin-rich fraction. The method was thin layer chromatography (TLC). The single compound standard was pure sinensetin (Sigma). The results and the fluorescence

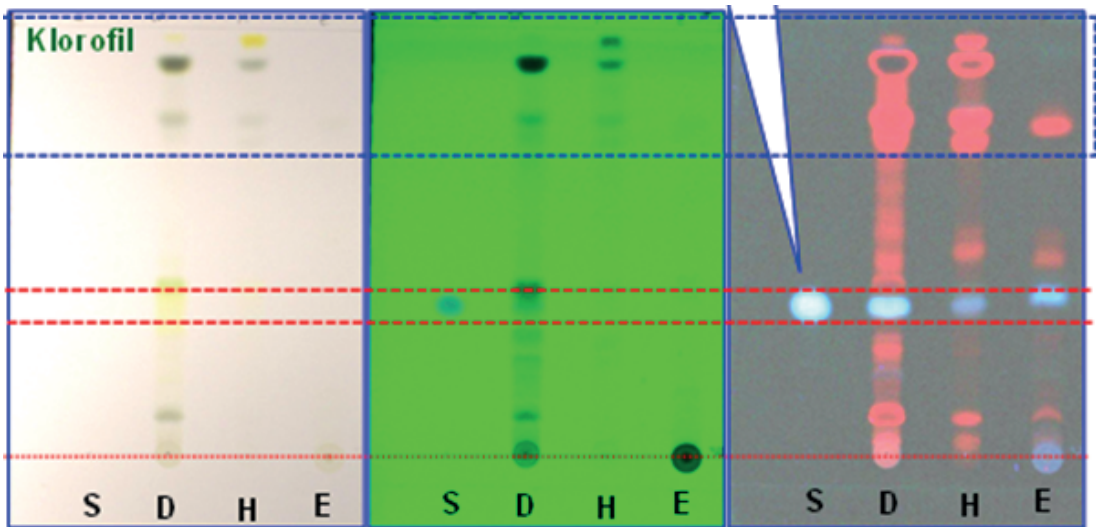

Figure 1: Visualisation of TLC results of sinensetin (S), a sinensetin-rich fraction (D), n-hexane fraction (H), and ethanol fraction (E) of *Orthosiphon stamineus* Benth under a solvent system of chloroform:ethyl acetate 15:1 [6].

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	1	0.37 Rf	3.7 AU	0.42 Rf	401.6 AU	100.00 %	0.48 Rf	5.3 AU	16067.3 AU	100.00 %	AutoGenerated1
2	1	0.07 Rf	4.7 AU	0.09 Rf	41.2 AU	2.99 %	0.10 Rf	2.1 AU	636.4 AU	1.14 %	unknown *
2	2	0.13 Rf	0.7 AU	0.15 Rf	157.6 AU	11.44 %	0.19 Rf	4.0 AU	3240.0 AU	5.79 %	unknown *
2	3	0.19 Rf	4.4 AU	0.21 Rf	52.2 AU	3.79 %	0.23 Rf	39.6 AU	1157.8 AU	2.07 %	unknown *
2	4	0.23 Rf	39.6 AU	0.25 Rf	119.9 AU	8.70 %	0.28 Rf	0.5 AU	2916.5 AU	5.21 %	unknown *
2	5	0.29 Rf	4.8 AU	0.30 Rf	22.3 AU	1.62 %	0.31 Rf	17.8 AU	320.8 AU	0.57 %	unknown *
2	6	0.32 Rf	18.4 AU	0.46 Rf	415.9 AU	30.19 %	0.51 Rf	1.2 AU	28407.9 AU	50.75 %	AutoGenerated1
2	7	0.54 Rf	14.4 AU	0.58 Rf	27.8 AU	2.02 %	0.62 Rf	2.0 AU	1097.5 AU	1.96 %	unknown *
2	8	0.68 Rf	1.0 AU	0.78 Rf	116.7 AU	8.47 %	0.80 Rf	34.1 AU	5532.1 AU	9.88 %	unknown *
2	9	0.80 Rf	94.4 AU	0.83 Rf	186.8 AU	13.55 %	0.85 Rf	37.4 AU	6114.3 AU	10.92 %	unknown *
2	10	0.86 Rf	138.6 AU	0.88 Rf	237.4 AU	17.23 %	0.92 Rf	0.6 AU	6556.4 AU	11.71 %	unknown *
3	1	0.13 Rf	0.3 AU	0.15 Rf	13.6 AU	3.10 %	0.17 Rf	0.1 AU	253.2 AU	1.57 %	unknown *
3	2	0.27 Rf	9.1 AU	0.34 Rf	35.5 AU	8.12 %	0.37 Rf	8.7 AU	1772.4 AU	10.99 %	unknown *
3	3	0.39 Rf	6.1 AU	0.47 Rf	130.2 AU	29.75 %	0.51 Rf	1.4 AU	5573.7 AU	34.57 %	AutoGenerated1
3	4	0.53 Rf	1.2 AU	0.56 Rf	13.3 AU	3.05 %	0.59 Rf	6.4 AU	391.1 AU	2.43 %	unknown *
3	5	0.74 Rf	29.5 AU	0.82 Rf	107.1 AU	24.46 %	0.85 Rf	38.5 AU	4650.0 AU	28.84 %	unknown *
3	6	0.85 Rf	39.0 AU	0.88 Rf	137.9 AU	31.52 %	0.91 Rf	0.1 AU	3484.4 AU	21.61 %	unknown *
4	1	0.07 Rf	47.2 AU	0.09 Rf	107.3 AU	20.75 %	0.10 Rf	4.9 AU	1666.0 AU	13.45 %	unknown *
4	2	0.16 Rf	0.2 AU	0.22 Rf	80.0 AU	15.46 %	0.25 Rf	12.7 AU	2183.7 AU	17.62 %	unknown *
4	3	0.25 Rf	12.7 AU	0.26 Rf	22.6 AU	4.38 %	0.28 Rf	0.4 AU	359.4 AU	2.90 %	unknown *
4	4	0.41 Rf	1.0 AU	0.46 Rf	123.0 AU	23.77 %	0.47 Rf	37.5 AU	3474.9 AU	28.04 %	AutoGenerated1
4	5	0.48 Rf	107.4 AU	0.49 Rf	112.7 AU	21.78 %	0.53 Rf	1.2 AU	2666.6 AU	21.52 %	unknown *
4	6	0.83 Rf	17.1 AU	0.87 Rf	71.7 AU	13.86 %	0.90 Rf	6.6 AU	2039.8 AU	16.46 %	unknown *

Figure 2: The calculation results of chromatogram peaks using Camag Scanner 3, Densitometer, on a maximum wavelength of sinensetin 334 nm.

were read on a silica gel GF 254 under direct observation, and UV 254 nm and UV 366 nm observation by means of TLC visualizer (Figure 1).

Table 1: Cell cycle observation of sinensetin-rich fraction using FACS.

Cell phase	Cell accumulation %)		
	Control	40 µg/mL	60 µg/Ml
Total	100	100	100
M1	12.04	11.36	8.79
Go-G1	39.85	32.66	32.42
S-phase	15.40	18.80	22.21
G2-M	17.71	18.32	17.95
M5	15.60	18.27	17.48

3.2. The cell cycle inhibition

The cytotoxic activity of the solid dispersion of sinensetin-rich fraction resulted from a modulation of cell cycle and apoptosis. The modulation of cancer cell cycle, such as T47D could be observed by using the profile of cell distribution on each phase, after the samples treated by using a sinensetin-rich fraction.

The analysis was done by calculating the percentage of cell population distributed in each phase of the cell cycle after treatment. The normal cell cycle has some differences on the number of chromosome sets, such as the chromosome sets of G1 phase is 2n. In the S phase, the chromosomes sets are between ranges of 2n-4n. On the G2 and M phase, the replications forms chromosome sets 4n perfectly. These chromosome sets also happen to T47D cells [7, 8]. The specific measurement has been obtained. Fluorochrome could intercalate DNA base. It is like propidium iodide. Therefore, the cells which have the different number of chromosome sets would give different fluorescence intensity. The higher number of chromosome sets, the higher fluorescence intensity would appear [9, 10].

The instrument used to read the intensity on each cell is FACS (Fluorescence Activated Cell Sorting) or flow cytometer. The data obtained is analyzed by facilitating of BD CellQuest. The profile of cell cycle and distribution of T47D cells after treatment are shown in Table 1.

The progressivity of cell cycle was regulated by some check point in each phase. This check point was the main control of cell proliferation. After treatment using solid disperse of sinensetin-rich fraction, the result showed an increasing of cell accumulation on S phase on the percentage of 18.80% (40µg/mL) and 22.21% (µg/mL) compared to cell control. It means that the S phase is the longest phase experienced by the cells. An

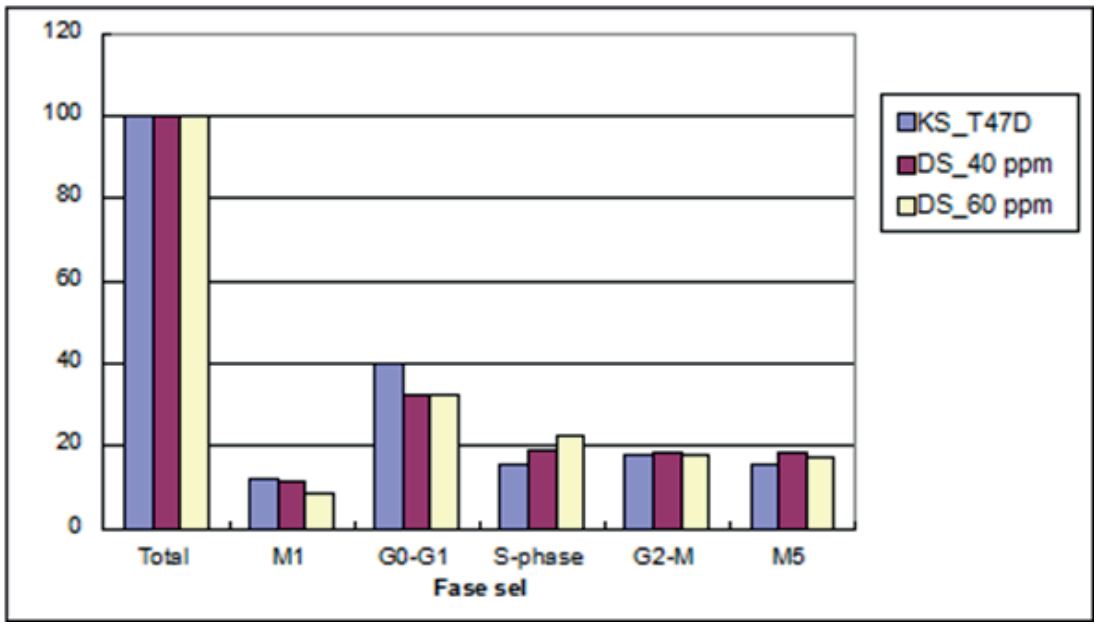

Figure 3: The histogram of T47D cell proliferation after receiving two concentrations of sinensetin-rich fraction in the solid disperse form .

inhibition of S phase (S arrest) resulted from a DNA elongation. It caused an inhibition on DNA synthesis process.

Acknowledgements

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