



# ASEAN FOOD SCIENCE AND TECHNOLOGY: COOPERATION AND INTEGRATION FOR DEVELOPMENT

PROCEEDINGS OF THE 8th ASEAN FOOD CONFERENCE  
8-11 October 2003, Hanoi, Vietnam

*Edited by*

Le Doan Dien, Nguyen Kim Vu, Nguyen Duy Lam

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# THE EFFECT OF SECANG WOOD (*CAESALPINIA SAPPAN* LINN) EXTRACTS ON THE MOUSE SPLENOCYTES AND K-562 CELLS PROLIFERATION

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**Abstract:** Secang wood is used as a raw material in many traditional medicines and healthy drinks. The specific component brazilin, an isoflavone, is known to have antioxidant and antibacterial activities. Phenolic compound which has antioxidative activity is expected that it can protect cell from damage and death. On the other hand, some of phenolic compounds had been proven that it had antiproliferative activity and toxic effect to cancerous cell. The aim of this research was to know the effects of secang wood extracts on the cell proliferation in vitro, especially mouse splenocyte and K-562 cell line.

This study showed that all of supplemented levels (0.1, 1, 5, 10 and 20 x C; which C was estimated equivalent to daily consumption dosage) of secang wood extracts (methanol, ethanol and water extract) resulted in higher splenocytes proliferative index than that of the control (the cells without any secang wood extracts added). The methanol extract resulted in the highest proliferative index, followed by ethanol extract and then water extract.

At low supplemented level (0.1 x C), the methanol, ethanol and water extracts showed to depress K-562 cells proliferation, but at the higher levels they were not toxic to the cells. Methanol and ethanol extracts, however, demonstrated very highly proliferative. This result suggested that there was a growth factor compound in secang wood extract which was high soluble in methanol and ethanol.

## INTRODUCTION

Secang wood (*Caesalpinia sappan* Linn) is not only commonly used as food colorants, but also has been used as a raw material in many traditional medicines for a long period of time, such as traditional healthy drinks. As a component of traditional healthy drinks, secang wood is known to be effective as antitussive, anti-cold, and antiinflammation. The major chemical components of secang wood are phenolic compounds tannin (tannic acid), gallic acid, resorzin, brazilin and sappanin (Heyne, 1987). Brazilin ( $C_{15}H_{14}O_2$ ), the yellow crystalline isoflavone, is known to have antioxidant and antibacterial activities (Subartati, 1983). If it is oxidized, it can be transformed into a reddish-brown brazilein, which is highly soluble in water.

Water extract of secang wood has a higher antioxidant index than that of BHA and BHT (Lim, 1997), expected it can protect cell from damage and death. Some phenolic compounds, such as tea catechin and curcumin, have immunoproliferative activity, were due to their antioxidant capacity. Meanwhile, these phenolic compounds showed antiproliferative capacity and toxic to cancer cells.

Because of its high potency to be developed as functional foods, it is necessary to investigate the effects of secang wood extracts on the cell proliferation. In this study, we observed its effects on mouse splenocyte and K-562 (chronic myelogenous leukemia) cell proliferation. Being expected, secang wood extract is not toxic on splenocytes representing as a normal cell, and it has an antiproliferative activity to K-562 cells. The amounts of cell that proliferated were assayed by MTT technique.

## MATERIALS AND METHODS

### Extraction

Secang woods flakes were purchased from local retailer in Bogor-Indonesia. They were then crushed by using a disc mill to obtain secang powder. In this study, there were three kinds of secang wood extracts (methanol extract, ethanol extract, and water extract) that assayed.

To obtain ethanol extract and methanol extract, secang powder were percolated in ethanol or methanol, which were containing 1% HCl each, for 24 hours at ambient temperature. The ratio of secang



powder to the solvent was 1:5 (w/v). The suspensions were then filtered by Whatman no.1 filter paper. The filtrates were concentrated and its solvent residue were eliminated by using a vacuum rotary evaporator at 50°C and then were freeze-dried.

To obtain water extract, secang wood powder water was boiled in aquades (1:10) during 10 minutes. After was filtered by Whatman no.1 filter paper, the filtrate was freeze-dried.

### Extract preparations

Secang wood extracts were dissolved in RPMI-1640 medium (Sigma, USA) and were sterilized by 0.22 µm Millipore membrane. This medium was also used to dilute the extracts. The supplemented levels of these extracts in the cell culture were 0.1 × C, 1 × C, 5 × C, 10 × C and 20 × C. C was estimated equivalent to daily consumption dosage of 'wedang secang' (5 g secang powder boiled in 250 ml water), without considering any loss amount during absorption. Table 1 showed the 1xC concentration levels of the three kinds of extracts.

### Splenocyte proliferation activity

The splenocyte stimulation assay was modified from Gery *et al.* (1972). Spleens from male BALB/c mice were removed aseptically and a single cell suspension was prepared by teasing apart spleens in RPMI-1640 medium. The cells were then treated with 0.85% ammonium chloride/l to lyse erythrocyte. The splenocytes were then resuspended in a sterile RPMI-1640 medium which was supplemented with gentamycin (1% v/v) and NaHCO<sub>3</sub> (0.2%; w/v). The splenocytes (50 µl; 1 × 10<sup>6</sup> sel/ml) were cultured in triplicates with 20 µl FBS and 80 µl secang wood extract for a total 30 h incubation in 5% CO<sub>2</sub> (37°C) chamber. The cells were cultured in 96 well flat-bottomed microplate.

The total amounts of proliferated cells were assayed by MTT technique. Briefly, six hours before incubation period ended, culture was added with 10 µl MTT (0.5% in PBS) solutions. At the end of the incubation period, 100 µl HCl-isopropanol 0.04 N was added to each well and then the optical density (OD) of the cultured cells was read at 570 nm using microplate reader. The proliferated cells were counted relative to the controls (the cells without any secang wood extracts added). The proliferation activity was expressed by proliferative index (P.I) as followed:

$$P.I = OD \text{ cultured cells with added extract} / OD \text{ control cells.}$$

### K-562 cell proliferation activity

The K-562 cells (50 µl; 1 × 10<sup>5</sup> sel/ml) were cultured in triplicates with 20 µl FBS and 80 µl secang wood extract for a total 24 and 48 hours incubation periods in 5% CO<sub>2</sub> (37°C) chamber. The cells were cultured in 96 well flat-bottomed microplate. After the incubation period, the total amounts of proliferated cells were assayed by MTT technique (as described previously).

## III. RESULTS AND DISCUSSION

### Extraction

Table 1. The yields of secang wood extracts by solvent extractions and the amount of extract that supplemented in the cell culture (g/ml medium) at 1x concentration level

Solvents	Yields extracts (%; w/w)	The 1 x C supplemented level of extract that in the cell culture (g/ml medium)
Ethanol	2.77	4.3 × 10 <sup>-5</sup>
Methanol	6.64	10.4 × 10 <sup>-5</sup>
Water	1.49	2.3 × 10 <sup>-5</sup>

Brazilin, the specific component in the secang wood, was high soluble in alcohol and water. This study showed that the highest extract yield was obtained from extraction secang wood powder by methanol, followed by the ethanol extract and the water extract (Table 1). Treated ethanol or methanol by acid (HCl 1%) was done in order to increase the extraction efficiency, because of its ability to denature plants cells membrane and to leach pigments out of the cells. Acid-treated methanol with 10% HCl produced the higher yields than that with 1% HCl (Metivier *et al.* 1980). In food research field, it was



ough to extract with 1% HCl, but needed 24 hours for extraction process (Francis, 1982). HCl-treated anol had less effective than that of methanol, but it was more appropriate for food application because the methanol toxicity (Markakis, 1982).

### Splenocytes and K-562 cells proliferation activity

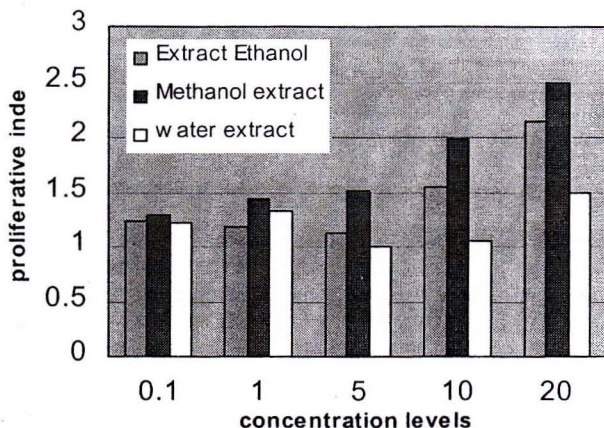


Figure 1. Proliferation of splenocytes that cultured 30 h with secang wood extracts

Figure 1 showed that the supplementation of methanol, ethanol and water extracts in cultured splenocytes resulted in higher proliferative index than that of the control (the cells without any secang wood extracts added). The supplementation with high level concentrations of secang wood extracts did not cause cell death or not toxic to these cells. The methanol extract resulted in the highest proliferative index, followed by ethanol extract. This result suggested that there was a proliferative cell stimulator compound which was high soluble in methanol and ethanol.

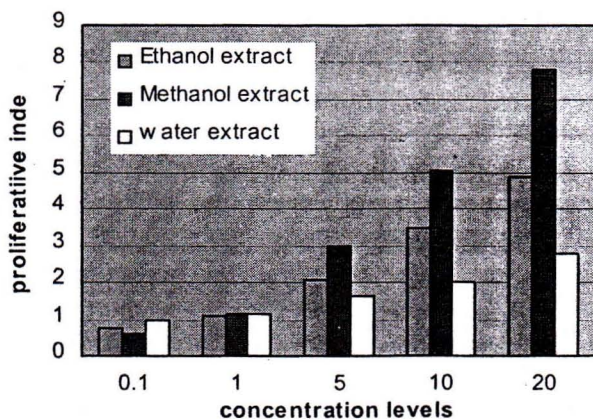


Figure 2. Proliferation of K-562 cells that cultured 24 h with secang wood extracts

Figure 2 showed that at low supplemented level (0.1 x C) the methanol, ethanol and water extracts suppressed K-562 cells proliferation, but at the higher levels they were not toxic to these cells. Methanol and ethanol extracts, however, demonstrated very highly proliferative. These results did not show any suggested bioactive compound which has immunoproliferative but antiproliferative properties to K-562 cells. Because of the high proliferative index was suggested that the unknown compound was a quinone and had ability as a growth-factor.

The study of Steinberg *et al.* (1994) reported that pyrroloquinoline quinone (PQQ; 4,5-dihydroxy-5,8-dimethyl-2,3,6,7-tetrahydro-1H-pyrido(2,3-f)quinoline-2,7,9-tricarboxylic acid) and PQQ-like compounds in animals and plants, was a growth factor and has antioxidant activity. The secang wood extracts had reddish-brown color, indicated that brazilin was oxidized to brazilein (quinone-like) suggesting has the ability as a growth factor. It needs further investigations.



PQQ was a redox cofactor for alcohol and glucose dehydrogenases. Because of this capacity, PQQ could be detected by nitroblue tetrazolium assay. Initially, considering the high proliferative index, there was a suspected compound that had a capacity as a redox cofactor that's like PQQ, which contributed to total amount of formazan formed. Figure 2 and Figure 3 showed that there were significantly difference of proliferative index resulted from 24 hours and 48 hours incubation times.

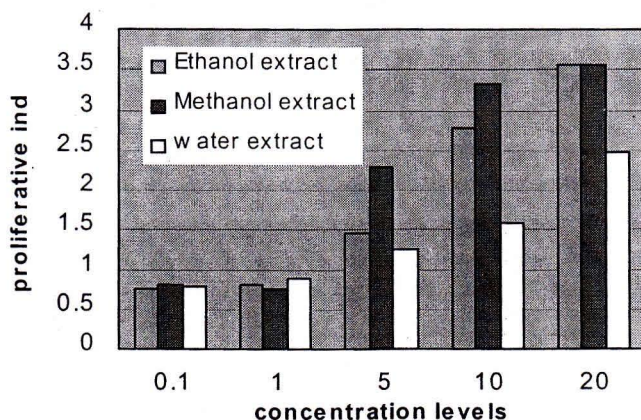


Figure 3. Proliferation of K-562 cells that cultured 48 h with secang wood extracts

Our work showed that the tannin content of the methanol extract was lower than that of the ethanol extract and the water extract (data is not shown). The tannin content of the water extract was the highest, approximately 4.5 higher than the methanol extract. On the other hand, Figure 1-3 showed that water extract had the lowest proliferative index. It suggested that the compound which was responsible on stimulating cell proliferation was not tannin.

#### IV. CONCLUSION

In vitro supplementation of secang wood extracts (methanol, ethanol and water extract) at high concentration level did not cause splenocytes that cultured death. It showed that these extracts were not toxic. However, at low supplemented level (0.1 x C) these extracts showed to depress K-562 cells proliferation, while at higher concentration levels showed to increase it. It recommended not exceeding to consumption secang wood extract.

Methanol extract showed the highest potency to increase cells proliferation, both splenocytes and K-562 cells, followed then by ethanol extract and water extract. Our suggestion that might be there was a growth-factor-like compound in secang wood extracts which had high polarity.

#### REFERENCES

- Francis, F.J., (1982), Analysis of Anthocyanins. In: Anthocyanins as Food Colors. Markakis, P. (ed.). Academic Press, New York.
- Gery, I., Gershon, R.K. and Waksman, B.H., (1972), Potentiation of the T-lymphocyte response to mitogens. J. Exp.Med. 136: 128-138.
- Heyne, K., (1987), Tumbuhan Berguna Indonesia (The useful plants in Indonesia). Translated. Badan Litbang Kehutanan, Jakarta.
- Lim, D.K., Choi, U. and Shin, D.H., (1997). Antioxidative activity of some solvent extract from *Caesalpinia sappan* Linn. Korean Journal of Food Sci and Tech; 28 (1) 77-82.
- Markakis, P., (1982). Anthocyanins as Food Additives. In: Anthocyanins as Food Colors. Markakis, P. (ed.). Academic Press, New York.
- Metivier, R.P., Francis, F.J. and Clydesdale, F.M., (1980). Solvent Extraction of Anthocyanins from Wine Pomace. J.Food Sci. 45: 1099-1100.
- Steinberg, F.M., Gershwin, M.E. and Rucker, R.B., (1994). Dietary pyrroloquinoline quinone: growth and immune response in BALB/c mice. J. Nutr. 124: 744-753.
- Suhartati, T., (1983). Pigment isolation from *Caesalpinia sappan* Linn (Isolasi Zat Warna dari Tumbuhan *Caesalpinia sappan* Linn). Department of Chemistry – Bandung Institute of Technology, Indonesia.