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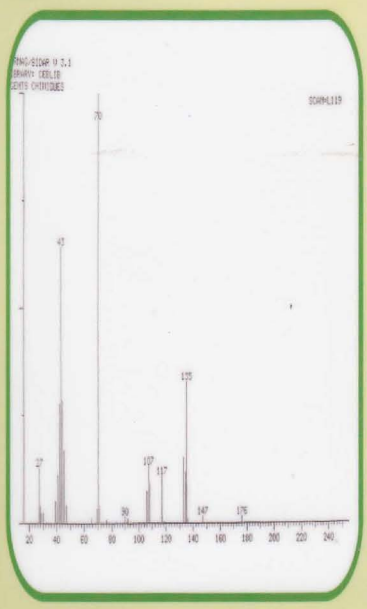

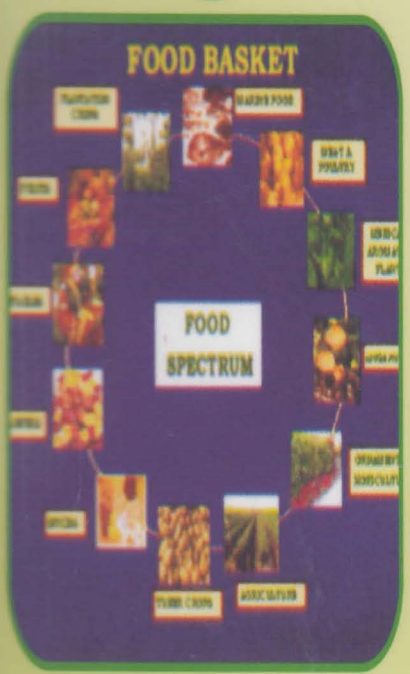

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THE EFFECT OF EXTRACTION SOLUTIONS AND INCUBATION TIME ON CHLOROPHYLL SOLUBILITY AND ANTIOXIDANT CAPACITY OF SUJI (*Pleomele angustifolia* N.E. Brown) LEAF EXTRACTS

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Abstract

Natural chlorophylls are lipophyllic that are due to the hydrophobic phytyl chain. Unphytyllated chlorophyll derivatives have been reported to be more hydrophyllic and have greater antioxidant capacity than phytyllated chlorophyll. These derivatives can be produced by chlorophyllase in leaves. Suji leaves (*Pleomele angustifolia* N.E. Brown) are commonly consumed as food colorant and used as traditional health promoter. This study first compared the efficiency of extraction solutions, namely Na_2CO_3 (0.1–0.5%), $NaHCO_3$ (0.1 – 0.5%), Na-citrate 12 mM, and incubation time from 1 to 60 minutes at 70-75^{0}C to produce extracts with high amount of both water-soluble chlorophyll content and antioxidant capacity. The effect of adding Tween 80 in the extraction solutions was also investigated. Conversion to phytil-free derivatives were verified by checking their insolubility in petroleum ether and measuring by spectrophotometer. Antioxidant capacity was measured by DPPH radical-scavenging activity method. This research showed that 0.75% Tween 80 in Na-citrate 12 mM as extraction solution combined with 30 minutes incubation time yielded the highest solubility and antioxidant capacity of suji leaf extract. We assumed that the emulsion capacity of Tween 80 used influenced the solubility of the leaf chlorophyll, which resulted in the increase the antioxidant capacity.

Keywords : suji (*Pleomele angustifolia* N.E. Brown), extraction, antioxidant capacity, chlorophyll

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INTRODUCTION

Chlorophylls are the most abundant natural plant pigments. Endo et al. (1985) first suggested that chlorophyll derivatives may bechain-breaking antioxidants by acting as effective electron donors. Chlorophyll and its derivatives have antioxidant capacity in vitro andex vivo (Kamat et al. (2000), Boloor et al. (2000), Hsu et al, 2005). Chlorophyllin, a water soluble and semi-synthetic sodium/copper derivative of chlorophyll, has potent antioxidant ability involving scavenging of various physiologically important ROS (Kumar et al., 2001). Antioxidant activity of natural chlorophylls was found to be significantly lower than commercial grade chlorophyllins (Ferruzzi et al., 2002).

Suji leaves are main source of natural green colorant for food application in Indonesia. Beside this, suji leaves are traditionally added to coconut oil and castor oil, and are used for traditional medicine to cure beri-beri disease (Heyne, 1987), fever, and bloody cough. Because of its high chlorophyll content, suji leaf is suggested to have high potential to be developed for functional food.

Antioxidant capacity in suji extract is suggested to be depended on the total chlorophyll content and the structure of chlorophylls. Traditionally, suji leaf extract is obtained by crushing the leaves in water. Because chlorophyll is liposoluble, the traditional extraction yield unstable chlorophyll suspension that easy to separate and aggregate. So, it is important to improve the extraction procedure in order to increase total soluble chlorophyll. The structure of chlorophyll also affects its antioxidant activity (Ferruzzi et al, 2002, Hsu et al., 2005). Although it is not yet clear, some studies showed

that phytyllated chlorophylls were less antioxidative than its unphytyllated. Unphytyllated forms are more polar than the phytyllated form. Elimination of phytol moiety can be achieved by action of chlorophyllase. We assumed that providing suitable conditions for chlorophyllase activity was suggested could increase antioxidant capacity of suji leaf extract.

MATERIAL AND METHODS

Material. Suji leaves (*Pleomele angustifolia* N.E. Brown) from local area (Bogor, West Java Indonesia), DPPH (1,1-diphenyl-2-picrylhydrazil) (Sigma), acetone p.a (E-Merck), petroleum ether p.a.(E-Merck).

Methods

Extraction. Suji leaves were washed and cleaned, and cut into small pieces prior to blending with extraction solutions. The extraction solutions were Na_2CO_3 (0.1 – 0.5%; w/v), $NaHCO_3$ (0.1 – 0.5%; w/v), Na-citrate 12 mM and aquades (as a control). The mixtures were incubated in waterbath at 75^0C for 0-60 minutes with 15 minutes interval for sampling. Incubation time was started at the time after the mixtures reached 70^0C. Each samples was filtered and heat-inactivated at 90^0C for 1 minutes immediately. The filtrates were used to analyze total chlorophyll, soluble chlorophyll and antioxidant capacity. For investigating the effect of using emulsifier on extraction solutions, Tween 80 was added in aquades, NaHCO3 0.5%, and Na-citrate 12 mM solutions then were analyzed as above.

Assesment of total chlorophyll and soluble chlorophyll. Chlorophylls in extract were separated by partition between acetone

and petroleum ether solvents. Extraction was done following AOAC (1995) method. Briefly, a quantity of the extracts were mixed with acetone. The mixture was shaken and allowed to stand overnight in a dark and cold place. The next day, the sample was centrifuged at 4000 rpm. A part of supernatant yielded were added with petroleum ether for determination of the soluble chlorophyll, and the remain supernatant was used for determination of the total chlorophyll. Deionized water was added to partition the chlorophyll derivatives into the petroleum ether phase. The acetone phase on lower layer was used for determination of the soluble chlorophyll. The total chlorophyll and the soluble chlorophyll were measured qualitatively at the maximum wavelength 660 nm and 655 nm, respectively.

Assesment of antioxidant capacity on DPPH. The ability of each suji extract to scavenge the stable DPPH* radical was determined by the method of Kubo et al. (2002), which was combined with that of Ferruzzi et al. (2002). One mL of 100 mM acetate buffer (pH 5.5), 1.87 ml of methanol, and 0.1 ml of methanolic solution of 3 mM DPPH were put into test tube. Then, 0.03 ml of the sample solution was added to the tube and incubated at 25^0C for 10 min. The absorbance at 517 nm was recorded. As control, 0.03 ml of methanol was added to the tube. Scavenging activity was expressed according to the degree of discoloration of DPPH solution.

RESULTS AND DISCUSSION

Extractions of fresh suji leaves using several solutions were conducted to obtain liquid extracts. Suji extract in powder form has been done by Nur and Rukmini (1992). They showed that the powder extract obtained from extraction using Na_2CO_3 solution was unstable, while ethanol extract failed to form powder due to the existing of an inhibitor component. In this research, the use of Na_2CO_3 and $NaHCO_3$ solutions were intended to create base conditions in order to prevent pheophytin formation. Na-citrate 12 mM has been reported of being able to increase chlorophyllase activity (Lopez, 1992).

Incubation of crushed suji leaves was conducted to give a chance for chlorophyllase to yield more chlorophyllides. Chlorophyllase (chlorophyll-chlorophyllido hydrolase; EC 3.1.1.14) act to hydrolyze phytol moiety of chlorophylls to form chlorophyllides which are more polar. In 35% acetone as a solvent, chlorophyllase activity of suji leaves was achieved at 39^0C, pH 7.4 and 30 minutes incubation time (Sibarani, 1994). Optimum conditions for chlorophyllase activity depend on solvent used. Olive fruit chlorophyllase in crude enzymatic extract showed maximum activity at 50^0C and the optimum pH was 8.5 in acetate-phosphate-borate buffer (Minguez-Mosquera et al., 1994).

Separation of phytylated and unphytylated pigment chlorophylls can be obtained by shaking an aqueous acetone solution with petroleum. The phytylated pigments pass into the petroleum layer while the unphytylated pigments remain in the acetone (Gross, 1991). As stated above, the unphytylated chlorophylls are more polar than the phytylated chlorophylls. Because of that, the amount of soluble

chlorophylls can be expressed by chlorophylls that remain in acetone layer. Figure 1 shows that, compared with aquades, all extraction solutions used could increase soluble chlorophyll. Generally, longer incubation time yielded more soluble chlorophyll.

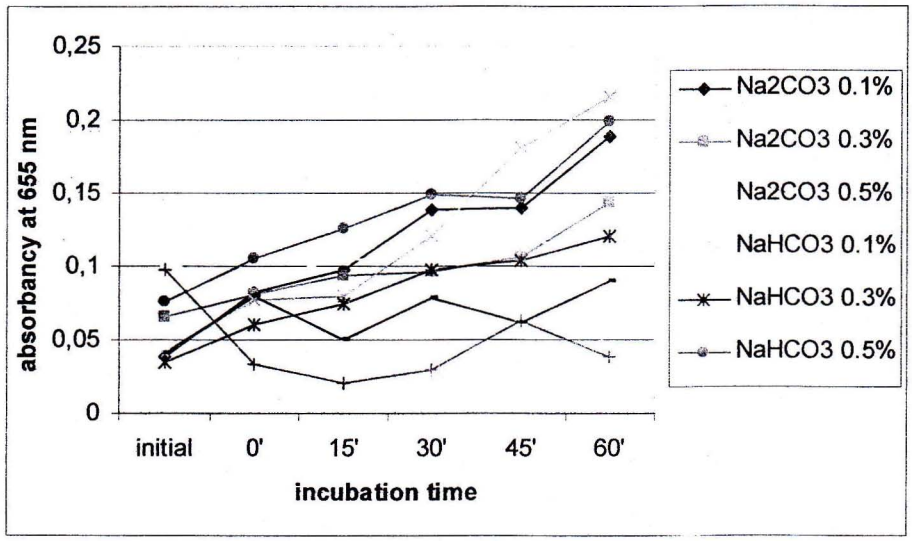

Figure 1. Absorbancy at 655 nm of acetone layer expressing the soluble chlorophylls in suji extracts yielded from different extraction solutions

Screening for antioxidant capacity of suji extracts was done using DPPH method. Antioxidant reacts with DPPH, which is a stable free radical, and convert it to α,α-diphenyl-β-picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract (Singh et al., 2002). Figure 2 shows that compared with aquades, using all concentrations of $NaHCO_3$ and Na-citrate as extraction solutions could increase antioxidant capacity of the extracts. However, Na_2CO_3 solutions did not seem to increase the antioxidant capacity significantly.

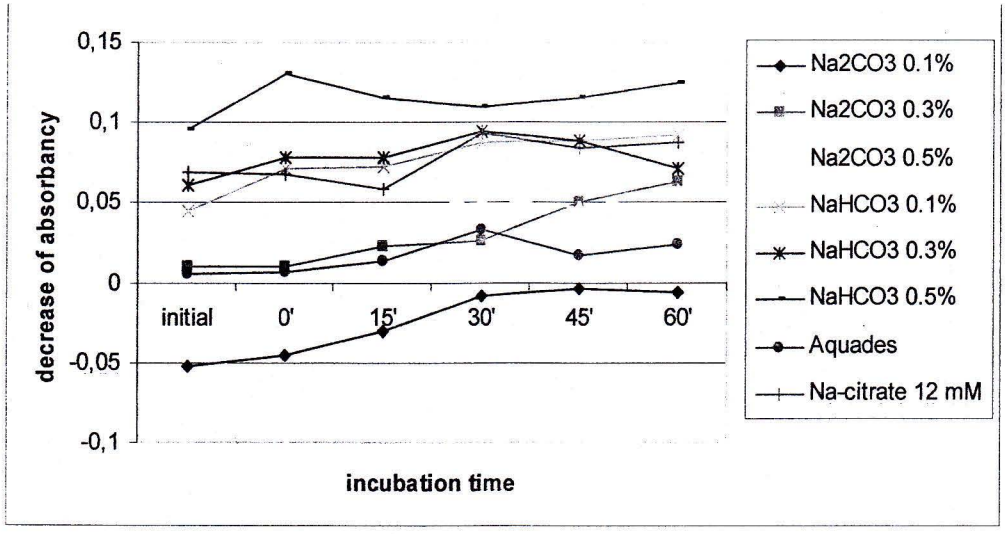

Figure 2. The degree of discoloration of DPPH solutions in suji extracts yielded from different extraction solutions

Natural chlorophylls are liposoluble. Because of that, adding emulsifier in the extraction solutions was assumed to stabilize chlorophyll suspensibility. Tween 80 has a high-HLB (hydrophylle-lipophylle balance) value. So, this emulsifier is suitable for oil/water emulsion system. The amount of Tween 80 that was added was 1% (w/v) in extraction solution, based on the ADI (Acceptable Daily Intake) of Tween 80 is 25 mg/kg body weight. Figure 3 showed that adding 1% Tween 80 (w/v) in several extraction solutions (aquades, $NaHCO_3$ 0.5% dan Na-citrate 12 mM) could significantly increase the amount of soluble chlorophyll. Adding Tween 80 in $NaHCO_3$ 0.5% and Na-citrate 12 mM did not increase the total chlorophyll (Figure 4), but this treatment could increase the antioxidant capacity of Na-citrate extract (Figure 5). The pH of 1% Tween 80 in Na-citrate 12 mM extract was 7,7, close to the optimum pH 7,4 for suji leaf chlorophyllase (Sibarani, 1994). So, this condition was more appropriate for chlorophyllase.

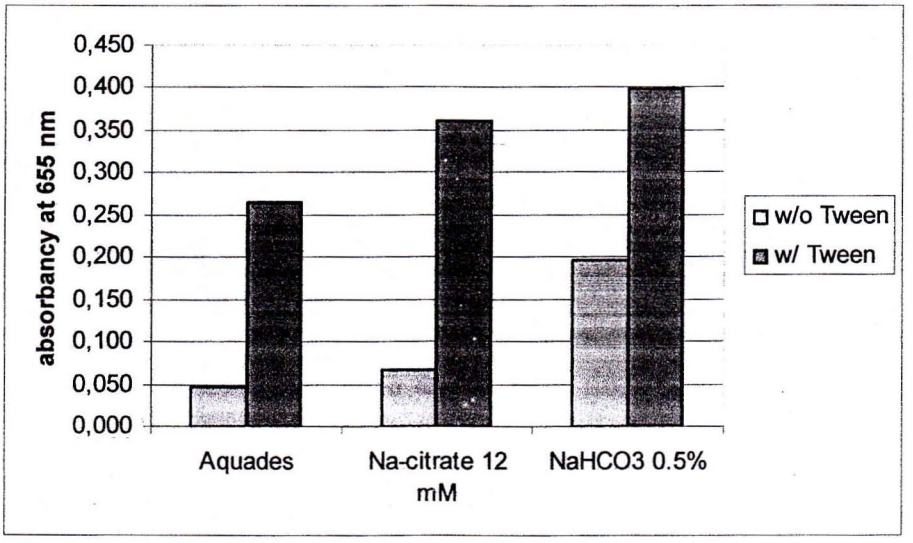

Figure 3. The effect of adding Tween 80 in extraction solutions on the average soluble chlorophylls of the extracts during incubation

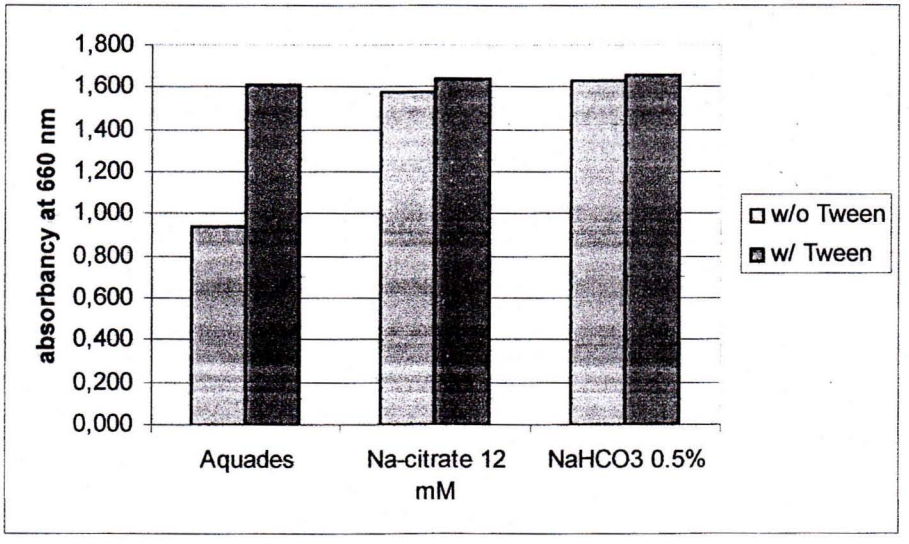

Figure 4. The effect of adding Tween 80 in extraction solutions on the average total chlorophylls of the extracts during incubation

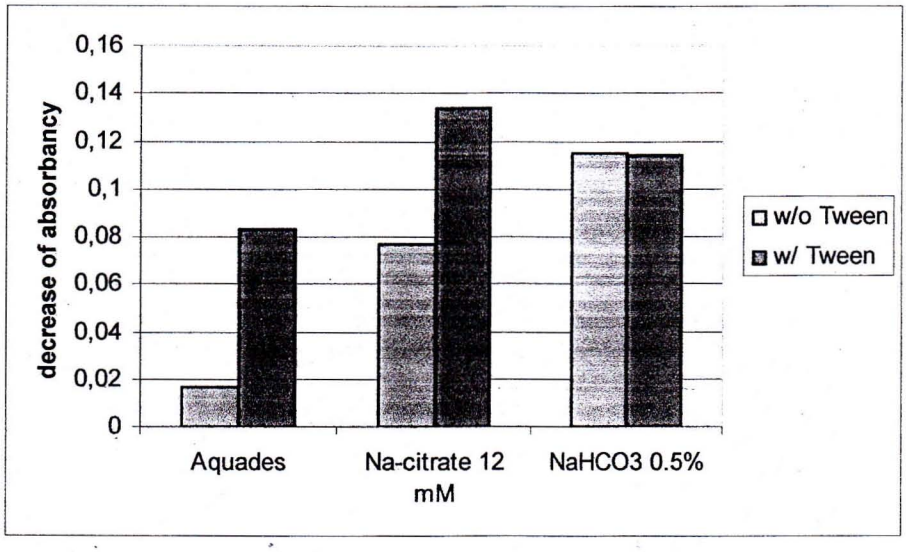

Figure 5. The effect of adding Tween 80 in extraction solutions on the average antioxidant capacity of extracts during incubation

The next step was experiment on concentration of Tween 80 in Na-citrate 12 mM. Figure 6 and 7 show that after 30 minutes incubation, the soluble chlorophyll and antioxidant capacity was relatively stable. Among the concentration range (0,25, 0,5, 0,75 and 1,0%), adding Tween 80 at 0,75% (w/v) combined with 30 minute incubation time showed the best performance of suji extract, i.e. the highest solubility and antioxidant capacity.

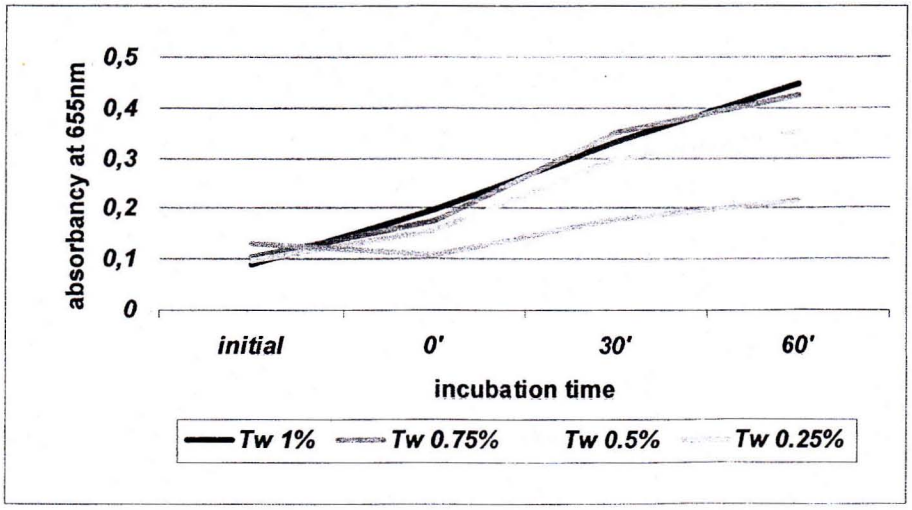

Figure 6. The effect of concentration of Tween 80 added in Na-citrate 12 mM extraction solution on the absorbancy of acetone layer expressing the soluble chlorophylls in suji extracts

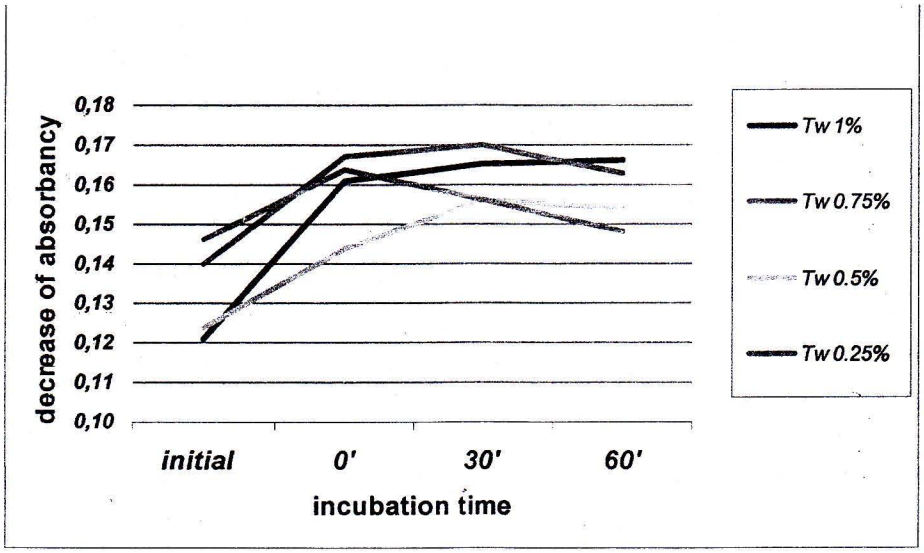

Figure 7. The effect of concentration of Tween 80 added in Na-citrate 12 mM extraction solution on the antioxidant capacity of suji extracts

CONCLUSIONS

Tween 80 0.75% (w/v) in Na-citrate 12 mM as extraction solution combined with 30 minute incubation time yielded the highest solubility and antioxidant capacity of suji leaf extract. We assummed that the emulsion capacity of Tween 80 used influenced the solubility of the leaf chlorophyll, which resulted in the increase of antioxidant capacity.

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