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PHYTOCHEMICAL CHARACTERIZATION AND TANNIN STABILITY TEST FROM KLUWEK (*Pangium edule Reinw*)

Siti Warnasih [a)], Uswatun Hasanah [a)]

[a)]*Universitas Pakuan, Bogor, Indonesia*
Corresponding Author: siti.warnasih@unpak.ac.id

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Abstract. One of the herbs that can be used as a source of natural dye is the kluwek which is brown. This study aims to determine the type of secondary metabolite compounds producing color, and to determine the stability of tannin in various conditions such as pH, oxidizing, UV rays, heating temperature, and storage using UV-Vis spectrophotometry method. This research includes preparation of kluwek, extraction with acetone:water (7:3) with maceration method, phytochemical test (tannin, flavonoid, carotenoid, and anthocyanin) and total tannins, and tannin stability test on various pH, oxidizing, UV rays, temperature and duration of heating, and storage using the UV-Vis spectrophotometry method. The results obtained are kluwek extract containing tannin, flavonoid, carotenoid, and anthocyanin compounds, and total tannins 2.80%. Based on the tannin content, kluwek extract stable at pH 6-7, heating 60-80 ^{o}C, and storage up to 9 days at temperature 27 °C, and kluwek extract unstable when contact with 1% H_2O_2, UV light, and if kept at cold temperature (10 ˚C).

Keywords: kluwek, natural dyes, phytochemicals, stability test, tannins

I. INTRODUCTION

The color of the food has an important role in consumer assessment, but the dye that is currently widely used is synthetic dyes. Azo synthetic dye in foods is not safe to eat because it affects health, Elbanna et al. [1] proved that mice given various food coloring treatments such as tartrazine, sunset yellow, carmoisine, and ponceau 4R showed that damage to various tissues of the body such as liver, kidney, small intestine, and others. Based on the above, it is necessary to increase the search for natural dye source.

One of Indonesia's biodiversity that can be utilized to be natural dye is *Pangium edule* Reinw or commonly called society with the name kluwek, kluwak, picung, or kepayang (Heyne [2]). Kluwek is a spice used in various cuisines such as rawon, brongkos, and konro soup. In addition to serving as flavoring, kluwek also gives a blackish brown color to the food. Chocolate from kluwek can be used as an alternative to synthetic dyes such as Chocolate Brown FH and Chocolate Brown HT.

Kluwek contains various substances such as beta-carotene, cyanide acid, hydnocarpic acid, kaulmograt acid, gloric acid, and tannins (Hilditch [3]; Mangunwardoyo [4]). Aside from being a colorant, tannin is also an antioxidant that acts as an antibacterial (Heruwati [5]). This causes kluwek dye is very good when applied in food because it can serve as a preservative. According to Artati and Fadilah [6], tannin is a polar polyphenol compound, soluble in glycerol, alcohol and hydroalcoholic, water and acetone. Tannin is insoluble in chloroform, petroleum ether and benzene. Based on research of Warnasih and Hasanah [7], water solvent can extract tannin from kluwek with yield of 19.42% with brown extract.

The problem faced on the use of natural dyes is the lack of stable color produced, because the easy natural dyes are damaged either due to oxidation reactions or degradation due to microorganisms. Some factors that cause damage to natural dyes are temperature, pH, oxidizing, UV rays, and others. Based on the studied background, this study aims to determine the secondary metholite compound of kluwek extract and to determine the stability of the dyestuff produced in various treatments, namely pH, oxidizer, UV rays, heating temperature, and storage by UV-Vis spectrophotometry method.

II. RESEARCH METHODS

The materials used are kluwek, water, acetone, Mg powder, concentrated HCl, 96% ethanol, amyl alcohol, $FeCl_3$ 10%, NaOH 2M, concentrated H_2SO_4, tanic acid standard, Folin Ciocalteu, Na_2CO_3, and H_2O_2 1%.

Equipment used include oven, weigh saucer, desiccator, analytical scales, Erlenmeyer, funnel, rotary evaporator, test tube, dropper, measuring cup, serological pipette, refrigerator, UV lamp, electric bath, pH meter and UV-Vis Spectrophotometer .

Preparation of Samples

Kluwek is cleaned of dirt, washed with running water, until clean, then drained. Kluwek is separated from the shell by pounding, then the meat is separated, and stoned to small and then put into the oven with a temperature of 50-60 ^{0}C until dry, then stored in a dry

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and tightly closed container. The simplicia is tested the water content and the ash content.

Kluwek Extraction

Simplicia kluwek as much as 25 grams was incorporated into the erlenmeyer and soaked using acetone solvent:water (7:3) of 250 mL containing 3 mL ascorbic acid 10 mM. Then macerated for 24 hours at room temperature while stirring using a stirer. The solution is filtered out, the residue is extracted again until the filled filtrate is clear. To separate the filtrate and residue was used centrifuge at 2000 rpm for 30 minutes and taken kluwek viscous extract using rotary evaporator vacum.

Phytochemical Test of Kluwek Extract

A. Tannin Test

Kluwek extract was added 3 drops of $FeCl_3$ reagent. The presence of tannin in the sample is indicated by the occurrence of a change in color to green or blackish black (Putra [8]).

B. Flavonoid Test

Kluwek extract of 2 mL in ethanol was heated for 15 minutes over a water bath then added 0.5 mL of concentrated HCl and 3-4 pieces of Mg powder. The presence of red or orange color indicates the presence of flavonoid compounds (Putra [8]).

C. Carotenoid Test

Kluwek extract of 2 mL is added 2 drops to 3 drops of concentrated sulfuric acid. The presence of blue or greenish-blue color indicates the presence of carotenoid compounds (Putra [8]).

D. Anthocyanin Test

Kluwek extract awaited 3 drops of 2M HCl then heated at temperature 78 ^{0}C for 5 minutes. Positive results when red color. And also added 2M NaOH drop by drop as observed changes occur. Positive results contain anthocyanins when the blue-green color fades slowly (Neliyanti and Nora [9]).

Determination of Total Tannin

Total tannin was performed by spectro photometric method, based on the color reaction between the sample with Folin Ciocalteu reagent using tanic acid as standard. Kluwek extract in each ethanol 96%, ethanol 70%, ethanol 40% and water dissolved in 100 mL flask, taken 1 mL into 10 mL measuring flask, added 7.5 mL of distilled water then 0.5 mL of Folin reagent Ciocalteu, and 1 mL of saturated Na_2CO_3 solution and squeezed with aquades then homogenized. After the solution was allowed to stand for 30 minutes, it was read on the spectrophotometer at 760 nm wavelength.

Tannin Stability Test of Kluwek Extract (Miksusanti [22])

Stability testing on oxidizing, light, heating, and storage temperature is performed at the initial pH of kluwek extract to maintain color stability. The initial pH conditions of the combined solution of the kluwek extract were then measured at various stability test parameters.

A. Stability at some pH

Kluwek extract dissolved in water, made a concentration of 500 ppm. To see the pH variation, the solution is adjusted to pH 1-8 with 1M HCl or 10% NaOH and stays for 30 minutes. Then tested its tannin content on various pH treatments.

B. Stability to the oxidizer

Kluwek extract is added 0.25 mL H_2O_2 (1%) (final volume of the solution is kept 10 mL) inserted into dark bottle. The mixture was measured at each contact time of 0, 3, 6, 9, 12 and 15 hours.

C. Stability against UV light

Kluwek extract is put into dark bottle and clear bottle then irradiated with 40 watt UV lamp (2500 lux light intensity) for 3 hours/day for 7 days. Measured tannin content for each treatment.

D. Stability against heating temperature

The kluwek extract was put into a dark bottle and incubated at 60, 80, 100, 120, and 150 ˚C for 90 minutes. Measured tannin content for each treatment.

E. Stability to temperature during storage

The kluwek extract was put into a dark bottle and stored at room temperature (27 ˚C) and cold temperature (10 ˚C), for 7 days. Measured tannin content for each treatment.

III. RESULTS AND DISCUSSION

Kluwek Simplicia Test Result

Before kluwek is extracted, characterization includes moisture content and ash content. The water content and ash content obtained are presented in Table 1.

Table 1. Water content and kluwek ash content

Sample	Water content (%)	Ash content (%)
kluwek simplicia	5,30 ± 0,01	1,95 ± 0,01

Based on the above data, the water content obtained after drying process is 5.30 ± 0.01%. Value of water content is in accordance with the required for the quality of simplicia that is less than 10% (Depkes [10]). The water content of the simplicia below 10% indicates that the simplicia to be used for the analysis has fulfilled the simplicia criteria, if the moisture content is more than 10% then the fruit is too moist and easy to decay because in a humid place it is easy to develop bacteria that cause decay (Depkes [10]).

According to Table 1, the ash content of kluwek is 1.95 ± 0.01%. Ash content shows the amount of mineral content in a sample. The higher the ash content so the higher the mineral present in the kluwek simplicia this is not so good because it will affect the color of the tannin with the high mineral.

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Rendement and Color of Kluwek Extract

The method of extraction is maceration with acetone:water (7:3) containing 3 mL ascorbic acid 10 mM for 3 x 24 hours. Maseration is one method of separation of compounds by immersion using organic solvents at room temperature. The maceration process is very beneficial in the isolation of natural material compounds because in addition to cheap and easy to do, by soaking the plant samples will occur breaking of walls and cell membranes due to pressure differences between inside and outside the cell, so that secondary metabolites present in the cytoplasm will dissolve in the solvent. The solvent that flows into the cell can cause protoplasm to swell and the cell content will dissolve in accordance with its solubility (Lenny[11]).

The use of mixed solvents of acetone and water aims to maximize tannin extract. Acetone solvents can minimize the interaction between tannins with proteins so that the tannins can be extracted all in the water phase and the protein can dissolve in acetone. The addition of ascorbic acid into the solvent aims as an antioxidant, so there is no oxidation in the tannin compound during the extraction process.

Table 2. Rendemen and color of kluwek extract

Sample	Rendemen (%)	Color
Kluwek extract	6,18	Brown

Be aware of the above data obtained a value of rendemen is 6.18% with brown extract, this is in accordance with Robinson [12] reinforce that states that tannins are soluble in water and solvents that are polar and produce a brown color.

Phytochemical Test Results of Kluwek Extract

Qualitative phytochemical test is to determine the content of phytochemicals in kluwek. Based on the experimental data showed that kluwek simplicia contains tannins, flavonoids, carotenoids and anthocyanins. The results of the test of phytochemical of kluwek simplicia extract on various solvents are listed in Table 3.

Table 3. Phytochemical content of kluwek simplicia extract

No.	Phytochemical test	Reagent	Result
1	Tannins	+ $FeCl_3$	(+) green color
2	Flavonoids	+ 0.5 mL concentrated HCl + Mg powder	(+) red color
3	Carotenoids	+ concentrated H_2SO_4	(+) green color
4	Anthocyanins	+ HCl 2 M heated 78^0C, 5 minutes	(+) blue color

In the phytochemical tannin test with ferric chloride solution will be formed green color this is due to a reaction involving tannin with Fe compound that will produce complex compounds with Fe^{3+} ions into green-colored polyphenols (Latifah [13]).

Flavonoid test was performed to determine the presence of flavonoid groups in kluwek. The addition of concentrated HCl to this test is intended to hydrolyze flavonoids into their aglycans, so that Mg metal can enter and form complexes that form a red color (Latifah [13]).

Carotenoids are a large group of hydrocarbons and xantofiles that give red, orange, or yellow color from various fruits and vegetables. Testing of carotenoids aims to identify the presence of color pigments in kluwek extract. Addition of sodium hydroxide aims to form complexes with isoprene groups in carotenoids to form a solution of green or blue (Harbone [14]).

Antosianin is one of the polar compounds of flavonoids and in large quantities found in fruits and vegetables. In the phytochemical test the addition of H_2SO_4 is intended to form methoxylation in anthocyanin to produce red color (Sundari [15]).

Total Tannins of Kluwek Extract

Tannin content on kluwek extract was analyzed quantitatively using spectrophotometric method. Based on the formation of blue colored compounds using Folin Ciocalteau reagents. This reactant oxidizes phenols to produce quinone compounds and forms a blue-tungsten molybdenum complex that can be detected by a UV-Vis spectrophotometer. The greater the concentration of phenolic compounds, the more phenolic ions that will reduce Folin-Ciocalteau reagent (Susanti [16]). Here is the result of tannin content analysis of kluwek extract.

Table 4. Tannin content of kluwek extract

Sample	Tannin content (%)
Kluwek extract	2,80

Based on Table 4, the tannin content of kluwek extract was obtained at 2.80%, this result is quite big compared to the previous research conducted by Sibuea [17] which determines the tannin content of water extract and ethanol extract with Sokhlet method that is equal to 8,73 ppm and 3,69 ppm, respectively. This indicates that the tannin content will be damaged in the Sokhlet method, and the best solvent for extracting tannins is water, this is because tannins are polar dyes and will dissolve well in the polar solvent.

Stability Tanin of Extract Kluwek

The dye stability test in the kluwek extract was done by looking at the stability of the tannin content, in some conditions, the variation of pH, the contact time with the oxidizer, the time of irradiation by UV rays, the heating temperature, the storage time of two conditions i.e. room temperature (27^oC) and cold temperature (10^oC).

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a. Stability of tannin content at the pH treatment

According to Figure 1, the graph of tannin content on the pH 6-7 treatment lies above the lower limit of the tannin content. Tannin content above the lower limit indicates that the tannin is stable at pH 6-7 (neutral pH). According to Okuda and Ito [18], the acidic atmosphere and the base of the tannins will be hydrolyzed so that it will break down the tannin core compound into a simpler compound. Levels of tannin are decreased in acidic pH atmosphere (pH 4 and 5), because when reacting with Folin Ciocalteu reaction conditions are not achieved so that the reaction of color formation is not optimal and cause the tannin level to fall. Here is the tannin content of kluwek extract on the pH treatment.

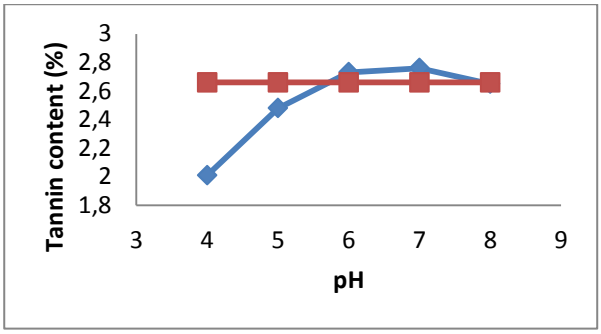

Figure 1. Graph of tannin content extract kluwek on the pH treatment

b. Stability of tannin content at contact time with oxidizer

Based on Figure 2, the tannin content at contact time of 0-9 hour oxidizer showed that the tannin level was far below the lower limit of 2.66%. The decline in tannin is caused by tannin as a natural antioxidant. The oxidiser will attack the phenolic group in the tannin core compound causing the tannin compound to decrease (Okuda and Ito [18]). The tannins that react with the oxidizing agent will become colored quinones and cause the color to become so thick that the absorbance is higher. Here is the tannin content of kluwek extract on the variation of the oxidator contact time

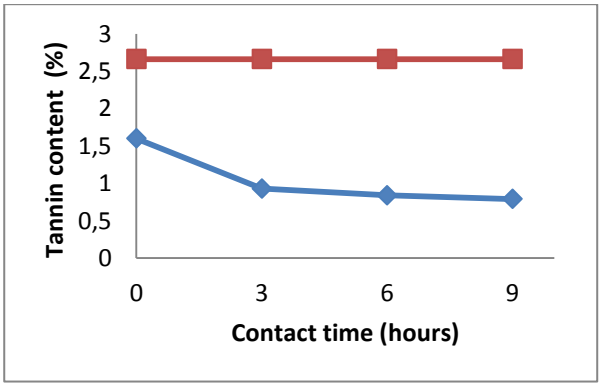

Figure 2. Graph of tannin content of kluwek extract at contact time with oxidizer

The relationship between tannin content and the contact time of the oxidizer illustrates that the longer oxidized the tannin content of the kluwek extract will decrease further. Tannin oxidation reaction as follows:

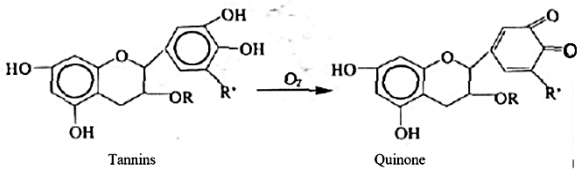

Figure 3. Reactions of Tanin Oxidation (Fajriati [19])

c. Stability of tannin content to the treatment of UV light exposure time

The UV light irradiation test was performed by irradiation of kluwek extract with UV light at a wavelength of 366 nm for 7 consecutive days with 3 hours/day irradiation time. The selection of 366 nm wavelengths is because these wavelengths include UV-A. It is the same as the UV rays that enter the earth.

According to Fig. 4, the tannin content of kluwek extract on the treatment of UV irradiation time on the 1st to 7th day is below the lower limit of the allowable tannin level of 2.66%. Levels of tannin are declining due to the flavonoid group contained in the tannin compound serves as a photoprotection agent because of its ability to absorb UV light, so the longer it absorbs the UV rays the tannin level will decrease (Saewan and Jimtaisong, [20]). The longer the kluwek extract receives UV exposure then the extract kinwak kluwek will decrease further. Here are the extracurricular tannin kluwek on variations of UV light exposure time.

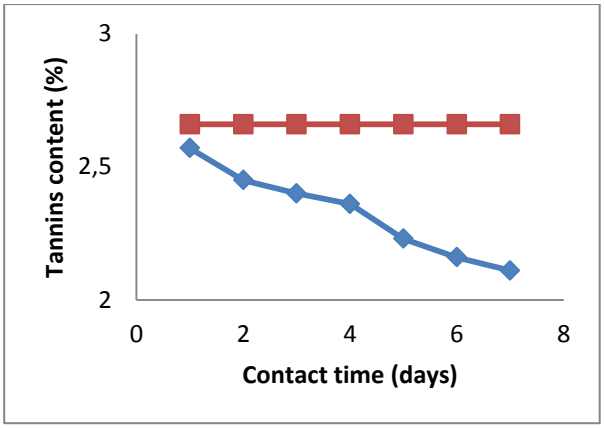

Figure 4. Graph of tannin content of kluwek extract at the time of UV light exposure

d. Stability of tannin content to the heating temperature

In this test, the kluwek extract was heated at different temperatures of 60, 80, 100, 120, and 150 °C. Based on Figure 5 at a heating temperature of 60-80 °C the tannin content is still above the lower limit of 2.66%, whereas at a temperature of 100-150 °C the

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tannin level begins to fall below 2.66%. Heating to 80 °C is still stable because the tannin content is still above the lower limit of the tannin 2,66%. Levels of tannins are getting down due to high heating temperature will damage tannins. Starting the heating temperature at 102 °C the tannins will break down into pyrogallol, pyrocatechol and phloroglucinol (Risnasari [21]). The higher the heating temperature the tannin content of kluwek extract will decrease further. The following tannin kluwek extract on variations of heating temperature.

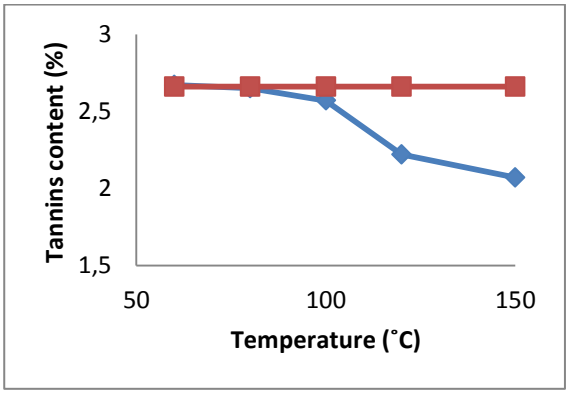

Figure 5. Graph of tannin content of kluwek extract at the heating temperature

e. Stability of tannins content of kluwek extract at room temperature storage (27 °C)

Based on Figure 6 the tannin content on the variation of room temperature storage time (27°C) to day 9 is still above the lower limit of 2.66% while on the 12th day and 15th it is beyond the lower limit. Storage of kluwek extract at room temperature (27°C) is too long will damage the tannins slowly because the nature of tannins easily oxidized and the color will become darker if exposed to direct light or left in the open (Risnasari [21]). The longer the storage time at room temperature (27°C) the tannin content of the kluwek extract will decrease further. The following tannin content of kluwek extract on the treatment of room temperature storage time.

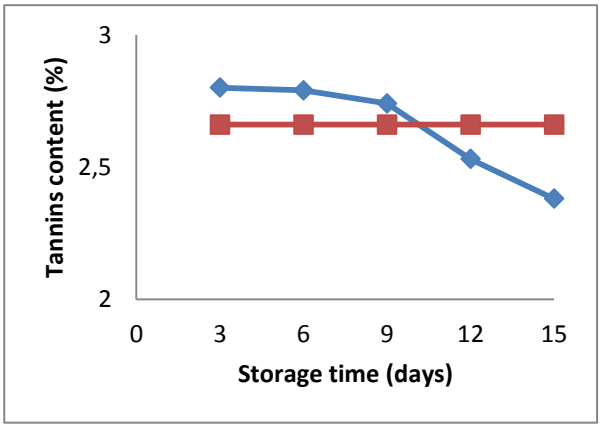

Figure 6. Graph of tannin content of kluwek extract at room temperature storage (27 °C)

f. Stability of tannin content of kluwek extract at cold temperature storage (10°C)

Based on Figure 7, the tannin content in all storage time treatment at cold temperature is lower than the lower limit level of 2.66%. Lower tannin content does not cause damage to tannins, but this is due to the reduced solubility. Tannins will become colloids in cold temperatures but will dissolve completely if dissolved by hot water (Risnasari [21]). Therefore, the tannin content decreases because the solubility decreases. The longer the storage time at cold temperatures the tannin content of kluwek extract will decrease further. The following are the levels of kluwek extract on variations in storage time in cold temperatures.

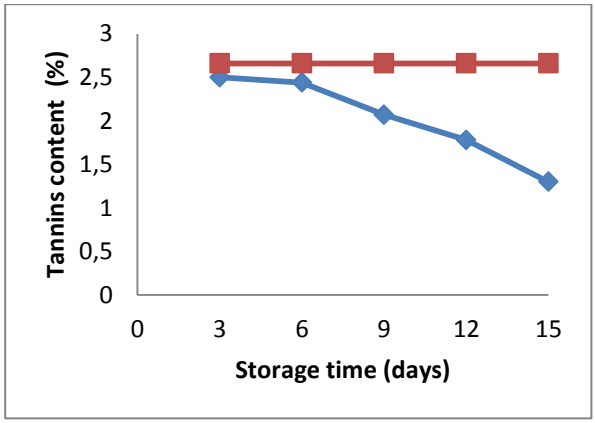

Figure 8. Graph of tannin content at cold temperature storage time (10°C)

IV. CONCLUSION

The conclusions of this study are as follows:

a. Kluwek extract contains tannin, flavonoid, carotenoid and anthocyanin compounds based on phytochemical test results, and contains a total tannin of 2.80%.
b. Tannin of kluwek extract is stable at pH 6-7, heating 60-80°C, and storage up to 9 days at temperature 27 °C, kluwek extract is unstable in contact with 1% H_2O_2 oxidizer, UV light, and if kept at cold temperature 10 ˚C.

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