

LABORATORY OF OREST PRODUCT CHEMISTRY FACULTY OF FORESTRY MULAWARMAN UNIVERSITY

PROTOKOL 010



STANDARD OPERATING PROCEDURE		
Title: Total Flavonoid Content Testing Method		Date:
		Created by:
		File:

1. OBJECTIVES

To provide instructions for performing a total flavonoid content test.

2. PRINCIPLE

The total *flavonoid content test* is carried out to determine the total flavonoid content in a sample using the colorimeter method.

3. REQUIRED COMPLETENESS

Laboratory suits, safety glasses, clean wipes/tissues.

3. MATERIALS AND TOOLS

Material	Tool
Plant extract sample	Test tube
Flavonoid standards (Example: Catechins)	Analytical balances
AlCl ₃	UV-Vis Spectrophotometer
NaNO ₂	Beaker glass
NaOH	Measuring cup
Distilled water	Micropipettes & Tips
Methanol	

4. PROCEDURE

4.1. PREPARATION OF TEST SOLUTION

- 4.1.1 Weigh 1 mg of extract, dissolve in 10 ml of distilled water
- 4.1.2 Weigh 1 mg of standard flavonoids, dissolve in 10 ml of distilled water
- 4.1.3 Weigh 10 gr of AlCl₃, dissolve in 100 ml of methanol
- 4.1.4 Weigh 5 grams of NaNO₂, dissolve in 100 ml of distilled water
- 4.1.5 Weigh 4 gr of NaOH, dissolve in 100 ml of distilled water

4.2. TOTAL FLAVONOID CONTENT TEST

- 4.2.1 To prepare the flavonoid standard curve, add solutions to each test tube as per the pattern in the table below;

Tube	Flavonoid Standard (μl)	Distilled water (μl)	NaNO ₂ (μl)	AlCl ₃ (μl)	NaOH (μl)
Tube 1	0	800	100	100	500
Tube 2	20	780	100	100	500
Tube 3	40	760	100	100	500
Tube 4	60	740	100	100	500
Tube 5	80	720	100	100	500
Tube 6	100	700	100	100	500

- 4.2.2 For the test sample, add solutions to each test tube as per the table below and repeat 3 times;

Tube	Flavonoid Standard (μl)	Distilled water (μl)	NaNO ₂ (μl)	AlCl ₃ (μl)	NaOH (μl)
Tube 1	100	700	100	100	500

- 4.2.3 Incubate the solutions for 10 minutes
- 4.2.4 Absorbance with a wavelength of 510 nm

4.2.5 Create a calibration curve from the standard absorption results of flavonoids, so that the value and regeneration formula are obtained;

4.2.6 Insert the average absorbance of the sample into the regression equation;

4.2.7 Calculate total flavonoid content using the formula;

$$C = c \times \frac{v}{m}$$

Where: C : Total flavonoid content (µg (standard flavonoids)/mg extract)

c : Concentration of sample taken (µg/ml)

m : Extract sample weight (µg)

v : Volume (ml)

4.2.8 Clean the work area and equipment if you have finished testing.

4.3. CAUTION

4.3.1 Conduct all weighing and testing in a low-light area to prevent rapid reaction of the solutions;

4.3.2 Prepare solutions one day before testing to stabilize reactions resulting from dilution;

4.3.3 Store solutions in a refrigerator;

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Validated by :