LABORATORY OF FOREST PRODUCT CHEMISTRY
FACULTY OF FORESTRY MULAWARMAN UNIVERSITY

PROTOCOL 009

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

1. OBJECTIVE
To provide instructions for performing a total phenolic content test.

2. PRINCIPLE
The total phenolic content test is conducted to determine the total phenolic content in a sample using a colorimetric method.

3. REQUIRED EQUIPMENT
Laboratory suits, safety glasses, clean wipes/tissues.

3. MATERIALS AND EQUIPMENT

Material	EQUIPMENT
Plant extract sample	Test tube
Phenol standards (Example: Galic acid)	Analytical balances
Folin-Ciocalteu Solution	UV-Vis Spectrophotometer
Sodium carbonate (Na_2CO_3)	Beaker glass
Distilled water	Measuring cup
	Micropipettes & Tips

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 phenol, dissolve in 10 ml of distilled water
4.1.3 Dissolve 1 ml of Folin-Ciocalteu solution in 10 ml of distilled water
4.1.4 Weigh 7.5 g of Na_2CO_3, dissolve in 100 ml of distilled water

4.2. TESTING *OF TOTAL PHENOLIC CONTENT*
4.2.1 To prepare the phenolic standard curve, add the solutions to each test tube in the order specified in the table below;

Tube	Phenol Standard (µl)	Distilled water (µl)	Folin-Ciocalteu (µl)	Na_2CO_3 (µl)
Tube 1	0	500	250	1250
Tube 2	20	480	250	1250
Tube 3	40	460	250	1250
Tube4	60	440	250	1250
Tube 5	80	420	250	1250
Tube 6	100	400	250	1250

4.2.2 For the test sample, add the solutions to each test tube as per the table below, and repeat this step three times;

Tube	Test Sample (µl)	Distilled water (µl)	Folin-Ciocalteu (µl)	Na_2CO_3 (µl)
Tube 1	100	400	250	1250

4.2.3 Incubate the solutions for 60 minutes.
4.2.4 Absorbance with a wavelength of 760 nm
4.2.5 Create a calibration curve from the absorbance results of the phenolic standards to obtain values and a regression equation.;
4.2.6 Insert the average absorbance value of the sample into the regression equation;

4.2.7 Calculate the total phenol content using the formula;

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

Where: C : Total phenol content (µg (standard phenol)/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 Weigh all solutions and conduct testing in a low-light area to prevent rapid reactions in the solutions;
4.3.2 It is advisable to weigh the solutions one day before testing to stabilize reactions occurring due to dilution;
4.3.3 Store solutions in a refrigerator;

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