

LABORATORY OF FOREST PRODUCT CHEMISTRY

FACULTY OF FORESTRY UNMUL



STANDARD OPERATING PROCEDURES

Title: Antioxidant Testing Methods

Date:

Created by:

File:

1. OBJECTIVE

To provide instructions for conducting antioxidant testing

2. PRINCIPLES

Antioxidant activity testing is uses the DPPH free radical scavenging method, based on the principle of working on samples (containing antioxidant compounds) that can reduce DPPH free radicals.

3. REQUIRED COMPLETENESS

Lab coat, mask, goggles, clean cloth/tissue.

3. MATERIALS AND TOOLS

Materials/reagents	Tools
Plant samples in the form of extracts, essential oils, liquid formulations	Tube 1.5 ml
Solvent (DMSO, ethanol, methanol, water, or mixtures).	Cuvette
Vitamin C (standard)	Analytical scales
DPPH (1,1-diphenyl-2-picrylhydrazyl)	Micropipette
	Yellow tip
	Blue tip

4. PROCEDURE

4.1. PREPARATION OF DPPH SOLUTION

- 4.1.1 Weigh DPPH using an analytical balance (DPPH: 27 mg/1000 ml);
- 4.1.2 Dissolve in ethanol and homogenize;
- 4.1.3 Incubate the solution in a refrigerator.

4.2. SAMPLE PREPARATION

- 4.2.1 Weigh the test sample and standard as needed using an analytical balance (3 mg/1 ml for 100 ppm);
- 4.2.2 Dissolve with a suitable solvent and homogenize;

4.3. ANTIOXIDANT TESTING

- 4.3.1 The dissolved sample is placed into the cuvette with 33 µl, then add 467 µl of the corresponding ethanol/solvent, and 500 µl of DPPH;
- 4.3.2 The mixture should total 1000 µl (1 ml);
- 4.3.3 Incubate the sample for 20 minutes in a low-light environment at a temperature of 24-27°C;
- 4.3.4 Antioxidant activity is determined through the decolorization of DPPH at a wavelength of 514-517 nm using a spectrophotometer.
- 4.3.5 The percentage of DPPH scavenging is calculated using the formula:

$$\% \text{ Damping} = \frac{A_0 - A_t}{A_0} \times 100$$

Where: A_0 = Average absorbance of negative control (without extract)

A_t = Average absorbance of the test sample (with extract)

- 4.3.6 Clean the work area and equipment used in testing

4.4. ATTENTION

4.4.1 The sample concentration can be adjusted as needed;

4.4.2 If the DPPH solution is still dark purple, add ethanol until the solution is light purple.

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Validated by :