LABORATORY OF fFOREST PRODUCTS CHEMISTRY AND RENEWABLE ENERGY
FACULTY OF FORESTRY MULAWARMAN UNIVERSITY

PROTOCOL

STANDARD OPERATING PROCEDURE		
		Date:
Title: Antimicrobial Testing Method		**Created by:**
		File:

1. PURPOSE
To provide instructions for antimicrobial testing implementation

2. PRINCIPLE
The antimicrobial activity test is conducted using the agar diffusion method by measuring the zone of inhibition of microbial growth that occurs due to the diffusion of substances acting as antimicrobials in solid media through a well

3. REQUIRED EQUIPMENT
Laboratory coat, mask, safety glasses, clean cloth/tissue, gloves.

3. MATERIALS AND EQUIPMENT

Materials/reagents	**Equipment**			
Plant samples in the form of extracts, essential oils, liquid formulations	1,5 ml tubes	Petri dishes (5 and 10 cm diameter)	Cotton swab	Laminar flow cabinet
Solvent (acetone, 40% ethanol or mixtures)	Test tubes	Beaker glass (various sizes)	Hot plate	
Test standards (chloramphenicol, chlorhexidine, etc.)	Analytical balance	Cork borer (6 mm diameter)	Cotton	
Aquadest	Micropipette	Cuvette	Aluminium foil	
Nutrient agar media (composition: nutrient broth media, glucose, and agar)	Yellow tip	Incubator	Inoculation loop/needle	
	Blue tip	Spectrophotometer	Autoclave	

4. PROCEDURE
4.1. MEDIA PREPARATION AND STERILIZATION
4.1.1 Weigh Nutrient Agar media with composition nutrient broth (8g/1000ml), glucose (1%), and agar (20g/1000ml);
4.1.2 Dissolve using distilled water, homogenize, and boil;
4.1.3 Sterilize the media along with necessary equipment using an autoclave at 121°C for 15 minutes.

4.2. MICROBIAL CULTURE
4.2.1 Microbial culture is done by transferring previously grown microbes from old media to new media;
4.2.2 Take 1 loop of microbe and inoculate on new media;
4.2.3 Incubate for 24 hours at 32°C.

4.3. SAMPLE PREPARATION
4.3.1 Weigh the sample according to needs (5 mg/1 ml for 100 ppm);
4.3.2 Dissolve with appropriate solvent and homogenize.

4.4. ANTIMICROBIAL TESTING
4.4.1 Use petri dishes as needed (diameter: 5 cm and 10 cm);
4.4.2 Pour sterile media into sterile petri dishes (5-7 ml for 5 cm diameter and 20-25 ml for 10 cm diameter) and allow to solidify;
4.4.3 Prepare microbial suspension by dissolving bacteria taken using an inoculating needle and homogenizing in sterile distilled water. The suspension should match the McFarland scale standard of 0.5 (transmittance 70-75%);
4.4.4 Pour suspension (25 µl for 5 cm diameter and 100 µl for 10 cm diameter) onto agar surface and spread using sterile cotton swab 3 times;
4.4.5 Make holes in the media using a sterile cork borer;

4.4.6 Put sample into the wells made (20 µl/as needed);
4.4.7 Each test should be conducted with three replicates;
4.4.8 Incubate for 24 hours at 32°C;
4.4.9 The final result of this test can be calculated in mm by measuring the diameter of the inhibition zones formed for each sample across three replicates and then averaging them.

4.5. ATTENTION

4.5.1 The preparation of microbial cultures should be done one day before testing;
4.5.2 Work related to microbes should be conducted aseptically and in a laminar flow cabinet;
4.5.3 Sample concentration can be adjusted as needed;
4.5.4 The number of holes made in the media can be adjusted according to the number of samples/requirements;

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Validated by :