Methodology 003

To assess the antimicrobial activity of the root bark extracts derived from Toddalia asiatica against both Gram-negative bacterium Escherichia coli and Gram-positive bacterium Staphylococcus aureus, alongside the fungal strain Aspergillus niger, utilizing the agar well diffusion method, you can follow this step-by-step procedure:

**Materials and Equipment:**

Toddalia asiatica root bark samples, solvents such as ethanol or methanol, sterile Petri dishes, nutrient agar plates (for bacteria) and Sabouraud agar plates (for fungi), inoculating loop, bacterial cultures of Escherichia coli and Staphylococcus aureus, fungal culture of Aspergillus niger, sterile pipettes, sterile agar well puncher or cork borer, incubator set at appropriate temperatures (37°C for bacteria, 25°C for fungi), micropipettes and sterile tips, sterile distilled water, ruler or calipers, aluminum foil, parafilm or laboratory film, disposal container for used materials and personal protective equipment (lab coat, gloves, safety goggles)

**Procedure:**

1. **Collection and Preparation of Toddalia asiatica Root Bark Extracts:** a. Collect fresh Toddalia asiatica root bark samples. b. Wash the root bark thoroughly to remove dirt and contaminants. c. Dry the root bark samples in shade to preserve their bioactive compounds. d. Grind the dried root bark into a fine powder.
2. **Extraction of Bioactive Compounds:** a. Weigh a specific amount of root bark powder. b. Prepare a suitable solvent (e.g., ethanol or methanol) and add it to the powdered root bark. The solvent-to-sample ratio can vary, but commonly used ratios are 1:5 or 1:10 (w/v). c. Allow the mixture to macerate for 24-48 hours at room temperature, shaking it occasionally. d. Filter the solvent extract through a filter paper to remove solid particles. e. Evaporate the solvent from the filtrate using a rotary evaporator or a vacuum concentrator to obtain the root bark extract. f. Reconstitute the extract in a small volume of sterile distilled water to achieve the desired concentration.
3. **Preparation of Microbial Cultures:** Escherichia coli and Staphylococcus aureus will be inoculated on separate nutrient agar plates using an inoculating loop. The second step will be to inoculate Aspergillus niger on Sabouraud agar plates. To obtain well – isolated colonies, the bacteria will be incubated at 370C for 24 hours and the fungal plates at 250C for 72 hours.
4. **Agar Well Diffusion Assay:** Each agar plate will then be labelled at the bottom with the name of the microorganism, the date and treatment type. The next step will be to sterilize a well puncher or a cork borer to create holes in the agar plates. They will then be filled with a known volume of the Toddalia asiatica root bark extract. Both positive and negative controls will be set, antibiotic disks for the positive control and sterile water for the negative control. Finally, the plates will be incubated the bacteria at 370C and the fungi at 250C.
5. **Measurement of Zone of Inhibition:** The clear zones which are zones of inhibition around each well will be measured after incubation using calipers. The measurements will then be recorded in millimeters.
6. **Data Analysis:** The average zone of inhibition will be calculated for each treatment. The results will then be measured against different antimicrobial activity.