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Chapter 14

Assessment of antibacterial efficacy of selected natural products: Methods, mechanisms, and applications

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1. Introduction:

Medicinal plants are a rich source of bio-resources, providing traditional remedies, modern drugs, dietary supplements, and components for synthetic drugs. Antibiotics, derived naturally, semi-synthetically, or synthetically, are crucial for combating bacterial infections by inhibiting or killing bacteria (Cowan, 1999; Tripathi et al., 2013 Yadav et al., 2021). However, bacterial resistance and the side effects of synthetic antibiotics pose significant challenges. Thus, exploring natural products like plant extracts is promising. Effective antimicrobial screening relies on methods such as disc/well diffusion and broth dilution. These techniques measure antimicrobial activity by determining the Minimal Inhibitory Concentration (MIC), the lowest concentration needed to prevent visible bacterial growth (Burt 2004; Gibbons,2008; Tripathi et al., 2018; Tortora et al., 2020).

Table 1: Plant and its antimicrobial compounds

Plant	Antimicrobial Compounds
Aloe vera	Phytosterols, fatty acids, indoles, alkanes, pyrimidines, alkaloids, organic acids, aldehydes, dicarboxylic acids, ketones, alcohols, phenolic acids, and polyphenols, particularly non-flavonoid polyphenols
Curcuma longa	Curcumin is a polyphenolic substance.

Syzygium aromaticum	Beta-caryophyllene, vanillin, crategolic acid, bicornin, gallotannic acid, methyl salicylate, eugenol (72–90% of essential oil), acetyleugenol, flavonoids (eugenin, kaempferol, rhamnetin, and eugenitin), triterpenoids (oleanolic acid, stigmasterol, and campesterol), and sesquiterpenes

2. Materials Required:

The materials required for this procedure are as follows:

1. Overnight grown bacterial cultures of *Escherichia coli*, *Klebsiella* sp., and *Staphylococcus aureus*.
2. Extracts of *Aloe vera*, *Curcuma longa*, and *Syzygium aromaticum*.
3. Nutrient broth and Luria Bertani (LB) broth.
4. Agar powder.
5. Ethanol.
6. Methanol.
7. Acetone.
8. Ethyl acetate.
9. A known or available antibiotic.
10. Tween 80.
11. Laminar airflow.
12. An incubator.
13. A weighing balance.
14. A pH meter.
15. An autoclave.
16. A magnetic stirrer.
17. A Bunsen burner.
18. Erlenmeyer flasks.
19. Test tubes.
20. A spreader.
21. An inoculating loop.
22. Petri plates.

3. Reagents and Media Preparation:

1. Nutrient Broth:

Ingredients: Mix 0.5% peptone, 0.3% beef or yeast extract, and 0.5% NaCl in distilled water to make the nutritional broth. Then, bring the pH down to 6.8 at 25 °C.

2. Luria Bertani (LB) Broth:

Ingredients: casein hydrolysate (1%), yeast extract (0.5%), NaCl (1%), distilled water (pH adjusted to 7.5 at 25 °C).

3. Agar Plates:

When preparing solid plates for the appropriate media, use 1.5% agar powder.

4. Positive Controls:

Prepared using discs of standard antibiotics with known concentrations

4. Preparations of Plant Extract:

a. *Curcuma longa* (Turmeric) Extract:

Process: Clean, cut, air dry, oven dry, grind, soak, filter, heat, store.

b. Aloe vera Extract:

Process: Wash, collect pulp, dry, powder, dissolve in ethanol, filter, evaporate, use.

c. *Syzygium aromaticum* (Clove) Extract:

Process: Dry, grind, dissolve in ethanol-methanol solution, incubate, filter, evaporate, dissolve in Tris-HCl buffer.

5. Procedure:

5.1. Agar Well Diffusion Method (*Curcuma longa* Extract):

1. Spread bacterial cultures over Nutrient Agar (NA) and LB Agar plates.
2. Create wells on plates and add plant extracts.
3. Incubate plates and measure inhibition zones.

5.2. Disc Diffusion Method (*Aloe vera* Extract):

1. Place filter paper discs impregnated with test compounds on agar plates.
2. Assess antibacterial activity by measuring inhibition zones.

5.3. Broth Dilution Method (*Syzygium aromaticum* Extract):

1. Emulsify clove oil with Tween 80.
2. Prepare a series of test tubes with different dilutions.
3. Transfer bacterial cultures and incubate tubes.
4. Assess growth and determine the Minimal Inhibitory Concentration (MIC).

6. Precautions:

1. Glassware should only be sterile.
2. The filtrate should be carefully allowed to evaporate until it becomes dry.
3. Shake each dilution well before taking an aliquot out for the next dilution.

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