Can Plant Extracts Fight Bacterial Infections?

Assessing Antimicrobial Activity of *Hedyotis diffusa* Secondary Metabolites Extracted using Different Solvents

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Introduction

- The potential of plant-derived antimicrobials is becoming an increasingly crucial research area due to the emergence of bacterial antibiotic resistance(1).
- *Hedyotis diffusa (family: Rubiaceae)* is a common herb used in Traditional Chinese Medicine, mainly for inflammatory conditions and cancer treatment(2).
- Antibiotic activity of plant antimicrobials which have neither reported resistance nor side effects are normally contributed by their secondary metabolites(3).

Aim

- To extract secondary metabolites of *Hedyotis diffusa* and investigate its antimicrobial activity against opportunistic human pathogens.

Methods

Sample Sourcing

Wild *H. diffusa* collected from Kluang, Johor, Malaysia

Sample Preparation

Washing and Air-Drying

Pulverisation

<0.05mm using grinder

Extraction

Maceration:
1:10 in ethanol/methanol/water
(150rpm, 27°C, three days)

Filtration

Remove plant residues using filter paper

Concentration

Ethanol/Methanol: Rotary evaporation
Aqueous: Freeze drying
Reconstituted in 10% DMSO

Filter sterilisation

Eliminate microorganisms using 0.45 µm syringe filter.

Antimicrobial Activity Evaluation

Bacterial Subculture

From glycerol stock culture of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli K12*.

Disc Diffusion Assay*

- Direct colony suspension: McFarland 0.5
- Positive control: Tetracycline
- Negative control: 10% DMSO

Broth Microdilution Assay*

Two-fold dilution of concentrated extracts + inoculum in 96-wells plate with growth and sterility controls **(Fig. 1)**
→ Minimum Inhibitory Concentration (MIC) **(Fig. 2)**

*in triplicates

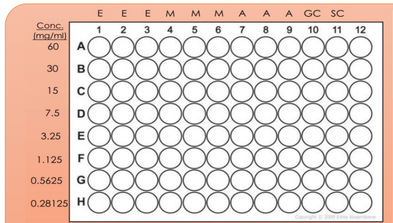

Figure 1 illustrates template of broth microdilution assay.

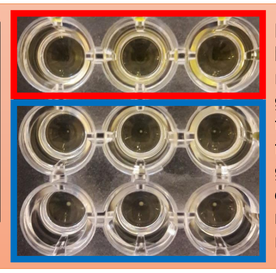

Figure 2 shows turbid button (blue box) represents microbial growth present and no turbidity (red box) indicating no microbial growth. Lowest concentration with no microbial growth recorded as MIC.

Results

- **Kirby-Bauer Disc Diffusion Assay**

Table 1 depicts the diameter of inhibition zone produced in disc diffusion assay on three opportunistic bacteria using three different types of *H. diffusa* extract (E: ethanol; M: methanol; A: aqueous) alongside positive(PC) and negative control(NC).

Bacterial Strain	Disc Content (10 µl)	Average diameter of inhibition zone (mm)
Staphylococcus aureus	PC	22.7
	NC	No inhibition
	E	
	M	
	A	
Staphylococcus Epidermidis	PC	23.0
	NC	No inhibition
	E	
	M	
	A	
Escherichia coli K12	PC	21.0
	NC	No inhibition
	E	
	M	
	A	

- **Broth Microdilution Assay**

Table 2 shows the minimum inhibitory concentration of three different *H. diffusa* extracts (E: ethanol; M: methanol; A: aqueous) on three bacterial strain tested determined from broth microdilution assay.

Bacterial Strain	Type of Extract	Minimum Inhibitory Concentration (mg/ml)
Staphylococcus aureus	E	15
	M	15
	A	No inhibition*
Staphylococcus epidermidis	E	15
	M	15
	A	60
Escherichia coli K12	E	No inhibition*
	M	
	A	

*within concentration range tested

Discussion

- Referring to Table 1, no significant inhibition zone were formed around discs impregnated with any of the three *H. diffusa* extracts (10 mg/ml) in Kirby-Bauer disc diffusion assay.
- This indicates *H. diffusa* extracts did not inhibit growth of *S. aureus*, *S. epidermidis* and *E. coli K12*, hence antimicrobial activity of *H. diffusa* is not proven (Table 1).
- A second trial was conducted using disc diffusion method with increased concentrations (10, 20, 40 and 60 mg/ml) and increased volume of 20 µl but returned negative results on antimicrobial activity as well.
- Table 2 illustrates both ethanol and methanol *H. diffusa* extracts exhibited MIC of 15 mg/ml on *S. aureus* and *S. epidermidis* in broth microdilution assay.
- Aqueous *H. diffusa* extract recorded an MIC of 60 mg/ml on *S. epidermidis* which was the highest concentration of extract tested (Table 2).
- No growth inhibition of *S. aureus* was observed by aqueous extract. Growth of *E. coli K12* was not inhibited by all three types of extract as turbid buttons were formed across all wells.

Conclusions

- Despite broth microdilution assay suggesting possible antimicrobial activity of *H. diffusa* extracts on *S. aureus* and *S. epidermidis*, the MIC values recorded are considerably high for practicality as antibiotic treatment.
- Modification to disc diffusion assay such as impregnation method of extracts may be useful to expect consistent results with broth microdilution assay.
- Further investigation on antimicrobial activity of *H. diffusa* extracts may be carried out by using solvents of different polarities to extract secondary metabolites then identify using thin layer chromatography and testing on other bacteria.

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