



and ampicillin against standard and clinical strains of *Staphylococcus aureus*



Norazian, M. H.; Sofiah, I.; Nurhaya, M. T.; Laina Zarisa, M. K. and Kahairi, A.
International Islamic University Malaysia, Kuantan, Pahang, Malaysia

INTRODUCTION

Antimicrobials combination therapy has been used to treat infections for decades, with the goal of achieving synergistic effects, producing wider spectrums of coverage and minimizing any toxicity effects of conventional antimicrobial agents (Odds, 2003). Plant metabolites are among the suitable candidates to be used as antimicrobial and synergistic agents, which can help mankind to curb the evolution of drug-resistant strains of microbes. *S. aureus* is commonly treated with the penicillin group antibiotics such as ampicillin. However, *S. aureus* resistance to these antibiotics is kept on increasing and the therapy produce serious side effects, such as hypersensitivity or anaphylactoid reactions. Curcuminoids, which are responsible for the yellow colour of turmeric possess bio-protective properties including promising antimicrobial activity (Goel, 2007; Foryst-Ludwig et al., 2004) with very low incidence of toxicity (Quereshi et al., 1992). For these reasons, our research effort turned to the antimicrobial combination study between ampicillin and curcuminoids in a view to enhance antimicrobial efficacy and developing safer drugs.

RESULTS

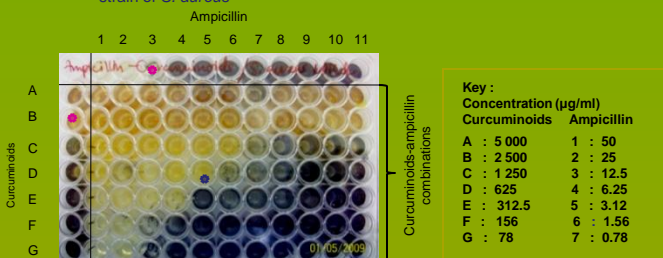
- The antimicrobial activity studies showed that the combination of ampicillin with curcuminoids fraction is likely to reduce the MIC of ampicillin compared with when tested alone against both strains of *S. aureus*.
- The results highlighted the occurrence of a pronounced synergism between 312.50 µg/ml of curcuminoids fraction and 1.56 µg/ml of ampicillin against the clinical strain with Fractional Inhibitory Concentration Index (FICI) of 0.25.

Table 1 : Antimicrobial activity of curcuminoids fraction from the rhizomes of *C. longa* alone and in combination with ampicillin.

Microbial strain	Combination	MIC (µg/ml)		FIC	FICI	Combined effect
		Alone	Combined			
<i>S. aureus</i> ATCC 25923	Curcuminoids fraction	25.00	25.00	1.00	1.5	Additive
	Ampicillin	0.24	0.12	0.5		
<i>S. Aureus</i> (clinical strain)	Curcuminoids fraction	2 500.00	312.50	0.125	0.25	Synergistic
	Ampicillin	12.5	1.56	0.125		

Fractional inhibitory concentration index (FICI) values < 0.5 are considered to indicate synergy; 0.5-4.0, additive; and >4, antagonism (Odds, 2003).

Figure 1 : Chequerboard assay of curcuminoids-ampicillin combination against clinical strain of *S. aureus*



(Addition of MTT revealed inhibited microbial growth as yellow colour whereas purple colour indicated the existence of microbial growth)

- indicate MIC point, i.e the lowest concentration without microbial growth.
- MIC point in combination

$$\begin{aligned} \text{FICI Index} &= \frac{\text{MIC of curcuminoids in combination}}{\text{MIC of curcuminoids alone}} + \frac{\text{MIC of ampicillin in combination}}{\text{MIC of ampicillin alone}} \\ &= \frac{0.125}{0.125} + \frac{0.125}{0.125} \\ &= 0.25 \text{ (synergistic effect)} \end{aligned}$$

CONCLUSION

In summary, this study shows that curcuminoids from *C. longa* extract have potential advantages as candidates for antimicrobial combination agents. Curcumin, demethoxycurcumin and bisdemethoxycurcumin are promising synergistic agents for antimicrobial combination therapy with ampicillin. The synergistic combination is found useful in combating *S. aureus* resistance towards ampicillin and minimizing the undesired effects of ampicillin.

MATERIALS AND METHODS

(1) Extraction of plant materials

200 g dried powdered rhizomes of *C. longa* was extracted consecutively with hexane and dichloromethane (DCM). Each extract was concentrated to dryness by using rotary evaporator. The DCM extract were then was fractionated through column chromatography to furnish curcuminoids fraction.

(2) Antimicrobial activity tests

Microbial strains

Two strains of *Staphylococcus aureus* were used, namely *S. aureus* ATCC 25923 and clinical isolate, which was obtained from patient diagnosed as having *S. aureus* infection at Hospital Tuanku Ampuan Afzan (HTAA), Kuantan.

Two antimicrobial assays

(a) Broth Microdilution Assay

This assay was performed to determine the MIC values of curcuminoids fraction and ampicillin.

(b) Chequerboard Assay

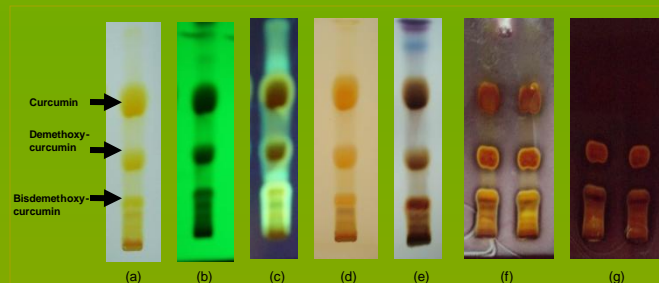
The antimicrobial activity of curcuminoids fraction in combination with ampicillin was determined according to the method described by Wei and Bobek, 2004.

(c) Bioautographic Agar Overlay Assay

This assay was performed according to the method described by Rahalison et al., 1991 with the aim to screen the antimicrobial active curcuminoids from the tested fraction, which responsible for antimicrobial synergistic effects.

- TLC Bioautographic assay of the DCM fraction has revealed three yellow pigments, which are curcumin, demethoxycurcumin and bisdemethoxycurcumin as the major antibacterial active compounds.

Figure 2 : Bioautographic profile of curcuminoids fraction from DCM extract of *C. longa* against *S. aureus*.



- Chromatogram viewed under visible light.
- Chromatogram viewed under UV₂₅₄ light.
- Chromatogram viewed under UV₃₆₆ light.
- Chromatogram after exposure to I₂ vapour.
- Chromatogram sprayed with Vanillin/H₂SO₄.
- Bioautogram against *S. aureus* ATCC 25923.
- Bioautogram against clinical strain *S. aureus*.

- Antibacterial compounds were detected as clear spots against pink background of viable organisms.
- Chromatographic condition : silica gel F₂₅₄, 0.2mm thickness, DCM:EA = 18:1.

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