Membrane Permeabilization and Antimicrobial Activity of Phospholipase A_2 from Ant venom

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ABSTRACT

Phospholipase A_2 (PLA_2), an enzyme commonly found in the venom of insects like ants, bees, wasps, and hornets, can break down phospholipids in cell membranes via hydrolysis, leading to membrane damage. In this study, the PLA_2 extracted from *Tetraponera rufonigra* venom was tested for its membrane permeabilizing ability and antimicrobial activity. To mimic cell membranes, we created liposomes made of phosphatidylcholine and phosphatidylethanolamine (POPC:POPE) at 1:1 ratio, encapsulated with a fluorescent dye called 5,6-carboxyfluorescein. These liposomes were tested with three substances, including antibiotic drug chloramphenicol, crude venom, and purified PLA_2. The results showed that crude venom and purified PLA_2 at 5 µg/mL caused a significant leakage in the liposomes, while chloramphenicol had little effect. These substances were also tested for their antimicrobial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* using broth dilution and agar dilution methods to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). As a result, crude venom had an MIC of 462.5 µg/mL against *E. coli*, while chloramphenicol was much more potent with an MIC of 3.12 µg/mL. Additionally, the disk diffusion method was used to measure the inhibition zones, indicating that the bacteria growth was inhibited. Crude venom of 440 µg exhibited a clear zone of 6.5 mm against *E. coli*, demonstrating its antibacterial activity. These findings suggest that crude venom could disrupt membrane and kill bacteria effectively, whereas the purified PLA_2 showed less effect suggesting that crude venom from *Tetraponera rufonigra*, which contains some proteins other than PLA_2 that may have antibacterial activity. Therefore, PLA_2 from ant venom might be promising for further research, especially in drug development.

INTRODUCTION

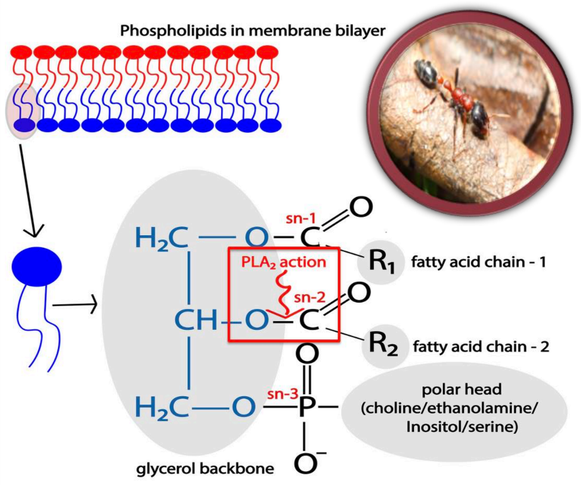


Figure 1 Catalytic action of PLA_2.

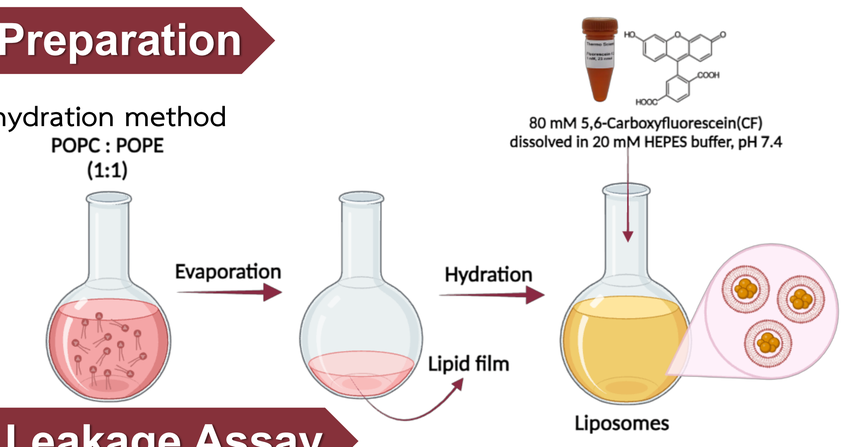
Phospholipase A_2 (PLA_2) is an enzyme commonly found in the venom of insects, including *Tetraponera rufonigra* (bicolor ant). This enzyme consists of a protein known as secreted Phospholipase A_2 ($sPLA_2$), which plays a crucial role in catalyzing the hydrolysis of phospholipid membranes at the sn-2 position.

This study aims to investigate the effect of PLA_2 on membrane permeability and its antimicrobial activity against *S. aureus* and *E. coli*. PLA_2 may hold significant potential for further research, particularly in the development of novel therapeutic agents.

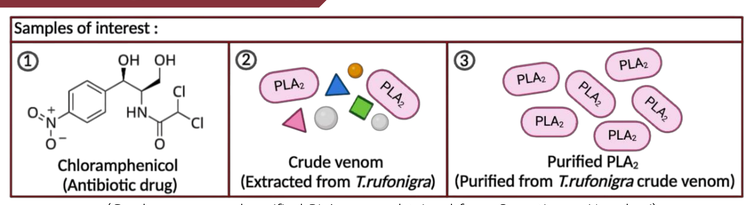
METHODOLOGY

Liposome Preparation

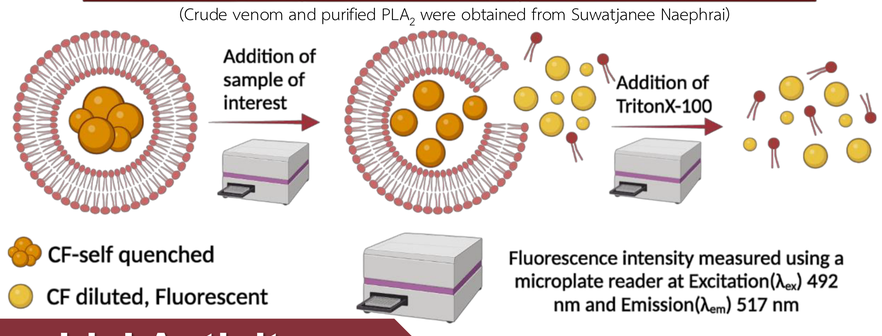
Thin-film hydration method



Liposome Leakage Assay

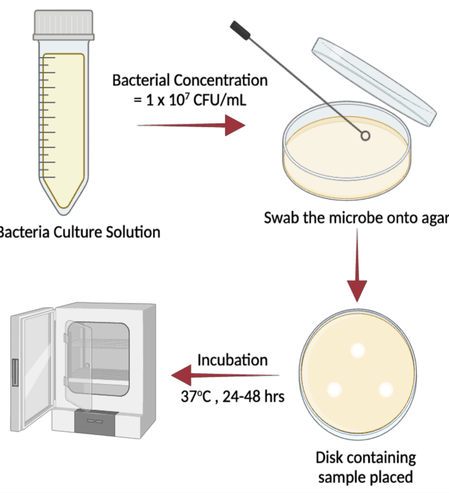


(Crude venom and purified PLA_2 were obtained from Suwatjanee Naephrai)

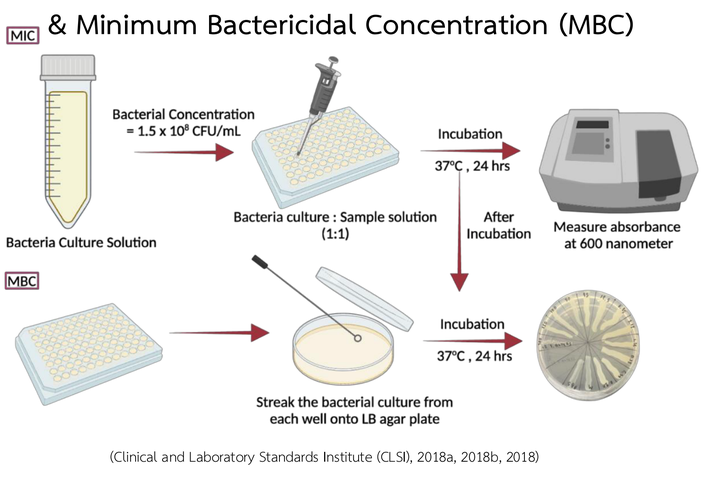


Antimicrobial Activity

Disk diffusion method



Minimum Inhibitory Concentration (MIC)
& Minimum Bactericidal Concentration (MBC)



(Clinical and Laboratory Standards Institute (CLSI), 2018a, 2018b, 2018)

RESULTS & DISCUSSION

Liposome Leakage Assay

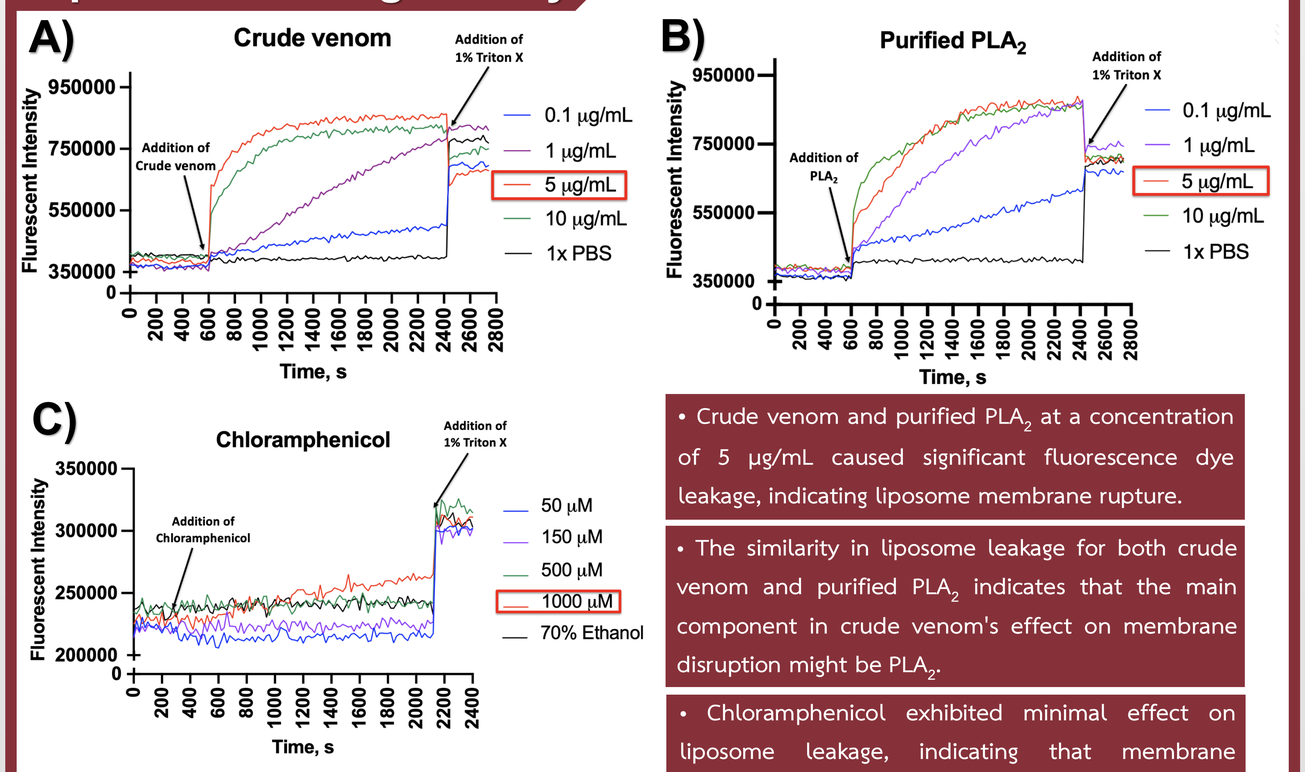


Figure 2. Effect of A) Crude venom, B) Purified PLA_2 and C) Chloramphenicol on Liposome Leakage.

- Crude venom and purified PLA_2 at a concentration of 5 µg/mL caused significant fluorescence dye leakage, indicating liposome membrane rupture.
- The similarity in liposome leakage for both crude venom and purified PLA_2 indicates that the main component in crude venom's effect on membrane disruption might be PLA_2.
- Chloramphenicol exhibited minimal effect on liposome leakage, indicating that membrane disruption might not be a primary action, but it might involve other mechanisms, e.g., inhibition of protein synthesis.

Antimicrobial Activity

Disk diffusion method

Figure 3. The disk diffusion method demonstrated the formation of an inhibition zone by *T. rufonigra* venom against *E. coli*.

Table 1. Antibacterial activity assay of *T.rufonigra* crude venom by disk diffusion method.

	Zone of inhibition in diameter (mm)	
	E. coli (-)	*S. aures* (+)
Crude venom (1, 5, 10, 50, 100 µg)	0.0	0.0
Crude venom (440 µg)	6.5	0.0
Chloramphenicol (positive control)	31.2	33.3
1x PBS (negative control)	0.0	0.0

Crude venom of 440 µg exhibited a clear zone of 6.5 mm against *E. coli*, demonstrating only a slight antibacterial activity.

MIC & MBC

Table 2. Antibacterial activity assay of *T. rufonigra* crude venom, purified PLA_2 and antibiotic drug using broth and agar dilution methods.

Bacteria Strains	MIC (µg/mL)			MBC (µg/mL)		
	Crude venom	purified PLA_2	Chloramphenicol	Crude venom	purified PLA_2	Chloramphenicol
E. coli (-)	462.5	>250	3.12	>3,700	>250	>200
S. aureus (+)	>3,700	>250	6.25	>3,700	>250	>200

Gram-positive/negative; Negative(-), Positive(+)

Crude venom exhibited antibacterial activity against Gram-negative bacteria (*E. coli*), with a MIC of 462.5 µg/mL, which was lower than that for Gram-positive bacteria (*S. aureus*). In addition, although PLA_2 should have the potential to damage bacterial cell membranes, it demonstrated low antibacterial potency. This suggests that targeting the cell membrane alone may not be an effective strategy for bacterial inhibition. Enhancing antibacterial potency may require a combination of multiple mechanisms.

CONCLUSIONS

- Crude venom and purified PLA_2 can induce membrane disruption, as evidenced by fluorescence dye leakage from liposomes. However, it exhibited low potency as an antibacterial agent.
- The disruption of the cell membrane may not be effective enough to inhibit bacterial growth on its own.
- PLA_2 can be used as an optional treatment to weaken the cell membrane, which may enhance the distribution and effectiveness of other drugs.

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