Effect of lupinifolin on the proteome of multidrug-resistant *Enterococcus faecium*

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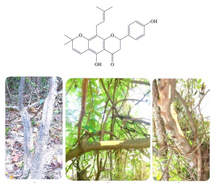
Introduction

Vancomycin-resistant enterococci (VRE) is a major concern of a global public health because it has been a leading cause of healthcare-associated infections. (Selleck *et al.*, 2019). Currently, bioactive compound have gained popularity and played a key role as an alternative treatment against infectious diseases, including antibacterial drugs (Atanasov *et al.*, 2021).



Albizia myriophylla Benth.

(Joycharat *et al.*, 2016)

- Lupinifolin is an prenylated flavonoid in Thai traditional herbs.
- The bioactive compound used in this study was isolated from *Albizia myriophylla* Benth. stem.
- Lupinifolin showed the potential antibacterial activity against Gram-positive bacteria including *Enterococcus* (Joycharat *et al.*, 2013; Joycharat *et al.*, 2016; Sianglum *et al.*, 2019).
- Lupinifolin is a promising new antibiotic.
- However, the mechanism of action underlying antibiotic effects of this compound is not yet understood.

Objective:

The aim of this study was to reveal the effect of lupinifolin against vancomycin-resistant *E. faecium* using proteomic analysis.

Material and methodology

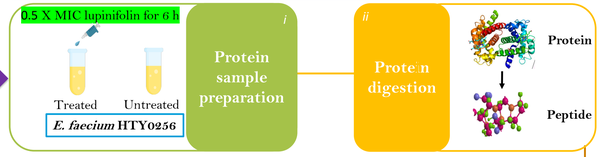
Antibacterial activity

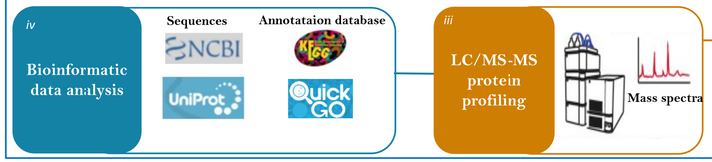
Bacterial strains

- Clinical isolate, *E. faecium* HTY0256 was colleted from urine sample.
- *E. faecalis* ATCC29212 was used as a quality control.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of lupinifolin, vancomycin and penicillin G were detected by broth microdilution method (CLSI, 2019)

Proteomic analysis





Results

Table 1 MIC and MBC of of lupinifolin of *E. faecium* HTY0256 and *E. faecalis* ATCC29212

Bacterial strains	Lupinifolin (µg/ml)		Vancomycin (µg/ml)	
	MIC	MBC	MIC	MBC
E. faecium HTY0256 (*vanA*)	4	8	256	>1024
E. faecalis ATCC29212	0.5	2	4	128

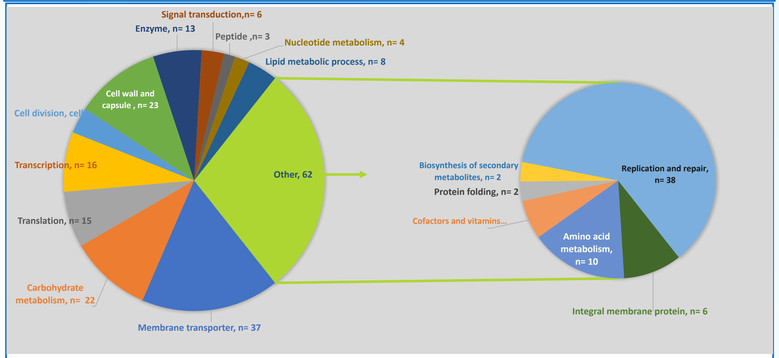


Figure 1. Proteomic profiling of *E. faecium* HTY0256 after lupinifolin exposure. KEEG pathway enrichment analysis of differentially expressed proteins (DEPs). DEPs were functionally sorted into 17 functional categories according to the gene ontology annotation.

- Proteins from same pathway probably carried out their biological function together, KEGG database was applied to analyze the biological pathways of the lupinifolin treatment, DEPs were mapped to KEGG pathway database in control vs treatment group.
- Among this pathways, the majority of pathways were related to transporter membrane, carbohydrate metabolism, call wall organization and replication, and DNA repair.
- The results suggested that lupinifolin mainly affected the transporter membrane, carbohydrate metabolism, call wall organization and replication, and DNA repair.

Results

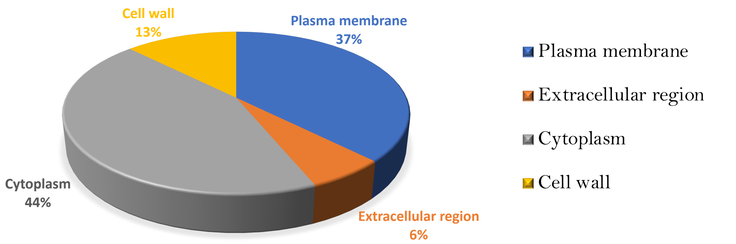


Figure 2. Identification and analysis of differentially expressed proteins (DEPs) between lupinifolin treatment and control. Identified proteins were groupd based on cellular component in the DEPs.

Conclusions

- These findings mainly suggests the comprehensive proteomic profiling related to action of lupinifolin against vancomycin-resistant *Enterococcus.*
- Our works provide further evidence to support therapeutic efficiency of lupinifolin which could lead to the development of a new effective drug for treatment of multidrug infections.

References

1. Selleck, E. M., Van Tyne, D., & Gilmore, M. S. (2019). Pathogenicity of enterococci. *Microbiology spectrum*, *7*(4).
2. Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: Advances and opportunities. *Nature Reviews Drug Discovery*, *20*(3), 200-216.
3. Sianglum, W., Muangngam, K., Joycharat, N., & Voravuthikunchai, S. P. (2019). Mechanism of action and biofilm inhibitory activity of lupinifolin against multidrug-resistant enterococcal clinical isolates. *Microbial Drug Resistance*, *25*(10), 1391-1400.
4. Joycharat, N., Thammavong, S., Limsuwan, S., Homlaead, S., Voravuthikunchai, S. P., Yingyongnarongkul, B. E., ... & Subhadhirasakul, S. (2013). Antibacterial substances from *Albizia myriophylla* wood against cariogenic *Streptococcus mutans*. *Archives of Pharmacal Research*, *36*(6), 723-730.
5. Joycharat, N., Boonma, C., Thammavong, S., Yingyongnarongkul, B. E., Limsuwan, S., & Voravuthikunchai, S. P. (2016). Chemical constituents and biological activities of *Albizia myriophylla* wood. *Pharmaceutical biology*, *54*(1), 62-73.

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