The Study on Bioactive secondary metabolites from *Phallusia nigra*

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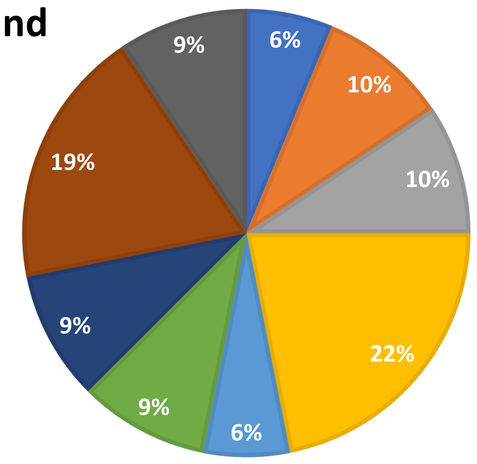
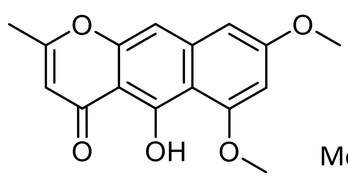
Introduction

Marine habitats are regarded as a rich source of molecules of biological functions. Either marine creatures or their symbiotics all show the ability to synthesize structurally diverse bioactive secondary metabolites, which has high potential in the field of medicine to treat human's diseases. *Phallusia nigra* is a tunicate that is abundant in the underwater area around Taiwan. Based on the survey in Reaxy database, over 30 secondary metabolites have been found from the genus of *Phallusia.* Among them, diketopiperazine alkaloids have showed potent inhibitory effect against SARS-CoV-2 M^{pro}[4], and naphthopyrones were regarded as bioactive compounds with cytotoxic and antibacterial[5]. The preliminary screening of the extracts of *P. nigra* showed clear inhibitory effect against the pathogenic indicators. Herein, we would like to report the study on the secondary metabolites from the tunicate*, P. nigra*, collected from Singda harbor by scuba diving.

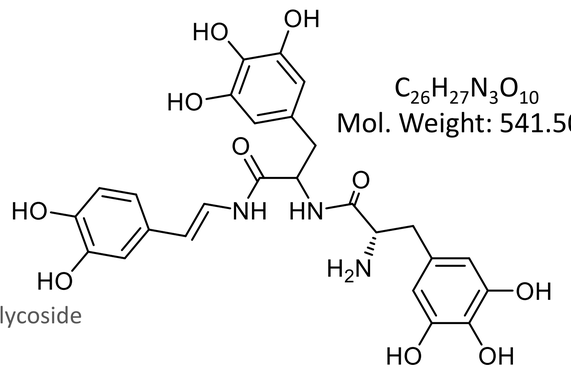
The MeOH layers showed strong inhibitory effect toward pathogenic bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Thus, following the bioassay guided fractionation and isolation, we separated the MeOH layer into 12 fractions. The anti-bacterial assay indicated that Frs. 6 and 7 also exhibited the antibacterial activity against *S. aureus* and *P. aeruginosa*. Moreover, Fr. 7 has stronger inhibitory effects than Fr. 6 does. So far, we keep to isolate and purify compounds in Fr. 7 for following antibacterial experiment.

Background



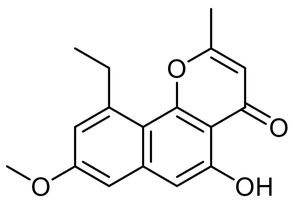


$C_{16}H_{14}O_5$
Mol. Weight: 286.279



$C_{26}H_{27}N_3O_{10}$
Mol. Weight: 541.507

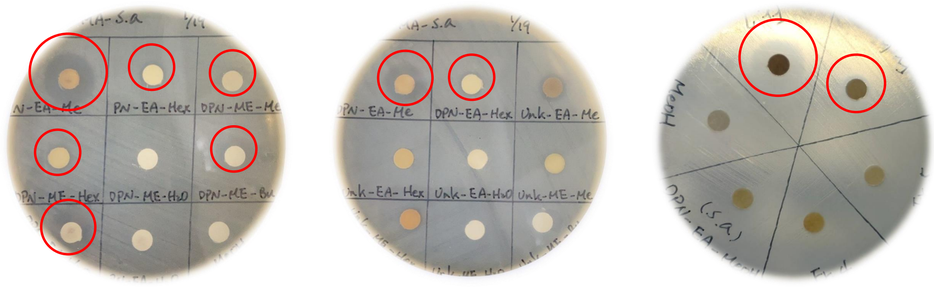
According to the Reaxy database, more than 30 secondary metabolites have been isolated from the genus of *Phallusia.* In the genus, steroids are the main type of compounds. Besides, terpenes, pyrones, and cyclopeptides were also found from this genus. Literature survey indicated that some secondary metabolites from *P. nigra*, such as terpenes, showed antioxidant[1], immunomodulatory[3], antibacterial[2] and antifungal[2] activities. Herein, we tried to isolate these secondary metabolites with diverse bioactivities from *Phallusia nigra*.



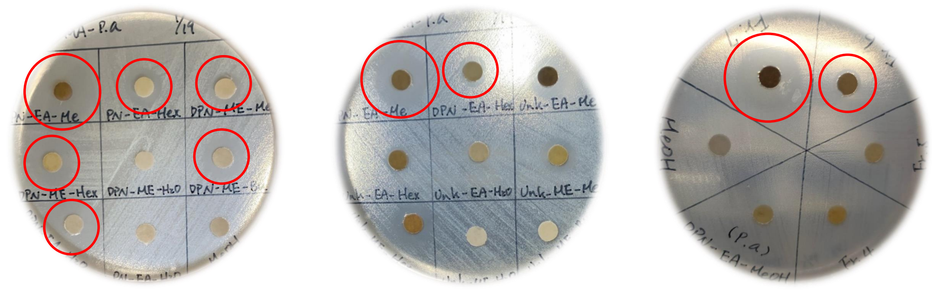
$C_{17}H_{16}O_4$
Mol. Weight: 284.307

Antibacterial Activity Assay

Staphylococcus aureus



Pseudomonas aeruginosa



Pathogenic bacteria	DPN-Me-Me	DPN-Me-Bu	DPN-Me-Hex	DPN-EA-DDW
S. aureus	-	-	-	+++
P. aeruginosa	++	+++	++++	++++

Pathogenic bacteria	DPN-EA-Me**	DPN-EA-Hex**	PN-EA-Me*	PN-EA-Hex*
S. aureus	+++	+	++++	+
P. aeruginosa	++++	++	+++++	+++

Pathogenic bacteria	Fr. 4	Fr. 5	Fr. 6	Fr. 7
S. aureus	-	-	+	+++
P. aeruginosa	-	-	+	+++

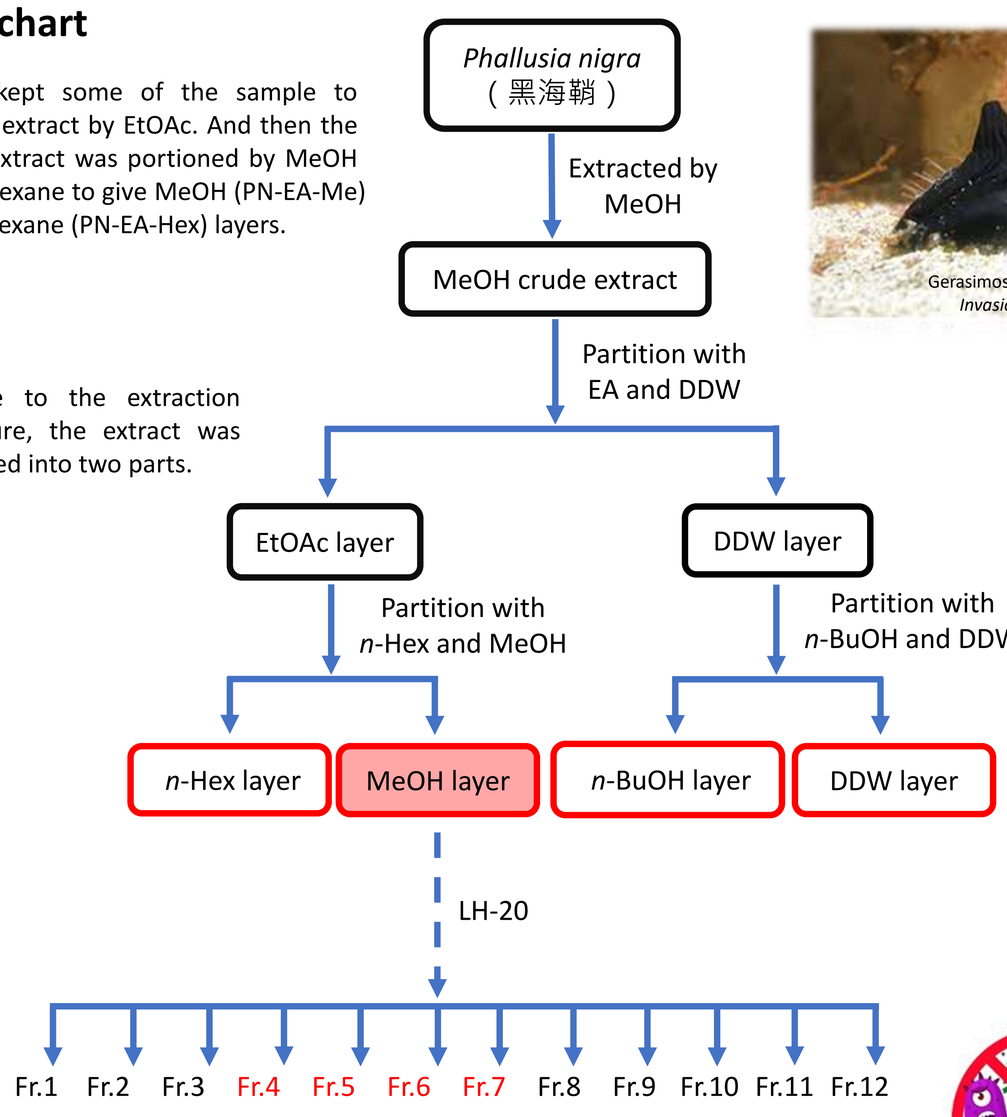
In the preliminary bioassay, all MeOH layers from either dried or wet *P. nigra* showed the inhibitory effect on pathogenic bacteria. Herein, we separated the MeOH layer of the dried sample into 12 fractions.

The enough amount of Fr. 4~7 were further evaluated the antimicrobial activity against *S. aureus* and *P. aeruginosa*. The results indicated that the Fr.7 exhibited the best ability toward these two pathogens mentioned above. In the future, we are paying our effort to the separation of bioactive compounds from Fr.7.

Flow chart

* We kept some of the sample to directly extract by EtOAc. And then the EtOAc extract was portioned by MeOH and n-Hexane to give MeOH (PN-EA-Me) and *n*-Hexane (PN-EA-Hex) layers.

** Due to the extraction procedure, the extract was separated into two parts.



Gerasimos Kondilatos et al. *Aquatic Invasions*, **2010**, *5*, 181-184

P. nigra was collected from Singda harbor by scuba diving. After freeze-drying, we extracted the dried sample with methanol to give the MeOH crude extract. Then, we further partitioned the MeOH crude extract with DDW and EtOAc to give DDW and EtOAc layers. After that, the EtOAc layer kept to partition with *n*-Hexane and MeOH to give the *n*-Hex and MeOH sublayers, and the DDW layer was partitioned with *n*-BuOH and DDW to give the *n*-BuOH and DDW sublayers.

By the bioactivity-guided fractionation and isolation, the MeOH sublayer with stronger antibacterial activity was subjected to column chromatography to separate into 12 fractions. By further antibacterial screening, Frs. 6 and 7 showed clear inhibitory effect against the pathogenic indicators. Next, we would try to isolate the compounds from these bioactive fractions.

Summary

In this poster, we reported one tunicate, *P. nigra,* which collected from Singda harbor by scuba diving. After a procedure of rough chromatography, we gain many crude extracts from different organic solvents. In the preliminary bioassay, the MeOH layers showed their inhibitory effects on pathogenic bacteria including *S. aureus* and *P. aeruginosa*. Further, we tried test the Fr. 4~7. Based on the results of secondary antibacterial assay, only Fr. 7 exhibited the best antibacterial activity against *S. aureus* and *P. aeruginosa.* In the future, we would like to isolate and purify more compounds for continue bioassay.

Reference

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[5] Abdelwahab, G. M., Mira, A., Cheng, Y. B., Abdelaziz, T. A., Lahloub, M. F. I., & Khalil, A. T. Acetylcholine esterase inhibitory activity of green synthesized nanosilver by naphthopyrones isolated from marine-derived Aspergillus niger. *PloS one*, **2021**,*16*, e0257071.