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INVESTIGATION OF ANTIOXIDANT ACTIVITY AND CHEMICAL FINGERPRINT OF MARINE POLYCHAETE BASED ON ATR-FTIR METABOLOMICS

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Abstract: Marine polychaete is an important component in marine benthic communities and can be widely found in Malaysia. To date, the information regarding its chemicals and biological activities of marine polychaete is still limited. This study aims to evaluate the chemical fingerprint of the marine polychaetes *Marphysa moribidii* and *Diopatra claparedii* using Attenuated Total Reflectance Fourier-Transform Infrared (ATR FTIR) metabolomics. The antioxidant activity including total phenolic content (TPC) and DPPH free radical scavenging activity was also evaluated. The results showed that *D. claparedii* has a higher amount of TPC (0.47±.0.03 mg GAE/g) compared to *M. moribidii* (0.30± 0.01 mg GAE/g). The DPPH activity tested at the concentration 5000 µg/ml showed percentage of inhibition expressed by *D. claparedii* and *M. moribidii* extracts were 38.80±11.70 and 25.54±7.35 respectively. The principal component analysis (PCA) score plot showed distinct clusters between *M. moribidii* and D. *claparedii.* Further investigation *via* PCA loading plot showed that the FTIR signals at 3340, 1090, 1047 and 880 cm^{-1} were contributed by *D. claparedii*. Meanwhile, the partial least square (PLS) analysis revealed several signals were correlated with the TPC including stretching vibration of C-H at 2854 cm^{-1}, C=C bonds at 1640 cm^{-1} and C=C bending at 1726 cm^{-1} showing the presence of aromatic ring deformations. This study provides the key important chemical fingerprint of different marine polychaetes (*D. claparedii* and *M. moribidii* sp.) that might be useful for the discovery of bioactive compounds from natural resources.

Keywords: Marine polychaete, *Marphysa moribidii*, *Diopatra claparedii*, ATR-FTIR metabolomics, antioxidant.

Introduction

Polychaetes are widespread and most abundant in marine environment. It can be important for the eco-toxicology testing organisms according to their small size and short life cycles. The developmental stages and the life cycle characteristics have been found to be useful in providing more sensitive results for monitoring of pollutants (Pachiappan *et al.,* 2015). Besides, marine polychaetes may have the potential as a source of bioactive compounds, but until now very limited information is recorded regarding its chemical constituents and biological activities. Earlier studies reported that marine polychaetes have been utilised for medical purposes including anti-oxidant and anti-wound-healing (Hussain *et al.,* 2018; Nazri *et al.,* 2019; Pei *et al.,* 2020). Thus, a study needs to be done to determine the chemical profile of polychaetes as well as the biological activities. Several polychaete species can be found in Malaysia including *Diopatra claparedii*, *D. neapolitana*, *Namalycastis rhodochorde*, *Vanadis minuta* and *Leiochrides australis* (Fielman *et al.,* 2001; Arshad & Idris, 2013). Recently, a new marine polychaete *Marphysa moribidii* from the west coast of Peninsular Malaysia was reported (Idris *et al.,* 2014).

Metabolomics can be defined as the study of all metabolites in organisms that unravels the chemical and biological correlations (Verpoorte *et al.,* 2007). Fourier-transformed infrared spectroscopy (FTIR) is one of the analytical tools that can be used in metabolomics research. Present study reported the chemical fingerprint of the two marine polychaetes (*Marphysa moribidii* and *Diopatra claparedii*) using FTIR metabolomics. In addition, the evaluation on their total phenolic content (TPC) and antioxidant activity was also carried out.

Materials and Methods

Chemical Reagents

Analytical organic solvents such as methanol and acetone were used for extraction and FTIR analysis. The chemicals 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), gallic acid and Folin-Ciocalteu were used for bioassay.

Sample Collection

D. claparedii sample was collected from the lower tidal flat zone of local mangrove during low tide and *M. moribidii* was collected at upper tidal flat zone of Pantai Kelanang, Morib mangrove in Selangor, Malaysia. The samples were ground and subjected to freeze dryer to remove excess water. The freeze-dried sample was kept at -20°C prior to analysis.

Sample Extraction

About 100 mg of freeze-dried sample in powder form was extracted with 1 mL methanol (ratio 1:10 w/v) and sonicated for 30 mins at room temperature. The supernatant was filtered using 1 mL of syringe through a 0.45 µm PTFE membrane filter (Nice®). The extraction procedure was repeated for three times. Five individual extractions were performed to obtain five biological replicates for each polychaete species. The samples were concentrated under vacuum using rotary evaporator. The dried extracts were kept in the freezer (4ºC) prior to analysis.

Total Phenolic Content

Using the Folin-Ciocalteu method, the total phenolic content was performed (Lee *et al.,* 2013). 20 µL of the polychaete extracts (3000 mg/mL concentration) were placed in 96-well microplate and 100 µL of Folin-Ciocalteu reagent was added. The mixtures were incubated for five minutes. Then, 80 µL of 7.5% sodium carbonate were added and incubated for two hours in the dark. The sample absorbance was measured at 750 nm using spectrophotometer. Both polychaete samples with five replicates were assayed and the results were presented as mg of gallic acid equivalents (GAE)/g of the sample based on a calibration curve prepared using gallic acid, assuming it is 100 % pure (Lee *et al.,* 2013).

DPPH Free Radical Scavenging Activity

The DPPH assay was used to ascertain the antioxidant activity of polychaete extracts. The test used a 50 µL of sample solution at the concentration of 5000 µg/mL in 96-well plate. Next, 100 µL of DPPH (60 µg/mL) was added to each well. The reaction mixture was incubated at room temperature for 30 mins. A micro-plate reader was used to measure sample absorbance at 517 nm. Five replicates were performed. The percentage inhibition was calculated as follow:

$$\% \text{ inhibition} = [(AB-AS)/AB] \times 100$$

where,

AB and AS are the absorbance of reagent blank and tested samples, respectively.

The results are reported as mean ± standard deviation of the percentage inhibition against DPPH at the concentration of 5000 µg/ml. Gallic acid was used as positive control (Abd Ghafar *et al.,* 2018).

ATR-FTIR Analysis

Infrared spectra were obtained from Nicolet™ iS™ 10 FTIR Spectrometer (Thermo Fisher Scientific, Madison, WI, USA). About 0.1 mL of the sample (1 mg/ml) was loaded onto

ATR crystal centre. Samples were dried for approximately 40 s at room temperature. Then, each spectrum was recorded between the wavelengths of 4000–600 cm^{-1} with 40 interferograms at a resolution of 4 cm^{-1}. Before each sample scan, a spectrum of the ATR crystal was recorded using the same instrumental conditions as background. The ATR plate was cleaned using acetone and a dust-free tissue after each sample scan. For FTIR analysis of the samples, five biological replicates with three technical replicates were performed. All FTIR data were converted to ASCII files and subjected to multivariate data analysis.

Multivariate Data Analysis (MVDA)

The multivariate data analysis (MVDA) including component analysis (PCA) and partial least square (PLS) were carried out using the SIMCA-P software (v. 14.1, Umetrics, Umeå, Sweden) with Pareto scaling applied to all the data. The PCA is a technique to reduce the dimensionality of such datasets, to increase interpretability but at the same time to minimize information loss. It is usually used for the metabolite fingerprint and classification purposes. In this study, the PLS model was used to evaluate the relationship between the chemical fingerprints from FTIR data with its total phenolic content (TPC) and DPPH activity. Partial least square model is often used in metabolomics, where predictive variables consist of many different measurements in an experiment (Maulidiani *et al.,* 2013).

Results and Discussion

Total Phenolic Content (TPC) and DPPH Activity of D. claparedii and M. moribidii

Table 1 showed the TPC and DPPH activity of *D. claparedii* and *M. moribidii*. The methanolic extract of *D. claparedii* showed higher TPC (0.47 mg GAE/g) compared to *M. moribidii* (0.30 mg GAE/g). The same pattern was also observed for the DPPH activity. The DPPH result of *D. claparedii* showed higher antioxidant activity compared to *M. moribidii*. The positive correlation was observed between DPPH activity and TPC of the marine polychaetes, and it can be concluded that TPC might have contributed to the DPPH activity.

Table 1: Total phenolic content (TPC) and DPPH activity of *D. claparedii* and *M. moribidii*

Species	TPC (mg GAE/ g sample)	DPPH Activity (at 5000 µg/mL)
D. claparedii	0.47±0.03	38.8011.70
M. moribidii	0.30± 0.01	25.547.35

FTIR spectra of D. claparedii and M. moribidii

In general, the two polychaetes showed the same pattern based on their FTIR spectra (Figure 1). In spite of that, the different intensity in the wavenumbers can be observed in their FTIR spectra. The FTIR spectra of *D. claparedii* and *M. moribidii* showed characteristic band with strong intensity at 1023 cm^{-1} resulting from C-O stretching (Figure 1). In addition, band in the area of 1391 cm^{-1} corresponding to CH_3 bending while band of 1700 cm^{-1} with weak intensity was also seen due to the C=O (amide). The signals at 2872-2965 cm^{-1} correspond to CH sp3 stretching for both symmetric signals at 2850 and asymmetric at 2920 cm^{-1}, respectively. The aforementioned are the characteristic signals of polysaccharides, lipids, and carbohydrates. In addition, a broad band in a range of 3055-3626 cm^{-1} with medium intensity is attributed to the functional groups of O-H and N-H stretch (water, alcohols, phenols, and amine). Detail identification of possible compounds in marine polychaete is presented in Table 2. In order to gain a clearer overview on the chemical fingerprint of the polychaete samples, the multivariate data analysis of PCA was conducted.

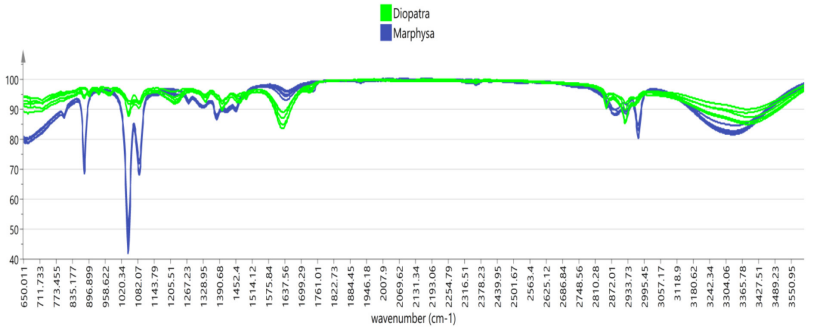

Figure 1: ATR-FTIR spectra overlay of *D. claparedii* and *M. moribidii*

Table 2: Tentative identification of compounds in marine polychaetes based on FTIR data

Wavenumber Range (cm^{-1})	Function Groups	Tentative Assignment	Reference(s)
3055-3626	O-H and N-H stretching	Alcohols, phenols, amine	Pei *et al.* (2020), Anguebes *et al*. (2016), Oliveira *et al*. (2016)
2872-2965	C-H stretching	Carbohydrates, lipids, phenols	Oliveira *et al*. (2016)
1570-1762	N-H bending vibrations, C=O bending vibrations	Amino acids, fatty acids, ester	Pei *et al.* (2020), Oliveira *et al*. (2016)
1315-1500	CH and OH bending	Alkene, carboxylic acids, alcohols, phenols	Pei *et al.* (2020), Oliveira *et al*. (2016)
1250-1020	C-N and C-O-C stretching	Amine, carbohydrates	Anguebes *et al*. (2016)
1045-1089	C-O and S=O stretching	Aliphatic ether, primary and secondary alcohols, sulfoxide	Anguebes *et al*. (2016)
898-980	C=C bending	Alkene	Oliveira *et al*. (2016)
880	C-H bending	1,2,4-trisubstituted, 1,3-disubstituted	Oliveira *et al*. (2016)
650-680	C-Br stretching	Halogen compounds	Pei *et al.* (2020)

Principal Component Analysis (PCA)

While there were strong visual variations between the spectra, multivariate analysis was used to further evaluate the samples for a non-biased interpretation of the findings. Principal component analysis (PCA) is one of the methods most widely used among statistical methods. In this study, the PCA model was constructed using all the analytical samples. The first two PC's explained variance of 87.33%. The PCA is considered to be a good model with values of R2X (cum) and Q2 (cum) of 0.873 and 0.797, respectively. The score plot serves to identify any groupings in the data set while the loading plots can be used to identify the wavenumbers that are responsible for the separation of the samples. Figure 2 showed the PCA score plot of the two polychaete samples generated based on its FTIR. It showed a clear discrimination between *D. claparedii* and *M. moribidii* extracts. The loading plot (Figure 3) showed that the wavenumbers at 3340, 2940, 2854, 1090, 1047

and 880 cm^{-1} were the characteristics of *D. claparedii* while the wavenumbers at 2900, 1726 and 1640 cm^{-1} appeared to be more prominent in *M. moribidii*.

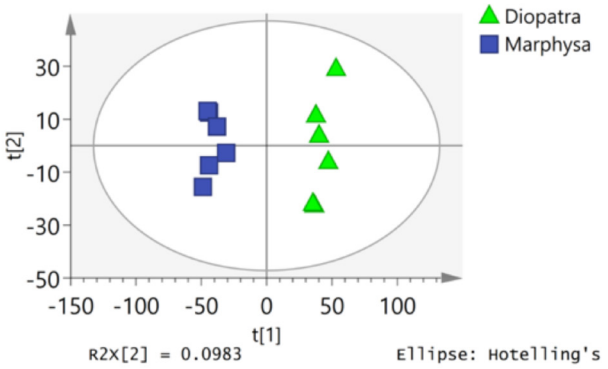

Figure 2: Principal component analysis (PCA) score plot of *D. claparedii* versus *M. moribidii*

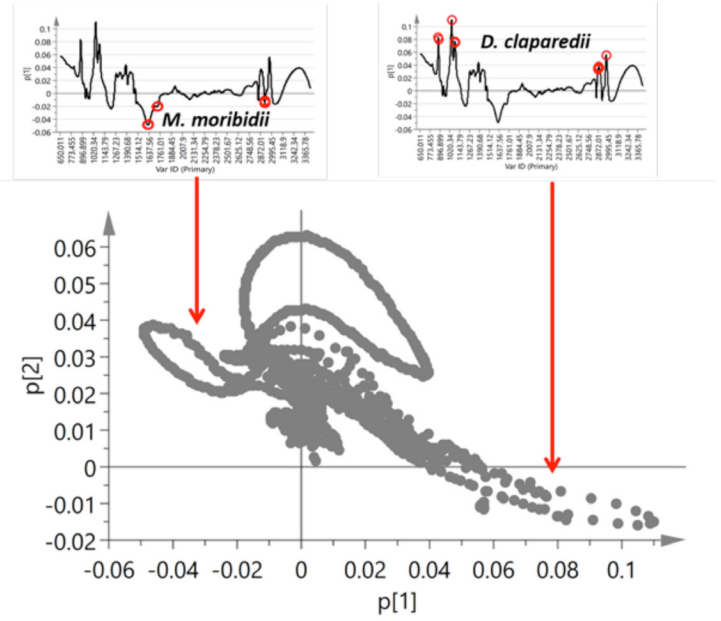

Figure 3: Principal component analysis (PCA) loading plot of *D. claparedii* versus *M. moribidii*

Partial Least Square (PLS) Analysis

PLS model was conducted to evaluate the relationship between the FTIR signals with TPC and DPPH activity. The X variables represented the wavenumbers, while the Y variables represented TPC and DPPH activity. The results are presented in PLS biplot, a single graphic representation was produced, combining the score and the loading plots.

A distinct difference between the two types of polychaete species, *D. claparedii* and *M. moribidii,* was observed based on the PLS biplot (Figure 4). The PLS analysis revealed several wavenumbers correlated with the TPC

and DPPH activity including C=C and C=O vibration at 1631 cm^{-1}, bending (δ) vibration of C–H and the stretching vibration of aromatics (-C=C-) at 1400 cm^{-1}, and signal at 881 cm-1 due to aromatic ring vibration (Oliveira *et al.,* 2016). The signal at 2926 cm^{-1} indicated the presence of C-H stretching vibration of methyl and methoxy groups and stretching vibration of -CH3 or -CH2 groups in carboxylic acid. In addition, the OH wagging (OH of phenolics) can be identified based on the FTIR signal at 3379 cm^{-1} (Oliveira *et al.,* 2016). Furthermore, the TPC and DPPH activity positioned at the same quadrant in the right side of PLS biplot indicated that the TPC has positive correlation with DPPH activity. Thus, it can be concluded that the antioxidant activity of polychaetes was closely related to the amount of phenolics. This finding is in agreement with a previous study which reported that TPC showed positive correlation to DPPH activity (Abd Ghafar *et al.,* 2018).

The PLS model was shown to be a good model indicated by its R2Y (cum) value of 0.602. Although the Q2 (cum) value of 0.325 is slightly low, the validity of PLS model was confirmed by permutation test and supported by the results from observed and predicted plots. The permutation test result for TPC showed R2 and Q2 values of 0.106 and -0.239, respectively. Meanwhile the permutation test results for DPPH activity showed R2 and Q2 values of 0.33 and -0.243, respectively. Both TPC and DPPH activity of observed and predicted plots showed R2 values > 0.5. Thus, the PLS model used in this study met the criteria of good model and can be used for prediction purposes.

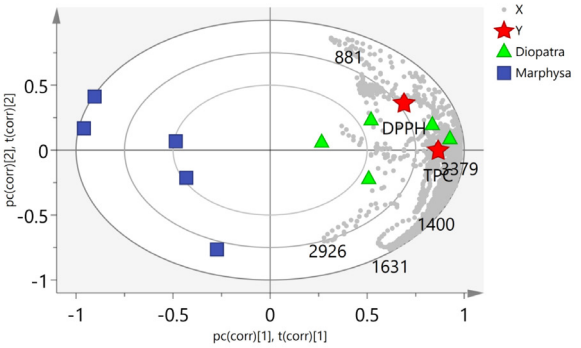

Figure 4: PLS Bi-Plot of *D. claparedii* versus *M. moribidii* correlates with its TPC and DPPH activity; The wavenumbers at 3379, 2976, 2926, 1631, 1400 and 881 cm^{-1} correspond to TPC and DPPH activity

Conclusion

The ATR-FTIR metabolomics was successfully applied to determine the chemical profile of two marine polychaete species (*D. claparedii* and *M. moribidii*). Both polychaetes showed weak antioxidant activity. The PLS model showed some FTIR signals at 3379, 2976, 2926, 1631, 1400 and 881 cm^{-1} were positively correlated to its TPC and DPPH activity. The outcome of this study provides preliminary information on the chemical profiles and biological activity of marine polychaetes in Malaysia.

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