Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 13(4): 2275-2285, August 2019

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Peptide profile and free radical scavenging activity of the low molecular weight peptide fraction from whole body extracts of *Tympanotonus fuscatus* var radula (Linnaeus) and *Pachymelania aurita* (Muller)

Queensley EGHIANRUWA[1,2*], Grace OPARINDE[3] and Omolaja OSONIYI[3]

[1]*Department of Molecular Biology and Biotechnology, Pan African University Institute of Science, Technology and Innovation, Juja, Kenya.*
[2]*Department of Biochemistry, FBMS, University of Uyo, Uyo, Nigeria.*
[3]*Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile Ife, Nigeria.*
[*]*Corresponding author; E-mail: qaeghianruwa@uniuyo.edu.ng; Tel: +2348024195501*

ABSTRACT

Low molecular weight peptides are among the most active bioactive components that have been isolated from marine molluscs. Currently, peptides isolated from molluscs as well as their synthetic structural analogues are in clinical trials as anticancer compounds and have been approved for use in pain management. These Bioactive peptides have also been demonstrated to exhibit antioxidant activities. This study aimed at isolating and profiling the low molecular weight peptide fractions from extracts of two common marine molluscs; *P. aurita* and *T. fuscatus* obtained from the Niger-Delta region of Nigeria. This study also investigated the free radical scavenging activity of the isolated fraction. Fourier Transform Infrared Spectroscopy analysis was carried out to determine the chemical entities and common functional groups found in the extracts. The Low molecular weight peptides were isolated using Molecular sieve chromatography, while the peptide profiles were established using SDS-PAGE and Thin Layer Chromatography (TLC). The free radical scavenging potential of the peptides was also assessed qualitatively using TLC, with DPPH as stain. The results show the presence of several low molecular weight peptides in the extracts of both *P. aurita* and *T. fuscatus*. Yellow spot on a purple background, indicating antioxidant activity was observed in the TLC plates sprayed before the chromatographic run. This activity was lost on further separation, indicating a synergistic effect. Further studies to effectively separate these fractions into individual peptides, investigate the synergistic effect observed as well as further explorations of possible bioactivity of these peptides is required.

Keywords: *Tympanotonus fuscatus* var radula, *Pachymelania aurita*, peptides, FT-IR, free- radical scavenging.

INTRODUCTION

Bioactive proteins have been defined as specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence health as well as play an important role in human health (Sharma et al., 2011). Bioactive peptides have been subject of intensive investigation including peptides derived from marine sources (Hamed et al., 2015). Cyclic

DOI: https://dx.doi.org/10.4314/ijbcs.v13i4.31

and linear peptides discovered from marine animals have increased our knowledge about new potent cytotoxic, anticancer, antimicrobial, and many other properties with novel chemical structures associated with novel mechanisms of pharmacological activity. These findings support the choice of marine peptides for research as sources of novel compounds for biomedical research (Anand et al., 2012).

Literature on marine molluscs reveals that among the vast number of bioactive compounds that have been isolated from the mollusca, the most active of them are the cyclic peptides, depsipeptides and low molecular weight peptides (Benkendorff, 2010). In general, bioactive peptides derived from marine organisms contain 5 to 40 amino acid residues depending on amino acid composition and sequence (Ibanez et al., 2011). The numbers of research publications reporting significant bioactivity exerted by high molecular weight proteins are few (Scotti et al., 2001). Furthermore, Lower molecular weight peptides have been demonstrated to possess stronger antioxidant activity compared to the higher molecular weight peptides (Pangestuti et al., 2017) and this may be due to improved contact ability with membrane lipids and/or permeability by the lower molecular weight peptides (Rajapakse et al., 2005; Pangestuti et al., 2017).

Methods of choice to purify crude extracts and to obtain specific peptide classes according to their molecular weight include centrifugation or ultrafiltration using appropriate membranes. To achieve further separation, these procedures are followed by gel and ion exchange chromatography techniques as well as RP-HPLC (Ngo et al., 2012). Purification procedures for cyclic peptides and depsipeptides isolated from marine creatures usually include initial extraction with Methanol (MeOH), partitioning of this extract with organic solvents of increasing polarities to render varied organic fractions and chromatographic steps on silica or Sephadex LH-20 columns and the use of RP- HPLC for the final purification (Anand et al., 2012).

Hence this study was aimed at using simple chromatographic methods to isolate the low molecular weight peptides from extracts of *P. aurita* and *T. fuscatus*, two commonly found molluscs in the Niger Delta area of Nigeria, which have been found to possess exploitable biological activities including analgesic, anti-inflammatory and antiproliferative activities.

MATERIALS AND METHODS

Collection and identification of molluscs

Fresh *Tympanotonus fuscatus* var radula and *Pachymelania aurita* samples were purchased in the month of July from the Oron Beach market (GPS coordinates: 4°49´37.6´´N 8°14´04.4´´E) in Oron, Akwa Ibom State, while still in the shells, and identified based on external morphology by a Zoologist from the Obafemi Awolowo University, Ile-Ife, Nigeria. Molluscs were transported alive to the laboratory in the mud that forms their natural environment. In the Laboratory, the molluscs were washed thoroughly to remove mud and then deshelled to collect both their flesh and hemolymph. The sample was stored as 50 g aliquots, in ziplock bags, at -20 ^{o}C until required.

Preparation of extracts

The alcohol extracts of *T. fuscatus* and *P. aurita* were prepared using the method described by Eghianruwa et al. *(*2019). To prepare the aqueous extracts, 200 g of mollusc flesh in its hemolymph was homogenized with 2000 ml of Phosphate buffered saline; PBS, pH 7.2 (0.1 M Sodium chloride in 0.025 M Sodium dihydrogen orthophosphate with 0.1 M PMSF) using a blender. The homogenate was left to extract for 48 hrs at 4 ^{0}C after which it was centrifuged at 10,000 g using a cold centrifuge, freeze dried and stored at 4 ^{o}C.

Fourier Transform Infrared Spectroscopy (FT-IR) Analysis of the mollusc extracts

10 mg of the powdered extracts of *T. fuscatus* and *P. aurita* were mixed with 100 mg of dried Potassium bromide (KBr) and compressed to prepare a salt disc (10 mm

diameter). The salt disc was inserted into the IR sample holder and the spectrum run, scanning between 600 – 4000 wave number (cm-1) at a speed of 1 micron/ min and with a programmed slit opening and air as reference. The infra-red spectra, indicating the frequencies of different components and functional groups present in each sample, was then analyzed.

Isolation of low molecular weight peptide fraction from the aqueous extracts

The isolation of the low molecular weight fraction from the aqueous extracts was carried out as described by Eghianruwa et al. (2011) using a calibrated Sephadex G100 molecular sieve column. The void volume of the column was determined with a fresh solution of blue dextran (2 mg/ml) in PBS, pH 7.2, the elution of which was monitored at 620 nm. Crude extract (5 ml) was applied on the column and fractions (2 ml) collected at a flow rate of 20 ml/hr and the elution monitored at 280 nm. The fractions in the low molecular weight peptide were pooled and stored at 4 °C.

Isolation of peptide fraction from the alcohol extracts

The peptide fractions from the alcohol extracts of *P. aurita* and *T. fuscatus* were obtained via molecular sieve chromatography on a Sephadex LH20 column. Sephadex LH20 resin (40 g) was swollen at room temperature in 200 ml of absolute methanol for 5 hrs. The slurry was stirred every hour during this time and fine particles were removed by decantation. The slurry was packed into a column (10 × 1.5 cm) and the column equilibrated with 300 ml PBS, pH 7.2. Crude aqueous extract (2.5 ml) of either *P. aurita* and *T. fuscatus* were applied on the column and eluted with 1 column volume of methanol and fractions (1 ml) were collected at a flow rate of 10 ml/hr. Peptide-containing fractions were detected by means of TLC.

Peptide detection in alcohol extracts

Analytical thin layer chromatography (TLC) was used to detect the peptide containing fractions from the Molecular sieve experiment using the method as described by Osoniyi and Onajobi (1998). The TLC was carried out on aluminium-backed silica 60 F254 gel plates (10 cm X 8 cm), using a solvent system of butanol: acetic acid: water (3: 1: 1) as the mobile phase while the detection stain was 0.2% Ninhydrin in ethanol. Plates were activated in an oven at 100-120 °C for 1 hr and allowed to cool before use. The solvent system was prepared fresh 15 min before each run, placed in the tank, swirled and allowed to saturate the tank before the run. 5 µl of each fraction obtained from the molecular sieve procedure was spotted on the plate and allowed to dry before placing in the tank for the run. After the run, the plate was air dried, sprayed with the ninhydrin stain and then dried in the oven at 100 °C for 15 min. Spots were analyzed and Rf values calculated using the formula:

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Peptide profile of the isolated low molecular weight peptide fractions

The peptide profile of the low molecular weight fraction obtained from the aqueous extracts was determined using SDS-PAGE on 10% gels in Tris-glycine buffer, pH 8.9 (Eghianruwa et al., 2011). The proteins were stained with Coomassie Brilliant Blue R, while the peptide profile of the peptide fractions from the alcohol extracts of *P. aurita* and *T. fuscatus* was determined using analytical TLC.

Evaluation of the antioxidant activity of the peptide fractions

The isolated peptide fractions from the alcohol extracts of *P. aurita* and *T. fuscatus* were screened qualitatively for antioxidant activity using Analytical TLC on silica gel

plates using a method as described by Mandal et al. (2013). Antioxidant activity is recognized by yellow spots on the chromatogram. 2 µl of the fractions were spotted and run on analytical silica gel 60 F254 TLC plates. The plates were oven dried at 100 °C and sprayed with DPPH solution (0.2% (wt/vol), MeOH). The plates were examined 30 min after spraying. 10 mg/ml each of ascorbic acid and Trolox were used as reference standards. Positive results are seen as yellow spots against a purple background.

RESULTS

FT-IR spectral analysis

When the extracts were passed into the FT-IR, the functional groups of the components were separated based on its peaks ratio (Table 1). The results of FT-IR analysis confirmed the presence of amines, carbonyl amides, alkanes, alkenes, aromatic groups and alkyl halide in the extracts of *P. aurita* and *T. fuscatus* with peptides and hydrocarbon derivatives as their predominant chemical groups.

Isolation of low molecular weight peptide fractions

Three Major protein peaks were obtained in the molecular sieve chromatography procedure on PAAQ, two of which were low molecular weight peaks (Figure 1) all low molecular weight peaks was pooled separately. The elution profile for the molecular sieve chromatography of TFAQ on Sephadex G-100 is presented in Figure 2**.** Two major protein peaks were obtained of which only one was a low molecular weight peak. The results of the molecular sieve chromatography of the crude alcohol extract of *P. aurita* and *T. fuscatus* is illustrated in Figure 2. Three peptide peaks were detected for each extract.

SDS-PAGE fingerprint profile of the isolated low molecular weight peptide fraction from the aqueous extracts

SDS-PAGE analysis of the isolated peptide fraction from Peak I obtained from PAAQ (Figure 3A) and the isolated from TFAQ (Figure 3B) and revealed several peptide spots. Only one peptide spot was observed in Peak II obtained from PAAQ (Figures 3A)

TLC fingerprint profile of the isolated peptide fractions from the alcohol extracts

Each isolated peak was observed to contain several peptide molecules (Figure 4). However, peak 2 obtained from both extracts appear to possess similar peptides.

Rf Values of the different bands in PAAC1 (P1) were 0.34 ± 0.01, 0.67 ± 0.00 and 0.8 ± 0.00. Bands in PAAC2 (P2) had Rf values of 0.34 ± 0.01, 0.525 ± 0.02, 0.65 ± 0.02, 0.78 ± 0.00 and 0.86 ± 0.00 while bands in PAAC3 (P3) had Rf values of 0.45 ± 0.01, 0.815 ± 0.02, 0.96 ± 0.00 and 0.98 ± 0.00.

For the alcohol extract of *T. fuscatus*, Rf values for the various peaks were calculated to be 0.22 ± 0.02, 0.35 ± 0.02, 0.515 ± 0.01, 0.645± 0.01 and 0.79± 0.01for bands in TFAC1 (T1); 0.21 ± 0.01, 0.355 ± 0.03, 0.505 ± 0.03, 0.585 ± 0.02 and 0.69 ± 0.02 for bands in TFAC2 (T2) and 0.21 ± 0.01, 0.355 ± 0.03, 0.505 ± 0.03, 0.585 ± 0.02 and 0.69 ± 0.02 for bands in TFAC3 (T3).

Antioxidant activity of the peptide fractions

TLC chromatograms used in evaluating the Antioxidant activity of the peptide fractions obtained from TFAC and PAAC are shown in Figures 5. On spotting the pooled factions without separation via the TLC, yellow spots were observed after spraying with DPPH. However, on spraying the plates with DPPH after a TLC run, no spots were observed, indicating a loss of activity.

Table 1: FTIR spectral peak values and functional groups obtained for the whole body extract of *P. aurita* and *T. fuscatus*.

EXTRACT	FUNCTIONAL GROUPS	PEAK VALUE(CM^{-1})
PAAQ	>C=O stretch of amide carbonyl (Amide 1)	1622
	-NH stretching Vibration	3138
	-NH bending Vibration (Amide 2)	1579
	SP^2-CH stretch of alkene	3053
	SP^3-CH Stretch of alkane	2983
	C-N stretch of an amine	1334
	C-O stretch of an acid conjugated to a carbonyl	1226
	-CH_2 bend of alkane	1471
	C-Cl alkyl halide	607
PAAC	>C=O stretch of amide carbonyl (Amide 1)	1624
	-NH stretching Vibration	3213
	NH bending Vibration(Amide 2)	1575
	SP^2-CH stretch of alkene	3155
	SP^3-CH Stretch of alkane	2976
	C-N stretch of an amine	1336
	C-O stretch of an acid conjugated to a carbonyl	1226
	-CH_2 bend of alkane	1471
	C-Cl alkyl halide	607
	N-CH3 (aromatic)	2813
TFAQ	>C=O stretch of amide carbonyl (Amide 1)	1627
	-NH stretching Vibration	3284
	-NH bending Vibration (Amide 2)	1575
	SP^2-CH stretch of alkene	3045
	SP^3-CH Stretch of alkane	2845
	C-N stretch of an amine	1263
	C-O stretch of an acid conjugated to a carbonyl	1118
	-CH_2 bend of alkane	1471
	C-Cl alkyl halide	671
	C-Br alkyl halide	509
TFAC	>C=O stretch of amide carbonyl (Amide 1)	1624
	-NH stretching Vibration	3168
	NH bending Vibration(Amide 2)	1575
	SP^2-CH stretch of alkene	3653
	SP^3-CH Stretch of alkane	2981
	C-N stretch of an amine	1338
	C-O stretch of an acid conjugated to a carbonyl	1226
	-CH_2 bend of alkane	147
	C-Cl alkyl halide	607

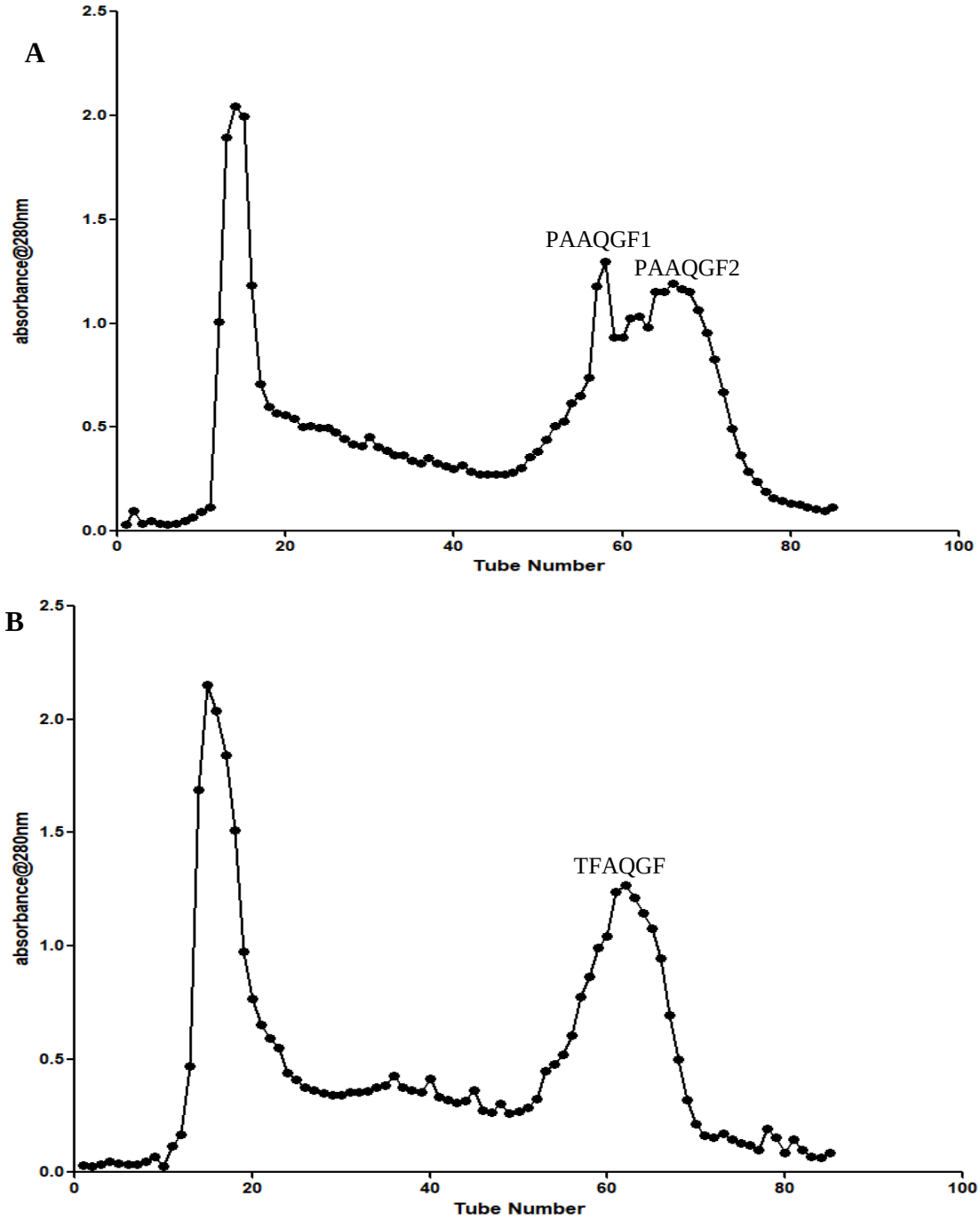

Figure 1: Molecular sieve elution profile of the crude aqueous extract of A). *Pachymelania aurita* and B). *Tympanotonus fuscatus* on Sephadex G-100.

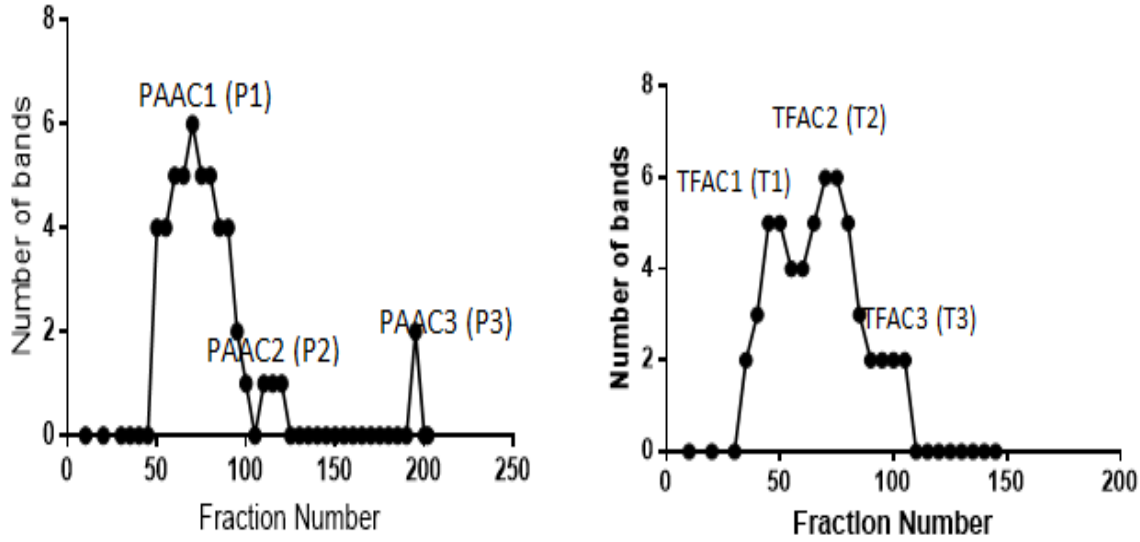

Figure 2: Molecular sieve elution profile of the crude alcohol extract of A) *Pachymelania aurita* and B) *Tympanotonus fuscatus* on Sephadex G-100.

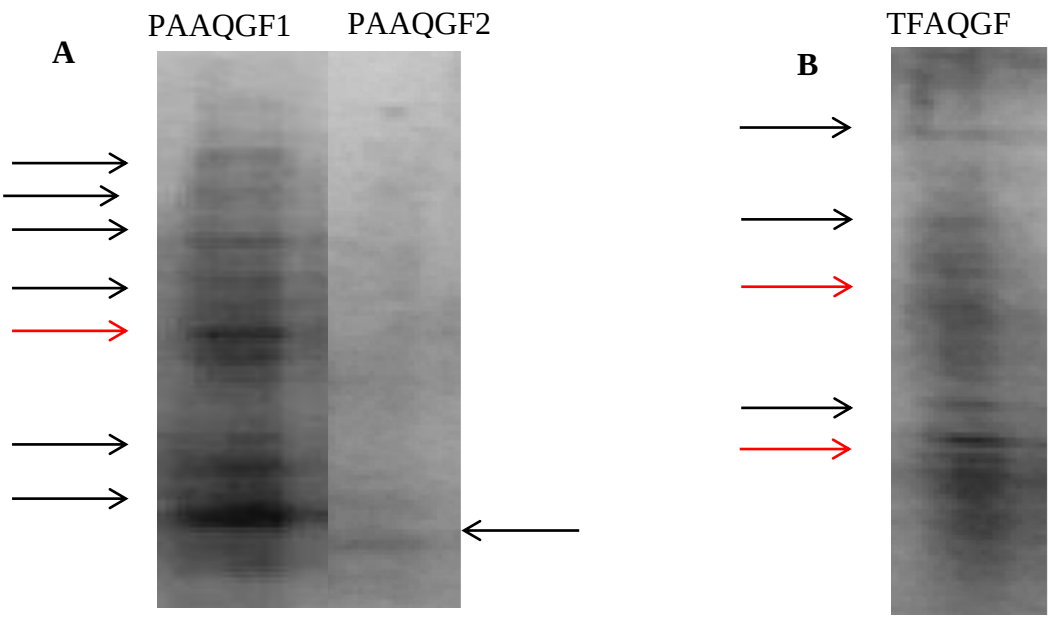

Figure 3: SDS-PAGE profile of the peptide peaks obtained from the molecular sieve chromatography of PAAQ (A) and TFAQ (B). Red arrows indicate regions of multiple bands.

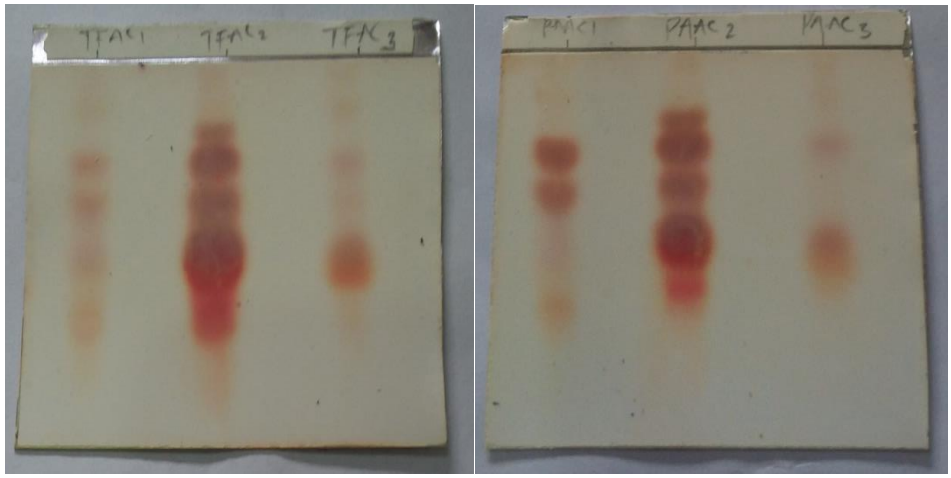

Figure 4: TLC chromatogram fingerprint profile of the three peaks pooled from the molecular sieve chromatography separation of the alcohol extract of *P. aurita* and *T. fuscatus*.

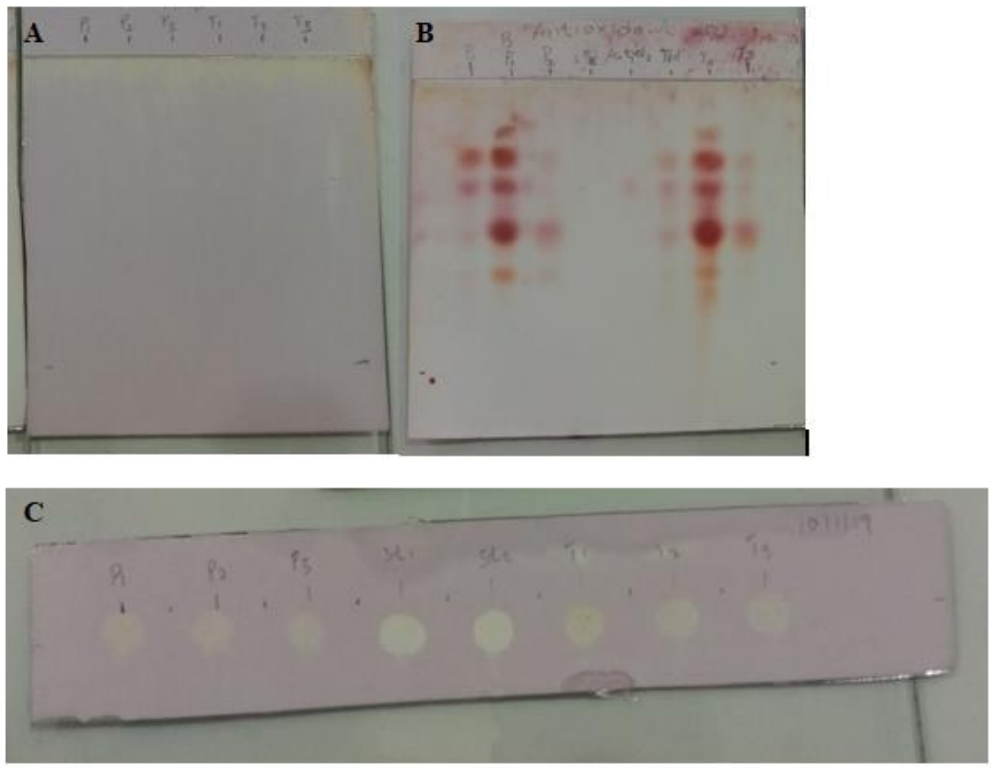

Figure 5: Plate A and B show qualitative assessment of the antioxidant activity of the peptide fraction of PAAC and TFAC after a TLC run to separate the peptides. P1, P2 and P3 indicate the peptide peaks obtained from PAAC while T1, T2 and T3. Plate A was sprayed with DPPH solution while plate B was sprayed with Ninhydrin in ethanol. Plate C shows the qualitative assessment of the antioxidant activity of the peptide fraction of PAAC and TFAC without a TLC run to separate the peptides.

DISCUSSION

A large number of natural products are used as alternate medicine for diseases of man and other animals since most of them are without side effects when compared with synthetic drugs. Identification of the chemical nature of phytochemical compounds present in these natural products will provide some information on the different functional groups responsible for their medicinal properties (Ashokkumar and Ramaswamy, 2014). Fourier transform infrared spectrophotometer (FTIR) is perhaps one of the most powerful tools for identifying the types of chemical bonds (functional groups) present in compounds (Nithyadevi and Sivakumar, 2015). This non-destructive technique has been used in a practical form to analyze natural products and identify the chemical identities in a wide range of compounds (Priya et al., 2010; Periyasmy et al., 2013).

In this study, FTIR analysis of the extracts of *P. aurita* and *T. fuscatus* revealed the presence of bioactive compounds signals at different ranges and showed the presence of similar functional groups in all the extracts. It confirmed the presence of functional groups like amides which indicate peptide linkages and thus the presence of amino acid containing molecules (protein or peptide). Characteristic bands found in the infrared spectra of proteins and polypeptides include the Amide I and Amide II. These arise from the amide bonds that link the amino acids. The absorption associated with the Amide I band leads to stretching vibrations of the C=O bond of the amide, absorption associated with the Amide II band leads primarily to bending vibrations of the N—H bond.

FT-IR also showed the presence of stretch and bending vibrations of alkenes and alkanes, alkyl halides and aromatic moieties and the CO stretch of an acid conjugated to a carbonyl. These are an indication of the presence of hydrocarbons that maybe the R-group of peptides or proteins, carbohydrate moieties or the tail of fatty acids.

In this study, Molecular sieve chromatography was the main technique used for the isolation of the peptides. Molecular sieve chromatography presents an excellent means of separation of the purification of low molecular weight peptides due to its ability to separate the components of a mixture by size. This Technique has been widely employed for the separation of low molecular weight peptides from Marine extracts (Benkendorff, 2010; Anand et al., 2012; Ngo et al., 2012).

A number of studies have been carried out for the separation of active fractions and thus their fingerprinting, using SDS-PAGE and TLC techniques (Patil et al., 2004; Hajimehdipoor et al., 2009; Marimathu et al., 2012). This is carried out in order to distinguish the active fractions between different species and from adulterant. In the present study, the SDS-PAGE and TLC profile for *T. fuscatus* and *P. aurita* was carried out and which can be applied to distinguish the peptide fraction of each species. Fingerprint identification profile tests are required to confirm the presence of the active constituents and potential adulterant in Traditional drugs and extracts especially when such extracts maybe employed for further studies (Marimathu et al., 2012). Furthermore, fingerprint profiles can be used to determine if variations in peptide production occurs during seasons or changes in the environment of marine species.

Separation has been proven to not always be beneficial in respect to bioactivity of natural products. In some cases mixtures of peptides, amino acids, and sugars show higher bioactivity (e.g. Antiproliferative and antioxidant activity) than single purified peptides (Sarmadi and Ismail, 2010). From the antioxidant studies on the crude extracts (in press), it was observed that the alcohol extracts of *P. aurita* and *T. fuscatus* exhibited antioxidant activity comparable to the standard, ascorbic acid. However, the peptide fraction exhibit lower antioxidant activity compared to the standard (as indicated by the lower yellow colour intensity) and antioxidant activity was completely lost on further separation using TLC. This indicates that the peptide fraction only exhibits antioxidant

activity when they are not separated and would exhibit higher activity when they are in solution with other non-peptide components in the extract. These components maybe simple inorganic molecules (FT-IR analysis revealed the presence of alkyl halides) needed for proper functioning of the peptides.

Conclusion

This study has been able to demonstrate the presence of several low molecular peptides in the extracts of both *P. aurita* and *T. fuscatus*. These peptides also proved to possess slight free-radical scavenging activity. Further studies to effectively separate these fractions into individual peptides, investigate the synergistic effect observed as well as further explorations of possible bioactivity of these peptides is required.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All laboratory procedures were carried out in the Laboratory of OO. EQ, OG and OO designed the study, wrote the protocols, managed the literature searches and performed all analysis. EQ also wrote the first draft of the manuscript, which was edited and proof read by all other authors.

ACKNOWLEDGEMENTS

The Authors would like to acknowledge Prof I.A.F. Adeniyi of the Department of Zoology, Obafemi Awolowo University, Ile-Ife, for identifying the samples used.

REFERENCES

Anand TP, Chellaram C, Kuberan G, Archana H. 2012. Bioactive Peptides from Marine Sources-A Review. *Indian J. Innov. & Dev*., **1**(S8): 61- 64.

Ashokkumar R, Ramaswamy M. 2014. Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. *Int. J. Curr. Microbiol. App. Sci*., **3**(1): 395-406.

Benkendorff K. 2010. Molluscan biological and chemical diversity: secondary metabolites and medicinal resources produced by marine molluscs. *Biol. Rev*., **85**(4): 757-775. DOI: https://doi.org/10.1111/j.1469-185x.2010.00124.x

Eghianruwa QA, Odekanyin OO, Kuku A. 2011. Physicochemical properties and acute toxicity studies of a lectin from the saline extract of the fruiting bodies of the shiitake mushroom, *Lentinulaedodes* (Berk). *Int. J. Biochem. Mol. Biol*., **2**(4): 309-317.

Eghianruwa QA, Osoniyi O, Wachira S, Maina N, Mbugua R, Imbuga M. 2019. In vitro antiproliferative studies of extracts of the marine molluscs: Tympanatonus fuscatus Var radula (linnaeus) and Pachymelania aurita (muller). *Int. J. Biochem. Mol. Biol*., **10**(1): 1– 8.

Hajimehdipoor H, Khanavi M, Zahedi H, Abedi Z, Kalantari, KN. 2009. Fingerprint study of *Thymus spp*. by TLC. *J. Med. Plants*, **8**: 19-24.

Hamed I, Ozogul F, Ozogul Y, Regenstein JM. 2015. Marine Bioactive Compounds and Their Health Benefits: A Review. *Comp. Rev. Food. Sci. & Food Safety*, **14**: 446- 465.

Ibanez E, Herrero M, Mendiola JA, Castro-Puyana M. 2011. Extraction and Characterization of Bioactive Compounds with Health Benefits from Marine Resources: Macro and Micro Algae, Cyanobacteria, and Invertebrates. In *Marine Bioactive Compounds: Sources, Characterization*, Hayes M (ed). Springer: New York; 58–62. DOI: https://doi.org/10.1007/978-1-4614-1247-2_2

Mandal S, Patra A, Samanta A, Roy S, Mandal A, Mahapatra TD, Pradhan S, Das K, Nandi DK. 2013. Analysis of phytochemical profile of *Terminalia*

arjuna bark extract with antioxidative and antimicrobial properties. *Asian Pac. J. Trop. Biomed*., **3**(12): 960–966. DOI: https://doi.org/10.1016/S2221-1691(13)60186-0

Marimuthu J, Antonisamy A, Essakimuthu P, Narayanan J, Anantham B, Tharmaraj RJJM, Arumugam S. 2012. Phytochemical characterization of brown seaweed Sargassum wightii. *Asian Pac. J. Trop. Dis*., **2**: S109–S113. DOI: https://doi.org/10.1016/s2222-1808(12)60134-0

Ngo DH, Vo TS, Ngo DN, Wijesekara I, Kim SK. 2012. Biological Activities and Potential Health Benefits of Bioactive Peptides Derived from Marine Organisms. *Int. J. Biol. Macromol;* **51**(4): 378-383. https://doi.org/10.1016/j.ijbiomac.2012.06.001

Nithyadevi J, Sivakumar R. 2015. Phytochemical Screening and GC-MS, FT-IR Analysis of Methanolic Extract Leaves of *Solanum torvum* Sw. *IJRSB.*, **3**(9): 61-66.

Osoniyi RO, Onajobi FD. 1998. Effect of pH and buffer concentration on the biosynthesis of Prostaglandin-like compounds by *Ocimum gratissimum* leaves. *Nigerian J. Biochem. and Mol. Biol.*, **13**: 14-47.

Pangestuti R, Kim S. 2017. Bioactive Peptide of Marine Origin for the Prevention and Treatment of Non-Communicable Diseases. *Mar. Drugs*, **15**: E67. DOI: https://doi.org/10.3390/md15030067

Patil S, Narayanan S, Eibl G, Jolly CI. 2004. Evaluation of antimitotic activity of *Rotula aquatica* (Lour): A traditional herb used in treatment of cancer. *Indian J. Exp. Biol.*, **42**: 893-899.

Periyasamy N, Srinivasan M, Balakrishnan S. 2012. Antimicrobial activities of the tissue extracts of *Babylonia spirata* Linnaeus, 1758 (Mollusca: Gastropoda) from Thazhanguda, southeast coast of India. *Asian Pac. J. Trop. Biomed*., **2**(1): 36-40. DOI: https://doi.org/10.1016/s2221-1691(11)60186-x

Priya ER, Ravichandran S, Ezhilmathi R. 2013. Bioactive proteins from pipefishes. *J. Coast Life Med.*, **1**(1): 1-5. DOI: https://doi.org/10.12980/jclm.1.2013c384

Rajapakse N, Mendis E, Byun H, Kim S. 2005. Purification and *in vitro* antioxidative effects of giant squid muscle peptides on free radical-mediated oxidative systems. *J. Nutr. Biochem*., **16**: 562- 569. DOI: https://doi.org/10.1016/j.jnutbio.2005.02.005

Sarmadi BH, Ismail A. 2010. Antioxidative Peptides from Food Proteins: A Review. *Peptides, **31***(10): 1949-1956. DOI: https://doi.org/10.1016/j.peptides.2010.06.020

Scotti PD, Dearing SC, Greenwood DR, Newcomb RD. 2001. Pernin: A Novel, Self-Aggregating Haemolymph Protein from the New Zealand Green-Lipped Mussel, *Perna canaliculus* (Bivalvia: Mytilidae). *Comp. Biochem. Physiol. B: Biochem. Mol. Biol*., **128**(4): 767-779. DOI: https://doi.org/10.1016/s1096-4959(01)00301-3

Sharma S, Singh R, Rana S. 2011. Bioactive Peptides: A Review. *Int. J. Bioauto*., **15**(4): 223-250.