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IDENTIFICATION OF THE BIOACTIVE COMPOUNDS OF SKIN MUCUS FROM ASIAN SWAMP EEL (*Monopterus albus*) USING LIQUID CHROMATOGRAPHY QUADRUPOLE-TIME-OF-FLIGHT MASS SPECTROMETRY

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Abstract

Asian swamp eels have been widely accepted as sources of food, especially among various Asian cultures. However, their potential values as novel sources of therapeutic agents have not been widely appreciated. Like most other tropical fishes and amphibians, the outer integumentary system of *Monopterus albus* is covered with mucus layers, which may act as a mechanical and biochemical barrier for their skin. The biochemical components of these mucus layers may have certain compounds that may be medically beneficial to human. The current study was interested to screen the bioactive compounds of skin mucus from the tropical Asian swamp eel (*Monopterus albus*) using Liquid Chromatography Quadrupole-Time-Of-Flight Mass Spectrometry (LC-QTOF-MS), for this purpose, eel skin mucus extract was used for LC-QTOF-MS analysis. The screening results for the bioactive compounds revealed different bioactive compounds which possess multiple biological properties mainly anticancer, antimicrobial, anti-inflammatory and antioxidant activities. In conclusion, the current study illustrated that eel skin mucus contain different bioactive compounds which might be consider as therapeutic-promising agents.

INTRODUCTION

Asian swamp eel, *Monopterus albus* (*M. albus*) belongs to synbranchidae family under the order of synbranchiformes [1]. It is native to the tropical and subtropical areas of northern India, China, Malaysia, Thailand, Indonesia, Philippines and possibly north-eastern Australia [2]. Asian swamp eel covered with a thick layer of mucus which is continuously replaced to protect the eel against microbial invasion [3].

Liquid chromatography quadrupole time-of-flight mass spectrometry performed very well, with sensitivities and specificities of >95 %. An interesting powerful analytical technique that has excellent screening capabilities is high-resolution mass [4] Liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOFMS). The use of LC-QTOF-MS has emerged as a valuable technique for the identification of by-products from emerging pollutants [5] due to the unique combination of high selectivity and structural information derived from accurate-mass MS and MS/MS spectra, as well as novel software implementations, which allow the comparison with empirical formulae databases [6]. Thus, the potential of LC-QTOF-MS was evaluated in terms of both qualitative and quantitative capabilities. [7].

Time of flight mass spectrometry provides new possibilities of the exact molecular mass together with the isotope peak pattern provide the molecular formula of a peak for search in theoretical databases as an important parameter for substance identification. [8]. Structural information about an unknown peak can be obtained from the fragment spectra generated by collision induced dissociation (CID) in single stage instruments [9, 10] or by CID in hybrid quadrupole time-of-flight instruments (LC–QTOF-MS) [11, 12]. In a previous work, an accurate mass CID spectra library of more than 2500 toxicologically relevant

substances was described for use in systematic toxicological analysis by LC–QTOF-MS and proved to be very useful for substance identification [13].

MATERIALS AND METHODS

Materials

The solvents and chemicals used for the mobile phase were purchased as follows: Formic acid and acetonitrile (LC–MS grade), and ammonium acetate (HPLC grade) from Fisher scientific (Schwerte, Germany), ammonium formate (LC–MS grade) from Agilent Technologies, water (HPLC grade) and formic acid (99+% for analysis) from Acros Organics (Geel, Belgium). All other solvents and reagents used for sample preparation were obtained from Merck (Darmstadt, Germany) in analytical grade purity.

Methods

Sample Collection and Extraction

Malaysian eels (*Monopterus albus*) were purchased from eel farm in Pekan, Pahang, Malaysia. The eel skin mucus (ESM) was collected from healthy eels by gently scraping the surface of the eel skin and then it was homogenized with 2 volumes of distilled water using homogenizer, followed by centrifugation at 13,000 rpm for 30 min, the supernatant was freeze-dried for 5 days. The dried substance was weighed and dissolved in methanolic to form methanolic extract, after that, the dissolved substance was filtered using 0.22µm syringe filter to be ready for use [14].

Identification of the bioactive compounds in ESM methanolic extracts using Liquid Chromatography quadrupole time-of-flight Mass Spectrometry (LC-QTOF-MS).

The analysis of identifying the bioactive compounds in ESM methanolic extracts was conducted with a QTOF-LCMS instrument (Waters VION Ion Mobility QTOF MS). The solvents used for the mobile phase were as follows: solvent name A (water + 0.1 % Formic Acid), solvent B: (acetonitrile) for gradient grade solvents as shown in **Table 1** and ammonium acetate was used for HPLC grade Column: C-18. All measurements were performed with a Q-TOF LC-MS instrument. The QTOF-MS was operated with an electrospray positive and negative ionization mode, the mass resolution of (100-1000) *m/z* measure the frequency of 10,000 transients s^{-1}, the low collision energy was 4.00 eV while the high collision energy ramp started with 10.00 eV and ended with 45.00 eV.

Table 1. The Gradient grade of solvents in the mobile phase using LC-QTOF-MS

Time (min)	Flow Rate (mL/min)	Solvent A (%)	Solvent B (%)	Curve
0.00	0.600	99.0	1.0	Initial
0.50	0.600	99.0	1.0	6
10.00	0.600	65.0	35.0	6
13.00	0.600	0.0	100.0	1
15.00	0.600	99.0	1.0	1

RESULTS AND DISCUSSION

LC-QTOF-MS screening was done to identify the non-volatiles compounds, as GCMS identify only the volatile compounds. LC-QTOF-MS results showed different types of compounds with different activities (**Figure 1 and Table 2).**

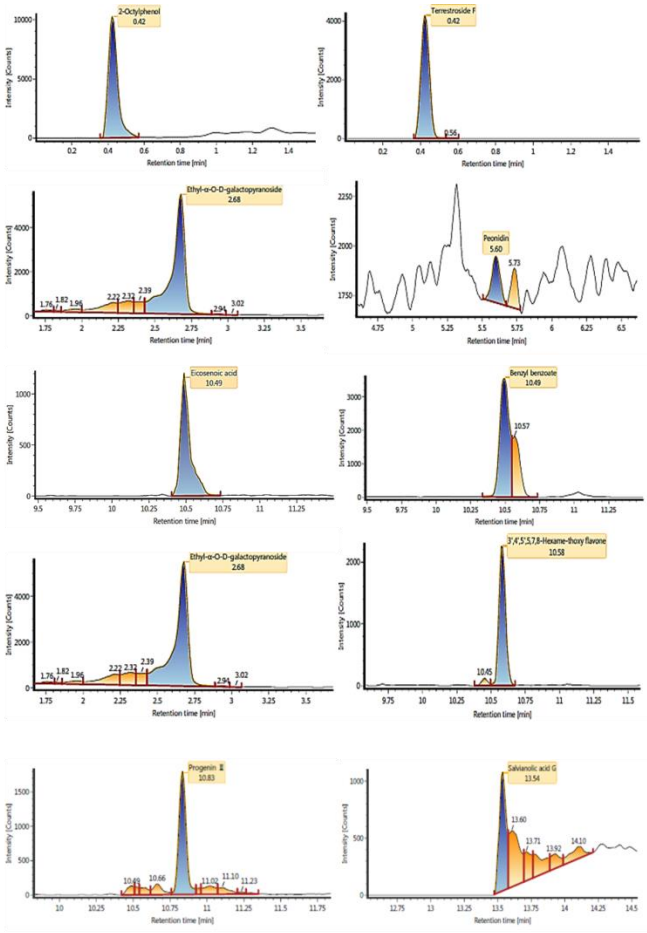

Figure 1. Chromatogram measured single-stage mass spectrum of isotope peak of the identified compounds in ESM methanolic extract with the peaks of [M+H].

LC-QTOF-MS results indicated the presence of different bioactive groups such as saponins, polyphenols, fatty acid, flavonoids, glucoside and cardiac glycoside. Terrestroside F compound was detected which is saponin and it has been reported that saponins have different biological activities such as, antioxidant, antifungal, antiviral, hypoglycaemia, hypocholesterolaemia and immunostimulant [15]. Peonidin also was detected which is anthocyanin and anthocyanins are polyphenols with antioxidant properties and prevent the risk of diabetes, arthritis, cardiovascular disease and cancer [16]. Eicosenoic acid was also identified which is a fatty acid and as it has been recorded that fatty acids have antibacterial and antifungal activities [17]. 6′-O-Palmitoyl-sitosterol-3-O-β-D-glucoside was also detected and as it has been reported that it possesses antimicrobial activity [18]. 3',4',5',5,7,8-Hexame-thoxy flavone is flavonoid and flavonoids are components of phenols, which has great antioxidative biological

activities [19]. **Table 3** shows the compounds detected in ESM methanolic extracts and it shows their biological activities.

Table 2. The compounds detected in ESM methanolic extracts using LC-QTOF-MS

Compound name	Formula	Observed mass (Da)	Observed *m/z*	Mass error (mDa)	Mass error (ppm)	RT (min)	Adduct
2-Octylphenol	$C_{14}H_{22}O$	206.1657	229.1549	-1.4	-6.1	0.43	+Na
Terrestrosid F	$C_{33}H_{54}O_7$	562.3857	585.3749	-1.2	-2.1	0.43	+Na
Ethyl-α-O-D-galactopyranoside	$C_8H_{16}O_6$	208.0950	231.0842	0.3	1.2	2.68	+Na
Peonidin	$C_{16}H_{13}O_6$	301.0711	302.0783	-0.1	-0.5	5.60	+H
Eicosenoic acid	$C_{20}H_{38}O_2$	310.2888	311.2960	1.6	5.1	10.49	+H
Benzyl benzoate	$C_{14}H_{12}O_2$	212.0834	251.0466	-0.3	-1.2	10.49	+K
6′-O-Palmitoyl-sitosterol-3-O-β-D-glucoside	$C_{50}H_{88}O_7$	800.6513	823.6406	-1.7	-2.0	10.55	+Na
3',4',5',5,7,8-Hexame-thoxy flavone	$C_{21}H_{22}O_8$	402.1316	403.1389	0.2	0.4	10.58	+H
Progenin II	$C_{40}H_{64}O_{11}$	720.4446	743.4339	-0.2	-0.3	10.83	+Na
Salvianolic acid G	$C_{18}H_{12}O_7$	340.0570	363.0463	-1.3	-3.5	13.54	+Na

Table 3. The biological activities of the bioactive compounds detected in ESM methanolic extracts using LC-QTOF-MS

Compound name	Biological Activity	Reference
2-Octylphenol	It has been reported that Octylphenol has estrogenic activity in rats	[20]
Terrestroside F	It has been proved that Saponine F named as terrestroside F.	[21]
	Terrestroside Falong is new type saponin.	[22]
Ethyl-α-O-D-galactopyranoside	It has been proven that acacetin-7-0-P-D-galactopyranosidel has high therapeutic index against HIV.	[23]
	α-D-Galactopyranoside has a potent inhibitory activity against intracellular α-galactosidases Debaryomyces hansenii UFV-1.	[24]
Peonidin	Peonidin like many anthocyanidins, has shown inhibitory and apoptotic effects against cancer cells	[25]
Eicosenoic acid	Eicosenoic acid has anti-inflammatory which cause a 10% reduction of NO levels	[26]
Benzyl benzoate	Benzyl benzoate has oestrogenic effect against human breast cancer cell line (MCF7).	[27]
6′-O-Palmitoyl-sitosterol-3-O-β-D-glucoside	It has modulating the immune response. It is effective in modulating the behavior of T-helper cells.	[28] [29]
	It has anti-inflammatory effect.	[30]
3',4',5',5,7,8-Hexame-thoxy flavone	Nobiletin (5,6,7,8,3',4'-hexamethoxy flavone) exhibits anticancer invasive properties.	[31]
Progenin II	Progenin II associated with cytotoxicity to mammalian cells	[32]
	Progenin II has cytotoxicity activities against human melanoma (A375-S2), human cervicoma Hela cell line and murine pneumoepithelial carcinoma (L929).	[33]
Salvianolic acid G	It has been proven that salvianolic acids have therapeutic potential on cancer treatment.	[34]

CONCLUSION

This study was able to provide relevant and useful information about skin mucus from Asian swamp eel (*Monopterus albus*), the objective was accomplished by providing the required information about the bioactive compounds detected in ESM extract using LC-QTOF-MS which showed different types of compounds with different biological activities including anticancer, antioxidant and antimicrobial activities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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