Chemical Composition and Antioxidant Activities of Catfish Epidermal Mucus

Nurul Mariam Hussin
Malaysia Ministry of Health, Putrajaya, Malaysia

Sharifudin Md. Shaarani and Mohd Rosni Sulaiman
Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Sabah, Malaysia

Abdul Hamid Ahmad and Charles S. Vairappan
Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Sabah, Malaysia
Email: nurulmariamhussin@gmail.com

***Abstract*—The antioxidant activity of *Clarias gariepinus* and *Clarias sp.1* epidermal mucus were determined. Aqueous extracts of *C.sp1* contained relatively higher levels of protein concentration [602.04mg g^{-1} fresh weight] than other extracts while the organic extracts (aqueous phase) of *C.sp1* gave higher levels of protein concentration (43.50mg g^{-1} fresh weight) than dichloromethane phase for both species. The chemical composition analysis revealed the values of moisture, ash, crude protein, fat and carbohydrate contents present in both species. The DPPH scavenging effect of all extracts and standards on the DPPH radical decreased in order of: ascorbic acid > BHT > dichoromethane phase extract of *C. gariepinus* > aqueous phase of *C. gariepinus* > acidic extract of *C.sp1* > acidic extract of *C. gariepinus* > dichloromethane phase extract of *C.sp1* > aqueous phase extract of *C.sp1* > aqueous extract of *C. gariepinus* > aqueous extract of *C.sp1* at the concentration of 100ug/ml, respectively. Reducing power activities in all extracts increased with increasing concentration except for acidic extract of *C. gariepinus* which decreased at concentration of 100ug/ml. The Ferric Reducing Antioxidant Power (FRAP) also increased as the concentration increased for all extracts. However, all extracts showed lower scavenging activity, reducing power and FRAP activities than BHT and ascorbic acid at the same concentrations. This preliminary information suggest that mucus from these fish species may be a source of novel antioxidant agents for fish and human health related applicants.**

***Index Terms*—catfish, epidermal mucus, chemical composition and antioxidant**

I. Introduction

A layer of mucus is secreted by specialized goblet cells present in the epidermal layer [1]. Proposed roles in respiration, ionic and osmotic regulation, reproduction, excretion, disease resistance, communication, feeding, nest building and protection potentially make mucus a highly multifunctional material [2].

Catfish are good sources of epidermal mucus [2] and they are also lean and highly nutritious fish rich in vitamins, proteins and minerals and low in carbohydrates [3], [4] that have positive impacts on human health. Their habitat can be found at Tropical South and North America, Africa and Asia, including Malaysia in the freshwater environments [5]. Channel catfish (*Ictalurus punctatus*), Blue catfish (*Ictalurus furcatus*), African catfish (*Clarias gariepinus*), Walking catfish (Family: Clariidae) and Shark catfish (Family: Pangasiidae) are some example of catfish species. In some developing country such as Turkey, catfish is generally consumed fresh, relatively cheap and it is an important source of cheap, high-quality protein [4].

To date, little known about the antioxidant properties of the epidermal mucus of catfish species. In the current study, *Clarias gariepinus* and *Clarias sp.1* epidermal mucus samples were extracted with acidic, organic and aqueous solvents where a variety of compounds including peptides, secondary metabolites, hydrophobic, aqueous and acid soluble compounds could be obtained and examined. The purpose of this study was to gain a better understanding of the antioxidant compounds of epidermal mucus in these fish species and to identify potential sources of fish to derive antioxidant compounds for fish and human health related.

II. Materials and Methods

A. Fish for Mucus Collection

Healthy and adult (both sexes) African catfish (*Clarias gariepinus*), was obtained from fish pond in Hatchery Universiti Malaysia Sabah, Sabah, Malaysia while the local catfish (*Clarias sp.1*), was obtained (wild catch) from Bongawan River, Sabah, Malaysia. All fish were allowed to settle down in two different tanks in the laboratory for 3 days before experimentation.

B. External Mucus Collection

Mucus was collected as described by Abdul Manan *et al.* [6] and Nagashima *et al.* [7] with slight modification. Live whole fish were cleaned by washing them with distilled water to remove any apparent dirt. Fish were then weighed and place into an enclosed clean plastic bag

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(12'' X 18'', ventral side of the body facing downward), one fish per bag. An estimated amount of distilled water (v / v fish / distilled water 1: 1) was added and transferred into a freezer (- 20 ℃) for 2hrs to induce a hypothermic stress condition where stressed fish will naturally secrete copious amounts of mucus [6]. The mucus was collected by carefully scrapped from the dorsal body using a clean plastic spatula. Ventral skin mucus was not collected to avoid blood, urino-genital, instestinal dan sperm contamination [7]-[10]. The mucus was centrifuged at 12, 000 x g for 10 min to get the bottom gel-like layer and the upper watery (supernatant) layer was discarded. The mucus harvest was immediately frozen at – 20 ℃ to prevent any bacterial contamination. The mucus samples obtained from individual fish of each species were pooled to yield one mucus sample per fish species.

C. Determination of Chemical Composition

Analysis of chemical composition was done in triplicate according to standard method of AOAC [11]. Water content (moisture) of each sample was determined by conventional method. 1.5g samples were dried at 105 ℃ overnight until reaching constant weight. Ash content was determined by using the basic method in the furnace oven at 550 ℃ overnight. The crude protein (N X 6.25) content was determined by the Kjeldahl procedure by using KJELTEC 2300 analyzer. All samples were digested (1.0 g each) with 12 ml of concentrated sulfuric acid in the presence of a catalyst, 7.0g of potassium sulphate and 0.8g of copper suphate. 1.5 gram of homogenised samples were placed in a Soxhlet extractor (Soxtec 2050 analyzer) for lipid content determination. 90 ml of petroleum ether (40–60% boiling point) were passed through, in hot extraction mode at 135 ℃ for 1h 45 minutes. The extract was dried in an oven at 105 ℃, until constant weight. The carbohydrate content was calculated by using the following formula:

Carbohydrate content (%) =

$$100 - (\text{moisture} + \text{ash} + \text{protein} + \text{lipid}) \quad (1)$$

D. Mucus Extraction

The acidic extracts of mucus were prepared using a slightly modification of the method Diamond *et al.* [12] and Subramaniam *et al.* [13]. 0.5g from the pooled mucus sample of each species was mixed with 50ml of 10 % (v/v) acetic acid and boiled for 5 min. The mixture was then cooled in ice, homogenized and centrifuge at 12, 000 x g for 30 min at 4 ^{O}C. The pellet was discarded and the supernatant was partially purified using a Thermo Scientific HyperSep Reversed-Phase C18 cartridge. Prior to the addition of supernatant, the cartridge was activated with 30ml of methanol and equilibrated with 10 ml of 10% (v/v) acetic acid. The cartridge was washed with 10 ml of 0.1% (v/v) trifluoroacetic acid (TFA) after loading the supernatant and then eluted with 40 ml of an acetonitrile/water/TFA (80.0:19.9:0.1, v/v/v) mixture. The eluates were freeze dried and resuspended in 18.2MΩ.cm Mili-Q ultra pure water (UPW). The organic extracts of mucus were prepared as described by Hellio *et al.* [8] and Subramaniam *et al.* [12]. 50ml of 95% ethanol was added to the pooled mucus sample (0.5g) and centrifuge at 12, 000 xg for 30 min at 4 ℃. The supernatant was decanted and the pellet was re-extracted two more times. The ethanol extracts were combined and evaporated under vacuum at 37 ℃. The extract was resuspended in 50ml of UPW and partitioned four times with 200ml (4 x 50ml) of dichloromethane (DCM). The aqueous phase was freeze dried, while the DCM phases (organic) were pooled and evaporated under vacuum at temperature below to 40 ℃. The dried samples obtained from the aqueous and organic phases were re-dissolved in UPW and 5% (v/v) dimethyl sulphoxide (DMSO) respectively.

To prepare aqueous extracts, the pooled mucus was suspended with stiring in UPW for 2 hours at 4 ℃. After centrifugation (30 min, 12, 000 x g, 4 ℃) and filtration, the supernatant was lyophilised to give the aqueous extract. These three phases were stored at –20 ℃ before use.

E. Determination of Antioxidant Activity

1, 1-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity:

The method reported by Garcia *et al.* [14] was followed. 1 ml of standard/sample was mix with 1ml of a daily-prepared solution DPPH at 0.1mM in methanol. The mixture was then kept in the dark at room temperature for 30 min. The reduction of DPPH radical was measured at 515nm and a blank was run in the same way by using UPW instead of sample, and sample control was also made for each sample by adding methanol instead of DPPH solution. The scavenging activity was calculated as follows:

DPPH radical scavenging activity (%)=

$$[1-(A_{sample})/A_{blank}]\times 100 \quad (2)$$

Reducing power: The reducing power of epidermal mucus samples were determined according to the method Garcia *et al.* [14] which originally adapted from Oyaizu [15]. 2ml of standard/sample was added to 2ml of 0.2mM phosphate buffer (pH 6.6) and 2ml of 1% potassium ferricyanide. The reaction mixture was incubated at 50 ℃ for 20 min. After incubation, 2ml of 10% trichloroacetic acid was added. The mixture was centrifuged at 1500 x g for 10 min. The supernatant (2ml aliquot) was mixed with 2ml UPW and 0.4 ml of 0.1% ferric cloride. The absorbance of the resulting solution was recorded at 700 nm after 10 min. An equivalent volume of UPW instead of sample was used as control. Analyses were carried out in triplicates.

Ferric Reducing Antioxidant Power (FRAP): The FRAP assay was carried out according to Li *et al.* [16]. Briefly, FRAP reagents were prepared by mixing acetate buffer (3.1g sodium acetate and 20ml acetic acid per litre, pH 3.6), a solution of 10mM TPTZ in 40mM HCl, and 20mM $FeCl_3$ at 10:1:1(v/v/v). The mixture was incubated at 37 ℃ for 10min. Then 1.0ml FRAP reagent (Fe^{3+}-TPTZ mixture) was added to 1.0ml of standard/sample and incubated in test tubes covered with aluminium foil at 25 ℃ for 30 min. Absorbance value was measured at 593nm.

F. Protein Quantification

The protein concentration of the acidic, organic and aqueous mucus extracts were determined based on the method of Bradford [17] using bovine serum albumin (BSA) as the standard.

G. Statistical Analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey's test by using statistical package of social science (SPSS) version 22.0 for windows. The values are mean ± SD of three replicate of experiments. P values < 0.05 were considered as level significance.

III. Results

A. Chemical Composition

The moisture, ash, crude protein, fat and carbohydrate contents of *C. gariepinus* and *C.sp1* epidermal mucus were shown in Table I. The moisture, fat and carbohydrate contents of *C. gariepinus* epidermal mucus were higher than *C.sp1* epidermal mucus. The ash and protein contents were higher in *C.sp1* epidermal mucus. The results showed that different species of catfish will give different amount of chemical composition.

TABLE I. Moisture, Ash, Protein, Fat and Carbohydrate Contents of *C. gariepinus* and *C.sp1* Epidermal Mucus

	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
C. gariepinus	90.01 ± 3.33^{c}	1.60 ± 1.03^{a}	6.34 ± 1.69^{b}	0.59 ± 0.07^{a}	2.25 ± 1.33^{a}
C.sp1	88.15 ± 0.32^{c}	2.98 ± 0.47^{a}	7.92 ± 0.63^{b}	0.47 ± 0.07^{a}	0.64 ± 0.23^{a}

All values are mean ± standard deviation of triplicate experiments. ^{abc} Different superscripts indicate significant (P<0.05) differences between samples.

Table II shows protein concentration of *C. gariepinus* and *C.sp1* epidermal mucus extracts. Water extracts for all samples gave the highest amount of protein followed by acidic extracts, ranging from 198.81 to 602.04mg g^{-1} fresh weight.

TABLE II. Protein Concentration of *C. gariepinus* and *C.sp1* Epidermal Mucus in Different Extracts (mg g-1 Fresh Weight)

	C.gariepinus	*C.sp1*
Acidic extract	312.35 ± 47.29$_{b}$	198.81 ± 80.03$_{ab}$
Organic extract:	23.12 ± 0.10 $_{a}$	21.26 ± 6.31 $_{a}$
DCM phase	32.19 ± 0.54 $_{a}$	43.5 ± 15.05 $_{a}$
Aqueous phase		
Water extract	335.11 ± 22.14$_{b}$	602.04 ± 402.38$_{c}$

All values are mean ± standard deviation of triplicate experiments. ^{abc} Different superscripts indicate significant (P<0.05) differences between samples.

B. Antioxidant Activities

The DPPH scavenging effect of all extracts and standards on the DPPH radical (Fig. 1(a)) decreased in order of: ascorbic acid (96.93%) > BHT (65.40%) > dichoromethane phase extract of *C. gariepinus* (54.52%)>aqueous phase of *C. gariepinus* (54.34 %) > acidic extract of *C.sp1* (53.93%) > acidic extract of *C. gariepinus* (53.27%) > dichloromethane phase extract of *C.sp1* (53.24%) > aqueous phase extract of *C.sp1* (53.12%) > aqueous extract of *C. gariepinus* (49.91%) > aqueous extract of *C.sp1* (38.28%) at the concentration of 100ug/ml, respectively. Reducing power activities in all extracts (Fig. 1(b)) increased with increasing concentration except for acidic extract of *C. gariepinus* which decreased at concentration of 100ug/ml. The Ferric Reducing Antioxidant Power (FRAP) (Fig. 1(c)), also increased as the concentration increased for all extracts. However, all extracts showed lower reducing power and FRAP activities than BHT and ascorbic acid at the same concentrations.

IV. Discussion

Epidermal mucus of both species had higher amount of moisture range from 88.15 to 90.01%. The 'slipperiness' of fish (mucus is synonymous with slime) is because of the high water (moisture) content and the presence of high-molecular-weight, gel-forming macromolecules [2]. Most of the constituents of mammalian mucus are also found in fish mucus where glycoproteins are the major macromolecular. In addition, a variety of the other materials has been identified where glycosaminoglycans, lysozyme, immunoglobulins, complement, carbonic anhydrase, a range of lectins and calmodulin have all been found in fish mucus [2]. Mucus from several species of fishes contained up to 20 times more lipid per unit area than human sebum and from previous study, its revealed free fatty acids which may provide antioxidant agents and protection againts bacterial and fungal attack [18].

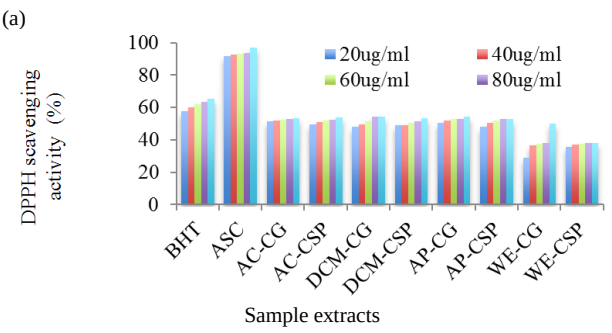
Due to screen epidermal mucus for a variety of antioxidant activities, a comprehensive suite of extraction methods was used. Acidic, organic and water extracts were prepared from the epidermal mucus of *C. gariepinus* and *C.sp1*. In order to obtain a basic peptide or protein enriched extract of the mucus, the mucus was extracted with acidic solvent, which is acetic acid [12], [13]. Solvent partioning of the ethanol extract resulted in the preparation of sub-fractions containing polar (aqueous) and non polar (dichloromethane (DCM)) compounds [13]. Water extraction method was used to prepare an extract containing all the water soluble compounds in the mucus, such as glycoprotein, lysozyme and proteases.

One of the methods to evaluate the antioxidant property of catfish epidermal mucus is to determine their ability to scavenge free radicals. Free radicals are involved in initiating and propagating lipid oxidation, and hence, food antioxidants would play an important role in scavenging these radicals [19]. In current study, the ability of catfish epidermal mucus to scavenge DPPH radicals was studied. DPPH free radicals (stable) and commercially available organic nitrogen radicals which can accept an electron or H• to become a stable molecule. DPPH has absorbance at 517nm which disappear upon reduction by an antiradical compound. Lower absorbance of the reaction mixture indicated higher DPPH radical-scavenging activity [14]. The scavenging activities of all samples were concentration-dependent. All samples

showed increasing of DPPH scavenging activity with increasing concentrations. Water extract of *C.sp1* epidermal mucus had the lowest DPPH scavenging activity, 38.28% at 100ug/ml. Among the different extracts of *C. gariepinus* and *C.sp1* epidermal mucus, dichoromethane phase extract of *C. gariepinus* exhibited the highest DPPH radical scavenging activity value at all concentrations tested (54.52% at 100ug/ml). However, all samples showed lower radical scavenging activity than BHT and ascorbic acid at the same concentration. The results obtained suggest that all sample extracts contained some peptides that were electron donors and could react with free radicals to convert them to more stable products and terminate the radical chain reaction.

Reducing power activity is a determination of ability of samples extracts to reduce iron (III). Reducing power of all samples was concentration-dependent. Increased absorbance of the reaction mixture indicated increased of reducing power. Higher reducing power have better abilities to donate electron where the presence of reducers causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form, therefore measuring the formation of Perl's Prussian blue at 700nm can monitor the Fe^{2+} concentration [19]. The yellow colour of test solution changes to various shades of green and blue, depending on the reducing power of each sample extracts. Reducing power of DCM phase (organic extract) of *C.sp1* epidermal mucus was similar to that ascorbic acid (0.14, 0.15), respectively. Water extract for both species showed higher reducing power than other extracts, (*C.gariepinus*, 0.12; *C.sp1*, 0.10).

The FRAP assay was based on electron-transfer which is commonly used for the routine analysis of antioxidants. The assay is based on the mechanism that the colourless TPTZ-Fe (III) reacts with electron donating antioxidants to generate the coloured TPTZ-Fe (II). The ferric reducing antioxidant power (FRAP) of *C. gariepinus* and *C.sp1* epidermal mucus was measured (Fig. 1(c)). Generally, the reducing power for all samples was concentration-dependent. A little different in the reducing power capacity was showed between *C. gariepinus* and *C.sp1* epidermal mucus extracts. However, all samples showed lower reducing power activities than BHT and ascorbic acid at the same concentrations. Different studies have reported that there is a direct correlation between antioxidant activities and reducing powers of some bioactive compounds [20], [21], [22].



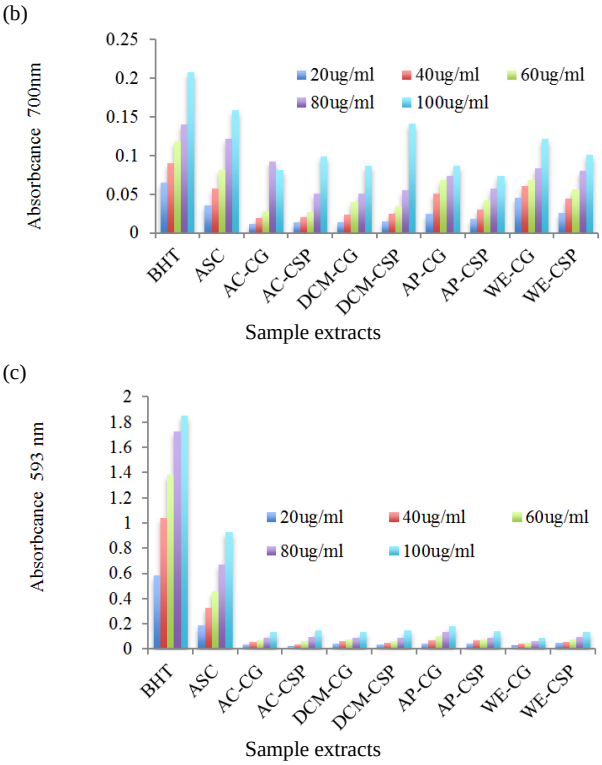


Figure 1. Scavenging effect on DPPH free radical (a), reducing power (b) and ferric reducing antioxidant powers (c) of *C. gariepinus* and *C.sp1* epidermal mucus extracts at different concentration, 0 to 100ug/ml. BHT and ascorbic acid were used as positive controls. Values presented are the mean of triplicate analyses. BHT, butylated hydroxytoluene; ASC, ascorbic acid; CG, *C. gariepinus*; CSP, *C. sp1*; AC, acidic extract; DCM, dichloromethane phase; AP, aqueous phase; WE, water extract.

V. Conclusion

In this study, the chemical composition and antioxidant activities of two different catfish species are presented. A significant variation in the chemical composition and antioxidant activities were observed among the catfish species examined. Of the screened mucus extracts, the acidic and water extracts of both catfish species exhibited the most potent activity indicating that basic biocative peptides or acidic soluble proteins could be the key as antioxidant agent. The present study also showed that *C. gariepinus* and *C.sp1* could be a potential source of novel antioxidant components for fish and human health related applications.

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Nurul Mariam Hussin was born in Malacca, Malaysia on March 9, 1984. She gained her bachelor degree in biochemistry (2006) at Universiti Putra Malaysia and finished her master's degree in plant biotechnology (2009) at the same university. She is a Scientific Officer (Biochemistry) since 2010 at Malaysia Ministry of Health. Currently, she is persuing her doctor of philosophy degree at University Malaysia Sabah. Her current research title is "Purification and Identification of Proteins and Other Novel Bioactive Compounds in Epidermal Mucus of Various Sabah Catfish Species".
Abdul Hamid Ahmad is an associate professor and a lecturer at Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Sabah, Malaysia.
Charles S. Vairappan is a professor and director of Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Sabah, Malaysia.
Sharifudin Md. Shaarani is a professor and dean at Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Sabah, Malaysia.
Mohd. Rosni Sulaiman is an associate professor and a lecturer at Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Sabah, Malaysia.