Original Research Article

Chemical Composition and Antioxidant Activity of Extracted Lipid from *Pangasius Pangasius:* A Complementary Analysis

ABSTRACT

Extraction of lipids from *Pangasius Pangasius* employed a unique simple route like the solvent extraction method used where ethanol is a diluent and chloroform acts as a solvent. The prime objective of this manuscript is to determine the chemical composition and antioxidant activity of lipids from *Pangasius Pangasius*. The chemical composition determination by Gas Chromatography Mass Spectrometry (GC-MS). The most abundant lipid components were Dodecanoic acid 1,2,3 Propanetriyl ester 86.67 % found in GC-MS which have strong bactericidal properties. For determination of the antioxidant activity, the Ultraviolet-Visible Spectrometer (UV) was introduced. The degree of hydrolysis, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity the antioxidant activity can be estimated. The control value of the UV is 1.297 a. u. and the absorption value by sample minimum value is 1.168 a. u. and the maximum value is 1.208 a. u. The inhibition rate maximum of 0.0995 % and a minimum of 0.0686 % by the human body. The absorption of UV light by the sample is near the control value which indicates lipids contain a little percentage of antioxidant activity. The sample

contains a small percentage of 0.33 of L (+) ascorbic acid 2,6 di-hexa-decanoate which is an antioxidant agent that reduces free radicals in the human body.

Keywords: Antioxidant activity, Chloroform, Ethanol, *Pangasius Pangasius,* Solvent extraction.

1. Introduction

Fish is an invaluable source of vital vitamins essential for the proper functioning of the human body [1]. Fish liver oil, in particular, is an extraordinary reservoir of vitamins A and D where Vitamin A maintains healthy skin and promotes bone development and vitamin D plays a pivotal role strength of teeth and bones [1]. It is noteworthy that oily fish, in particular, constitute an excellent source of vitamin D [1]. A large number of peptides produced from hydrolyzed dietary proteins have antioxidative properties [2]. Antioxidative action has been observed for fish protein hydrolysates, such as skin gelatin hydrolysate from Alaska Pollack [2], yellowfin sole [3] and phytochemicals [4]. Hydrolyzed fish protein may also be a promising source of anticancer peptides [5], angiotensin I-converting enzyme (ACE) inhibitors [6], an anti-anemia drug [7] and a component of microbial growth media [8] considering preliminary evidence. On the other hand, not much is known about the antioxidative properties of collagen peptides derived from *pangasius pangasius* or striped catfish [9]. The *pangasius hypophthalmus* fish is a popular food source because it is rich in nutrients, including fat, protein, vitamins and minerals, all of which have a substantial positive impact on human health [9]. Pangasius boasts minerals, vitamins A, B, and E, and these antioxidants efficiently reduce oxidative stress [9]. Dietary sources of *pangasius hypophthalmus* fish include vitamins and minerals that function as antioxidants, thereby mitigating the effects of oxidative stress [10]. *Pangasius hypophthalmus* oil's omega-3 content improves zinc transfer to the cell membrane which assists in regulating insulin, slows down its breakdown, and improves insulin sensitivity [10]. In food preparation items, gelatin can be utilized as a stabilizer and emulsifier. Ethical and theological arguments have shown that fish

gelatin is a more efficient substitute for animal gelatin [11]. Usually, freshwater fish are utilized as a source of fresh gelatin [11]. However, numerous investigations have demonstrated that after the edible portions of some freshwater fish are removed, a significant amount of by-product remains [12]. High gelatin yields are therefore anticipated from fish such as tilapia [12], Nile perch [13] and striped catfish (*Pangasius sutchi fowler*) [14]. Gelatin extraction and the characterization of several freshwater fish as substitute sources of gelatin revealed that the gelatin derived from *pangasius pangasius* catfish had the best rheological characteristics when compared to the other fish [14]. Its characteristics include a total amino acid content, a melting point of 32.0 ^{0}C, a gelling temperature of 12.0 ^{0}C, a melting strength of 273.58 g and a viscosity of 36.5 cP [14].

The lipids extracted from *Pangasius pangasius* and *Pangasius sutchi* have undergone nutrient content analysis. The fishes' specific gravity, refractive index and viscosity coefficient were recorded at 0.97 ± 0.01 and 0.94 ± 0.02 (at 30.0 ^{0}C), 4.88 ± 0.25 and 5.49 ± 0.30 (at 30.0 ^{0}C), and 448.96 ± 2.5 and 421.76 ± 2.1, respectively. Chemical characterization of the lipids in both fish species has yielded significant results [14]. Furthermore, *P. pangasius* and *P. sutchi* have demonstrated carbohydrate, protein, cholesterol and lipid contents of 1.89 ± 0.35 % and 0.75 ± 0.11 %, 35.0 ± 2.1 % and 38.37 ± 2.5 %, 17.46 ± 1.15 % and 13.79 ± 0.37 % and 10.01 ± 1.11 % and 6.16 ± 0.54 %, respectively. Adequate levels of humidity, solids and cinder percentages were discovered. Furthermore, *P. pangasius's* mineral levels such as calcium, phosphorus and zinc were inferior, while iron levels were greater than those of *P. sutchi* [15]. The fatty acid compositions of *P. pangasius* and *P. sutchi* consisted amount of lauric acid 13.36 and 4.26 %, palmitic acid 26.15 and 29.32 %, oleic acid 46.07 and 59.16 % and stearic acid 14.40 and 7.24 % correspondingly [15]. Additionally, consuming pangasius fish provides a rich source of essential proteins which are crucial for combating protein insufficiency. In contrast to other fish, pangasius is economically viable and offers a higher concentration of protein and beneficial

cholesterol [16]. In Bangladesh, two distinct varieties of the revered Pangas fish can be discovered- the first being the cultivated Pangas and the latter being the natural Pangas that thrive in the rivers. While the former is raised in household-installed tanks and ponds, the latter is preferred by the majority of the population owing to its delectable taste [16]. However, as a result of its scarce availability, the natural river pangasius catfish comes with a hefty price tag.

Freshwater catfish or *Sperata aor* are highly preferred by consumers and make a substantial contribution to the production of fisheries in tropical rivers [16]. Nutritional profiles of pangasius catfish, both wild (*Pangasius pangasius*) and farmed (*Pangasius hypophthalmus*) were gathered from several river and culture pond sources in Bangladesh [17]. The indigenous Pangas exhibited an elevated protein content of 26.06 ± 1.27 % and fat content of 14.79 ± 2.47 %, in contrast to the hybrid Pangas which demonstrated a protein content of 23.18±2.11 % and a fat content of 11.11±1.75 % [18]. It has been stated that the muscles of farmed pangasius are exported to more than 80.0 nations worldwide, including the US, Germany and the Netherlands where frozen fillets devoid of skin and bone are much sought after [19]. The fillets exhibited elevated moisture levels ranging from 80.0 % to 85.0 % coupled with diminished protein levels ranging from 12.60 % to 15.60 % and lipid levels hovering between 1.10 % and 3.00 % [20]. A study was designed to ascertain the proximate composition of fatty acids, amino acids and mineral contents of frozen fillets from Pangasius catfish (*Pangasius hypophthalmus*) imported to Poland, Germany and Ukraine to establish the nutritional composition of these fish [21]. However, due to its superior taste compared to farmed pangasius and the wild pangasius (Pangasius pangasius) species of riverine catfish is in high demand locally [22]. For this point of the study chemical composition and antioxidant activity of lipids from *Pangasius pangasius* profiling is the prime object of this manuscript.

2.0 Materials and Methods

2.1 Materials

The DPPH (2,2-diphenyl-1-picrylhydrazyl), ethanol and chloroform were procured from Sigma-Aldrich, Germany and DI water was collected from the ACCE Lab plant. The *Pangasius Pangasius* was also collected from the local market of Kushtia-7003, Bangladesh.

2.2 Preparation of lipid sample from Pangasius Pangasius

The lipids of *Pangasius Pangasius* can be extracted by solvent extraction process. In the solvent extraction process, the collected pangas fish were cut into small pieces and then the small pieces of fish were cleaned with water and DI water. After cleaning the fish sample, it was ground in a hand-grinding machine. Then 20.00 grams of mesh fish sample is taken in a bucket and then the 750.00 ml of chloroform and 250.00 ml of ethanol are taken in that bucket to maintain 3:1 (v/v). Then the chloroform, mesh fish sample and ethanol are mixed well. Then the total mixture is divided into four conical flasks in the same equivalent ratio. Then the all-conical flask is shaken well by an orbital shaker (RJH5005, Richard James Hilfiger, UK). The speed of the shaking should be 300.00 rpm maximized.

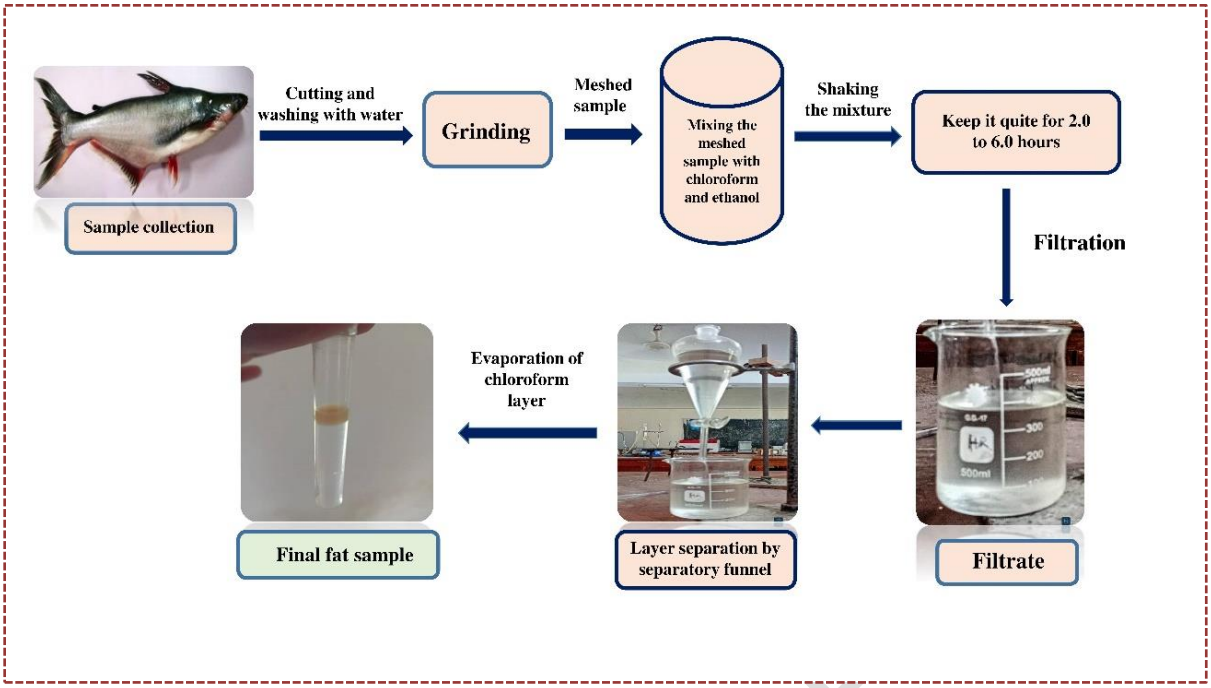

Fig. 1. Preparation route of lipid sample from *Pangasius Pangasius*

The shaking process is continued for 20.0 hours for complete extraction. Then the well shake is kept quiet for two hours for complete separation of chloroform and ethanol layer. The chloroform layer contains the lipid of *Pangasius Pangasius*. Then the two layers are separated by a separating funnel. The ethanol layer is collected in a bucket and another chloroform layer is collected in a beaker. Then the chloroform layer evaporated by nature in the shade. In the evaporation process, the extra heat or sun rays cannot be used because the fat can be broken. After the complete evaporation, the lipids of pangas appear. The route is illustrated in Fig. 1. The lipid sample is collected in a refrigerator for analysis of chemical composition by GC-MS and antioxidant test by UV.

3. Characterization

3.1 Gas Chromatography Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was carried out with Clarus ® 690 gas chromatograph (PerkinElmer, CA, USA) using a column (Elite-35, 30m length, 0.25 mm diameter, 0.25 µm thickness of film) and it was equipped with Clarus ® SQ 8 C mass spectrophotometry (PerkinElmer, CA, USA). 1.0 µL

sample was injected (split less mode) and pure Helium (He) 99.99% was used as a carrier gas at a constant flow rate (1.0 mL/min) of 40.0 mins runtime. The sample was analyzed in EI (electron ionization) mode at high energy at 70.0 eV. Though inlet temperature was constant at 280.0 °C, column oven temperature was set at 60.0 °C (for 0.0 min), raised at 5.0 °C per minute to 240.0 °C and held for 4.0 mins. The sample compounds were identified by comparing them to the National Institute of Standards and Technology (NIST) database [23].

3.2 Ultraviolet-Visible Spectrometry Analysis

The antioxidant test can be done by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity method [24]. Various concentration of the sample was prepared with DPPH such as 25.0 µl, 50.0 µl, 100.0 µl, 150.0 µl, 200.0 µl, 250.0 µl, 300.0 µl. The concentration of the blank sample was sorbed in a fixed wavelength at 517.0 nm. The Ultra violate light is absorbed by the lipid sample which estimates the antioxidant activity of the lipid sample. The antioxidant activity can be estimated by the inhibition of free radicals.

Inhibition rate (%) = {(blank absorbance - sample absorbance)/ blank absorbance}x 100%

Where blank is the absorbance of the control reaction which contains all reagents except the test compound. Sample absorbance containing test compound.

4.0 Result and Discussion

4.1. Chemical composition of lipid from Pangasius Pangasius

Pangasius Pangasius lipids are grey colour solid fat substances. Initially, during the extraction process, it forms a transparent liquid but after evaporation, it forms a solid grey colour. The identified compounds by GC-MS, according to their retention time in the column, the result is shown in Table 1.

Table 1. Chemical composition of the extracts from the sample of *Pangasius Pangasius*

Serial No.	Retention time (RT)	Compounds	Molecular Weight	Molecular Formula	Area (%)
1	7.44	propionic acid, 4-hydroxy-3-hexyl ester	174.0	$C_9H_{18}O_3$	0.02
2	21.73	benzoic acid,4,4- (1,2-dioxo-1,2-ethanediyl) bis-dimethyl ester	326.0	$C_{18}H_{14}O_6$	0.05
3	21.95	propanoic acid, 2-methyl,2-(hydroxymethyl)-1-propyl butyl ester	216.0	$C_{12}H_{24}O_4$	0.09
4	24.64	diethyl phthalate	222.0	$C_{12}H_{14}O_4$	0.02
5	28.27	Hepta cosanoic acid, 25-methyl, methyl ester	438.0	$C_{29}H_{58}O_2$	0.02
6	29.69	l-(+)-ascorbic acid 2,6-dihexa decanoate	652.0	$C_{38}H_{68}O_8$	0.33
7	33.19	bis(2-ethylhexyl) phthalate	390.0	$C_{24}H_{38}O_4$	12.37
8	37.36	dodecanoic acid,1,2,3-propanetriyl ester	638.0	$C_{39}H_{74}O_6$	86.67

In GC-MS analysis, twenty-four (24.0) compounds were identified from the sample. The bioactive compounds identified from the sample were represented by their retention time (RT), molecular formula, molecular weight and peak area (%) in Table 1 and Fig. 2. represent the distinct GC-MS chromatogram. This chromatogram representation of the vertical line indicates the peak area, where the chemical compound can be identified compared with the National Institute of Standards and Technology (NIST) database. The horizontal line represented the retention time in minutes. The samples were run in the column for 40.0 minutes the sample was analyzed in electron ionization mode at high energy at 70.0 ev. The different peaks are formed at different retention times which represent the different chemical compositions.

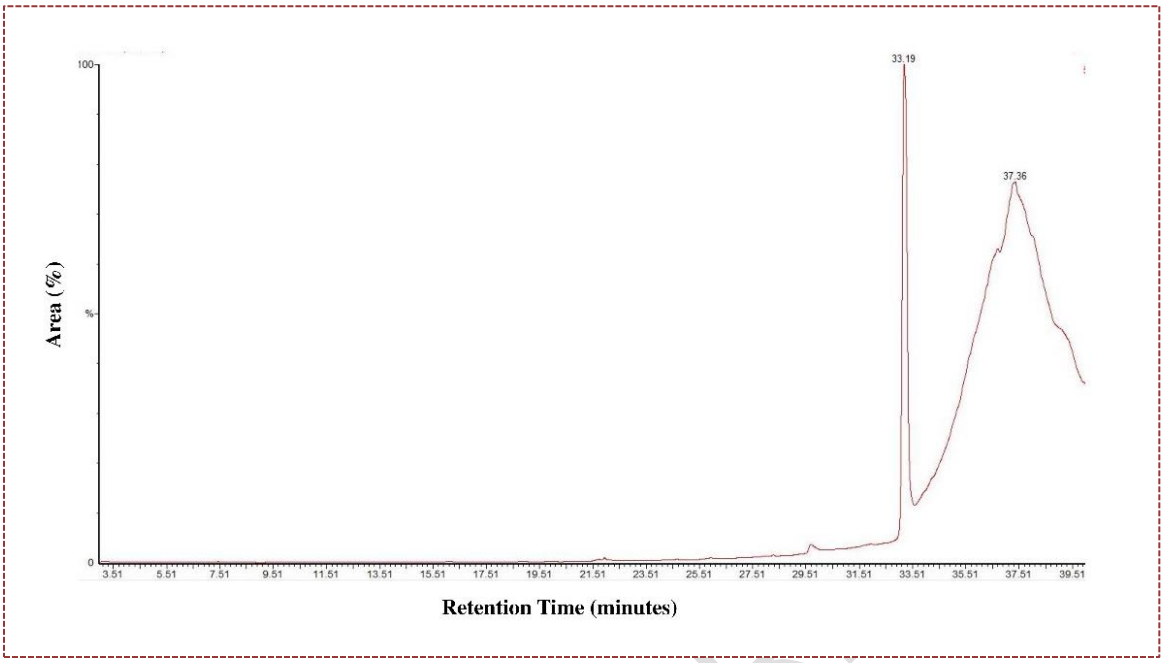

Fig. 2. Retention time vs area curve from GC-MS

First times, we observed propionic acid, 4-hydroxy-3-hexyl ester at the 7.44 retention time. It's useful in the food industry for the preservation of foods such as it is used to inhibit bacterial growth and used as a preservative for food [25]. Benzoic acid,4,4- (1,2-dioxo-1,2-ethanediyl) bis-dimethyl ester has an antimicrobial property which is beneficial for food and beverages and strong antibacterial property at pH 2.5 to 4.0 [26]. Propanoic acid, 2-methyl,2-(hydroxymethyl)-1-propylbutyl ester known as methyl ethyl ketone peroxide acts as a strong oxidizing agent [27]. It's also used in the curing of unsaturated polymer resin as a catalyst. Effect of diethyl phthalate use in plastic and personal care products. It also has a hazardous property [27]. It causes male reproductive, female reproductive, liver and kidney damage and causes cancer [27]. Hepta cosanoic acid, 25-methyl, methyl ester a long-chain fatty acid. It is an energy source, structure source for cell membranes and also signalling molecules [27]. L (±) ascorbic acid 2,6-dihexadecanoate has an antioxidant property, it is also used for the reduction of free radicals

from the human body. It also possesses antibacterial, antitumor and wound-healing properties. It is used for various pathogenic diseases [28].

Bis-2-Ethylhexyl is a plasticizer, it is intended use of food contact application. But it also has some side effects that cause cancer, birth defects or other reproductive harm [29]. It also interferes hormone system. Dodecanoic acid,1,2,3-propanetriyl ester is also known as glyceryl laurate [29]. It is used as an emulsifier, stabilizer, and also flavoring agent. It has also an antimicrobial property which extends the shelf life of products [29]. The major component from the extract is Dodecanoic acid, 1,2,3-Propanetriyl Ester at 86.67%, the second percentage from the extract is Bis-2-Ethylhexyl Phthalate 12.37% and the third is (±) Ascorbic acid 2,6-Dihexa decanoate at 0.33 %.

4.2. Antioxidant Properties Analysis

DPPH Radical Scavenging Activity is expressed where DPPH is widely acceptable for the determination of free radical scavenging activities of antioxidants [30]. The DPPH radical's reduction capacity can be determined by an absorbance of 517.0 nm induced by antioxidants. The increasing of ultra-violet light with concentration indicates the increasing antioxidant property of this compound. However, during analysis of this compound, the absorption rate had been decreased with concentration. So, the compound has less antioxidant properties. The UV visible spectrum analysis concentration vs absorbance of *Pangasius Pangasius* extracts is shown in Fig. 3.

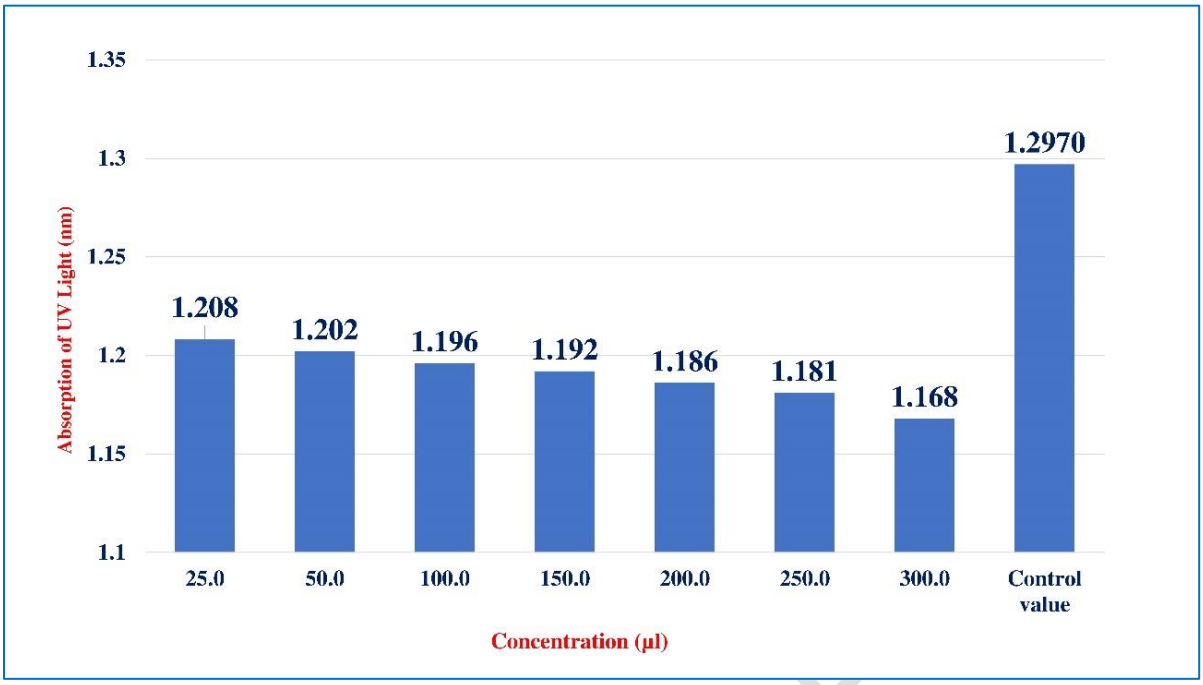

Fig. 3. The UV visible spectrum analysis concentration vs absorbance of *Pangasius Pangasius* extracts

The absorbance capacity of the lipid sample is shown in Fig. 3. It expressed that the increasing the concentration of 25.0 µl o 300.0 µl the absorbance decreased from 1.208 a.u. to 1.168 a.u. respectively. So, the low absorbance indicates that antioxidant properties are decreased expressed in Table 2.

Table 2. Absorption of ultra violates light by the extract from *Pangasius Pangasius*

Observation number	Concentration (µl)	Absorption of UV light (a.u)
1	25.0	1.208
2	50.0	1.202
3	100.0	1.196
4	150.0	1.192

5	200.0	1.186
6	250.0	1.181
7	300.0	1.168
8	Control value	1.297

The exact antioxidant properties are illustrated by the inhibition rate which accumulated in Table 3. So, by calculating the inhibition rate, we can determine of antioxidant activity of the sample.

Inhibition rate (I)%={1-(A) sample /(A) control}*100

Here, A= absorption and I= inhibition rate

From the above equation, the inhibition rate is determined. If the inhibition rate increases with concentration indicates the absorption rate decreases. If the inhibition rate increases the antioxidant property of the compound also decreases.

Table 3. Free radical scavenging activity (DPPH) of lipids from *Pangasius Pangasius*

Observation Number	Concentration (µl)	Inhibition rate (%)
1	25.0	0.0686
2	50.0	0.0732
3	100.0	0.0778
4	150.0	0.0809
5	200.0	0.0856
6	250.0	0.0894
7	300.0	0.0995
8	Control value	0.0000

Table 3 shows that the concentration increased from 25.0 µl to 300.0 µl. The inhibition rate increased from 0.0686 to 0.0995 % respectively. So, the high-concentration antioxidant properties of the extracts decreased and with the low-concentration, the low inhibition rate of the antioxidant properties of the extracts increased.

Conclusion

In the analysis of lipids from *Pangasius Pangasius,* twenty-four (24.0) bioactive chemical compounds were identified by GC-MS. The first major compound is Dodecanoic Acid, 1,2,3-Propanetriyl Ester 86.67 % is a strong antibacterial agent and also a plant metabolite. It is used in soaps and cosmetics. The second major compound is Bis (2-Ethylhexyl) Phthalate 12.37 % which is a plasticizer for resins, in pesticides and as a solvent for ink. The third is L (+) Ascorbic acid 2,6-Dihexa decanoate observed at 0.33 %. Ascorbic acid is helpful for wound healing cells of the human or animal body, to increase the absorption of iron from plant foods and to support the immune system. It works as an antioxidant to protect cells against free radicals. The overall findings of this study could be useful for *Pangasius Pangasius*. Thus, *Pangasius Pangasius* chemical compounds contain little percentage of antioxidants and are more anti-bacterial and pharmaceutical functions.

References

[1] Bechtel, P. J., & Oliveira, A. C. (2006). Chemical characterization of liver lipid and protein from cold-water fish species. Journal of Food Science, 71(6), S480-S485.

[2] Kim, S. K., Kim, Y. T., Byun, H. G., Nam, K. S., Joo, D. S., & Shahidi, F. (2001). Isolation and characterization of antioxidative peptides from gelatin hydrolysate of Alaska pollack skin. Journal of agricultural and food chemistry, 49(4), 1984-1989.

[3] Jun, S. Y., Park, P. J., Jung, W. K., & Kim, S. K. (2004). Purification and characterization of an antioxidative peptide from enzymatic hydrolysate of yellowfin sole (Limanda aspera) frame protein. European Food Research and Technology, 219, 20-26.

[4] Islam, M. R., Ahmed, S., Sadia, S. I., Sarkar, A. K., & Alam, M. A. (2023). Comprehensive review of phytochemical content and applications from cestrum nocturnum: A Comparative Analysis of Physicochemical Aspects. Asian Journal of Research in Biochemistry, 13(4), 43-58.

[5] Picot, L., Bordenave, S., Didelot, S., Fruitier-Arnaudin, I., Sannier, F., Thorkelsson, G., ... & Piot, J. M. (2006). Antiproliferative activity of fish protein hydrolysates on human breast cancer cell lines. Process Biochemistry, 41(5), 1217-1222.

[6] Bougatef, A., Nedjar-Arroume, N., Ravallec-Plé, R., Leroy, Y., Guillochon, D., Barkia, A., & Nasri, M. (2008). Angiotensin I-converting enzyme (ACE) inhibitory activities of sardinelle (Sardinella aurita) by-products protein hydrolysates obtained by treatment with microbial and visceral fish serine proteases. Food chemistry, 111(2), 350-356.

[7] Dong, Y. L., Sheng, G. Y., Fu, J. M., & Wen, K. W. (2005). Chemical characterization and anti-anaemia activity of fish protein hydrolysate from Saurida elongata. Journal of the Science of Food and Agriculture, 85(12), 2033-2039.

[8] Baehaki, A., Nopianti, R., & Anggraeni, S. (2015). Antioxidant activity of skin and bone collagen hydrolyzed from striped catfish (Pangasius pangasius) with papain enzyme. J. Chem. Pharm. Res, 7(11), 131-135.

[9] Azizah, N., Ochiai, Y., & Nurilmala, M. (2020). Collagen peptides from Pangasius fish skin as antioxidants. In IOP Conference Series: Earth and Environmental Science (Vol. 404, No. 1, p. 012055). IOP Publishing.

[10] Ramezani, F., & Khan, M. M. (2024). Effect of Pangasius Hypophthalmus Fish Extract on Blood Sugar and Uric Acid Levels in Alloxan-Induced Rattus Norvegicus.

[11] Pradarameswari, K. A., Zaelani, K., Waluyo, E., & Nurdiani, R. (2018, April). The physico-chemical properties of pangas catfish (Pangasius pangasius) skin gelatin. In IOP Conference Series: Earth and Environmental Science (Vol. 137, No. 1, p. 012067). IOP Publishing.

[12] Grossman, S., & Bergman, M. (1992). U.S. Patent No. 5,093,474. Washington, DC: U.S. Patent and Trademark Office.

[13] Muyonga, J. H., Cole, C. G. B., & Duodu, K. G. (2004). Extraction and physico-chemical characterisation of Nile perch (Lates niloticus) skin and bone gelatin. Food hydrocolloids, 18(4), 581-592.

[14] Pradarameswari, K. A., Zaelani, K., Waluyo, E., & Nurdiani, R. (2018, April). The physico-chemical properties of pangas catfish (Pangasius pangasius) skin gelatin. In IOP Conference Series: Earth and Environmental Science (Vol. 137, No. 1, p. 012067). IOP Publishing.

[15] Islam, R., Paul, D. K., Rahman, A., Parvin, T., Islam, D., & Sattar, A. (2012). Comparative characterization of lipids and nutrient contents of Pangasius pangasius and Pangasius sutchi available in Bangladesh. J Nutr Food Sci, 2(2), 1-6.

[16] Alam, S. M. D., Karim, M. H., Chakrabortty, A., Amin, R., & Hasan, S. (2016). Nutritional characterization of the Long-Whiskered catfish Sperata aor: A commercially important freshwater fish of Bangladesh. International Journal of food science and Nutrition Engineering, 6(1), 1-8.

[17] Monalisa, K., Islam, M. Z., Khan, T. A., Abdullah, A. T. M., & Hoque, M. M. (2013). Comparative study on nutrient contents of native and hybrid Koi (Anabas testudineus) and Pangas (Pangasius pangasius, Pangasius hypophthalmus) fish in Bangladesh.

[18] Chakma, S., Rahman, M. A., Siddik, M. A., Hoque, M. S., Islam, S. M., & Vatsos, I. N. (2022). Nutritional Profiling of Wild (Pangasius pangasius) and Farmed (Pangasius hypophthalmus) Pangasius Catfish with Implications to Human Health. Fishes, 7(6), 309.

[19] Karl, H., Lehmann, I., Rehbein, H., & Schubring, R. (2010). Composition and quality attributes of conventionally and organically farmed Pangasius fillets (Pangasius hypophthalmus) on the German market. International journal of food science & technology, 45(1), 56-66.

[20] Orban, E., Nevigato, T., Di Lena, G., Masci, M., Casini, I., Gambelli, L., & Caproni, R. (2008). New trends in the seafood market. Sutchi catfish (Pangasius hypophthalmus) fillets from Vietnam: Nutritional quality and safety aspects. Food Chemistry, 110(2), 383-389.

[21] Kulawik, P., Migdal, W., Tkaczewska, J., Gambus, F., Szczurowska, K., & Özoğul, F. (2016). Nutritional composition of frozen fillets from Pangasius catfish (Pangasius hypophthalmus) and Nile tilapia (Oreochromis nilotcus) imported to European countries. Annals of Animal Science, 16(3).

[22] Rahman, M. A., Ullah, M. R., Kabir, M. A., Alam, M. A., Rahman, M., & Hossen, M. F. (2020). Artificial propagation of indigenous yellowtail catfish (Pangasius pangasius): experiences and challenges. Aquaculture, 523, 735215.

[23] Zilani, M. N. H., Islam, M. A., Biswas, P., Anisuzzman, M., Hossain, H., Shilpi, J. A., ... & Hossain, M. G. (2021). Metabolite profiling, anti-inflammatory, analgesic potentials of edible herb Colocasia gigantea and molecular docking study against COX-II enzyme. Journal of Ethnopharmacology, 281, 114577.

[24] Baehaki, A., Nopianti, R., & Anggraeni, S. (2015). Antioxidant activity of skin and bone collagen hydrolyzed from striped catfish (Pangasius pangasius) with papain enzyme. J. Chem. Pharm. Res, 7(11), 131-135.

[25] Propionic Acid - an overview | ScienceDirect Topics.” Accessed: Mar. 12, 2024. [Online]. Available: https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/propionic-acid.

[26] Benzoic Acid - an overview | ScienceDirect Topics.” Accessed: Mar. 12, 2024. [Online]. Available: https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/benzoic-acid

[27] Weaver, J. A., Beverly, B. E., Keshava, N., Mudipalli, A., Arzuaga, X., Cai, C., ... & Yost, E. E. (2020). Hazards of diethyl phthalate (DEP) exposure: A systematic review of animal toxicology studies. Environment international, 145, 105848.

[28] Fig. 2h: L-(+)-Ascorbic acid 2,6-dihexadecanoate,” ResearchGate. Accessed: Mar. 12, 2024. [Online]. Available: https://www.researchgate.net/figure/h-L--Ascorbic-acid-2-6-dihexadecanoate_fig9_281776251

[29] Green, R., Hauser, R., Calafat, A. M., Weuve, J., Schettler, T., Ringer, S., ... & Hu, H. (2005). Use of di (2-ethylhexyl) phthalate–containing medical products and urinary levels of mono (2-ethylhexyl) phthalate in neonatal intensive care unit infants. Environmental health perspectives, 113(9), 1222-1225.

[30] Sánchez-Moreno, C. (2002). Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food science and technology international, 8(3), 121-137.