

## Identification and Antibacterial Activities of Composition Compounds from Liver and Texture of *Hypophthalmichthys molitrix*

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In this investigation, the liver and muscle tissues of *Hypophthalmichthys molitrix* were extracted of their lipid and fatty acid contents using the method of Bligh & Dyer. Chloroform and *n*-butanol were used as extraction solvents for semi and polar lipids, respectively. After removal of the solvents, the GC/MS spectra of the fractions were obtained. Identification of constituents was made by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples. Seven compounds representing 99.8 % of the liver chloroform phase of *Hypophthalmichthys molitrix* were identified among them palmitic acid (16.7 %) and cholesta-3,5-diene (42.5 %) were the major ones. Eight compounds representing 97.9 % of tissue chloroform phase were identified among them palmitic acid (26 %) and oleic acid (12 %) were the major ones. One polar compound representing 99 % of the liver *n*-butanol phase and no polar compound representing of muscle *n*-butanol phase. Two antibacterial activities were reported in tissue chloroform phase.

**Key Words:** *Hypophthalmichthys molitrix*, Antibacterial, GC/MS Palmitic acid.

### INTRODUCTION

Considering the rapid world population growth and the spread of cardiovascular, brain and psychological diseases, the need for drug synthesis and preventing drug is strongly felt. The best sources for such compounds are the natural plant and animal resources, of which the aquatic resources such as the various species of the fishes is at the first rank<sup>1</sup> because of its rich content of fatty acids like omega-3.

Silver Carp, Thick forehead or White thick forehead (with the scientific name of *Hypophthalmichthys molitrix* and the Persian name of Phytophague) from the family of Cyprinidae, the genus of *Hypophthalmichthys* and the species of *molitrix*<sup>2</sup>.

This fish is found in the southern shores of the Asian side of the Pacific, from Amour to Perel river in southern China. The main species of the warm water breeding fishes is Silver Carp. This is because of the high potential of this fish for rapid growth and its adaptation to the environment, its good taste and the low demanding diet of the fish which requires little attention and facilities. This fish feeds on Planktons and planktons can be produced from all natural fertilizers. Therefore, Silver Carp are among the breeding fishes with a high economical return<sup>3</sup>.

Silver Carp has a dense and fat body resulting in a high ratio of meat *versus* the bone. The stomach contains a sharp line called keel, the head is fairly big, the eyes are fairly small and lower than the horizontal body line and the space between the eyes is very wide<sup>2</sup> (Fig. 1).



Fig. 1. The appearance features of the Silver Carp

The fatty acids are really the linear carboxylic acids that exist mostly in neutral fats and oils in the form of ester and are carried in plasma in the form of free fatty acids. The carbon chain can be saturated or unsaturated<sup>4</sup>. The wax of the saturated and unsaturated fatty acids of omega is under the group of unsaturated fatty acids<sup>5</sup> like:  $\omega$ -3,  $\omega$ -6,  $\omega$ -9,  $\omega$ -7. The structure of the fatty acid of  $\omega$ -9 (oleic acid) and  $\omega$ -6 (linoleic acid).

## EXPERIMENTAL

The test process consists of: sampling, extraction by Bligh & Dyer, concentration phases, essence analysis by GC/MS, identification of the components and finally the determination of antibacterial activities<sup>6</sup>.

The liver and tissues of this fish were obtained from the center for reproduction of aquatic animals of Isfahan in March of 2006. The extraction process was conducted by the use of chloroform and normal butanol solvents, for the liver and tissues separately. During these processes, the chloroform, methanol and water were first added to the samples to solve the fatty acids in the phase of chloroform and the polar compounds in the phase of methanol-water. Then using the normal butanol (a perfect polar solvent) all the polar components of the methanol-water phase are separated and after a complete concentration by a rotary machine the oily essences are diluted in pure ether and is injected into the GC/MS machine. The produced spectra have been identified by the two methods of adjustment

by eight peak and the comparison of the retention indices (RI) with the standard quantity<sup>7</sup>.

On each essence, in a completely sterilized plantation environment, the antibacterial properties test was done. These bacteria were of the type of *bacillus* and *cocus* and with positive and negative grams. Positive gram (G+) is an indicator of cellular wall with multiple layers and high resistance against by sides and the negative gram (G-) means a cellular wall with few layers and low resistance (Table-1).

TABLE-1  
SPECIFICATIONS OF THE FOUR BACTERIA GROUPS WITH  
POSITIVE AND NEGATIVE GRAMS

Bacteria	Gram	Disease
<i>Escherichia coli</i>	G-	Intestine infection, Inflammation of the digestion system, Nausea
<i>Bacillus subtilis</i>	G+	Soil bacteria (opportunistic), lung infection
<i>Staphylococcus aureus</i>	G+	Boil and blain
<i>Entero bacter</i>	G-	Intestine fever

The results of up to 24 h after plantation in the incubator and the diameter of the area empty of bacteria in millimeters were recorded.

## RESULTS AND DISCUSSION

In the chloroform phase, the silver Carp tissues (the second stage of the growth) eight compounds were identified 97.9 % that: 1,2-benzendi-carboxylic acid, *bis*(2-octyl ester) (37 %), palmitic acid (26 %), oleic acid (12 %) were the main compounds identified (Table-2).

TABLE-2  
IDENTIFIED COMPOUNDS IN THE CHLOROFORM  
STAGE OF THE TISSUE

Compound	%
1,2-Benzene dicarboxylic acid, <i>bis</i> (2-octyl ester)	37.0
Palmitic acid	26.0
Z-9-Octadecenoic acid	12.0
Stearic acid	5.2
Butyl hexadecanoate	6.5
Tri- <i>m</i> -cresyl phosphate	2.8
Octadecanoic acid, butyl ester	3.2
1,2-Benzene dicarboxylic acid, <i>bis</i> (2-hexyl ester)	5.2

From the seven compounds of the chloroform stage, with 99.8 % compounds like palmitic acid, 16.7 % 5-diene and 42.5 % cholesta-3 were of the most importance (Table-3).

TABLE-3  
IDENTIFIED COMPOUNDS IN THE CHLOROFORM  
STAGE OF THE LIVER

Compound	%
Palmitic acid	16.7
Linoleic acid	10.4
Stearic acid	5.2
2,6,10,14-Tetramethylpentadecan-7-ol	12.5
Cholesta-3,5-diene	42.5
4-Methyl piperidine	4.6
3-Octadecanone	7.9

Normal butanol phase lacks any polar compounds and in the normal butanol phase, the liver contained a compound of 99 % 3,5-diene cholesta<sup>8,9</sup>. The antibacterial effects were also studied and determined (Table-4). The diameter of the antibacterial area was determined in millimeter (Table-5).

TABLE-4  
ANTIBACTERIAL RESULTS OF THE LIVER AND  
TISSUES OF THE SILVER CARP FISH

Bacteria	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Entro bacter</i>
Liver chloroform phase	–	–	–	–
Liver butanol phase	–	–	–	–
Texture chloroform phase	–	+	+	–
Texture butanol phase	–	–	–	–

TABLE-5  
DIAMETER OF THE ANTI-BACTERIAL AREA IN THE  
CHLOROFORM PHASE OF THE TISSUE

Bacteria	Antibacterial limits (mm)
<i>Escherichia coli</i>	0
<i>Bacillus subtilis</i>	24
<i>Staphylococcus aureus</i>	7
<i>Entro bacter</i>	0

From these observations, it was determined that the Silver Carp fish contains fatty acids, ester, cetone, alcohol and cholesterol. Some of the necessary fatty acids for the human body are  $\omega$ -3,  $\omega$ -6 and  $\omega$ -9 in breeding

fishes exist 10.43 %  $\omega$ -6 and 12.22 %  $\omega$ -9. Considering that Phytophagous fish feeds on Phitoplanktons and the temperature has a great impact on its growth, the fishing time for it is after 4 years of breeding and with weight of at least 2.0 to 2.5 kg. This is one of the best species of the fish for human nutrition. Because fish compounds are vastly used in curing diseases like heart and brain diseases and cancers like breast cancer, psychological disorders like depression, schizophrenic or schizophrenic and addiction to the drugs and alcohol. It is highly recommended by the doctors that every person must use 2 to 3 meals of seafood and fish in a week<sup>1</sup>. Considering the antibacterial tests on fishes, it has become clear that the fatty acids have very little antibacterial effect and in most cases they are not antibacterial.

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