UNIVERSITI PUTRA MALAYSIA

INVESTIGATION OF MALACHITE GREEN RESIDUES IN LOCAL AND IMPORTED *PANGASIUS HYPOPTHALMUS* SOLD IN SELANGOR MARKETS

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CERTIFICATION

It is hereby certified that I have read this project paper entitled "Investigation of malachite green residues in local and imported *Pangasius hypopthalmus* sold in Selangor markets", by Nur Ain Syahira binti Ishak and in my opinion it is satisfactory in term of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999- Final Year Project.

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DEDICATIONS

This project paper is dedicated to Allah S.W.T., who had created me and made all things possible throughout this project.

To my family,

My mother, Noryati binti Jaafar.

My FYP partner, Hasni Nabilah and friends whose been supporting me.

And to all my teachers and lecturers who have committed themselves towards the noble cause of education. Thank you for your continuous support and care.

Without your help, I may not reach this moment. Your time spends with me is the most greatest gift of all.

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First and foremost, I would like to thank Allah S.W.T for giving me chance to breath and strength physically and mentally throughout my life and to be aware of the desire for knowledge as well as to improve myself and to contribute this knowledge with objective as a purpose to contribute the country in economic and intellectual field to become more successful in the future.

I would like to express my biggest gratitude to by beloved supervisor Dato' Dr Mohamed Shariff Mohamed Din for his invaluable knowledge sharing, time, support and guidance throughout this research. His motivation and knowledge had contributed to the completion of this research project.

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LIST OF ABBREVIATIONS

%	: Percent
MG	: Malachite green
LMG	: Leucomalachite green
MPRLs	: Minimum Required Performance Limit
ºC	: Degree Celsius
EU	: European Union
FAO	: Food and Agriculture Organization
NRA	: National Registration Authority
VASEP	: The Vietnam Association of Seafood Exporters and Producers
FDA	: Food and Drug Administration
ppb	: parts per billion
ASEAN	: The Association of Southeast Asian Nations
DOF	: Department of Fisheries Malaysia
ELISA	: Enzyme-linked immunosorbent assay

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek Ilmiah Tahun Akhir

SIASATAN KE ATAS SISA MALEKIT HIJAU PADA *PANGASIUS HYPOPTHALMUS* TEMPATAN DAN IMPORT YANG DIJUAL DI PASARAYA SEKITAR SELANGOR

Oleh

Nur Ain Syahira binti Ishak

2017

Penyelia: Dato' Dr Mohamed Shariff Mohamed Din

Malekit hijau adalah sejenis pewarna *N*-methylated diaminotriphenylmethane yang ratanya digunakan sebagai rawatan penyakit dalam sektor akuakultur serata dunia. Malekit hijau mudah teroksida ke leuko-malekit hijau yang menetap di dalam otot dan organ dalaman selama beberapa minggu. Apabila ikan yang

terdapat sisa malekit hijau dimakan, ia boleh membawa musibah kepada manusia kerana ia adalah toksik dan karsinogenik. Kajian ini bertujuan untuk mengesan sisa malekit hijau di dalam ikan segar *Pangasius hypopthalmus* tempatan dan fillet import sejukbeku dari Vietnam. Satu set ikan tempatan dan import fillet telah dibeli daripada tujuh pasaraya sekitar Selangor. Sisa malekit hijau di dalam otot ikan telah dianalisa menggunakan enzyme-linked immunosorbent assay (ELISA) dan liquid chromatography tandem mass spectrometry (LC-MS). Graf standard bagi ELISA tidak mempunyai pelarasan yang linear iaitu 0.5355, dan menyebabkan ketidaktepatan dalam pengiraan sisa malekit hijau dan leuko-malekit hiijau. Sebaliknya, graf standard LC-MS mempunyai pelarasan linear dan memberi keputusan sisa malekit hijau dan leuko-malekit hijau terdapat di antara 1.19 hingga 4.09 µg/kg di dalam tujuh sampel (4 tempatan dan 3 import). Had prestasi minimum (MPRLs) bagi malekit hijau dan leuko-malekit hijau yang ditetapkan oleh European Union (EU) adalah 2 ppb menunjukkan terdapat beberapa sampel di dalam kajian ini mempunyai sisa lebih tinggi.

Kata Kunci: Malekit hijau, *Pangasius hypopthalmus, sisa, ELISA, LC-MS*

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Project.

INVESTIGATION OF MALACHITE GREEN RESIDUES IN LOCAL AND IMPORTED *PANGASIUS HYPOPTHALMUS* SOLD IN MARKETS IN SELANGOR

By

Nur Ain Syahira binti Ishak

2017

Supervisor: Dato' Dr Mohamed Shariff Mohamed Din

Malachite green is an *N*-methylated diaminotriphenylmethane dye widely used for therapeutic purposes in the aquaculture sector across the world. Malachite green can easily oxidize to leuco-malachite green (LMG) which remains in muscles and internal organs for several weeks. If fish with MG residues are consumed, it may be harmful to humans as it is toxic and carcinogenic. This study was aimed to detect MG residues in local freshwater fish *Pangasius hypopthalmus* and imported frozen fillet of the same species from Vietnam. A set of local fish and imported fillet were purchased from seven supermarkets in Selangor. The muscle was analysed for MG residues using enzyme-linked immunosorbent assay (ELISA) and liquid chromatography tandem mass spectrometry (LC-MS). The ELISA standard curve did not have a linear

calibration with correlation coefficients lower than 0.9999 (0.5355), causing inaccuracy of calculating the MG concentration. Whereas LC-MS standard curve was near 1 (0.999) and showed residues of MG and LMG ranging from 1.19 to 4.09 µg/kg concentration in 7 samples (4 locals and 3 imported). The minimum required performance limit (MPRLs) for MG and LMG in fish set by the European Union is 2 ppb which means the detected levels in the present study were higher in some samples with an indication that MG is being used indiscriminately in aquaculture.

Keywords: ***Malachite green, Pangasius hypopthalmus, residues, ELISA, LC-MS***

1.0 INTRODUCTION

1.1 Study background

Malachite green (MG) is a dye with multiple purposes. It is generally used in aquaculture to control external fungus and protozoan infection in fish (Liu et al., 2009). Malachite green is not included in the veterinary drugs list, but many farmers prefer to use MG for treatment because it is relatively cheap, easily available and highly efficient. However, MG is carcinogenic (Hidayah et al., 2013) and its use has been banned in several countries such as US and Europe (Conti et al., 2015), as well in ASEAN countries (ASEAN, 2013). Thus, it is important to investigate the presence or absence of MG in fish as it is of safety concern that the fish consumed should be free of MG residues. *Pangasius hypopthalmus* is a commonly cultured and popular fish consumed in Malaysia. The leading producers in exporting Pangasius fillet are Vietnam and Thailand (FAO, 2006). The main export product is sutchi catfish or *Pangasius hypophthalmus* originated from freshwater farming in Mekong Rivers, Vietnam where the fish is raised in floating cages, rivers or ponds (Orban et al., 2008). There are several reports claiming the presence of MG residues and other harmful chemicals in imported Pangasius fillet from Vietnam (Southern Shrimp Alliance, 2012)

1.2 Justification

The use of the MG in aquaculture industry is a common practice for the treatment of a broad range of infections. However, studies have shown that the MG is carcinogenic and mutagenic (Hidayah et al., 2013). Thus, the present study was conducted to determine the presence of MG in the muscle of local and imported *Pangasius hypopthalmus* that are sold in the market. This study will provide useful information whether the fish sold in the local markets are safe for human consumption.

1.3 Objectives

1. To identify the presence of MG residues in locally cultured and imported *Pangasius hypopthalmus* sold in the market.
2. To compare the sensitivity and specificity between enzyme-linked immunosorbent assays (ELISA) and liquid chromatography tandem mass spectrometry (LC-MS) in detecting MG residues.

1.4 Hypothesis

There should be absence of MG residues in local and imported *Pangasius hypopthalmus* sold in the market.

2.0 LITERATURE REVIEW

2.1 Aquaculture industry in Malaysia

Report by FAO (2008) indicates that Malaysia began practicing aquaculture in the 1920's with extensive polyculture of Chinese carps in ex-mining pools. Brackish water aquaculture has been dominating mostly in the western coastal areas in Malaysia where culture of the blood cockles (*Anadara granosa*), shrimps, and marine fish were practiced. For freshwater aquaculture, pond culture has predominated throughout the country. In 2009, culture of catfish (*Clarias batrachus*) was on the top of the list of freshwater aquaculture species in Malaysia, followed by red Tilapia (Oreochromis spp.) and river catfish (*Mystus nemurus*). Aquaculture industry is important as security of food supply and improves self-sufficiencies of the country.

2.2 Malachite Green

Malachite green is an N-methylated triphenylmethane dye that is useful for controlling fungal and protozoan infections in fish. Malachite green has been proven as the most effective remedy in 1960s for ectoparasites (*Ichthyophthirius multifiliis*) as well as against fungus *Saprolegnia* sp. The use of malachite green has become attractive to the aquaculture industry since early 1930s as it has high efficiency, affordable, and is readily available. Malachite green is used for

industrial purpose as a dye in silk, wool, cotton, leather, paper and acrylic as well as food colouring agent and food additives as early in 1933 (Hidayah *et al*., 2013).

Malachite green is available in green crystalline powder form, soluble in water and easily deactivated by light (Culp & Beland, 1996; Mitrowska *et al.,* 2007). Malachite green can be easily oxidized into reducing form; leuco-malachite green (LMG) by photo-oxidative demethylation (Mitrowska *et al*., 2007). Leuco-malachite green is also known as 4,4′ -Benzylidenebis (N,N-dimethylaniline) which is very toxic and deposited in fatty tissues of aquatic animals. Leuco-malachite green remains in fatty tissues for more than 10 months after treatment due to its lipophilic nature (Jiang et al*.,* 2009). Leuco-malachite green impose hazardous impact to human health due to its mutagenic and carcinogenic properties (Hidayah *et al*., 2013). However, LMG is able to slowly oxidize to MG during freezer storage (Stammati et al., 2005). Malachite green and LMG residues in food are reduced by cooking with oil, baking, boiling or when microwave at different temperatures. However, increase in temperature does not support the full breakdown of the residues (Mitrowska et al., 2007)

The restriction in using MG and LMG has been implemented earlier in European countries, Canada and United States compared to Asian countries. As cited in Hidayah *et al*. (2013), the commonly referred MG and LMG residues laws and regulations are Commission of Codex Alimentarius, Commission of European Communities (EU) and National Registration Authority (NRA).

Maximum residue limits (MRLs) and minimum required performance limit (MPRLs) varies among regulations. However, many countries prefer to follow Commission of European Communities (EU) regulation which is simpler and meets the food safety requirements. The MPRLs of MG and LMG is 2.0 µg/kg in meat and seafood products as documented by European Commission in 2007 (Hidayah et al., 2013).

2.3 Striped catfish (*Pangasius hypophthalmus)*

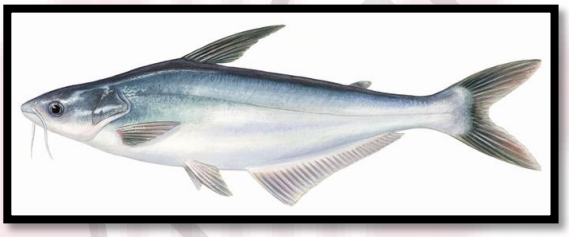

Table 1 Striped catfish (*Pangasius hypopthalmus)* classification

Kingdom	Animalia
Phylum	Chordata (Notochord group of animals)
Class	Actinopterygii (ray-finned fishes)
Order	Siluriformes (prominent barbels)
Family	Pangasiidae (shark catfish)
Genus	*Pangasianodon*
Species	*P. hypophthalmus*

According to FAO (2010), the introduction of striped catfish (*Pangasius hypophthalmus*) to the world started in the Mekong River, Vietnam. Traditionally, the production started as capture-based aquaculture that could be due to its prolific spawning, and production of numerous larvae that are easily harvested from the flowing river. At present, striped catfish is reared intensively in ponds and cages. Vietnam is the largest single major exporter of striped catfish fillets worldwide. The demand for the fish in the market has expanded due to its low price and desired qualities; white flesh, delicate texture, and lack of horizontal bones. Simultaneously, the farming of striped catfish increased due to its traits of fast grower, high yield and low cost (Wang & Hsieh, 2016). Pond culture of striped catfish has become predominant in the Mekong River since 2003 besides the other farming systems (Phan et al., 2009).

Based from Southern Shrimp Alliance report of 2012, the residues of harmful chemicals persists in seafood export products from Vietnam. The Vietnam Association of Seafood Exporters and Producers (VASEP) formally claimed that the Ministry of Agriculture and Rural Development has banned the use of several prohibited chemicals in fish and shrimp farming. The VASEP also reported that there was no chemical violation claims from the European Union (EU) or the United States. However, it was an erroneous claim due to reported veterinary drugs residues in early 2012 from Vietnamese shrimp by the U.S. Food and Drug Administration (FDA). There were also reports from Japan, Canada

and Australia that rejected Vietnamese shipments due to the presence of chemical residues found in fish or shrimp from the same report.

In Malaysia, there are no studies of MG residues in striped catfish. Striped catfish is among one of the popular cuisine in Malaysia which has high demand in market. As in Vietnam, striped catfish in Malaysia are also reared in pond and cages and sold in local markets.

3.0 MATERIALS AND METHOD

3.1 Experimental Design

A piece of local striped catfish and a pack of Vietnam-imported frozen fillet of the same species were bought from seven different supermarkets located in Selangor with the total of 14 samples. A part of muscle devoid of the skin and bones was dissected from the fish. For the Pangasius fillet, a small portion of the fillet was cut. Both samples were then blended separately to fine paste appearance. After that, all samples were kept frozen under -84°C until the sample extraction procedure was done.

3.2 Muscle analysis

3.2.1 Malachite Green/LMG ELISA Test Kit

The MaxSignal® Malachite Green/LMG ELISA Test Kit (Bioo Scientific Corporation, Austin, TX) was used for detection of MG in the fish muscle. The kit has the capacity for 96 determinants or testing of 42 samples in duplicate. The 14 samples of fish muscles were screened for MG residues using the ELISA test kit. The ELISA kit has the detection limit of 0.1 µg/kg for detection of the combination of MG and LMG in the muscle. However, the ELISA analysis procedure directly converts LMG present in the muscle to MG.

3.2.1.1 Sample preparation

All 14 fish muscle samples were blended or homogenized into fine paste consistency. Two grams of homogenized muscle sample was put into a 15 mL Falcon tube. One ml of 1X Sample Extraction Buffer A, 0.4 ml of 1X Sample Extraction Buffer B was added and was swirled for 15 seconds to allow the buffers to coat the tissue. At this moment, the muscles started to change colour into white or grey colour. Six ml of acetonitrile was added and the solution was manually shaken to ensure the acetonitrile penetrated all the tissue. The sample was vortexed manually for 3 minutes to ensure overall mixing of sample. The sample was centrifuge for 15 minutes at 4,000 rpm. Then, 2 ml of the upper layer of acetonitrile layer was slowly transferred to a new tube containing 300 mg of

MG Clean Up Mix. The sample was immediately vortexed at maximum speed for 1 minute and incubated at room temperature for 3 minutes. The sample was vortexed again for additional 30 seconds after incubation. After that, the sample was centrifuge again for 10 minutes at 4,000 rpm. One ml of the supernatant was slowly transferred to a 1.5 ml micro-centrifuge tube and dried using oven 37°C overnight. For the dried residue, 100 µl of 1X Oxidant Solution was added and was vortexed vigorously for 30 seconds. The sample was centrifuge again for 10 seconds to force all the solution to the bottom of the tube. The sample was incubated at room temperature for 15 minutes. The colour intensity of the solution in tube begins to weaken from orange-pink to clear. Extra 5 minutes incubation time was given if the colour persisted. Next, 400 µl of 1X Sample Extraction Buffer C was added to sample, swirled, 50 µl of n-hexane then was added and was vortexed vigorously for 1 minute. The sample was centrifuge at maximum speed for 10 minutes. Then, the upper hexane layer was discarded and left to evaporate so as to avoid hexane residues in the sample. Finally, 90 µl of the lower aqueous layer was used for the ELISA assay.

3.2.1.2 ELISA Testing Protocol

Ninety µl of MG Standard (negative control, 0.05, 0.15, 0.5, 1.5, and 4.5 ng/mL) was added into each duplicate wells. Ninety µl of each samples were added next in duplicate into different sample wells. Freshly prepared 30 µl 1X

MG-Biotin Conjugate was added into each well and mixed thoroughly for 1 minute. The plate was then incubated for 30 minutes at room temperature in the dark. Liquid from the wells was then discarded and the plate was washed with 250 µl of 1X Wash solution three times and the plate was dried with paper towels. Next, 100 µl of freshly prepared 1X Streptavidin-HRP was added to each well and mixes thoroughly for 1 min. The plate was incubated at room temperature in dark for 15 minutes. Then, liquid was discarded from the plate, washed and dried (repeat previous step). One hundred µl of TMB substrate was added into each well and incubated for 15 minutes at room temperature in the dark. After incubation, 100 µl of Stop Buffer was added to each well as to stop the enzyme reaction. The plate was read on a plate reader with 450 mm wavelength right after addition of Stop Buffer.

3.2.2 Liquid Chromatography tandem mass spectrometry (LC-MS)

Liquid Chromatography tandem mass spectrometry is an analytical technique which functions to identify, characterized and quantify compounds within complex substances especially food contaminants. Liquid Chromatography tandem mass spectrometry is one of the most commonly used method for MG and LMG detection. It has higher sensitivity, accuracy and specificity compared to ELISA.

3.2.2.1 Sample preparation

The sample preparation procedure was referred to Ding et al. (2007). Two grams of 14 homogenized samples was weighed and put into a 50-ml Falcon tube. Milli-Q® ultra-pure distilled water was added 600 µl into the each samples. Two mL of 5% hydroxylamine HCl was added to the samples, vortexed and stirred for 10 minutes. Next, acetonitrile was added and stirred to ensure well distribution of the chemicals in the sample. The sample was centrifuge at 6500 rpm for 10 minutes. The supernatant was extracted and filtered by using 0.45 µm syringe filter until the solution becoming clear. Finally, 4.9 ml of the supernatant was inserted into a glass tube and 0.1 ml of d_6-LMG internal standard was added resulting to 5 ml solution. The solution was then sent for LC-MS analysis.

3.2.2.2 Sample analysis

Ten concentration of d_6-LMG standard solution (0.05, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 ppb) were prepared and analysed by LC-MS for calibration. Samples were then inserted into a glass vial and put into the panel for the analysis. Fifteen minutes were taken for one sample analysis and the analysis was repeated four times for each sample.

3.3 Statistical analysis

Statistical analysis was performed by using SPSS version 22. All analysis was analysed by using independent T-test.

4.0 RESULTS

4.1 Enzyme-linked immunosorbent assay (ELISA)

The results from ELISA plate reader showed the absorbance value MG from each plate. Before determine the concentration of MG residues in each samples, the MG standard curve was constructed for calibration by plotting the relative absorbance value against the MG standard concentration in ng/ml. Relative absorbance (%) value, was calculated by using the following equation:

$$Relative\ absorbance\ (\%) = \frac{absorbance\ standard\ (or\ sample)\ \times 100}{absorbance\ zero\ standard}$$

**absorbance zero standard= absorbance value of negative control.*

Following is the relative absorbance (%) value obtained from the MG standard (ng/mL) plotted in the graph:

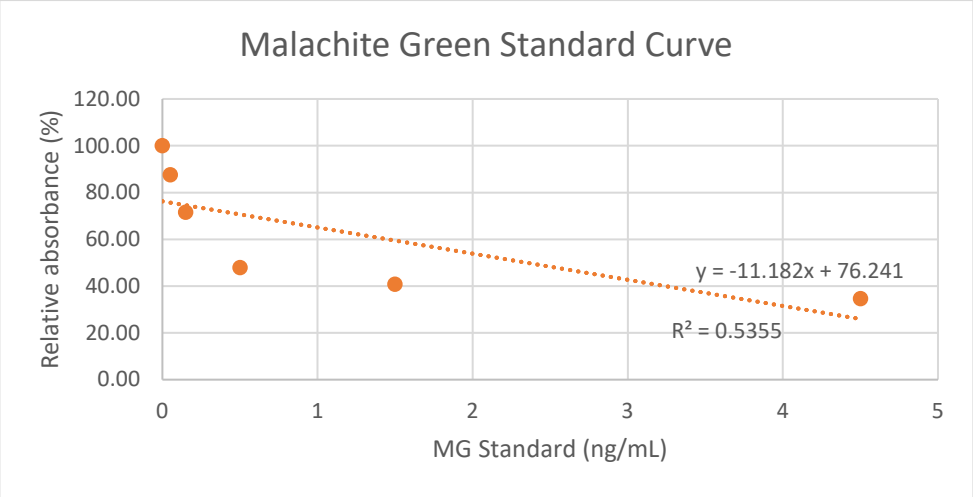

Figure 1 Malachite Green Standard Curve for ELISA

Regression line was plotted based from the distribution of relative absorbance (%) distribution in the graph. Based on the regression line, graph equation and R-squared (R^2) can be obtained. R^2 also known as coefficient of determinants is a statistical measure to determine the relationship of the data to regression line (Frost, 2013). When R^2 value is near to 1, it shows that the regression line nearly fits the data making it reliable to be used. However, in this

study, the R^2 obtained was too low (0.5355) and not reliable for correct interpretation.

4.2 Liquid Chromatography tandem mass spectrometry (LC-MS)

Liquid chromatography tandem mass spectrometry was able to separately detect MG and LMG residues from the sample. Malachite green and LMG standard was first analyzed for calibration. The MG and LMG standard curve was plotted based on the standard concentration (ppb) and area ratio obtained from the analysis.

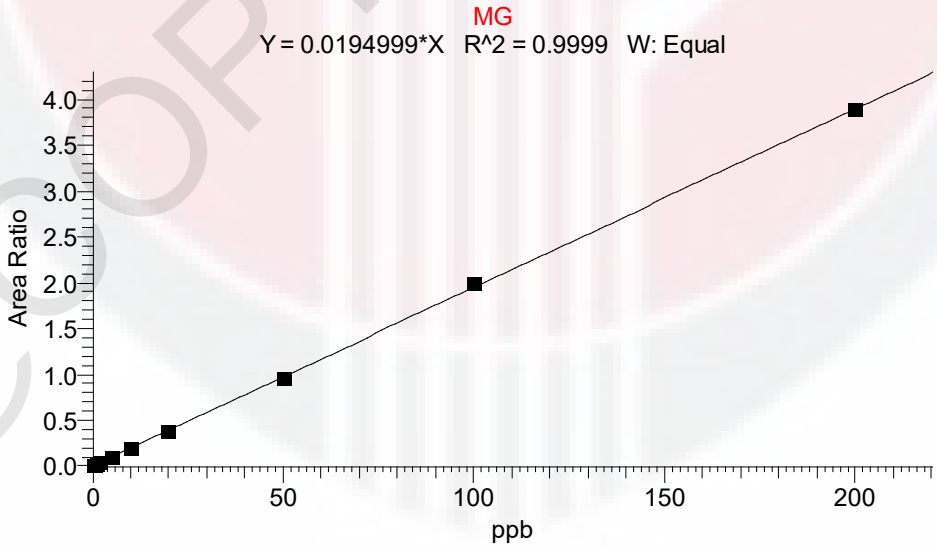

Figure 2 MG standard calibration curve for LC-MS

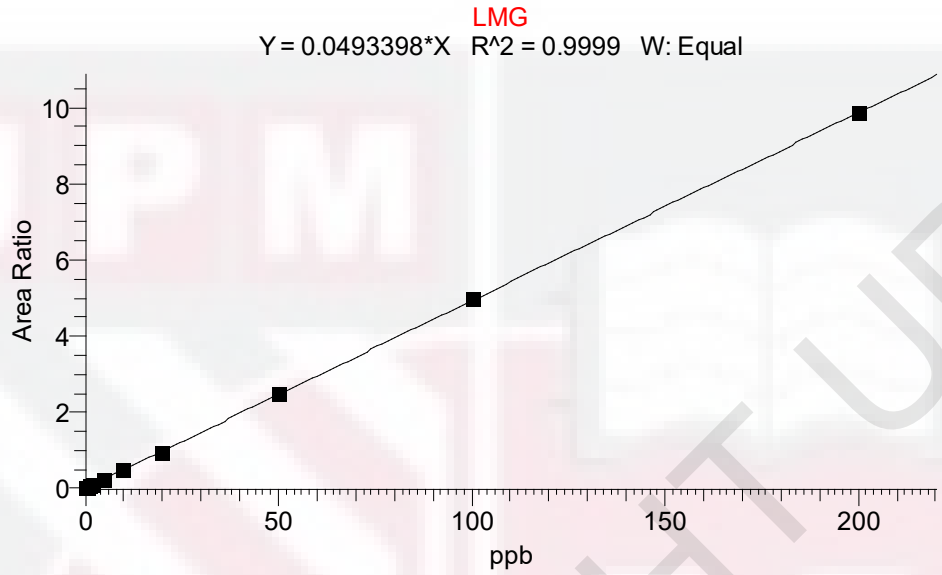

Figure 3 LMG standard calibration curve for LC-MS

Both of the calibration curves showed a correlation coefficient (R^2) of 0.9999 which is acceptable to be interpreted. Thus, the results from the samples are reliable and can be used for analysis.

From the 14 fish muscle samples obtained from seven different markets, 7 samples show positive residues of MG and LMG. The results are shown in Table 2.

Table 2 Levels of MG and LMG residues detected from locally cultured and imported fIllet of *Pangasius hypopthalmus* purchased from the local market.

Markets	Fish type	MG (µg/kg)	LMG (µg/kg)	MG + LMG (µg/kg)
Market 1	**Local**	**1.765**	**ND**	**1.765**
	Import	**ND**	**ND**	**ND**
Market 2	**Local**	**ND**	**ND**	**ND**
	Import	**ND**	**ND**	**ND**
Market 3	**Local**	**2.164**	**0.349**	**2.513**
	Import	**1.52**	**0.206**	**1.726**
Market 4	**Local**	**ND**	**ND**	**ND**
	Import	**ND**	**ND**	**ND**
Market 5	**Local**	**0.761**	**0.428**	**1.189**
	Import	**0.840**	**0.884**	**1.724**
Market 6	**Local**	**ND**	**ND**	**ND**
	Import	**ND**	**ND**	**ND**
Market 7	**Local**	**3.509**	**0.528**	**4.094**
	Import	**1.643**	**0.528**	**2.171**

ND: Not detected

Based from this results, there were 3 samples (Market 3 local, Market 7 local and import) showed levels of residues higher than the MRPLs (2.0 µg/kg), while the other 4 samples are within safe limits (<2.0 µg/kg). Statistical analysis was done as to determine the difference between MG and LMG combination in the local fish and imported fillet from all 7 markets. The results showed significant different ($p<0.05$) between the variables.

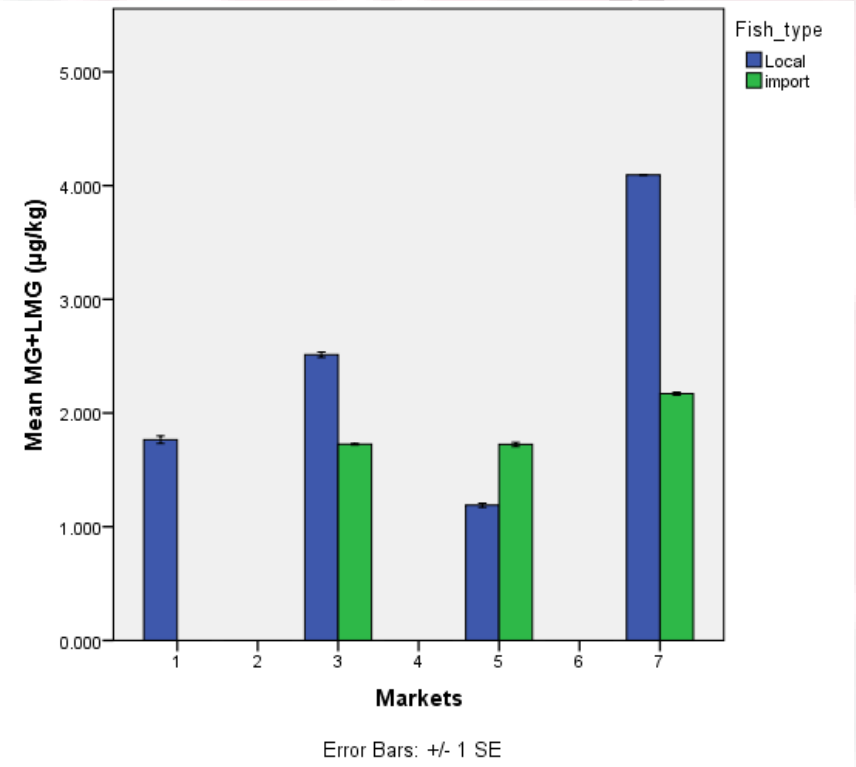

Figure 4 MG and LMG residues detected in local fish and imported fillet of *Pangasius hypopthalmus* samples obtained from 7 local markets

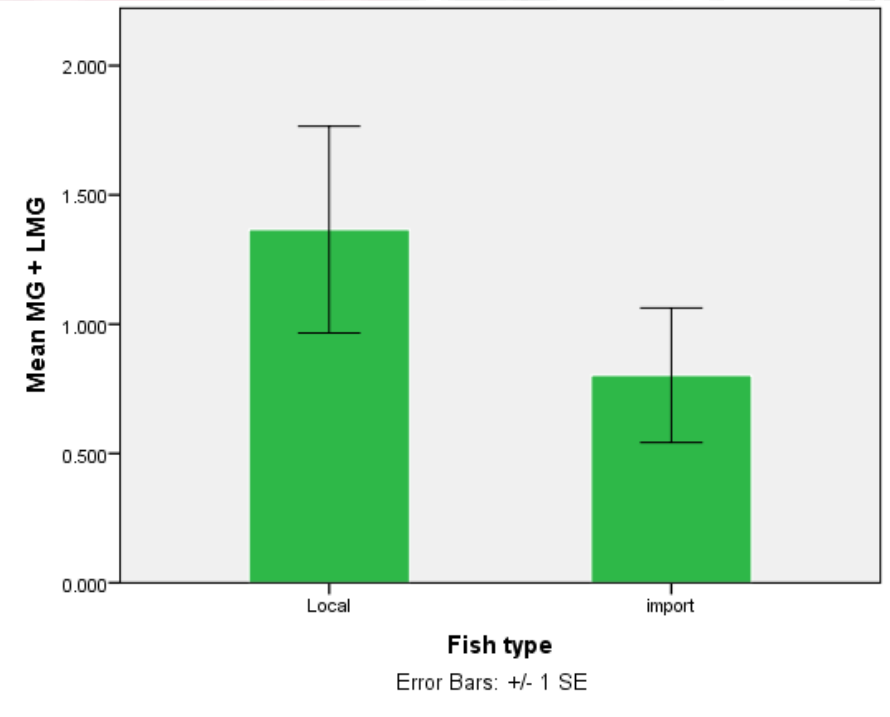
2.000
1.500
1.000
0.500
0.000
Mean MG + LMG
Local
import
Fish type
Error Bars: +/- 1 SE

Figure 5 Combination of MG and LMG residues detected in local fish and imported fillet of *Pangasius hypopthalmus* samples obtained from 7 local markets

The combination of MG and LMG residues in total local fish and imported fillet was also compared. Based from the graph (Fig. 5), shows that more local fish had residues than the imported fillet. However, statistical analysis did not show a significance ($p>0.05$) difference between these variables.

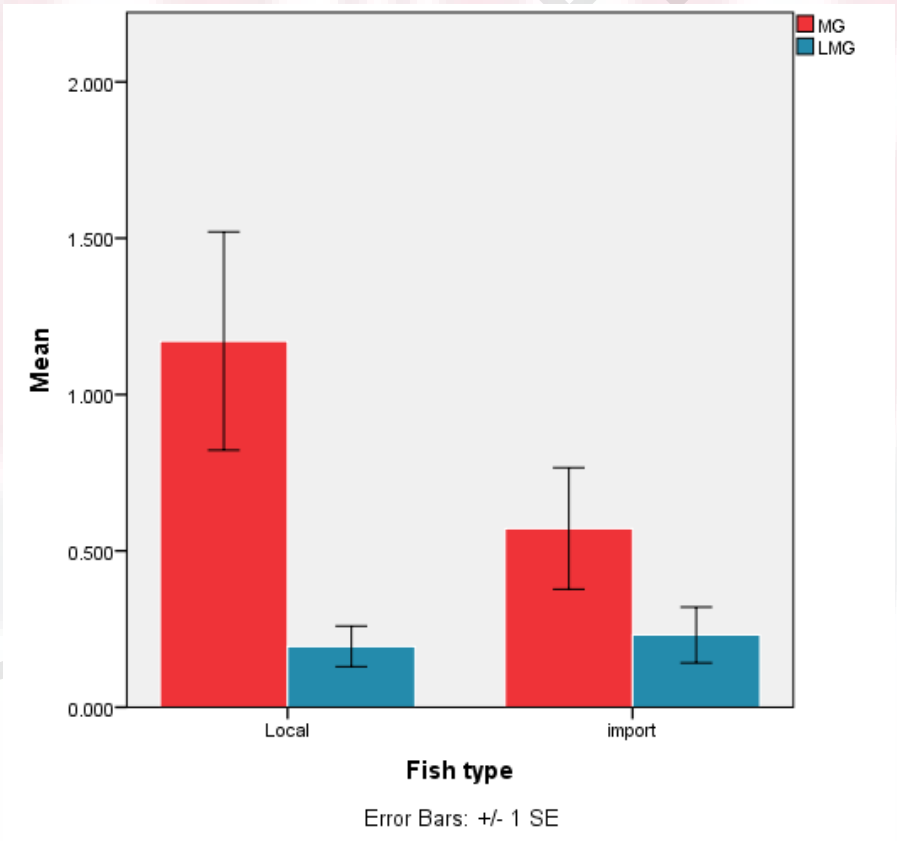

Figure 6 Difference between MG and LMG residues in local fish and imported fillet of *Pangasius hypopthalmus* samples obtained from 7 local markets

As shown in Figure 6, there were higher MG residues in both local fish and imported fillet compared to LMG. However, the statistical analysis did not show significant difference ($p>0.05$) between these variables.

5.0 DISCUSSION

The use of MG has been prohibited in aquaculture in most countries in the world (Srivastava et. al., 2004). According from 'Guidelines for the use of chemicals in aquaculture and measures to eliminate the use of harmful chemicals' by The Association of Southeast Asian Nations (ASEAN) in 2013, MG has been prohibited in all ASEAN countries including Malaysia. In the international level, Europe, Canada and United States had implemented earlier rules on the restriction of MG for aquaculture sector (Hidayah et. al., 2013).

Even though Malaysia has prohibited the use of MG for therapeutic purposes, there are no rules for the use of MG in Malaysia Food Regulation 1985. According to the Department of Fisheries Malaysia (DOF), the effort to enforce the regulation into the Malaysia Food Regulation is ongoing. For local fish farms that are registered with the Department of Fisheries Malaysia, an "Aquaculture

Residue Monitoring Program" is been done twice a year to monitor the presence of harmful chemical residues in fish sold in the market. This program is to control the usage of MG and prevent from human consuming fish with residues of MG. The method used for MG detection in fish muscle by DOF is LC-MS/MS which has higher specificity and selectivity for analyte analysis as compared with the method use in the present study (LC-MS). The higher sensitivity in the LC-MS/MS process is due to the double filtrations used to reduce noise produce by other substances in the sample.

Two methods were used for MG detection in the present study which are ELISA and LC-MS. As mentioned in the objectives, this study was aim to compare the ability and sensitivity of these methods for MG detection. Based from the results, the ELISA analysis was unable to show reliable result due to calibration error. The calibration error might be due to several factors during sample preparation and sample analysis procedure. During sample preparation, the sample might have been contaminated that can cause interruption during the analysis. During sample analysis, some inaccuracy might occur during incubation period, reagents preparations or contaminated sample.

On the other hand, LC-MS showed good calibration curve (R^2=0.9999) giving reliable results. The LC-MS is able to separate and analyses two types of analyte in this study which are MG and LMG. From the results, there were 7 samples positive for MG residues from total of 14 samples of which some contain

higher than the MPRLs stated by EU. Local striped catfish sample gave higher content of MG and LMG concentration compared to the imported pangasius fillet from Vietnam. Even though MG has been prohibited in Malaysia and Vietnam, the presence of these positive residues might be due to several factors. For local striped catfish, the sources of the fish may be taken from farms that are not registered with the DOF. These unregistered farms are not monitored by DOF for harmful residues, and use MG independently. Samples for LC-MS analysis need to be free from contamination by filtrating the solution until it becomes clear. Contamination of samples could interrupts detection of analytes of interest.

From the review prepared by Hidayah et. al, (2013), we can compare the two methods used in this study based on the advantages and disadvantages. The ELISA method is commonly used for screening of diseases or detection of substances. It is also a rapid assay system and suitable for large number of samples. However, ELISA test kit is expensive as the plate and reagents are used once only, thus it requires correct procedure during analysis. The quality of the ELISA kit depends on the quality of antibody itself to determine its reliability and sensitivity. On the other hand, LC-MS has been used as confirmatory analysis due to its highly specificity, selectivity and sensitivity. The results shown are highly accurate with good calibration curve. The LC-MS method is less laborious as most of the analysis is done by the instruments. However, the analysis is more expensive as it requires purchasing and maintenance of the instruments. The

system maintenance as well as result interpretation need an experienced personnel as this require skills.

6.0 CONCLUSION

There are several methods available for the detection of MG in aquatic species. Most common method analyses are the LC-MS and ELISA. However, ELISA analysis alone may not able to provide specific results as it is usually used for rapid screening. The LC-MS is used as confirmatory analysis for the MG or other substances detection due to higher sensitivity and it is more specific. Thus, for the detection of low levels of MG or LMG, the use of LC-MS is recommended. Furthermore, fish farmers should be made aware of the dangers of using MG for treating fish as it is carcinogenic and can be harmful to consumers. There should be a continuous regular monitoring process in place to ensure that fish sold in the market is safe for human consumption.

7.0 RECOMMENDATION

Increasing the sample size with different aquatic species are needed to obtain more significant results. Samples should be obtained from different areas throughout Malaysia so as to confirm the prohibition of MG usage. Other than muscles used as sample for MG analysis, internal organs such as liver, kidney and gills should also be analyzed to determine the distribution of the residues.

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