# ANALYSIS, IDENTIFICATION, AND FORMULATION OF METALLOTHIONEIN EXTRACTS ON NUMEROUS VARIETIES OF PADDY LEAVES

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***ABSTRACT***

*Background: Vegetable materials, such as rice, corn, bean, and soybean have metallothionein proteins contained in their roots, stems, leaves, flowers, and fruits. Of those vegetable materials, paddy leaves have the most numerous metallotionein proteins. However, the varieties which produce the highest metallotionein proteins have not yet been found. The roles of metallothionein proteins are to bind and to detoxify heavy metals. Objectives: to analyze, identify, and formulate the metallothionein extracts contained in numerous varieties of paddy leaves. An experimental research method. Each vegetable material of numerous varieties of paddy leaves is made into simplicia and is then processed into infusion. ELISA method is used as a technique to measure the protein level of each infusion. The metallothionein protein levels contained in numerous varieties of paddy leaves are then identified to determine the one with the highest metallothionein protein level. Extract formula is taken from the highest metallothionein level. Results :the highest metallothionein protein level is contained in paddy leaves of IR begendit (28.832 ng) while the lowest one is in paddy leaves of IR-64 SS (0.358 ng). The extracts of Paddy leaves of IR begendit with the highest protein level are then formulated into metallothionein extracts. Conclusion: Analysis on metallothionein levels of numerous varieties of paddy leaves with the highest protein level is contained in paddy leaves of IR begendit (28.832 ng) while the lowest one is in paddy leaves of IR-64 SS (0. 358 ng).*

*Keywords: paddy leaves, metallothionein*

## PENDAHULUAN

The development of science, technology, and modernization has not only positive but also negative impacts. One negative impact is the use of Pb in many industries which influences the health of human bodies. Thus, preventive efforts regarding to those negative impacts should be well undertaken.

One preventive effort is due to the use of metallothionein protein. Metallothionein protein has the ability to bind and detoxify heavy metals. Metallothionein is a protein (polypeptide) which has small molecular mass (4-8kDa), contains 26-33% of cysteine amino acid (Cys), and has no aromatic amino acid or histidin. (Hijova, 2004).

Metallothionein is a metal-binding protein functioning in metal binding or enclosing process within the tissues of each living thing. Metallothionein may strongly and efficiently bind heavy metals because of containing "thiol" group (sulfidril, SH) in a great amount. Cys' sulfidril residues may bind 1 metal ion for 2 or 3 SH residues. Each Cys' metal ion binding coordination forms a structure of tetrahedral tetrathiolate. Cys residues are required in detoxifying heavy metals by binding cations from those transitional metals (Cheung RCK, et al, 2001, Jiang S, et al, 2013). Metallothionein protein is rich with sulfidril clusters which are covalently bound with Pb within tissues

through blocking reaction which is then entered to a detoxification process.(11) (Murray et al, 2006).

Some literatures state that metallothionein is positively correlated with zinc's micronutrients (Santosa B, et al. 2013; Lee S. M. et al. 2013; Manahan SE, 2002) and are greatly found in plants. Metallothionein contained in plants is first identified in 1987, including EcMT (Early cysteine Metallothionein) within wheat embrios. At present, more than 140 Metallothionein sequences have been recorded from numerous plant species (Jiang S, et al, 2013). Metallothionein proteins are greatly found in vegetable materials, such as soybean, rice, corn, and bean contained in their roots, stems, leaves, flowers, and fruits. A research conducted by Santosa et al., in 2016 on metallothionein level contained in vegetable materials, such as yang terdiri atas soybean, rice, corn, and bean contained in their roots, stems, leaves, flowers, and fruits are found the highest in paddy leaves by 1.35 ng. Metallothionein level of 1.39 ng is proven significant to reduce the number of basophilic stipling.(12). Santosa B, Subagio HW. Metallothoinein is not only found in various levels of tissues and organs but also in cytoplasm and nucleus (Krężel A ,et al. 2007; Steven R, 2000).

A preventive effort regarding to the negative impacts of Pb has ever been conducted by Santosa B (2016) using vegetable extracts, such as rice, corn, soybean, and bean containing metallothionein in their roots, stems, flowers, and fruits. The research results show that paddy leaves have the highest metallothionein level and are proven effective to prevent kidney disorders resulted from Pb exposures (Santosa B, 2015; Santosa B, 2016). In that research, the metallothionein level has not been identified that it comes from paddy varieties. Thus, concentration of metallothionein extracts taken from numerous varieties of paddy leaves are considered worth to identify as well as the highest one with metallothionein content.

## METODE

This research uses an experimental method. Samples are taken from numerous varieties of paddy leaves. Each variety is made into simplicia which is then processed into infusion. ELISA is used as a technique in measuring the protein level contained in each infusion of those vegetable materials. Those vegetable materials are then identified to determine the one with the highest metallothionein protein level. Extract formula is taken from that with the highest metallothionein level.

## HASIL DAN PEMBAHASAN

### Metallothionein Levels

Metallothionein level is examined through the processes of material collection, simplicia making, infusion making, and the use of ELISA to determine the level. The examination stages are as follows:

1. Materials collection regarding to numerous varieties of paddy leaves' extracts.

The numerous varieties of paddy leaves in this research are obtained from Boja, Godong, and Purwadadi. Those areas are selected due to the existing planting season. Those materials are then classified based on their varieties. There are 16 varieties of paddy leaves. Some of them are read glutinous rice, black glutinous rice, white glutinous rice, red rice, Chiherank rice, umbuk rice, Ciliwung rice, IR-bagendit rice, and IR-64 rice from Godong, Serang rice and IR-64 rice are from Purwodadi, Boja red rice, umbul rice, Suka Mandi rice, IR 64 SS rice, and IR rice from Boja. Those rice varietas are separated and classified due to the preparations for simplicia making shown in figure 1.

| | | |
|---|---|---|
|  |  |  |
| Black glutinous rice | Red glutinous rice | White glutinous rice |

| | | |
|---|---|---|
|  |  |  |
| Red rice | *Chiherank* rice | *Serang* rice |
|  |  |  |
| Umbuk rice | *Ciliwung rice* | *IR-bagendit rice* |
|  |  |  |
| IR-64 rice | *IR-64 rice* | *Bj red rice* |
|  |  |  |
| Umbul rice | *Suka Mandi rice* | *IR-64 SS rice* |

| |
|---|
|  |
| IR (Bj) rice |

**Figure 1.Varieties of paddy leaves**

2. Vegetable simplicia making

Simplicia is a natural material used as cure and has not yet been processed.

How to make simplicia:

Clean the materials. Separate each part of the materials. Slightly slice them as shown in figure 2. Finally, put those simplicia collected based on their parts into Beaker glasses

Figure 2. Simplicia of Vegetable materials.

3. Preparations for Infusion making

Infusion is liquid supply made by extracting *simplicia* using water with the temperature 90-96 $^0$C for 15 minutes. Infusion is typically used to figure out the substantial contents in vegetable materials.

Working Procedures:

Put 100 g of simplisia into container A, add it with 1 liter of distilled water and then Close. Add container B (as water bath) with water as needed that container A is partially immersed in the water bath (container B). Heat it for 15 minutes. The time calculation starts when the temperature in container A reaches 90$^o$C. Occasionally, stir and then filter the supernatan as infusion. Figure 3 below shows the processes of making infusion.

Stage infusa

Before filtered

infusa

Figure 3. Infusion Making Processes

4. Metallothionein level Determination using ELISA method

The obtained infusions of vegetable materials are then analyzed using ELISA method to figure out the metallothionein level.

The principles of ELISA method to figure out the metallothionein level are: cover the microtiter plate with a specific antibody for MT, put the standards or samples into the mikrotiter plate wells meeting the preparations of specific polyclonal biotin conjugated antibody for MT and add the conjugated Avidin for horseradish peroxidase (HRP) in each micro plate well and then incubate. Add TMB substrate solution to each well. Only wells containing MT, biotin-conjugated antibody, and avidin-conjugated enzyme show color changes. The reaction of substrate enzyme is ended with the addition of sulfuric acid solution, and the color changes are measured with spectrophotometer at 450 nm ± 2 nm waves long. MT concentrations within samples are then set by comparing samples' Optical Density with standard concentration.

Examination Results

The metallothionein examination results are obtained by calculating the levels of samples and standards using ELISA reader. metallothionein concentration / level is shown with a unit of ng in 100 ul sample/infusion.

Based on figures of table 1, the highest metallothionein level is contained in paddy leaves of IR bagendit (28.832 ng) while the lowest metallothionein level is in paddy leaves of IR-64 SS (0.358 ng). Thus, based on varieties of paddy leaves, IR bagendit has the highest metallothionein level.

Some literatures state that metallothionein is greatly found in plants. Metallothionein contained in plants is first identified in 1987, including EcMT (Early cysteine Metallothionein) within wheat embrios. At present, more than 140 Metallothionein sequences have been recorded from numerous plant species (Jiang S, et al, 2013). Rice, corn, soybean, and bean plants have quite high metallothionein content while the highest one is contained by paddy leaves (Santosa B et al., 2016). In this research, paddy leaves of IR bagendit have the highest metallothionein level. A research conducted by Santosa B (2015) states that metallothionein content of 1.39 ng is quite significant to reduce the impacts of Pb exposures regarding to heme bio-sintesis. Liquid extracts of paddy leaves with a dosage of 1.34 ng significantly develop hematopoiesis regarding to Pb exposures (Santosa B et al., 2015). Findings of the highest metallothionein level contained in paddy leaves of IR bagendit are expected beneficial to prevent people from Pb exposures. Metallothionein is a protein rich of sulfidril clusters which strongly binds heavy metals. Thus, the results of this research are expected beneficial to prevent people from the negative impacts of heavy metals

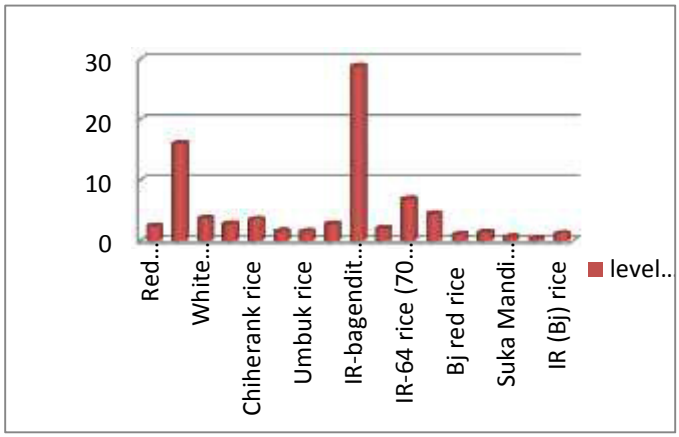


Figure 4. MT levels in varieties of paddy leaves

Tabel 1. MT Kaai of numerous varieties of paddy leaves

| No | Type of paddy leaves | Absoption | Level of mt (ng) |
|---|---|---|---|
| 1 | Red glutinous rice | 0.729 | 2.472 |
| 2 | Black glutinous rice | 1.252 | 16.136 |
| 3 | White glutinous rice | 0.842 | 3.800 |
| 4 | Red rice | 0.761 | 2.831 |
| 5 | *Chiherank* rice | 0.827 | 3.613 |
| 6 | *Serang* rice | 0.652 | 1.652 |
| 7 | *Umbuk* rice | 0.647 | 1.601 |
| 8 | *Ciliwung* rice | 0.758 | 2.797 |
| 9 | IR-bagendit rice | 1.500 | 28.832 |
| 10 | IR-64 rice | 0.695 | 2.113 |
| 11 | IR-64 rice (70 days) | 1.052 | 6.942 |
| 12 | IR-64 rice (40 days) | 0.897 | 4.516 |
| 13 | Bj red rice | 0.599 | 1.119 |
| 14 | *Umbul* rice | 0.635 | 1.478 |
| 15 | *Suka Mandi* rice | 0.548 | 0.627 |
| 16 | IR-64 SS rice | 0.440 | 0.358 |
| 17 | IR (Bj) rice | 0.614 | 1.267 |

## SIMPULAN DAN SARAN

### Simpulan

Analysis on metallothionein levels on numerous varieties of paddy leaves with the highest protein level is contained in paddy leaves of IR begendit (28.832 ng) while the lowest one is in paddy leaves of IR-64 SS (0.358 ng).

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