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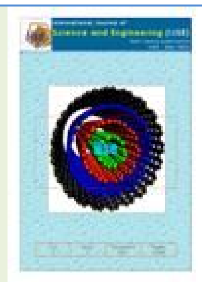

THE ANALYSIS, IDENTIFICATION, AND FORMULATION OF METALLOTHIONEIN EXTRACT AVAILABLE IN ROOTS, STEMS, LEAVES , FLOWERS , AND GRAINS OF RICE, CORNS, BEANS , AND SOYBEANS

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***Abstract* –**Vegetable materials are easily found around us but are frequently considered useless. The *Metallothionein* content in vegetable materials such in roots, stems, leaves, flowers, and fruit of rice, corns, beans, and soybeans has not been investigated before. *Metallothionein* protein has the role to bind heavy metals and serves as means of detoxification of heavy metals. This studi investigated to analyze, identify, and formulate *metallothionein* extracts from vegetable materials such as in roots, stems, leaves, flowers, and fruit of rice, corns, beans, and soybeans. The Experimental, each vegetable material available in roots, stems, leaves, flowers and fruit of rice, corns, beans, and soybeans was made into simplisia (crude medication) and then was processed into infuse. ELISA method was conducted as the measurement techniques upon protein level of each vegetable material infuse. The levels of *Metallothionein* protein available in vegetable materials were identified to determine the highest. The extract formula was taken from the highest levels of *metallothionein*. The result showed that the average *metallothionein* protein level from the entire vegetable materials was 0.62 ng: the highest in rice leaves of 1.4 ng and the lowest in bean flowers of 0.2 ng. The rice leaves were further formulated into *metallothionein* Extract. The analysis of *metallothionein* levels of vegetable materials such as in roots, stems, leaves, flowers, and fruit of rice, corns, beans and soybeans found that the highest level was in rice leaves of 1.354539 ng, and the lowest was in bean flowers of 0.22478.

***Key Words* -** Vegetable, metallothionein..

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I. INTRODUCTION

Metallothionein was first discovered in 1957 as a protein which contained a lot of sulfur and had a close relationship with zinc. There was significant positive correlation between zinc and *metallothionein* at any ages (Santosa B, et al. 2013; Lee S. M. et al. 2013; Manahan SE, 2002).

Metallothionein is a protein (polypeptide) which has small molecular mass (4-8kDa) containing cysteine (Cys) amino acid of 26-33%, having no aromatic or histidine amino acids (Hijova E, 2004). *Metallothionein* is classified based on amino acid composition, the amount of amino acids, the number and distribution of Cys amino acid in sequences, similarity of sequences, and phylogenic relationships. *Metallothoinein* was not only found in various levels of tissues and organs but also in cytoplasm and nucleus (Krężel A ,et al. 2007; Steven R, 2000).

The role of *metallothionein* in metal detoxification mechanism is related to the ability of *metallothionein* to bind metals which have toxic characteristics (Kosnett MJ, 2004). *Metallothionein* is a metal-binding protein functioning in the process of metal binding or confinement in every living tissue. Thus, *metallothionein* can be used as indicator for metal pollutions (RCK Cheung, et al, 2001; Jiang S, et al, 2013).

Metallothionein can strongly and efficiently bind metals since it contains "thiol" (sulfhydryl , SH) in large quantity (Reddy MS et al.2014). Sulfhydryl residue of Cys is able to bind 1 metal ion for 2 or 3 SH residues. Binding coordination of each Cys metal ion will form tetrahedral tetrathiolate structures (Abouhamed M, et al,2007). Cys residues are urgently required for heavy metal detoxification by binding cations of transitional metals (Cheung RCK, et al, 2001; Jiang S, et al, 2013).

Some literatures stated that *metallothionein* was easily found in many plants. *Metallothionein* in plants was first identified in 1987 as EcMT (Early cysteine Metallothionein) in wheat embryos. Now, there are more than 140 metallothionein sequences which were recorded from various sorts of plant species (Jiang S, *et al,* 2013; Aydemir, T.B, et al, 2005).

Of those various types of plants, no one has significantly found the highest levels of *metallothionein*. Thus, it is necessary to conduct a study upon the analysis of metallothionein levels in various sorts of plants such as rice (Yuan J,et al 2008), corns, beans, and soybeans which are available in their roots, stems, leaves, flowers, and fruit. After knowing the highest levels of metallothionein, it is expected that extracts are able to produce and used as preventive effort from heavy metal exposure.

II. MATERIAL AND METHOD

This research used experimental method that samples were taken from roots, stems, leaves, flowers, and fruit of the vegetable materials such as rice, corns, beans, and soybeans that each was simplified and then processed into infuse (Wahyuningsih MSH, 2013). ELISA method was conducted as the measurement techniques upon the protein level of each vegetable material infuse. *Metallothionein* protein levels available in vegetable materials were further identified to determine the highest level of *metallothionein*. Extract Formulation was then taken from the highest levels of *metallothionein*.

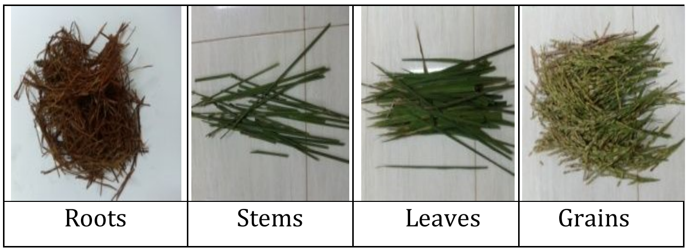
III. RESULTS AND DISCUSSION

Metallothionein Levels

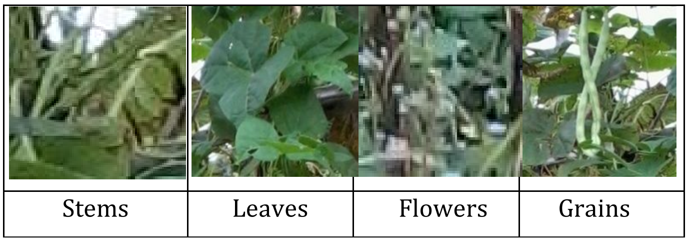
Metallothionein levels were investigated through the process of collecting vegetable material, making simplisia and infuse, and determining levels using ELISA. Stages of the test results were as follows:

1. Collecting material for vegetable extract (roots, leaves, stems, flowers, fruit and grains of rice, corn, beans, and soybeans).
   Vegetable materials in the form of roots, leaves, stems, flowers, fruit, and grains of rice, corns, beans, and soybeans were obtained from Boja and Bandungan. Those areas were selected based on the available seasonal plants, then these materials were further separated and grouped according to their parts such as roots, stems, leaves, flowers, and grains for the preparation of making *simplisia* shown in Figure 1 below:

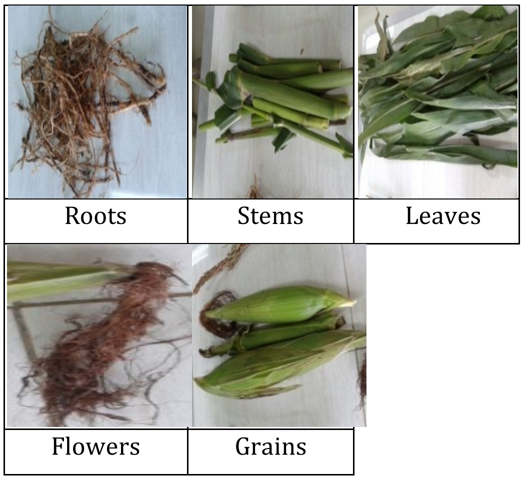
1. RICE



2. BEANS



3. CORNS



4. SOYBEAN

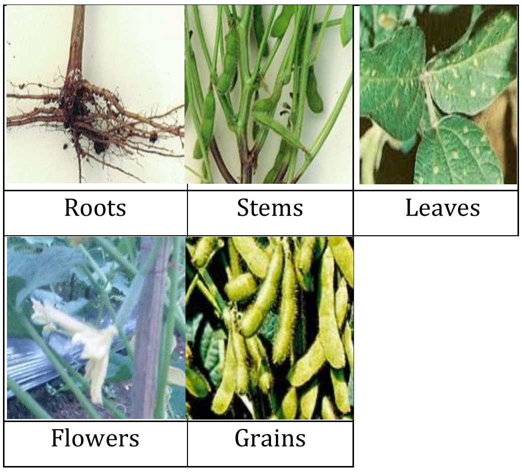


Figure 1. Rice, Beans, Corns, and Soybeans

2. Making vegetable simplisia
Simplisia is the natural material used as medicine and has not undergone any processes yet. Vegetable materials were cleaned and then separated based on their parts (leaves, roots, stems, flowers, fruit, and grains of rice, corns, soybeans, and beans). They were further sliced in tiny pieces as shown in Figure 2. The simplisia which was collected based on its parts was put into glass beaker.

Figure 2. Vegetable Simplicia

3. Preparation for making infuse
Infuse is a liquid supply made by extracting simplisia with oada water at the temperature of 90 - 96^{o}C for 15 minutes. Infuse is generally used to find substances available in vegetable materials.
Procedures:
Weighed simplisia as much as 100 g, put it into container A, add it with 1 liter of distilled water, and then close. Add water in container B (as water bath) as required that container A was partially submerged in container B. Heat for 15 minutes it, calculated when the temperature of pot A inside part reaches 90^{o}C while occasionally stir it, then filter, and supernatant as infuse. Figure 3 below is the process of making infuse.

Figure 3. Stages of Infuse Process

4. Metallothionein level determination of ELISA
The obtained vegetable Infuse were then analyzed to determine the *metallothionein* levels using ELISA method. The Principles of ELISA method to determine the levels of metallothionein were that *micro titer plate* was previously coated with specific antibodies against *MT*. Standards or samples were then added into the *wells* of *micro titer plate* which were appropriate for the preparation of specific polyclonal conjugated-biotin antibody for MT and conjugated Avidin for horseradish peroxide (HRP) was added to each *well micro plate* and then incubated. The *TMB* substrate solution was then added to each *well*. Only wells containing MT, conjugated biotin antibody and Avidin conjugated enzyme would exhibit color changes. Substrate enzyme reaction was finally added with sulfuric acid solution and the color changes were measured with spectrophotometer at 450 nm ± 2 nm wavelength. MT concentrations in samples were then determined by comparing the *optical density* of samples with standard concentration.

5. Test results
The Test results of *metallothionein* levels were obtained by calculating samples and standards with ELISA reader. *Metallothionein* concentrations /levels were shown in units of ng in 100 ul samples/infuse.

Based on figure 4 and table 1, the highest *metallothionein* level was in rice leaves (1.35 ng) and the lowest *metallothionein* level was in bean flowers (0.23 ng). Based on vegetable material types, the highest *metallothionein* level was in rice leaves (1.35 ng), corn roots (0.91 ng), Bean stems (0.67), and soybean flowers (0.73 ng). Some literature stated that *metallothionein* was easily found in many plants. *Metallothionein* in plants was first identified in 1987, and called as EcMT (Early cysteine *metallothionein*) in wheat embryos. Now, more than 140 *metallothionein* sequences were recorded from various sorts of plant species (Wu JP et al, 2007; Jiang S, et al, 2013). Based on these results, it showed that rice, corns, beans, and soybeans had relatively high of *metallothionein* content.

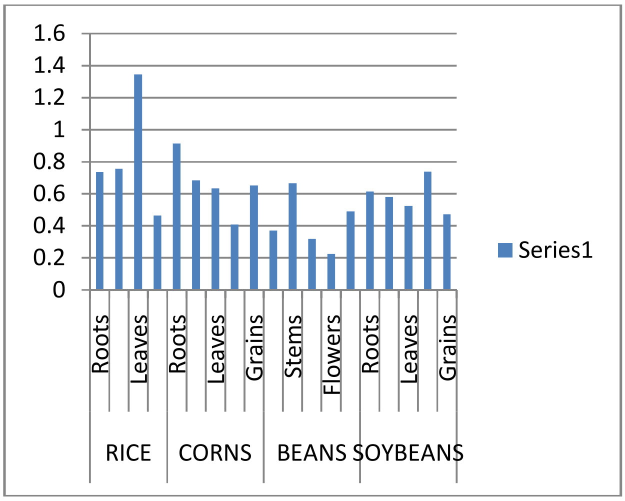


Figure 4. MT levels in rice, corns, beans, and soybeans

Table 1. vegetable *metallothionein* Concentrations

N0	Material Types		MT CONSENTRATION (ng)
1	RICE	Roots	0.736439
		Stems	0.756864
		Leaves	1.34539
		Grains	0.46457
2	CORNS	Roots	0.913193
		Stems	0.685044
		Leaves	0.632877
		Grains	0.40845
		Roots	0.654134
3	BEANS	Roots	0.372254
		Stems	0.666911
		Leaves	0.31976
		Grains	0.22478
		Roots	0.489622
4	SOYBEANS	Roots	0.613788
		Stems	0.57995
		Leaves	0.524178
		Grains	0.739661
		Roots	0.47187

Research conducted by Santosa B (2013) stated that 1.39 ng metallothionein content could significantly reduce the impact of Pb exposure upon heme biosynthesis. Metallothionein is protein which is rich of *sulfhydryl* groups that strongly bind heavy metals. Thus, the results of this research could be used as preventive effort upon the impact of heavy metals.

IV. CONCLUSION

The analysis of *metallothionein* levels of vegetable materials such as rice, corns, beans, and soybeans, in their roots, stems, leaves, flowers, and fruit found that the highest level was in rice leaves of 1.34539 ng and the lowest was in bean flowers of 0.22478 ng.

REFERENCES

1. Abouhamed M, Wolff NA, Lee WK, Smith CP. Knockdown of endosomal/lysosomal divalent metal transporter 1 by RNA interference prevents cadmium-metallothionein-1 cytotoxicity in renal proximal tubule cells. Am J Physiol Renal Physiol 2007;293:705-12
2. Aydemir, T.B., Raymond, K.B., Cousins, R.J., (2005). Zinc supplementation of young men alters metallothionein, zinc transporter, and cytokine gene expression in leukocyte populations. Nutritional Genomics Laboratory, Food Science and Human Nutrition Department, Center for Nutritional Sciences, University of Florida, Gainesville;FL 32611-0370
3. Cheung RCK, Chan MHM, Ho CS, Lam CWK and Lau ELK., (2001).Heavy metal poisoning clinical significance and laboratory investigation.Asia pasific Analyte Notes. BD Indispensable to Human Health. Hong Kong;7(1):22-34
4. Hijova E. Metallothionein and zinc: their functions and interactions. Bratislavia Med J 2004;105:230-4.
5. Krężel A, Hao Q, Maret W.The zinc/thiolate redox biochemistry of metallothionein and the control of zinc ion fluctuations in cell signaling. Archives of Biochemistry and Biophysics. 2007;463(2):188–200
6. Jiang S, Rui Guo, Zhang Y, Zou Y, and Ren J.,(2013).Heavy metal scavenger metallothionein mitigates deep hypothermia-induced myocardial contractile anomalies: role of autophagy.Am J Physiol Endocrinol Metab; 304: 74 - 86.
7. Kosnett MJ. Heavy Metal Intoxication & chelators. In Katzung B.G:Basic & Clinical Pharmacology. 9th ed. Boston, New York:Mc Graw Hill 2004:970-81
8. Lee SM, McLaughlin JN, Frederick DR, Zhu L , Thambiayya K , Wasserloos KJ.,(2013). Metallothionein-induced zinc partitioning exacerbates hyperoxic acute lung injury. Am J of Physiology - Lung Cellular and Molecular Physiology;304: 350-60.
9. Manahan SE.Toxicological chemistry and biochemistry. Lewis Publishers. 3rd ed. New York Washington 2002
10. Reddy MS, Prasanna L, Marmeisse R, and Tachet LF. Differential expression of metallothioneins in response to heavy metals and their involvement in metal tolerance in the symbiotic basidiomycete Laccaria bicolor. Microbiology 2014;160: 2235-42
11. Steven R, Davis and Cousin J.R.,(2000). Metallothionein Expression in Animal: A Physiological Perspective on Function. American Society for Nutrition Sciences, Food Science and Human Nutrition Departement, University of Florida, Gainesville FL.; 32611-0370.
12. Santosa B, Hertanto WS, Henna R, Lisyani S., (2013).Effect of zinc suplementation prevention (Zn) to metallothionein and heme biosynthesis in rat Rattus novegicus before Pb-exposed. **Internat. J. of Sci. and Eng.**2013;5(2):15-17
13. Wahyuningsih MSH. Pengumpulan, pemilihan simplisia, pembuatan ekstrak dan identifikasi golongan senyawa herbal. in:Penyiapan sampel dari bahan herbal. FK UGM 2013
14. Wu JP, Ma BY, Ren HW, Zhang LP, Xiang Y, Brown MA. Characterization of metallothioneins (MT-I and MT-II) in the yak. J Anim Sci 2007; 85: 1357-62
15. Yuan J, Chen D, Ren Y, Zhang X, Zhao J. Characteristic and Expression Analysis of a Metallothionein Gene, OsMT2b, Down-Regulated by Cytokinin Suggests Functions in Root Development and Seed Embryo Germination of Rice1[OA]. Plant Physiol. 2008;146:1637–50