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Beneficial of Clover Leaf (*Marsilea crenata*) as Bio-adsorbent of Heavy Metal Contamination in Red Mussels (*Masculita senhausia*)

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Abstract. Clover (*Marsilea crenata*) is one type shrub plant that is widely used as a vegetable in Surabaya, Indonesia, rich in phytochemical compounds. One of them is polyphenols which have the ability to chelate metals. Chaempherol, quercetine, routine, flavonols are types of polyphenols that can be used as chelating agents for heavy metals because they have functional groups to bind metals. The objective of the research was to study the effect of clover leaf flour concentration and contact time on decreasing heavy metal contamination in red mussels. This study used a factorial completely randomized design with two replications. The first factor were the concentration of clover leaf flour (1%, 3%, 5%) and factor II were the contact time (0 minutes as a control, 60 minutes, 90 minutes, 120 minutes). The data from this research analysis using ANOVA (analysis of variant) and DMRT (duncan't multiple range teste). The best treatment was concentration of 5% clover leaf flour and contact time 120 minutes can reduce copper from 47.49 ppm to 19.69 ppm (decrease 58.54%), lead from 21.31 ppm to 9, 67 (decrease 54.67%), mercury from 0.54 ppm to 0.26 ppm (decrease 52.32%) in red mussels.
Keyword: bio-adsorbent, clover leaf, heavy metal, red mussel

1. Introduction

Clover plants (*Marsilea crenata*) contain phytochemical compounds such as terpenoids, steroids, saponins, polyphenols, flavonoids, tannins, alkaloids and phenol components [1]. Research by [2], using the *Marselia quadrifolia* plant showed that there were polyphenol compounds namely kaempferol, xypsilotinin, dihydroxyscirpusin, quercetin, hegoflavone, caffeic acid, ethyl caffeate, cyperusphenol, mesocyperusphenol, scirpusin, routine[3], marsiline, triacontan, hentriacontan, methyl amine, methyl amine marsileagenin[4], flavonol, diglycoside, glucocyl flavones, glucosylxanthones and beta-sitosterol.

According[5], metal chelating agents can come from organic components, namely groups of phenolic compounds or polyphenols. Organic components can function as metal chelating agents because of the presence of carboxyl groups and one phenolic group or two adjacent hydroxyl groups reacting with metal ions and forming a stable complex. The presence of phytochemical content, especially the flavonoids in clover, can be used as heavy metal chelating on aquatic biota, one of them is red mussels. Based on research conducted [6], that Pb levels in red mussels from Balongdowo village, Sidoarjo were 4.24 ppm. Another study conducted by [7], stated that the content of Hg mussels raw rice at Sidorajo was 1.796 ppm. The copper metal levels in green mussels (*Perna viridis*) from Gresik amounted to 58,010 ppm [8]. The maximum security limit stipulated by the National Standardization Agency SNI 7387: 2009 for Pb at bivalve, mollusk and sea cucumber is 2 mg/kg and in Hg is 1.0 mg/kg. The copper (Cu) threshold set by the World Health Organization (WHO) and the

Food and Agriculture Organization (FAO), namely 800-1200 ppb [9]. According to the Decree of the Director General of BPOM No. 03725/ BSK/VII/89 the maximum limit of copper on foodstuffs is 20 ppm. The high level of pollutant of Pb, Hg and Cu heavy metals in red mussels needs to be reduced.

One method to reduce the levels of heavy metals that exceed the threshold is the adsorption method. Heavy metal adsorption occurs because of the interaction between active groups of adsorbents, so that the chemical structure of the adsorbent will affect the adsorption process. Adsorption on "enceng gondok" biomass occurs mainly in active groups, such as carboxyl (-COOH) and hydroxyl (-OH)[10].

The adsorption process is influenced by several factors including adsorbent concentration, surface area, temperature, particle size, contact time, adsorbent media shape, ability of adsorbents to absorb and bind heavy metals, and types of adsorption[11],[12],[13]. In this study variations in the concentration of clover flour and contact time of clover flour with red mussels as a factor determine the adsorption process.

The time to reach equilibrium in the process of metal absorption by an adsorbent ranges from a few minutes to several hours. The research on the influence of weight and contact time of lead (II) adsorption by adsorbents from guava stem bark (*Psidium guajava* L). The best results of the study were a decrease in lead metal (II) of 53.04% in the addition of adsorbent weight of 3 grams 100 ml and the duration of contact time was 90 minutes [14].

2. Methodology

2.1. Materials and tools

The main ingredients used in this study were clover leaves and red mussel. Supporting materials for analysis are Aquades, 0.02 N HCl, 6 M HgO HCl, K2SO4, H2SO4, 5% Na2CO3 (sodium carbonate), tanic acid, ethanol 96%, follin ciocalteu 50%, H3BO3 (boric acid), NaOH-Na2S2O3 (sodium thiosulfate), mother liquor Hg, KMnO4, SnCl2.2H2O, 65% HNO3, H2O2, matrix modifier solution, HClO4, alcohol, 0.2% methylene, 0.2% methylene blue, filter paper, filter cloth.

The tools used include: baking sheet, weighing bottle, porcelain exchange rate, furniture, cabinet dryer, kjeldahl pumpkin, porcelain cup, measuring flask, oven, desiccator, shaker, Atomic Absorption Spectrophotometer (AAS), mercury analyzer, UV-VIS spectrophotometer, erlenmeyer, clamp pliers, magnetic stirrer, vortex, hot plate, acid chamber, incubator, test tube, alarm cup, glass funnel, measuring cup, analytic balance, scales, blenders, basins, knives, sheets, kjeldah flasks, heaters, tools distillation, condenser tube, 80 mesh sieve, volume pipette.

2.2. Data Analisis

Data analisis in this study uses a Completely Randomized Design (CRD) which is arranged in a factorial pattern, consisting of two factors, where factor A consists of three levels and factor B consists of three levels. Data obtained from the results of the analysis were processed using Analysis Of Variance (ANOVA) and further tests using Duncan (α=5%), so that there was an interaction and influence between each treatment.

3. Result and Discussions

3.1. Raw material

The raw material used in this study was clover leaves flour from fresh clover plants and fresh red mussel meat. Fresh clover carried out phytochemical analysis and initial analysis of clover flour with parameters including water content, ash content, yield and total phenol. The results of the analysis can be seen in Table 1.

The results of fresh clover phytochemical screening using distilled water solvent showed that there were phytochemical compounds namely terpenoids/steroids, flavonoids, saponins and polyphenols. According to the study of [1], states that the phytochemical screening on

Marsilea quadrifolia using distilled water solvent shows the presence of tannins, flavonoids, phenolic components, steroids, terpenoids and saponins.

Table 1. Screenning result of fitochemical fresh clover leaves

Parameter	Method	Results	Description
Terphenoid/steroid	Thin Layer Chromatography	+	There was a red or purple red color
Flavonoid	Thin Layer Chromatography	+	There was an intense yellow colored stain with the appearance of ammonia vapor stains
Saponin	Exhaust Test	+	There was a froth formed when dripping with hydrochloric acid
Pholyphenol	Ferricloride Tests	+	There was a bluish-green dark color

The yield of clover leaf flour was calculated by comparing the initial weight of the material used with the weight of the powder obtained. The results of the analysis of clover flour were 14.35 ± 0.045% smaller than the results of [15], study of the yield of clover leaf simplycia which was 15.36%. The difference in yield analysis results can be caused by several factors such as the place of growth, weather conditions and the drying method. [8], reported that phenol compounds have been known to have various biological effects such as antioxidant activity, free radical schavanger, metal chelating, dampening the formation of singlet oxygen and electron donors. According to [16], research, total phenol testing aims to determine the total phenolic compounds contained in the sample. The results showed that total phenol in clover flour was 64.78 ± 0.297 mg TAE/gram[17]. Total phenol level of 165.0 ± 50.9 mg TAE/gram was greater than the results of a total phenol in this study. This difference is caused by several factors that is natural condition of the compound, sample particle size, storage conditions and time, comparison the number of solvents and samples [18], and the influence of the environment where plants grow, climate, soil quality and water quality. Red mussels in this research carried out an initial analysis heavy metal contamination including copper (Cu) content, lead level (Pb) and mercury levels (Hg) (Table 3).

Table 3. The result of analysis heavy metal contamination fresh red russels meat

Parameter	**Result of Analysis**	**Refference**
Protein content (%)	9,82±0,170	11,54±0,05 (Nurjanah dkk, 2014)
Cu content (ppm)	47,49±0,386	58,01 (Rofiananda, 2017)
Pb content (ppm)	21,31±1,963	23,32 (Sari dkk, 2017)
Hg content (ppm)	0,54±0,027	1,796 (Purwanto dkk,2000)

In research [16] explained that mineral composition in marine invertebrate animals is influenced by eating habits, age, sex, climate, and habitat conditions. This statement is also explained that eating habits of an organism can affect the ability to absorb minerals contained in the environment. According [17], heavy metal content is influenced by the concentration of heavy metals in water, pH, water pollution levels in the form of COD (chemical oxygen demand), water animal species or species [18], body weight/size and life phase (eggs and larvae).

4. Level Copper (Cu) in Red Mussels

Based on the results of variance analysis it is known that there is a real interaction ($p<0.05$) between the treatment of differences in the concentration of clover leaf flour and the contact time with the levels of copper (Cu) red mussels. Each treatment significant differences. The effect of contact time and the concentration of clover leaf flour on decreasing Cu metal contamination in red mussels can be seen in figure 1.

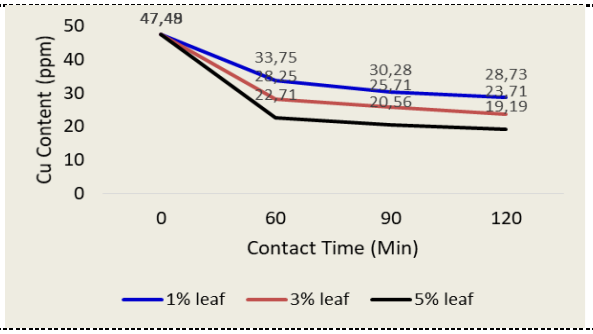

Figure 1. Effect of clover leaf flour concentration and contact time on copper (Cu) levels in red mussels.

The maximum limit of copper metal content in foodstuffs determined by SNI 7387: 2009 and Decree of Director General of BPOM No. 03725/BSK/VII/89 at 20 ppm. Copper metal content after treatment showed that with immersion in the concentration of 5% clover leaf flour and contact time of 120 minutes it could reduce copper metal by 19.67 ppm. So that with this treatment red mussels have been safe to consume because the levels of copper metal are below the maximum limit.

5. Level Lead (Pb) in red mussels

Figure 2 shows that the higher concentration of the cloverleaf flour and the contact time of immersion, the lead content of red mussels was decreases. This is because the flavonoid compound is assumed to be quercetin which has a functional group C-OH, C-O, C=O which can bind to lead metal to form a lead quercetin complex. According research by [10] states that the adsorption process of Pb (II) with the guava bark adsorbent (*Psisdium guajava* L) can occur due to the chemical reaction between the adsorbate molecules and the surface of the adsorbent which can be assumed to be a complex formation. [10] states that immersion time can affect the adsorption power by the adsorbent which is indicated by reduced Pb (II) levels. The ability to adsorb the guava bark (*Psidium guajava* L.) in adsorbing Pb (II) reached the optimum time in the contact time of 90 minutes.

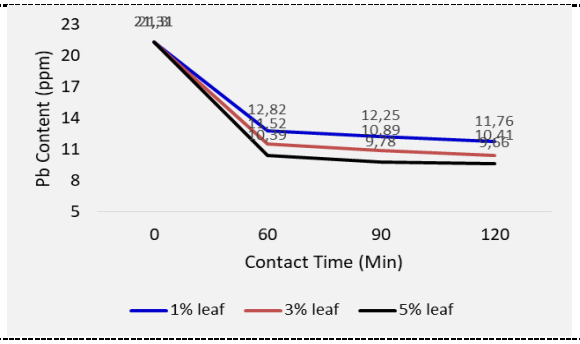

Figure 2. Effect of clover leaf flour concentration and contact time in red mussel lead (Pb) levels

The maximum limit of lead metal content in foodstuffs determined by SNI 7387: 2009 and Decree of Director General of BPOM No. 03725 / BSK/VII/89 at 2 ppm. The levels of lead metal after treatment showed that with immersion in the concentration of 5% clover flour and contact time of 120 minutes it could reduce the copper metal by 9.67 ppm. So that with this treatment red mussels still exceed the safe limit for consumption because their lead metal content is still above the maximum limit.

6. Level Mercury (Hg) in red mussels

Figure 3 shows that the higher concentration of the clover leaf flour and the longer of contact time, the mercury content of red mussels decreases. This is because the flavonoid compound is assumed to be

quercetin which has a functional group C-OH, C-O, C=O which can bind to metal mercury to form a quercetin-mercury complex. According to [17] organic components can function as metal chelating agents because of the presence of a carboxyl group and two adjacent hydroxyl groups reacting with metal ions to form a stable complex. The higher concentration of the clover leaf flour, the more functional groups present in the solution can reduce mercury levels in red mussels.

According to [18], that the decrease in mercury content in immersion is thought to be due to the release of metal ions from the structure of shellfish proteins which are then dissolved (leaching) from shellfish in an effort to balance concentrations in shellfish. This is in accordance with [19] which states that mercury contact time with the adsorbent surface significantly affects the efficiency of the adsorption process. In the case of the use of biochar adsorbents and activated carbon adsorbents, the increasing of contact time amount of mercury adsorbed on the surface of the adsorbent is increases.

The maximum limit of mercury metal content in foodstuffs set by SNI 7387: 2009 is 1 ppm. The levels of metal mercury after treatment showed that with immersion in the concentration of 5% clover leaf flour and contact time of 120 minutes it could reduce mercury metal by 0.26 ppm. So that with this treatment red mussels are still safe to consume because their metal levels are still below the maximum limit set.

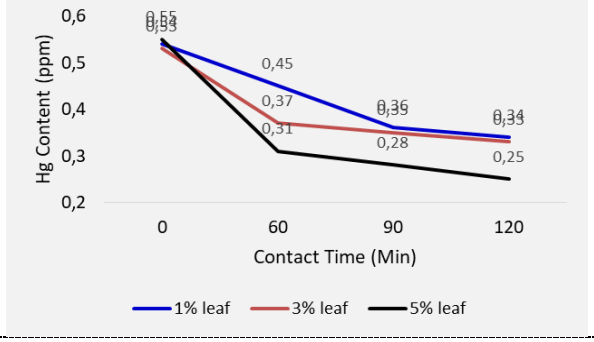

Figure 3. Effect of clover leaf flour concentration and contact time in red mussel mercury (Hg) levels

7. CONCLUSION

The results of the phytochemical analysis of fresh clover leaves indicate that clover contains compounds of flavonoids, polyphenols, steroids/terpenoids and saponins. The analysis of red mussels obtained protein content 9.82±0.17%; copper content of 47.49±0.386 ppm; lead levels of 21.31±1.96 ppm and mercury levels of 0.54±0.027 ppm. The best treatment was found the concentration of 5% clover leaf flour and the contact time of 120 minutes can reduce the contamination of heavy metals in red mussels copper 19.69 ppm, lead 9.66 ppm, mercury from 0.54 ppm to 0.26 ppm (down 52.32%). The maximum limit of mercury in food is 1 ppm.

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