VOLUME 17 NO. 1 |JANUARY 2023
P-ISSN: 1411-1616 | E-ISSN:2549-8215
Accredited by Kemenristekdikti No. 10/E/KPT/2019 (S3)

# JURNAL ILMIAH BERKALA
# SAINS DAN TERAPAN KIMIA

Rice husks from different varieties as source of silica (Mosa et al, pp. 4)

JURNAL ILMIAH BERKALA
SAINS DAN TERAPAN
KIMIA

PUBLISHED BY:
CHEMISTRY DEPARTMENT, FACULTY OF MATHEMATICS AND NATURAL SCIENCES
LAMBUNG MANGKURAT UNIVERSITY, INDONESIA

## *PUBLISHER*

Chemistry Department, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University

## *MAILING ADDRESS*

Jl. A. Yani Km. 36 Banjarbaru, Kalimantan Selatan , Indonesia 70714
Phone: 0511-4773112, 0511-4782899
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## *FIRST EDITION: 2007*

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**Jurnal Ilmiah Berkala: Sains dan Terapan Kimia**
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# The Effect of Rice Varieties on Silica Purification from Rice Husk Ash as Adsorbent for Mn(II)

# Kajian Pengaruh Varietas Padi pada Pemurnian Silika Dari Abu Sekam Padi Sebagai Adsorben Mn(II)

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## ABSTRACT

The influence of rice types on the purification of rice husk ash was investigated by extracting silica and purifying it with a 12% NaOH solution, followed by the coprecipitation technique. Rice husk samples were from Mbay (Nagekeo Regency) with two different varieties, Ciherang and Inpari 42, and from Soa (Ngada Regency) with Ciherang variety. The silica produced in this research was characterized using UV Vis spectrophotometry to obtain the surface area value. The surface acidity value was calculated using the acid-base titration method. Using XRF, the purity of silica was determined. Ciherang Mbay, Ciherang Soa, and Inpari Mbay had surface area values of 18.397 m2/gram, 18.347 $m^2$/gram, and 18.491 $m^2$/gram, respectively. The surface acidity value of the three samples was 45.1 mmol/gram, 45.5 mmol/gram, and 44.7 mmol/gram, respectively. The purity of silica from rice husk ash Ciherang Mbay, Ciherang Soa, and Inpari Mbay samples based on XRF was 98.6%, 98.3%, dan 99.2%, respectively. Silica with the highest purity (Inpari Mbay variety) was applied as an adsorbent in the adsorption process of manganese (Mn(II)) metal ion with an adsorption capacity of 212.76 µmol/gram and adsorption energy of 38.165 kJ/mol through chemical adsorption.

**Keywords**: purification, rice varieties, silica, adsorption, manganese

## ABSTRAK

*Pengaruh varietas padi terhadap proses pemurnian silika dari abu sekam padi telah dikaji melalui ekstraksi silika dengan metode pelarutan menggunakan larutan NaOH 12% yang dilanjutkan pengendapan dengan asam sulfat. Sampel sekam padi yang digunakan berasal dari daerah Mbay (Kab. Nagekeo) dengan dua varietas padi yang berbeda yaitu Ciherang dan Inpari 42 serta dari daerah Soa (Kab. Ngada) dengan varietas Ciherang. Silika yang dihasilkan dikarakterisasi menggunakan spektrofotometri UV Vis untuk mendapatkan nilai luas permukaan, sedangkan nilai keasaman permukaan ditentukan dengan metode titrasi asam basa serta dikarakterisasi kemurnian silika menggunakan XRF. Nilai luas permukaan silika yang dihasilkan dari sampel Ciherang Mbay, Ciherang Soa dan Inpari Mbay berturut turut adalah 18,397 $m^2$/gram, 18,347 $m^2$/gram dan 18,491 $m^2$/gram serta nilai keasaman permukaan dari ketiga sampel berturut turut adalah 45,1 mmol/gram, 45,5 mmol/gram dan 44,7 mmol/gram. Kemurnian silika dari sampel abu sekam padi varietas Ciherang Mbay, Ciherang Soa dan Inpari Mbay berdasarkan hasil XRF berturut-turut adalah 98,6%, 98,3% dan 99,2%. Silika dengan kemurnian tertinggi (varietas Inpari Mbay) diaplikasikan sebagai adsorben pada proses adsorpsi ion logam Mn(II) dengan nilai kapasitas adsorpsi sebesar 212,76 µmol/gram dan energi adsorpsi sebesar 38,165 kJ/mol sehingga dapat diindikasikan bahwa terjadi proses adsorpsi secara kimia.*

**Kata Kunci:** *permurnian, varietas, silika, adsorpsi, mangan*

**Received**: January 27, 2022; **Accepted**: January 4, 2023: **Available online**: February 15, 2023

## 1. INTRODUCTION

The rice husk is the outermost layer of the rice grain, which has a high carbon and ash content and is resistant to weathering. Rice husk is composed of cellulose (34-44%), lignin (20-30%), ash (13-39%), and water (8-15%) (Trivana *et al*., 2015). Rice husk ash also contains silica, small amounts of alkali, and metal impurities. When burned at high temperatures (600°C) in a controlled manner, rice husks will produce silica ash which can be used in various chemical processes. Typically, the silica percentage of rice husk ash is between 94 and 96%. (Sriyanto and Darwanta, 2017).

Silica is a compound commonly found in minerals such as quartz sand. Sand has high crystallinity and contains many impurities, reducing its ability as an adsorbent (Mujiyanti and Kunarti, 2010). Therefore, silica was synthesized from rice husk to increase its qualities and replace silica derived from nature. Silica extracted from rice husk is also utilized in producing other silica-based substances. Wogo and Ndoen (2020) synthesized a composite of immobilized silica EDTA-Ag and chitosan to manufacture antibacterial plastics that could kill Escherichia coli and Staphylococcus aureus bacteria up to 99.99%. Ngatidjo *et al*. (2011) also utilized silica from amine-modified rice husks as an adsorbent for copper metal ions. In addition, research on silica from rice husks was carried out by Valentine *et al*. (2019), who produced chitosan membranes by adding rice husk ash silica as an additive to a casting solution to reduce Cu heavy metal.

Silica purification from rice husk ash is essential due to its wide range of applications. Additionally, the quality of the generated silica affects its applicability. Trivana *et al*. (2015) synthesized sodium silicate from rice husk. Based on EDS, the sodium silicate did not contain impurities in the form of C atoms and metal impurities. Harimu *et al*. (2019) obtained silica from processing rice husk ash using NaOH (89.09%) and $H_2SO_4$ (94.94%). Sriyanto and Darwanta (2017) investigated the influence of acid type on the purification of rice husk ash, where $H_2SO_4$ (99.48%) purification is superior to HCl (99.35%) and $HNO_3$ (99.38%) purification.

Silica is one of the important elements for rice growth and is mostly taken from the growing medium. Hence, the Si content in each rice variety can vary. In addition to variations, the soil type affects the Si content in rice. Lede *et al*. (2021) have proved that changes in cultivars and soil types can affect the Si content in rice husk ash. The silica content of the Ciherang variety sample from Tarus (99.1%) and Pariti (98.4%) was higher than that of the Inpari variety sample from Pariti (98.1%). The samples taken from two rice-producing locations in Kupang Regency reveal that the soil type impacts the Si content in rice. The soil type in the Tarus area is vertisol soil with a rather high Si content.

Mn is one of the essential heavy metals because it builds bone structure and helps bone metabolism and enzyme work. Nonetheless, if the concentration within the body is excessive, it will be poisonous and cause harm to the central nervous system and lungs. Manganese consumption should not exceed 11 mg per day because it can produce signs of nervous system diseases. In dust, the manganese concentration should not be more than 5 mg/m3 because it might induce respiratory diseases (Syuhada, 2018). In addition, high quantities of manganese in the environment can contribute to air, water, and soil pollution. Therefore, improper handling of this substance can be dangerous to humans, animals, plants, and the environment. This research uses silica as an adsorbent to reduce Mn pollution in the environment.

## 2. MATERIALS AND METHODS

### *2.1. Materials*

The materials were rice husks of the Ciherang variety from Mbay (sample 1) and Soa (sample 2) and the Inpari 42 variety from Mbay (sample 3), $H_2SO_4$, NaOH, PP indicator, methylene blue solution, $MnSO_4.4H_2O$, HCl and distilled water. The equipment used was glassware, sieve, magnetic stirrer, analytical balance, oven, furnace, UV-Vis Spectrophotometer, X-Ray Fluorescence (XRF), and Atomic Absorption Spectrophotometer (AAS).

### *2.2. Preparation of Rice Husk Samples*

Rice husks were collected from Mbay (Nagekeo Regency) and Soa on Flores Island, East Nusa Tenggara Province, Indonesia (Ngada Regency). There were two rice varieties taken from Mbay, namely the Ciherang variety (sample 1) and Inpari 42 (sample 3), while from Soa, the Ciherang variety (sample 2) was used. Each rice husk sample was cleaned, washed, rinsed, and dried at 110°C for ± 1 hour. Furthermore, the husks are roasted until they turn black and then roasted using a furnace for ± 4 hours at 700°C. The sample was then pulverized and passed through a 100-mesh sieve. Fig. 1 depicts photographs of rice husks from each sample.

### *2.3. Silica Extraction of Rice Husk Ash*

a. Purification with NaOH Solution

Twenty grams of sample 1 were combined with 120 mL of 12% NaOH and heated at 85°C for 90 minutes with magnetic stirring. The sample was filtered, and the filtrate was neutralized with 0.5 M $H_2SO_4$ until a gel precipitate formed, followed by 18 hours of incubation. The gel precipitate was filtered, washed with 1000 mL of hot distilled water, and dried in a 110°C oven for five hours. The identical procedure was applied to samples 2 and 3.

b. Precipitation with H2SO4 Solution

One gram of silica purified with NaOH for each sample was added with 10 mL of 10.88% $H_2SO_4$. The solution was then left for 120 minutes and washed using 200 mL of distilled water. The solution was then filtered, and the precipitate was dried in an oven at 110°C for 2 hours and then weighed. The yield was determined.

$$\%Yield = \frac{Weight\ of\ silica}{Weight\ of\ sample} \times 100\%$$

### *2.3. Characterization of Silica*

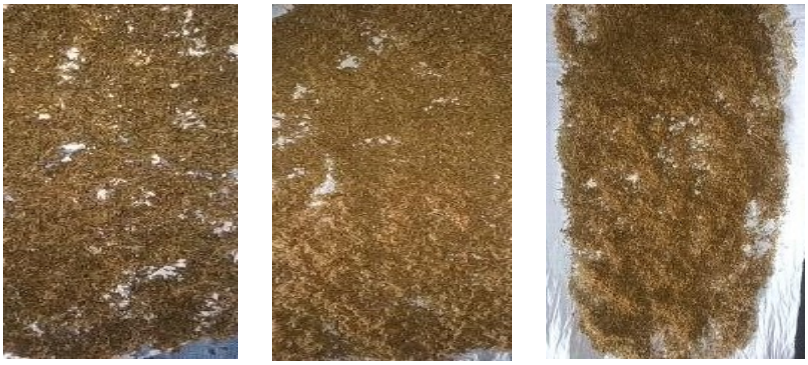
Determination of the surface acidity of silica was carried out by the titration method (Wogo *et al*., 2014). The surface area of silica was measured using a spectrophotometric technique employing methylene blue as an adsorbate (Wogo *et al*., 2014). Silica purified from rice husk ash samples 1, 2, and 3 were analyzed using an XRF instrument to identify the purity of Si in the sample (Sapei *et al*., 2015). XRF analysis was also carried out on rice husk ash from the three samples.

### *2.4. Silica Applications for Adsorption of Mn Metals*

A beaker was filled with 0.1 grams of silica from sample 3 (Inpari 42). Using a batch system, adsorption was performed by adding 50 mL of $MnSO_4.4H_2O$ solutions with concentrations of 10, 20, 40, 80, 100, 150, and 200 mg/L. The solution was stirred for one hour before being centrifuged at 2,000 rpm for 30 mins to separate the filtrate from the adsorbent. AAS was utilized to determine the amount of metal ions adsorbed by the filtrate. The adsorption capacity was calculated using the Langmuir adsorption isotherm equation, and the adsorption energy was calculated using the Gibbs free energy equation under standard conditions.

a b c

**Figure 1.** Rice husk of each variety (a) sample 1, (b) sample 2, (c) sample 3



a b c

**Figure 1.** Rice husk of each variety (a) sample 1, (b) sample 2, (c) sample 3

## 3. RESULTS AND DISCUSSION

### *3.1. Preparation of Rice Husk Samples*

In order to assist the pyrolysis process, sample preparation was performed. The coagulation procedure consisted of roasting or controlled burning until the rice husk changed color from brown to black to break down organic components, lowering the temperature and accelerating the ashing procedure. The following process was to remove the remaining organic compounds that makeup rice husks and completely oxidize all carbon to $CO_2$ gas and hydrogen to $H_2O$ vapor. The white color of the blasted ash suggests a high concentration of silica.

### *3.2. Extraction of Silica from Rice Husk Ash*

a. Purification with NaOH solution

NaOH was used in purifying silica from rice husk ash because silica has a high solubility at a pH above 9. This process produces a filtrate which is a solution of sodium silicate. When exposed to a sodium silicate solution, the skin will feel slippery, which is an early indicator of the solution's formation (Ngatidjo *et al*., 2011). The reaction for the formation of sodium silicate is as follows:

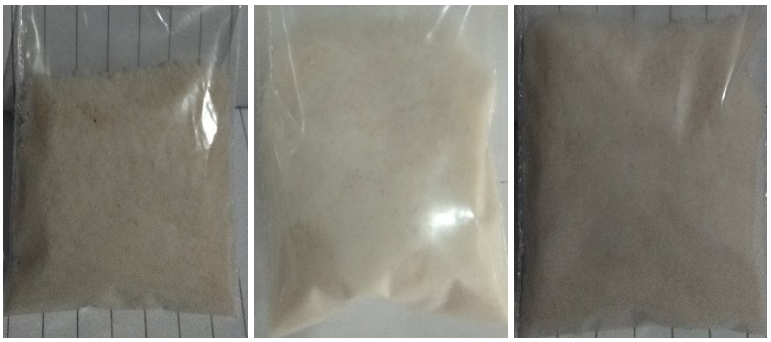
$$SiO_{2(s)} + 2NaOH_{(aq)} \longrightarrow Na_2SiO_{3(aq)} + H_2O_{(l).} \quad (1)$$

After the sodium silicate solution was formed, it was followed by silica gel formation by adding 0.5 M $H_2SO_4$ so that a neutralization reaction occurs, which formed silicic acid monomers and allowed the formation of a gel. The resulting gel was then allowed to stand and neutralized with hot distilled water. The following describes the neutralization process with sulfuric acid and washing with distilled water:

$$Na_2SiO_{3(aq)} + H_2SO_{4(aq)} \longrightarrow H_2SiO_{3(l)} + Na_2SO_{4(aq)} \quad (2)$$

$$H_2SiO_{3(s)} \longrightarrow SiO_{2(s)} + H_2O \quad (3)$$

Neutral silica gel was again dried to remove water. Silica purified using NaOH is presented in Fig. 2.



a b c

**Figure 2.** Silica purified with NaOH (a) sample 1, (b) sample 2, (c) sample 3

b. Precipitation with $H_2SO_4$ solution

The precipitation process with a 10.88% H2SO4 solution (Harimu, 2019) aims to reduce impurities in the form of metal oxides such as $Na_2O$, $K_2O$, and CaO produced in the extraction process with NaOH. The silica yield of the three samples is presented in Table 1.

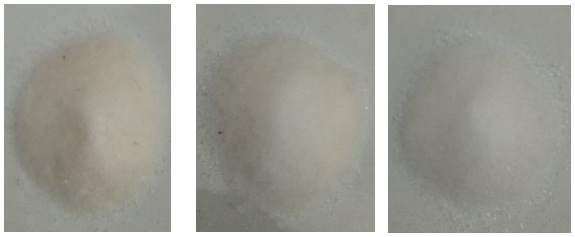
Based on Table 1, the yield of silica from similar rice varieties can differ depending on the sampling location. The Ciherang variety contains the least amount of silica compared to the Inpari variety. As demonstrated in Fig. 3, silica from sulfuric acid precipitation yields powder with finer granules and a whiter hue than silica through NaOH purification. The characterization results using XRF for the three samples are presented in Table 2.

According to Table 2, silica purity increased across all treatments. Silica with the highest purity was found in sample 3, namely from the processing of rice husk ash of the Inpari variety at 99.2%. Different levels of silica purity in the three samples demonstrated a correlation between rice variety and soil nutrient content.

Based on the different varieties, sample 1 (Ciherang Mbay) and sample 3 (Inpari 42 Mbay) had different levels of silica purity even though they were planted in the same area. It is possible that they share the same nutrients. Silica is the most abundant nutrient absorbed by rice plants, and it plays a crucial role in boosting photosynthesis and plant tolerance to biotic and abiotic stressors (Putri *et al*., 2017). Where rice varieties are continually developed into superior varieties that can produce quality rice plants and increase the resistance of these plants to biotic and abiotic stresses (Tampoma *et al*., 2017), this underlies the assumption of this study that rice varieties can affect the level of silica purity produced. In contrast, the influence of soil can be seen in samples 1 (Ciherang Mbay) and 2 (Ciherang Soa), which come from different areas. In addition, the silica content of the two samples varied. According to Makarim *et al*. (2007), rice plants absorb silica from the soil at a much higher rate than other elements such as potassium, nitrogen, phosphorus, and calcium. Therefore, the silica content of the soil can also affect the silica purity produced by processing rice husk ash.

**Table 1** Silica yield

| Sample | Yield (%) |
|---|---|
| Sample 1 | 82.91% |
| Sample 2 | 83.40% |
| Sample 3 | 82.80% |



a b c

**Figure 3.** Silica from the precipitation of (a) sample 1, (b) sample 2 and (c) sample 3

**Table 2** XRF data from rice husk ash and silica extraction using NaOH and $H_2SO_4$

| Compound | Parameters (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Rice Husk Ash | | | Purification eith NaOH | | | Precipitation with $H_2SO_4$ | | |
| | S1 | S2 | S3 | S1 | S2 | S3 | S1 | S2 | S3 |
| $SiO_2$ | 94.7 | 95.5 | 93.5 | 96.0 | 97.0 | 98.4 | 98.6 | 98.3 | **99.2** |
| $K_2O$ | 1.41 | 2.17 | 4.04 | 0.063 | 0.066 | 0.21 | - | - | - |
| CaO | 3.08 | 1.75 | 1.89 | 3.41 | 2.38 | 0.883 | 1.12 | 1.38 | 0.524 |
| MnO | 0.15 | 0.16 | 0.19 | 0.065 | 0.13 | 0.13 | 0.029 | 0.055 | 0.044 |
| $Fe_2O_3$ | 0.369 | 0.19 | 0.18 | 0.23 | 0.13 | 0.11 | 0.11 | 0.073 | 0.065 |
| CuO | 0.043 | 0.052 | 0.059 | 0.043 | 0.050 | 0.055 | 0.031 | 0.033 | 0.033 |
| ZnO | 0.02 | 0.036 | 0.022 | 0.036 | 0.083 | 0.10 | 0.005 | 0.051 | 0.039 |
| $Re_2O_7$ | 0.07 | 0.07 | 0.07 | 0.06 | 0.06 | 0.06 | 0.080 | 0.07 | 0.07 |
| $Eu_2O_3$ | 0.05 | 0.04 | 0.05 | 0.03 | 0.03 | 0.03 | - | 0.04 | - |

*3.3. Silica characterization*

Surface acidity is the amount of total acid (Bronsted acid and Lewis acid) on the surface of a solid expressed as the number of mmol of acid per sample weight (Widihati, 2008). Data on the silica surface acidity of the three samples are presented in Table 3.

According to the surface acidity table, there is no statistically significant difference between the surface acidity values of the three samples. The sample with the greatest surface acidity is number 2, with a value of 45,5 mmol/gram.

The surface area can be determined based on the sample's number of methylene blue molecules adsorbed at the optimum contact time. Surface area determination was carried out at a maximum wavelength of 660 nm and a standard curve with the regression equation, $y = 0.0904x - 0.0017$, with an $R^2$ value of 0.999. The surface area data of the three samples are presented in Table 4.

Based on Table 4, the surface area of the three samples was not significantly different. Surface area is known to play an important role in the adsorption process. The greater the surface area of an adsorbent, the more adsorbate can be adsorbed by the adsorbent (Wogo *et al*., 2014). The highest surface area was obtained from sample 3 of 18.491 $m^2/g$. The resulting surface area is higher when compared to a study conducted by Giri *et al*. (2014), who used the same method by utilizing silica gel from rice husk ash modified with diphenylcarbazide (Si-DPZida) to adsorb Cr(VI) ions with a surface area value of 4.4538 $m^2/g$.

**Table 3** Surface acidity value

| Sample Code | Ka (mmol/gram) |
|---|---|
| Sample 1 | 45.1 |
| Sample 2 | 45.5 |
| Sample 3 | 44.7 |

**Table 4** The surface area of the sample

| Contact time (minutes) | Surface Area ($m^2$/gram) | | |
|---|---|---|---|
| | Sample 1 | Sample 2 | Sample 3 |
| 30 | 16.686 | 18.111 | 17.875 |
| 40 | 18.275 | 18.121 | 18.418 |
| 50 | **18.397** | 17.485 | 18.142 |
| 60 | 17.987 | **18.347** | **18.491** |
| 70 | 17.711 | 18.141 | 18.480 |

### *3.4. Application of silica for adsorption of Mn metal*

The silica used for the adsorption of Mn metal ions was refined silica from sample 3 (Inpari 42 Mbay) with the highest silica content based on XRF data. The adsorption process was carried out on manganese metal ions with various concentrations between 10 to 200 mg/L with a contact time of 60 minutes according to the data for determining the sample's surface area.

The adsorption capacity was determined by the Langmuir adsorption isotherm equation. The slope value was used to calculate the adsorption capacity, and the intercept value to calculate the adsorption energy value. The adsorption capacity was calculated using the Langmuir isotherm equation and the curve showing the relationship between the amount of metal ion adsorbed on the adsorbent in an equilibrium state. The adsorption isotherm pattern curve is presented in Fig. 4.

Based on the curve in Fig. 4, the value of the adsorption capacity of the purified silica from sample 3 is 212.76 µmol/gram. This relatively high adsorption capacity indicates that the higher the silica purity, the better its application as an adsorbent. Silica has active groups on its surface in the form of silanol groups (-SiOH) and siloxane groups (Si-O-Si), both of which have an O atom, which is a hard base group (Wogo *et al*., 2014). Based on the HSAB theory, the $Mn^{2+}$ metal ion is a group of strong acids that tend to bind to stable hard bases.

The adsorption energy value was 38.165 kJ/mol, indicating that there was a chemical adsorption process that involves bonds between the adsorbate and the surface of the adsorbent. According to Adamson (1990), chemical adsorption (chemisorption) can occur if the adsorption energy produced is more than 20.92 kJ/mol.

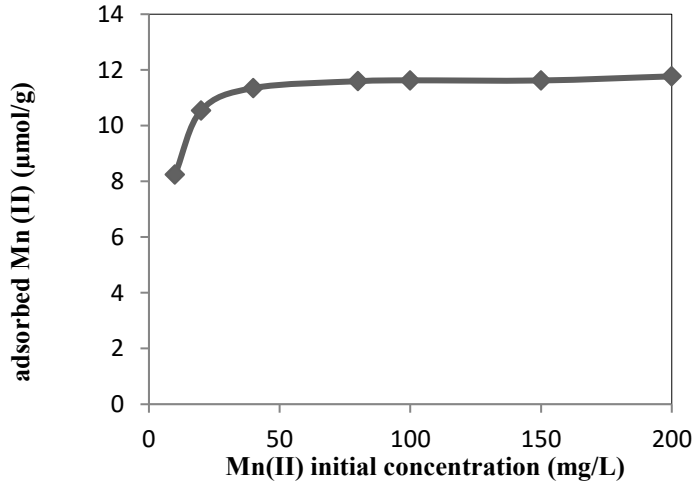



**Figure 4.** Graph of sample adsorption isotherm pattern 3

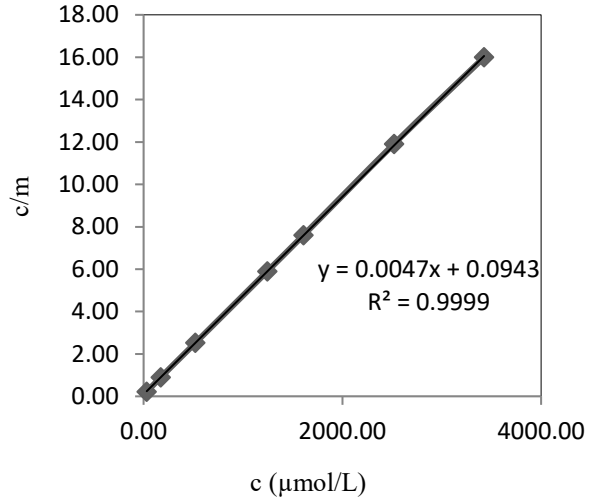



**Figure 5.** Graph for determining the adsorption capacity value of sample 3

## 4. CONCLUSION

Based on the research performed, it could be concluded that:

1. The yield of silica produced by each rice variety varies. The yield of silica was 82.91% for the Ciherang Mbay sample, 83.40% for the Ciherang Soa sample, and 82.80% for the Inpari 42 Mbay sample.
2. Based on the XRF, the highest silica purity was found in the Inpari 42 sample from the Mbay area, namely 99.2%. In contrast, the acidity and surface area of the three samples showed no significant difference.
3. The adsorption capacity for Mn metal ions was 212.76 µmol/gram and was chemical adsorption with an adsorption energy of 38.165 kJ/mol.

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JURNAL ILMIAH BERKALA SAINS DAN TERAPAN KIMIA

**Jurnal Ilmiah Berkala: Sains dan Terapan Kimia**
*Vol. 17 No. 1, Januari 2023*

# Corrosion Inhibition Mechanism Based on Adsorption Isotherm Model From Water Extract of Merkubung (*Macaranga gigantea*) Bark Extract on Mild Steel in Sulfuric Acid Solution

# Mekanisme Inhibisi Korosi Berdasarkan Model Isoterm Adsorpsi Ekstrak Air Kulit Kayu Merkubung (*Macaranga Gigantea*) pada Baja Lunak dalam Larutan Asam Sulfat

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## ABSTRACT

The phenolic chemicals found in water extract of *Merkubung* bark (WEMB) can be employed as corrosion inhibitors. The corrosion inhibition mechanism by WEMB can be studied using an adsorption isotherm model. The Langmuir adsorption isotherm model has an $R^2$ value that was closest to 1 one compared to the Freundlich, Temkin, Florry Huggins, and Frumkin adsorption isotherm models, as shown by an analysis of the adsorption isotherm model. Due to the interaction between the WEMB and the steel surface, the adsorption isotherm model reveals that the inhibitor of WEMB on the surface coating of mild steel tends toward chemisorption.

**Keywords**: extract, Merkubung, corrosion inhibitor, adsorption isotherm model, Langmuir, Freundlich, Temkin, Flory Huggins and Frumkin

## ABSTRAK

Ekstrak air kulit kayu merkubung (*Macaranga gigantea*) mengandung senyawa fenolik yang dapat digunakan sebagai inhibitor korosi. Mekanisme inhibisi korosi oleh ekstrak air kulit kayu merkubung (AEMB) dapat dipelajari menggunakan model isoterm adsorpsi. Berdasarkan hasil analisis model-model isoterm adsorpsi diperoleh model isoterm adsorpsi Langmuir memiliki nilai $R^2$ lebih mendekati 1 dibandingkan model isotherm adsorpsi Freundlich, Temkin, Florry Huggins, dan Frumkin. Model-model isoterm adsorpsi tersebut memberikan gambaran bahwa inhibitor AEMB dalam melapisi permukaan baja lunak cenderung berlangsung secara kemisorpsi dengan adanya interaksi tarik menarik antara AEMB dengan permukaan baja

**Kata Kunci:** *ekstrak merkubung, inhibitor korosi, model isoterm adsorpsi*

**Received**: September 14, 2020; **Accepted**: January 4, 2023: **Available online**: February 15, 2023

## 1. INTRODUCTION

The industrial world faces corrosion problems in steel due to exposure to acids, bases, and salts. Many industries have added a small number of substances known as corrosion inhibitors in process fluid streams to industrial pipes (Gusti *et al.*, 2019; Permanasari *et al.*, 2020; Sibarani *et al.*, 2021). Corrosion inhibitors are substances that can adsorb on metal or steel surfaces, thereby reducing the occurrence of the corrosion process. In general, the inhibitors used are synthetic inhibitors which are toxic and not environmentally friendly. As corrosion inhibitors, plant extracts are currently favored. Much research has been carried out on natural extracts as corrosion inhibitors because they are environmentally friendly (Akinbulumo *et al.*, 2020; D. R. Gusti *et al.*, 2020; Ogunleye *et al.*, 2020).

Natural extracts that have been studied with high corrosion inhibition efficiency and are recommended for use as corrosion inhibitors generally contain phenolic compounds (Gusti *et al.*, 2020). The turtledove plant (*Macaranga gigantea*) has been extensively studied and shown to contain phenolic compounds (Arung *et al.*, 2018; Sulastri *et al.*, 2020). Phenolic compounds contain many OH functional groups. The O atom in the OH group has a lone pair of electrons interacting with Fe on the steel surface (Gusti *et al.*, 2022). Therefore, the phenolic content of the Merkubung plant has the potential to be used as a corrosion inhibitor.

Corrosion inhibitors will be adsorbed to form a layer on the steel surface which can inhibit corrosion. Adsorption behavior can be studied with the help of the adsorption isotherm method, which provides information to estimate what happens in the adsorption layer (Ituen *et al.*, 2017; Jain *et al.*, 2017). Therefore, to find out whether WEMB extract has the potential as a corrosion inhibitor, a study was carried out using the weight loss method to calculate the efficiency of corrosion inhibition (Gusti *et al.*, 2017). The inhibitory effects of various plant extracts show different performances of each extract on metal surfaces (Lin *et al.*, 2021). Several adsorption isotherms models, such as the Langmuir, Freundlich, Temkin, Florry Huggins, and Frumkin, are utilized to evaluate whether the adsorption from WEMB extract is physical or chemical and how the WEMB extract interacts with the steel surface (Ituen *et al.*, 2017; Lin *et al.*, 2021).

## 2. MATERIALS AND METHODS

### *2.1 Materials*

The materials used were mild steel, merkubung wood sap, and $H_2SO_4$ (Merck). The tools used were thermometers, water baths, glassware (Pyrex), sandpaper grade 120, calipers, reflux tools, and hot plates.

### *2.2 Research Procedure*

The water extract of merkubung bark (WEMB) is prepared by employing a reflux technique to extract merkubung bark powder in water. The WEMB extract was filtered, and the filtrate was then collected. The filtrate was concentrated by heating at 50°C in a water bath to obtain a concentrated extract. About 1.25 g of WEMB extract was put into a 500 mL volumetric flask, then dissolved with 0.75 M $H_2SO_4$ to obtain an inhibitor solution concentration of 2.5 g/L. Inhibitor solutions were also prepared for a concentration of 2 g/L; 1.5 g/L; 1, and 0.5 g/L.

A drill with a diameter of 3 mm was used to drill holes in mild steel measuring ± 2 x 1 cm. The steel surface was smoothed with 120-grit steel sandpaper and cleaned with distilled water and acetone. The steel was then dried for ±5 minutes. The length and width of the steel were measured with calipers. Using an analytical balance, the mass of the steel was determined, and the initial mass ($W_1$) was estimated.

The mild steel was then immersed in a 0.75 M solution of sulfuric acid and a

solution of sulfuric acid containing WEMB extract, each with a concentration of 0.5; 1.0; 1.5; 2.0; and 2.5 g/L for 24, 48, and 72 hours, respectively. Mild steel was lifted and cleaned with distilled water and acetone, then dried. Dried mild steel was weighed and referred to as the final mass ($W_2$).

*2.3 Data Analysis*

The data were analyzed using the weight loss method, which compares the original weight of the steel before immersion to its final weight after immersion. Weight loss data can be used to calculate the corrosion rate and adsorption isotherm models (Yetri & Jamarun, 2015). The corrosion rate is calculated using the following equation (Gusti *et al.*, 2020):

$$CR = \frac{W1-W2}{A \times t} \text{ “} \quad (1)$$

CR is the corrosion rate (mg/cm$^2$ hour). $W_2$ is the final weight of steel (mg). $W_1$ is the initial weight of steel (mg). A is the steel surface area (cm$^2$), and t is the steel immersion time (hours).

The percentage of inhibition efficiency on steel corrosion is obtained by using the equation:

$$\%EI = \frac{CR1-CR2}{CR1} \; x \; 100\% \quad (2)$$

CR1 is the corrosion rate in the absence of an inhibitor (mg/cm$^2$ hour), and CR2 is the corrosion rate in the presence of an inhibitor (mg/cm$^2$ hour).

Adsorption isotherms were determined using the following equation (Ituen *et al.*, 2017; Nwabanne & Okafor, 2012):

Langmuir : $\frac{C}{\theta} = \frac{1}{Kads} + C$ (3)

Freundlich: $\text{Log } \theta = \text{Log } K_{ads} + n \log C$ (4)

Temkin : $\theta = \frac{-2.303 \log K}{2a} - \frac{2.303 \log C}{2a}$ (5)

Frumkin : $\log \frac{\Theta}{1-\Theta} C = 2.303 \log K + 2a\theta$ (6)

Florry Huggins : $\log \left( \frac{\theta}{C} \right) = \log K + x \log (1-\Theta)$ “ (7)

## 3. RESULTS AND DISCUSSION

*3.1. The Effect of Inhibitor Concentration of Merkubung Bark Aqueous Extract (WEMB) on Inhibition Efficiency and Corrosion Rate of Mild Steel*

The effect of WEMB extract inhibitor concentration and time on the inhibition efficiency and corrosion rate of mild steel is presented in Fig. 1 and Fig. 2. Fig. 1 and 2 demonstrate that as concentration increases, inhibition efficiency increases and corrosion rate decreases. As a corrosion inhibitor, WEMB extract is likely absorbed and forms a thin layer on the steel surface, preventing corrosive ions in the solution from reacting with Fe on the steel surface. The optimum inhibition efficiency value was found at an inhibitor concentration of 2.5 g/L in 72 hours with a value of 93.41%.

Soaking time affected the inhibition efficiency and corrosion rate of mild steel in a solution of aqueous extract of merkubung bark inhibitor at a concentration of 0.5; 1; 1.5; 2, and 2.5 g/L with variations in immersion time of 24, 48, and 72 hours. Fig. 1 and 2 show that the inhibition efficiency and corrosion rate of mild steel with corrosion inhibitor WEMB extract increased with increasing immersion time in sulfuric acid solution. WEMB extract increases corrosion inhibition efficiency on the surface of mild steel. It protects the steel surface from corrosive ion attack by forming a thin layer on the steel's surface. Gusti *et al.* (2019) reported that increasing the concentration of inhibitors and the length of immersion time will increase the inhibition efficiency and reduce the corrosion rate.

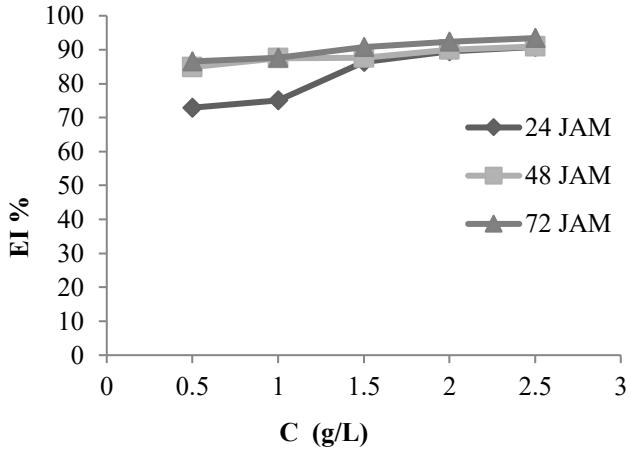


**Figure 1.** Effect of Concentration and Soaking Time of Mild Steel in the presence of WEMB Extract Inhibitors on the Efficiency of Corrosion Inhibition

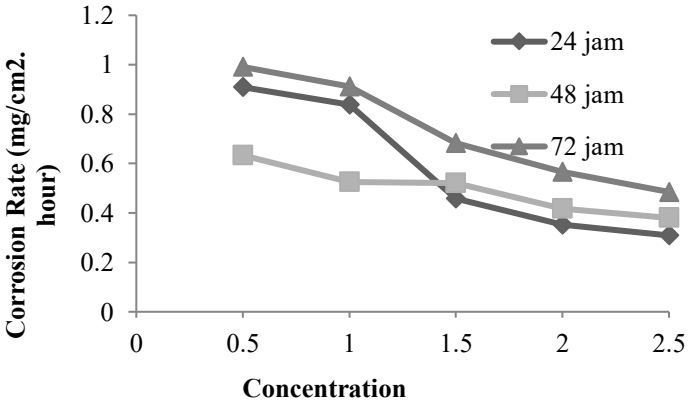


**Figure 2.** Effect of WEMB Extract Inhibitor Concentration on the corrosion rate of steel in sulfuric acid solution

*3.2. Adsorption Isotherm*

Adsorption isotherm parameters were used to investigate the behavior and mechanism of corrosion inhibitors. Inhibitor molecules can bind to metal surfaces through adsorption. Adsorption can be divided into physical adsorption (physisorption) and chemical adsorption (chemisorption) (Ituen *et al.*, 2017). The adsorption isotherm theory was applied by linear regression analysis for each adsorption theory, namely the theories of Langmuir, Freundlich, Temkin, Florry Huggins, and Frumkin.

Table 1 shows that linear regression analysis is used to determine the model's suitability for the research data by looking at the correlation coefficient ($R^2$). If the value of $R^2$ is close to 1, it can be concluded that there is an increasingly significant influence and a close relationship between variables.

*3.3. Langmuir Adsorption Isotherm*

Table 1 shows that the Langmuir approach with a regression value of 0.9996 is close to 1, so it is more acceptable to describe the adsorption properties of inhibitor molecules. The Langmuir adsorption isotherm indicates the presence of chemical bonds between the chemical groups of the secondary metabolites present in the inhibitor and the metals present in the mild steel, resulting in the formation of a surface passivation layer that is highly strong. Fig. 3 shows the information on adsorption K values. The adsorption K value at 48 hours in Table 1 shows the highest adsorption value compared to the adsorption K at 24 hours and 72 am. The adsorption K value describes the adsorption strength (Ituen *et al.*, 2017).

**Table 1** Adsorption isotherm values of aqueous extract of Merkubung bark on mild steel

| Adsorption Isotherm | Soaking Time | $R^2$ | K | |
|---|---|---|---|---|
| Langmuir | 24 hours | 0.9956 | 4,209 | |
| | 48 hours | 0.9996 | 15,72 | |
| | 72 hours | 0.9996 | 13,10 | |
| | | $R^2$ | K | n |
| Freundlich | 24 hours | 0.8971 | 1,261 | 0.15 |
| | 48 hours | 0.9325 | 1,147 | 0.04 |
| | 72 hours | 0.9331 | 1,124 | 0.05 |
| | | $R^2$ | K | *A* |
| Temkin | 24 hours | 0.8954 | 1,562 | 0.28 |
| | 48 hours | 0.9295 | 1,150 | 0.08 |
| | 72 hours | 0.9308 | 1,195 | 0.10 |
| | | $R^2$ | K | X |
| Flory Huggins | 24 hours | 0.8500 | 4,134 | 1.00 |
| | 48 hours | 0.8955 | 306.8 | 2.84 |
| | 72 hours | 0.8975 | 57,45 | 1.87 |
| | | $R^2$ | K | A |
| Frumkin | 24 hours | 0.9691 | 7446 | 6.21 |
| | 48 hours | 0.9648 | 2717 | 15.3 |
| | 72 hours | 0.9730 | 9512 | 13.9 |

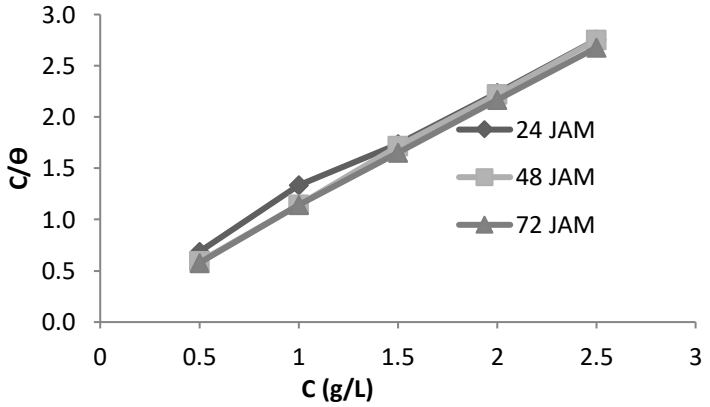


**Figure 3.** Langmuir adsorption isotherm for mild steel corrosion with immersion times of 24 hours, 48 hours, and 72 hours

### *3.4. Freundlich Adsorption Isotherm*

The Freundlich isotherm model shows that the inhibitor has a heterogeneous surface; each molecule has a different adsorption potential and assumes that adsorption occurs in a multilayer manner on the surface of the inhibitor. The value of n between 1.0 - 10.0 in this isotherm indicates that the adsorption process is going well and easily (Ituen *et al.*, 2017). Based on Fig. 4 and Table 1, the n values of the immersion times of 24, 48, and 72 hours were 0.15, 0.04, and 0.05, respectively.

### *3.5. Temkin Adsorption Isotherm*

The Temkin adsorption isotherm explains the interactions occurring in the adsorption layer (Ituen *et al.*, 2017). The degree of surface covering (θ) is related to the inhibitor concentration (C) in Equation 5, where K is the adsorption equilibrium

constant, and a is the molecular interaction parameter. Plotting θ against log C, as presented in Fig. 3, gives a linear relationship. The a value in Table 1 of the Temkin adsorption isotherm is positive. This shows the behavior of the inhibitor attraction on the mild steel surface (Nwabanne & Okafor, 2012).

### *3.6. Frumkin Adsorption Isotherm*

The values for the Frumkin adsorption parameters are presented in Table 1. In Frumkin adsorption, a which has a positive value indicates the attraction behavior of the inhibitor on the mild steel surface. A negative a value indicates inhibitor repulsion on the mild steel surface (Ituen *et al.*, 2017). Table 1 and Fig. 4 show that the a value of the Frumkin isotherm is positive, indicating the presence of attractive inhibitory behavior on the mild steel surface.

### *3.7. Flory Huggins Adsorption Isotherm*

The adsorption isotherm model is a substitution model. The constant parameter (x) in Equation 7 describes the substitution of molecular inhibitors for water (Ituen *et al.*, 2017). Fig. 5 and Table 1 show positive x parameter values, indicating that there is a lot of adsorbed extract from the merkubung bark aqueous extract because it can move more than one water molecule from the mild steel surface (Nwabanne & Okafor, 2012).

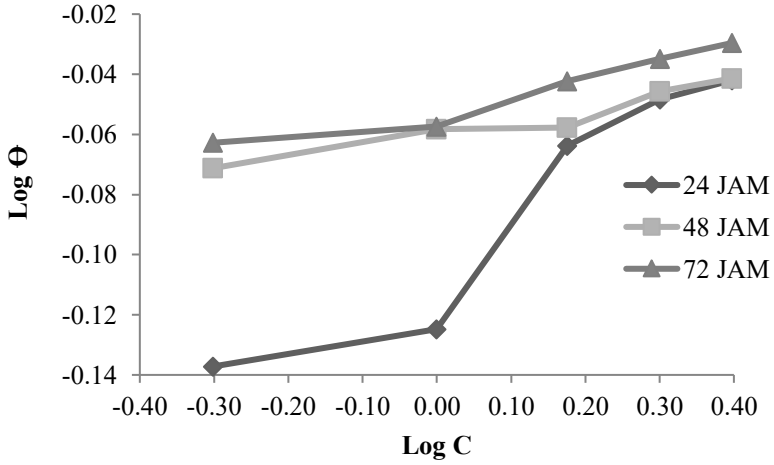


**Figure 4.** Freundlich adsorption isotherm for mild steel corrosion with immersion times of 24 hours, 48 hours, and 72 hours

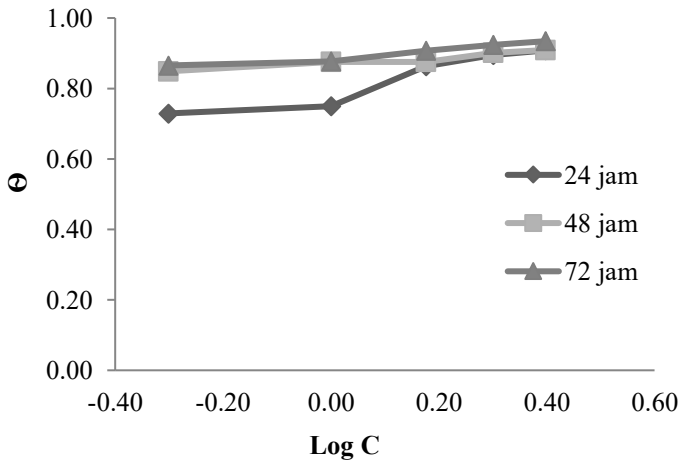


**Figure 3.** Temkin isotherms for adsorption of aqueous extract of merkubung bark on the surface

of mild steel with immersion times of 24 hours, 48 hours, and 72 hours

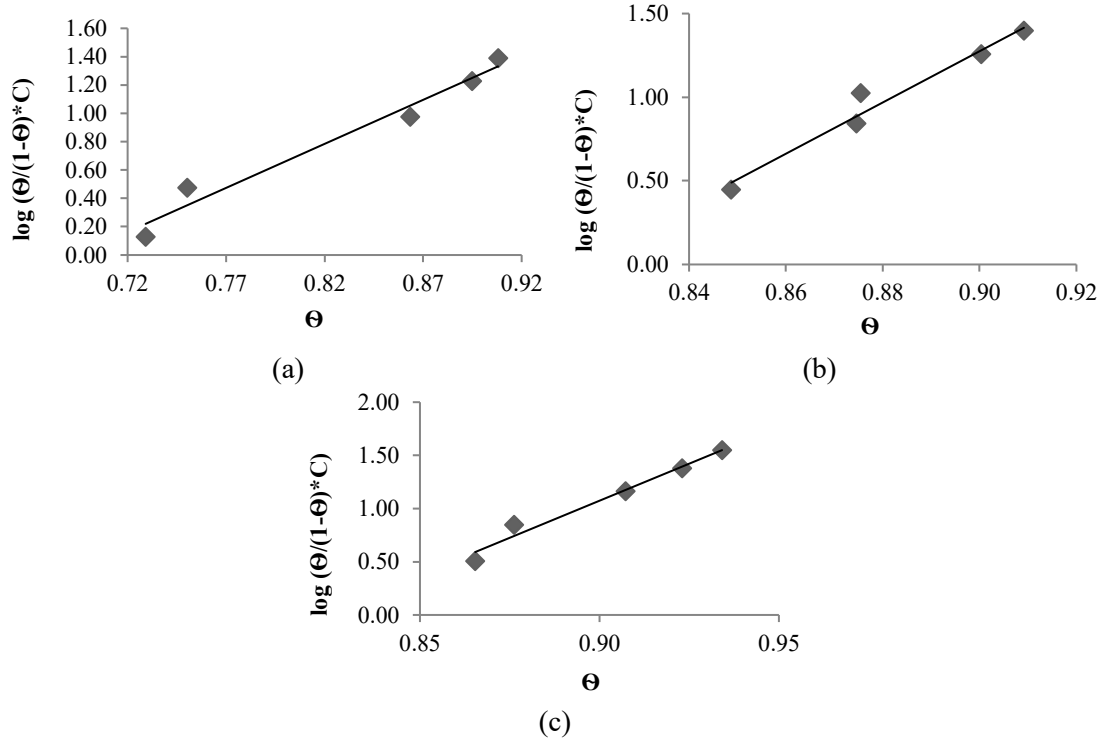


**Figure 4.** Frumkin isotherm for adsorption of aqueous extract of merkubung bark on a mild steel surface with an immersion time of (a) 24 hours, (b) 48 hours, and (c) 72 hours

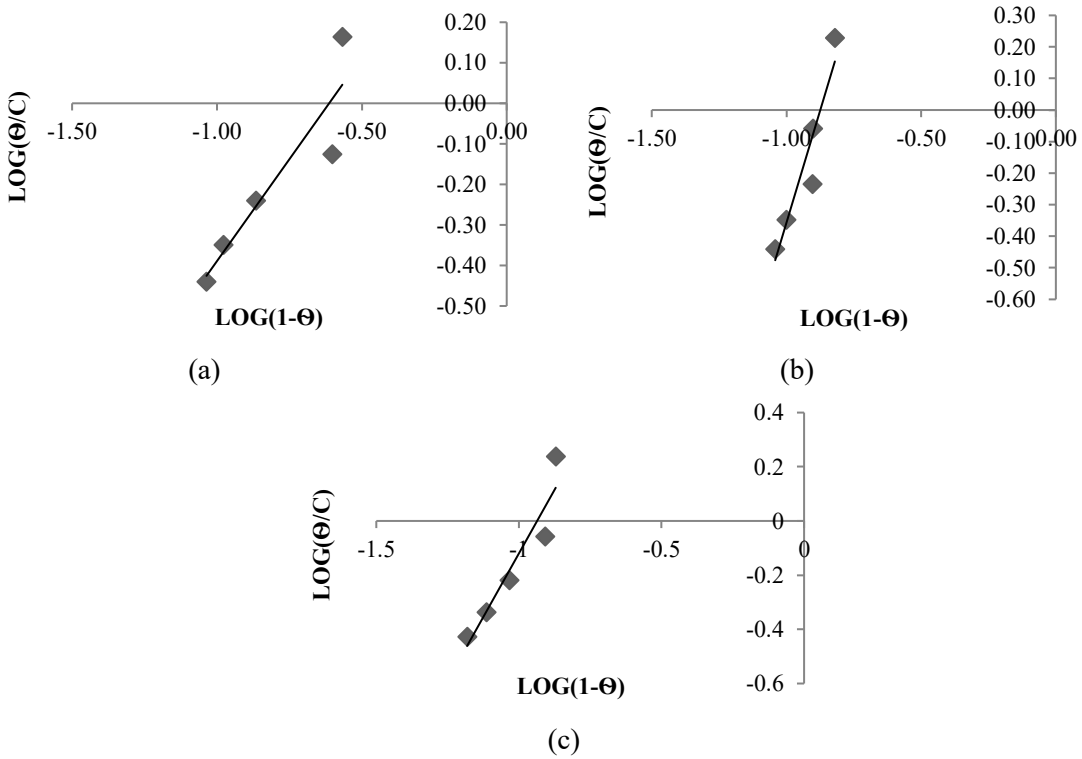


**Figure 5.** Isotherm Flory Huggins for adsorption of aqueous extract of merkubung bark on a mild steel surface with an immersion time of (a) 24 hours, (b) 48 hours, and (c) 72 hours

## 4. CONCLUSIONS

The inhibition efficiency increases, and the corrosion rate decreases with increasing WEMB extract concentration. The inhibition efficiency and corrosion rate of mild steel with the corrosion inhibitor WEMB extract increased with the longer immersion time in sulfuric acid solution.

Analysis of the adsorption isotherm model from the Langmuir, Freundlich, Temkin, Florry Huggins, and Frumkin adsorption isotherm models shows that the R2 value of the Langmuir adsorption isotherm model is closer to 1 than the other models. The adsorption isotherm models tested illustrate that the WEMB extract inhibitors in coating the surface of mild steel tend to chemisorb more due to the attractive interaction between the WEMB extract and the steel surface.

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JURNAL ILMIAH BERKALA SAINS DAN TERAPAN KIMIA
**Jurnal Ilmiah Berkala: Sains dan Terapan Kimia**
*Vol. 17 No. 1, Januari 2023*

# Antioxidant and Antiproliferative Activities of Methanol Extract from *Melaleuca cajuputi* subsp. Cumingiana [Turcz.] Fruit

# *Aktivitas Antioksidan dan Antiproliferatif Ekstrak Metanol Buah Melaleuca cajuputi subsp. Cumingiana [Turcz.]*

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## ABSTRACT

*Melaleuca cajuputi* subsp. Cumingiana [Turcz.] Barlow (*M. cajuputi*) is widely available in Banjarmasin. *M. cajuputi* contains phytochemical compounds in the form of polyphenols, including flavonoids, quinones, saponins, and alkaloids, that have antioxidant and antiproliferation activities. This research aimed to analyze the antioxidant and antiproliferation activity of *M. cajuputi* fruit methanol extract. The antioxidant activity was tested employing the DPPH method. The activities were observed in $IC_{50}$ and measured using the UV-VIS spectrophotometer at 517 nm. The antiproliferation was conducted using true experimental with a post-test. This study used 30 mature zebrafish (length >2.5 cm), which were grouped into four, namely the negative control group (DMSO 0.05%), the methanol extract group of *M. cajuputi* with concentrations of 18.5 ppm, 37 ppm, and 74 ppm. The bound variable in this study was antiproliferation activity in the tail of an amputated fish. Data analysis was measured by one-way ANOVA and post-hoc Tukey HSD tests. The phytochemical test indicates the presence of phenol compounds, quinones, flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids. The $IC_{50}$ of the methanol extract of *M. cajuputi* fruit was 15.50 ppm (95% CI 8.31- 32.72). The antiproliferation activity of zebrafish tails increased in the administration of 74 ppm ($p < 0.05$) *M. cajuputi* fruit extract on day four and day eight of measurement compared to negative controls. It can be concluded that the methanol extract of *M. cajuputi* fruit has antiproliferative activity against the growth of amputated zebrafish tails.

**Keywords**: antioxidant, antiproliverative, *Melaleuca cajuputi,* zebrafish, amputated tail.

## ABSTRAK

*Melaleuca cajuputi* subsp. Cumingiana [Turcz.] Barlow (*M. cajuputi*) merupakan tanaman yang mudah ditemukan di Banjarmasin, Kalimantan Selatan. *M. cajuputi* mengandung senyawa fitokimia polifenol antara lain flavonoid, kuinon, saponin, dan alkaloid yang diduga memiliki aktivitas antioksidan dan antiproliferasi. Penelitian ini bertujuan untuk mengetahui aktivitas antioksidan dan antiproliferasi ekstrak metanol buah *M. cajuputi*. Uji aktivitas antioksidan menggunakan metode DPPH. Aktivitas diamati di $IC_{50}$ dan diukur

menggunakan spektrofotometer UV-VIS pada panjang gelombang 517 nm. Uji antiproliferasi menggunakan true eksperimental dengan post-test. Hewan yang digunakan dalam penelitian ini adalah 30 ekor zebra fish dewasa (panjang > 2,5 cm) yang dikelompokkan menjadi 4 yaitu kelompok kontrol negatif (DMSO 0,05%), kelompok ekstrak metanol *M. cajuputi* dengan konsentrasi 18,5 ppm, 37 ppm dan 74 ppm. Analisis data diukur dengan uji one-way ANOVA dan Post-Hoc Tukey HSD. Hasil uji fitokimia menunjukkan adanya senyawa fenol, kuinon, flavonoid, alkaloid, saponin, tanin, steroid dan terpenoid. Ekstrak metanol buah *M. cajuputi* berada pada $IC_{50}$ sebesar 15,50 ppm (95% CI 8,31- 32,72). Aktivitas antiproliferasi ekor ikan zebra meningkat pada pemberian ekstrak buah *M. cajuputi* dalam konsentrasi 74 ppm ($p < 0,05$), baik pada hari ke-4 dan hari ke-8 pengukuran bila dibandingkan dengan kontrol negatif. Dapat disimpulkan bahwa ekstrak metanol buah *M. cajuputi* memiliki aktivitas antiproliferatif terhadap pertumbuhan ekor ikan zebra yang diamputasi.

**Kata kunci**: antioksidan, antiproliveratif, Malaleuca cajuputi, ikan zebra, ekor yang diamputasi.

**Received**: March 30, 2022; **Accepted**: July 7, 2022: **Available online**: February 15, 2023

## 1. INTRODUCTION

Indonesia is the second largest biodiversity country in the world, with various plants containing various natural active ingredients beneficial to the human body. One of the most common plant genera found in almost every area of South Kalimantan is the genus Melaleuca. There are about 300 species in this genus. One of the species found in South Kalimantan is *M. cajuputi* (Al-Abd *et al.*, 2015; Takao *et al.*, 2015).

This plant contains phytochemicals such as flavonoids, quinones, saponins, and alkaloids in polyphenols (Wardhani *et al.*, 2018a, Wardhani *et al.*, 2018b). These four compounds are found in cancer because they induce electrogenic compounds, protective enzymes with conjugated activity, autophagy, increase apoptosis rates, inhibit cell proliferation, and metabolize carcinogens and tumorigenesis. It is believed to have great prophylactic and therapeutic effects-regulates expression, inhibits lipid peroxidation, inhibits angiogenesis, and inhibit DNA oxidation (Ren *et al.*, 2003). In addition, the four compounds also serve as antioxidants.

Antioxidant compounds have a significant influence on the prevention and treatment of cancer. Antioxidants can suppress oxidative stress to reduce the activation of cancer cells' prosurvival factors, such as NFkB and AP-1, and increase the activation of the p53 tumor suppressor gene. Small levels of oxidative stress can induce the proliferation of cancer cells. Therefore, antioxidants are vital for cancer patients. Epidemiological studies have also shown that a diet rich in polyphenols affects cancer risk. In particular, these four polyphenolic compounds can exert anticancer effects through mechanisms that include antioxidant and antiproliferative activities as well as their effects on subcellular signaling pathways, apoptosis, and induction of cell cycle shortening (Zakaria *et al.*, 2011).

One of the animal models used for preliminary study on anticancer drugs is zebrafish (*Brachydanio rerio*). The early stages of regeneration in amputated zebrafish tail fins have similarities to the development of cancer cells. Hence, the growth of the amputated zebrafish tail can be used as a model to study the antiproliferative effect of the fruit extract of *M. cajuputi* (Muñoz *et al.*, 2009). This study was conducted to prove the antiproliferative activity of the methanol extract of the fruit of *M. cajuputi* in inhibiting the growth of the amputated zebrafish tail. A close relationship between cancer and antioxidants has also encouraged researchers to examine the antioxidant activity of the

methanol extract of *M. cajuputi* using the DPPH method.

## 2. MATERIALS AND METHODS

### *2.1. Materials*

The material used in this study was 1 kg of the young fruit of *M. cajuputi* (Fig. 1), methanol 50% technical grade, methanol grade p.a, DMSO, ketamine injection, sterile distilled water, Mayer reagent, Dragendorff reagent, Pb acetic acid 10%, NaOH, Gelatin 1%, FeCl3 3%, HCl benzene, chloroform, anhydrous acetic acid, and concentrated $H_2SO_4$ and DPPH.

### *2.2. Material collection and extraction*

The young fruit of *M. cajuputi* was obtained from Jalan Kayutangi, Banjarmasin city, South Kalimantan (3°17'50.1"S 114°35'07.8"E). Verification of the *M. cajuputi*'s fruit was conducted by the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, with certificate number 0.65a/LB.LABDASAR/III/2020. The young fruit of *M. cajuputi* was air-dried on paper at room temperature until dry. After drying, the fruit was crushed and sieved to become homogeneous simplicia. The simplicia was soaked in 50% methanol and 0.5% acetic acid. The immersion was performed three times. The thick methanol extract obtained was evaporated with a rotary evaporator at 60ºC, then re-evaporated with a water bath at 60ºC. It was then freeze-dried to obtain a concentrated extract (Dahlan, 2014).

### *2.3. Antioxidant Activity*

Two mL of each methanol extract of *M. cajuputi* fruit (concentrations: 1, 5, 10, 15, 25, and 50 ppm) was added with 2 mL of 50 ppm DPPH solution. The mixture was then incubated for 30 minutes in a dark room. The absorbance was measured using a UV-VIS spectrophotometer at 517 nm. The formula used to calculate % damping is as follows: (Tiwari *et al.*, 2011).

$$DPPH_{radical\ scavenging\ act.}(\%) = \frac{A_{ctrl} - A_{sample}}{A_{ctrl}} \times 100\%$$

The percentage of free radical attenuation in the fruit extract of *M. cajuputi* was used to determine the $IC_{50}$ value using probit analysis. The classification of $IC_{50}$ values was determined based on Table 1.

**Table 1** Classification of Antioxidant Activity

| No. | Value of $IC_{50}$ | Antioxidant activity |
|---|---|---|
| 1 | <50 | Very strong |
| 2 | 50-100 | Strong |
| | 100-150 | Moderate |
| 3 | 151-200 | Weak |

**Figure 1.** Young fruit of *M. cajuputi*

*2.4. Antiproliferation Test*

The sample was divided into four groups: the DMSO negative control group (0.05%, DMSO), the *M. cajuputi* group with concentrations of 18.5 ppm (EMFM 18.5), 37 ppm (EMFM 37), and 81 ppm (EMFM 81). The *M. cajuputi* group was added with 0.05% DMSO. This study obtained an ethical clearance letter from the Ethics Commission of the Faculty of Medicine, Lambung Mangkurat University No. 457/KEPK-FKUNLAM/EC/XI/2020.

Zebrafish were reared for one week at room temperature before receiving treatment to provide the same physical and psychological conditions. During maintenance, fish were fed 2-3 times a day. Before being treated with tail amputation, zebrafish were anesthetized with the anesthetic solution of ketamine at a dose of 2 mL/L for two minutes to reduce movement and stress. After being anesthetized, the fishtail was amputated by placing it on a petri dish with a little puddle of water and cut straight with a scalpel from top to bottom. Each of the amputated fish was put into four different aquariums containing 1 L of treatment solution. Each aquarium contains six zebrafish. The aquarium water was changed every two days. The length of the zebrafish's tail was measured at four and eight days after the amputation. Measurements were made using a caliper (millimeter scale).

*2.5. Data statistical analysis*

Data measurements of fishtail length were evaluated statistically. Normality and homogeneity analysis were performed. The normality of the data was based on the Shapiro-Wilk normality test (sample <50), and the homogeneity was based on the Rubin variance test. Since the study data was normal and evenly distributed, the parametric analysis was performed using a one-way ANOVA and a post-Tukey HSD test with a 95% confidence level ($\alpha = 0.05$) (Dahlan, 2014).

## 3. RESULTS AND DISCUSSION

*3.1. Results*

Based on the phytochemical tests (Table 2), it is known that the methanol extract of the fruit of *M. cajuputi* contains alkaloid compounds, flavonoids, polyphenols, saponins, quinones, steroids, terpenoids, and tannins. These results differed from Wardhani *et al.* (2018). The difference might be caused by the difference in each plant's growth site and soil nutrients. In this research, *M. cajuputi* was obtained from the Banjarmasin area, while Wardhani's research was obtained from the Palangkaraya area (Wardhani *et al.*, 2018a; Zafrial & Amalia, 2018).

After a phytochemical screening test, methanol extract was made into various concentrations and reacted with DPPH. Based on the probit analysis, the $IC_{50}$ value of the methanol extract of *M. cajuputi* subsp Cumingiana [Turcz.] Barlow fruit was 15.50 ppm (95% CI 8.31 - 32.72).

**Table 2**. The Phytochemical Screening Tests of *Melaleuca cajuputi*

| No | Compound Type | Reagen | Results | Wardhani *et al.* 2018 |
|---|---|---|---|---|
| 1 | Polyphenol | $FeCl_3$ 3% | + | + |
| 2 | Flavonoids | Pb acetate | + | + |
| | | Alkaline Reagent | + | + |
| 3 | Alkaloids | Mayer Reagent | + | - |
| | | Reagen Dragendorft | + | - |
| 4 | Quinone | Benzene + ammonia | + | + |
| 5 | Saponins | Aquadest | + | + |
| 6 | Steroids | *Libermann Burchard's test* | + | - |
| 7 | Terpenoids | *Salkowski's test* | + | - |
| 8 | Tannin | Gelatine Solution 1% | + | - |

Description:
(+) = contains secondary metabolites
( -) = does not contains secondary metabolites

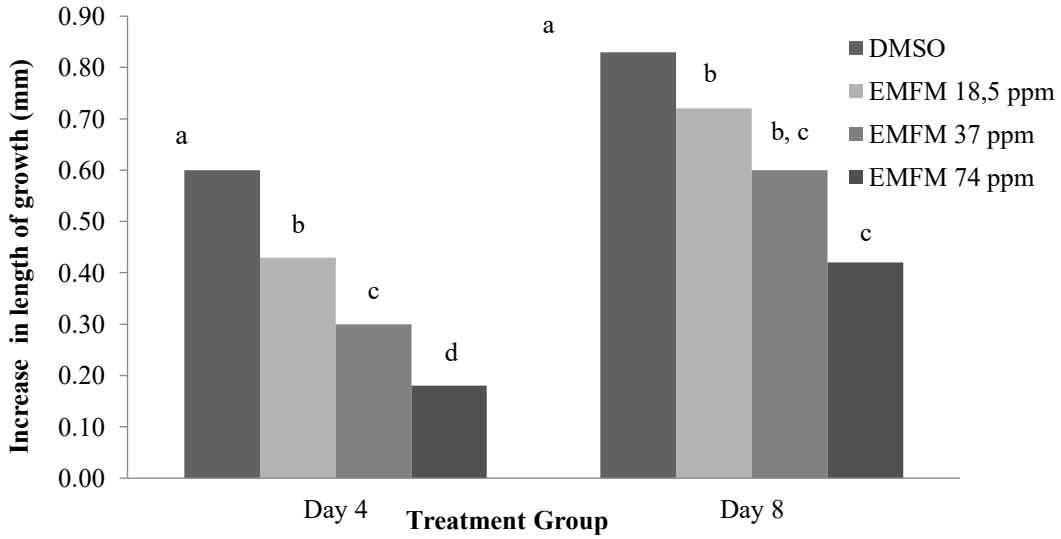


**Figure 2.** The average growth length of the amputated zebrafish tail in each treatment group (significant difference $p<0.05$)

Fig. 2 shows that on the fourth day of measurement, all treatments significantly differed in the length of zebrafish tail growth compared with the negative control. Furthermore, only EMFM 74 ppm had a significant difference with the negative control on the eighth day of measurement.

*3.2. Discussion*

EMFM inhibits the proliferation activity of zebrafish tails. There are three phases of regeneration in the growth of zebrafish tails. The first phase occurred 0-18 hours after amputation in the form of wound healing. Epithelial cells cover the wound by forming an epidermis on the wound. The second phase occurs through blastema formation (18-48

hours after amputation). In the last phase, regenerative growth occurred from 48 hours to 20 days after amputation. During this phase, patterns and differentiation occurred to restore the tissue architecture and function of the tail fin (Khoirunnisa & Sumiwi, 2019; Ren *et al.*, 2003). In this study, observations on the eighth day after amputation showed that the tissue architecture had not fully recovered in the negative control group and EMFM administration. The regeneration process after the zebrafish tail's amputation will return complete in 20 days after amputation (Khoirunnisa & Sumiwi, 2019).

The phytochemical screening of the methanol extract of *Melaleuca cajuputi* indicates the presence of antioxidant compounds, namely steroids, flavonoids, saponins, tannins, phenols, quinones, and terpenoids. In general, the antioxidant mechanism in steroids is not yet clearly known, but some steroids can have natural antioxidant activity, including estriol and 17β-estradiol (Mooradian, 1993). Flavonoids, saponins, and tannins have antioxidant activity by indirect mechanisms (Singh & Chaudhuri, 2018). Phenols, pheninones, tannins, terpenoids, alkaloids, and flavonoids are compounds that have antioxidant activity by the direct mechanism of donating hydrogen to free radicals (Grassmann, 2005; Nweze *et al.*, 2019).

In this study, DPPH acted as free radicals. The higher levels of hydrogen donor compounds, the higher the free radical damping activity, and the lower the $IC_{50}$ value (Kusuma, 2019; Shekhar & Anju, 2014; Widyastuti, 2010). In addition, flavonoid compounds, saponins, quinones, steroids, terpenoids, and tannins are believed to have antiproliferative effects. Flavonoids inhibit ornithine decarboxylase, which plays a role in polyamine biosynthesis, DNA synthesis, and protein synthesis. Flavonoid compounds can also inhibit the cell cycle, either in G1/S or G2/M, by inhibiting cyclin-dependent kinases (CDKs), which are key regulators of cell cycle development. In addition, the flavonoid compounds block growth factor receptors inhibit mitogen-activated protein kinase (MAPK) through tyrosine kinase (RTK) receptor signaling pathways, inhibition of DNA activity of topoisomerase I/II, decrease ROS, modulation of apoptosis signaling pathways, activation of caspase-9 and caspase-3, activation of endonuclease, and decrease in Mcl-1 protein (Achmad *et al.*, 2014; Khoirunnisa & Sumiwi, 2019; Man *et al.*, 2010; Zhao *et al.*, 2018).

Furthermore, the methanol extract of Melaleuca cajuputi contains quinones, steroids, terpenoids, saponins, and tannins. Quinones have pharmacological activity as an anticancer. The mechanism of quinones as chemopreventive contributes to inducing apoptosis by stopping cell cycles, regulating carcinogen metabolism, and the expression of ontogenesis (Achmad *et al.*, 2014). Saponins can inhibit cell cycles, trigger autophagy, inhibit angiogenesis, dis-sync the cytoskeleton, inhibit metastasis intrinsically and extrinsically, and activate apoptosis pathways (Man *et al.*, 2010). Both quinones and saponins potentially become antiproliferative through the inhibition mechanism of their cell cycles. Steroids and terpenoids can inhibit the primary mechanisms in cell proliferation and trigger apoptosis and autophagy of cancer cells (Man *et al.*, 2010; Zhao *et al.*, 2018). Tannin compounds have an effect in inhibiting the cancer proliferation process, while in the process, protein kinase will be activated. The protein kinase inhibits the signal transmission pathway from the membrane to the cell nucleus. Tannins inhibit the activity of tyrosine kinase receptors that play a role in the growth of cancer cell malignancies (Firdaus, 2016).

After amputation, the three main regeneration phases are activated in the growth of the zebrafish tail. The first phase occurs 0–18 hours after amputation in the form of wound healing. The epithelial cells will cover the wound by forming the wound epidermis and secreting factors such as Fgf20a and Activin-βA to induce the next phase of the regeneration process. Then, this process is followed by blastema formations (18-48 hours after amputation). In the last phase, regenerative growth occurs from 48 hours to 20 days after amputation. During this phase, patterns and differentiation occur to restore the tissue architecture and tail fin function (Chahar MK *et al.*, 2011; Abidin IZZ *et al.*, 2020). In this research, observations on the eighth day after amputation showed that the tissue architecture had not fully recovered in the negative control group and the extract treatment group with concentrations of 18.5 ppm, 37 ppm, and 74 ppm. It is in accordance with research by Abidin IZZ *et al.* (2020), which states that the regeneration process after the amputation of the zebrafish's tail will be completed 20 days after the amputation (Abidin IZZ *et al.*, 2020).

In this study, the exact amount of anti-proliferative active compounds in *M. cajuputi* is unknown. Therefore, further research is suggested to carry out quantitative tests for the phytochemical content of *M. cajuputi* and trials on human cancer cells.

## 4. CONCLUSION

*M. cajuputi* has an antioxidant and anti-proliferation activity based on the phytochemical test conducted (phenol compounds, quinones, flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids).

## ACKNOWLEDGMENT

We acknowledge Agung Biworo, Oski Illiandri, and Fujiati for their expertise in providing input on this research.

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JURNAL ILMIAH BERKALA SAINS DAN TERAPAN KIMIA

**Jurnal Ilmiah Berkala: Sains dan Terapan Kimia**
*Vol. 17 No. 1, Januari 2023*

# Isolation of Pinostrobin Compound in Temu Kunci (*Kaempferia Pandurata Roxb*) Rhizome

# Isolasi Senyawa *Pinostrobin* pada Rimpang Temu Kunci (*Kaempferia Pandurata Roxb*)

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## ABSTRACT

Isolation and identification of Pinostrobin compounds in Temu Kunci Rhizomes *(Kaempferia pandurata* Roxb) were conducted in this study. The isolation and identification methods used were extraction, crystallization & recrystallization, Thin Layer Chromatography, and (4) Infrared (IR) Testing. The extraction results were followed by crystallization and recrystallization processes. The recrystallization produced a yellow solid isolate. A Thin Layer Chromatography test was carried out to determine the purity of the isolate using the eluent Chloroform : n-hexane of 6:4, Chloroform : Ethylacetate of 7:3. A comparison of the Retention Factor (Rf), and the Rf value of the phinostrobin standard indicates that the positive test sample contained the Pinostrobin compound. The Infrared spectrum indicates that the sample belongs to the flavonol group. It is confirmed from the functional groups of the isolates that are compatible or identical to the functional groups in the pinostrobin compound.

**Keywords**: Temu Kunci Rhizome, Pinostrobin, Thin Layer Chromatography, Crystallization, IR Spectrophotometer.

## ABSTRAK

*Telah dilakukan isolasi dan identifikasi senyawa Pinostrobin dalam Rimpang Temu Kunci (Kaempferia pandurata Roxb). Secara umum metode isolasi dan identifikasi yang dilakukan yaitu: (1) Ekstraksi, (2) Kristalisasi & Rekristalisasi, (3) Kromatografi Lapis Tipis dan (4) Pengujian Infra Merah (IR). Hasil ekstrasi yang diperoleh dilakukan proses kristalisasi dan rekristalisasi. Hasil rekristalisasi menghasilkan isolat padat berwarna kuning. Untuk mengetahui kemurnian isolat dilakukanlah uji Kromatografi Lapis Tipis menggunakan eluen Kloroform:n-heksana = 6:4; Kloroform:Etilasetat = 7:3. Berdasarkan perbandingan nilai Faktor Retensi (Rf) dengan nilai Rf dari standard phinostrobin, mengindikasikan sampel uji positif mengandung senyawa Pinostrobin. Hasil spektrum Infra Merah dapat diduga bahwa sampel merupakan golongan flavonol, hal ini dilihat dari gugus-gugus fungsi dari isolat yang didapatkan sesuai atau identik dengan gugus-gugus fungsi pada senyawa pinostrobin.*

**Kata kunci:** *Rimpang Temu Kunci, Pinostrobin, Kromatografi Lapis Tipis, Kristalisas, Spektrofotometer IR.*

**Received**: September 13, 2022; **Accepted**: January 4, 2023: **Available online**: February 15, 2023

## 1. INTRODUCTION

The rhizome of Temu Kunci (*Kaempferia pandurata* Roxb) is widely used as a traditional medicine in Indonesia for dry cough, canker sores, large intestine disorders, bloated stomach, voiding dysfunction, cervicitis, dysentery, and tumours/cancer (Bail *et al.,* 2015). Based on research conducted in Bangkok, the temu kunci (*kaempferia pandurata* Roxb) rhizome extracted with diethyl ether resulted in a relatively large concentration of the flavonoid compound pinostrobin, namely 20 grams/800gram powder or 2.5% and 1% alpinetin (Handayani *et al.,* 2018). Pinostrobin acts as an antioxidant and relaxes smooth muscle. Due to the high concentration of pinostrobin, its isolation as a pure substance can be performed quickly. The polarity of pinostrobin is reduced due to intra-molecular hydrogen bonds between the carbonyl group at C-4 and the hydroxy group at C-5. Therefore, the extraction can be carried out with less polar solvents such as chloroform and n-hexane (Nugraha *et al.,* 2012).

Pinostrobin is a secondary metabolite compound of the flavonoid group. Pinostrobin is a non-polar compound. Therefore, non-polar compounds such as n-hexane, chloroform, and ether are needed for extraction.

Based on its structure, pinostrobin can be identified by UV-Vis spectroscopy. Pinostrobin consists of two absorption bands, band I (325 nm) and band II (287 nm) and bathochromic shift when added with an $AlCl_3$ shear reaction of 20 - 26 nm to indicate the presence of an OH substituent at the C-5 position. IR spectroscopy is used to identify the functional groups. RMI Proton and Carbon spectroscopy is used to see the type and number of H and C atoms of Pinostrobin, and Mass spectroscopy to see the Relative Molecular Mass (Mr) and fragmentation of 5-hydroxy-7-methoxy flavanone or pinostrobin (Silverstein *et al.,* 1981).

## 2. MATERIALS AND METHODS

### *2.1. Materials*

The materials used were 1.5 kg of Temu Kunci Rhizome, n-hexane, Chloroform, Aquades, and Ethyl Acetate. Tools used were analytical balance, TLC plate, separating funnel, rotary evaporator, filter paper, chromatographic chamber, glass jar, small capillary tube, hot plate, and oven.

### *2.2 Sample preparation*

Temu Kunci rhizomes were purchased from the local market. It was cleaned and thinly sliced. The thin slices were dried in an oven at 60°C for three days. Dried slices were mashed using a blender. Then maceration was carried out for three days using n-hexane as solvent. The next step was evaporating the solvent with a rotary evaporator.

### *2.3 Crystallization and Recrystallization*

The recrystallization process was carried out by adding n-hexane solvent to the thick extract and heating it. The recrystallization process was carried out five times.

### *2.4 IR Spectroscopy Test*

The isolation results were then identified using Perkin Elmer's FT-IR Frontier to analyze the functional groups. FT-IR needed a solid sample; therefore, sample preparation was needed. The sample was mixed with KBr powder (5 – 10% of KBr powder samples) and then made into KBr pellets (KBr pills) using the "mini hand press".

## 3. RESULTS AND DISCUSSION

About 1.5 kg temu kunci rhizomes purchased from the local market were cleaned from soil and other impurities with clean water. After cleaning, the rhizome was thinly sliced to facilitate faster drying. Thin slices of temu kunci rhizome were dried in an oven at 60°C for three days. The dried sliced temu kunci rhizome was mashed with a blender. From 1.5 kg of dry temu kunci rhizome, 135 grams of brown temu kunci powder was obtained.

The temu kunci powder was macerated using n-hexane solvent and carried out for three days. The maceration aims to remove all the chemical components contained in the simplicia. The solvent n-hexane was used because the compound isolated, pinostrobin, is non-polar. It can be seen from the structure of the pinostrobin. Its polarity is reduced due to intra-molecular Hydrogen bonds between the carbonyl group at C-4 and the hydroxyl group at C-5. Therefore, extraction can be carried out with less polar solvents such as n-hexane and chloroform (Harborne J.B., 1992). After maceration, a brown macerate was obtained. The obtained macerate was heated on an electric stove until boiled. The heating aims to dissolve the isolated compound. It was then filtered with filter paper in a hot state. A brown extract was obtained.

The next step was to evaporate the solvent by aerating it using a rotary evaporator. The result was a thick green extract. The viscous extract was recrystallized using n-hexane. The recrystallization process was carried out by adding n-hexane to the thick extract. The heating aimed to dissolve the viscous extract. The completely mixed solution was left for one night to form pure yellow crystals. The recrystallization was carried out five times. Recrystallization is a technique for purifying a solid substance from a mixture of impurities. It is carried out by recrystallizing the substance after being dissolved in a suitable solvent (Day and Underwood, 2001). N-hexane was chosen for recrystallization because the crystals formed, indicated to be Pinostrobin compounds, are non-polar. Therefore no-polar solvent is the suitable choice. It is also following the "Like Dissolve like" rule.

The purity of the yellow crystal was tested using Thin Layer Chromatography (TLC). First, the crystal was dissolved using chloroform. Chloroform was used because chloroform is semipolar. This compound can dissolve polar and nonpolar compounds. Qualitative and quantitative thin layer chromatography (TLC) analysis of curcumin used the stationary phase plate/ TLC plate. Prior to sampling, the stationary phase was activated by preheating at 110°C for 15 minutes. It aims to increase the absorption power of the stationary phase. The TLC plate was made of 3 cm x 3 cm, and the start and finish lines were determined. The starting line is where the sample is pointed and the start of the bearer. The starting line is spaced between one sample and another so that the spots formed do not overlap to avoid difficulties in analysis. In this study, the distance of the sampling point was 1 cm. The finish line is the final boundary line of the solvent during the carrier process. The starting line was 1 cm from the plate's base, and the finish line was 1 cm from the end of the plate.

Thin layer chromatographic analysis was performed with eluents of Chloroform, Chloroform : n-hexane of 6:4, and Chloroform : Ethylacetate of 7:3. It showed a single spot, indicating that the isolated compound is pure (Markam 1988) with Rf values of 0.84 and 0.64, respectively. This Rf result was then compared with the Rf value of the standard Pinostrobin compound with the same eluent and ratio. The comparison obtained the same value for two eluent systems, namely chloroform and chloroform : ethyl acetate

(7:3). This indicates that the isolated compound is identical to the standard compound, which is a pinostrobin compound. The isolation results were then identified using Perkin Elmer's FT-IR Frontier to see the functional groups.

Identification by IR spectroscopy obtained a wave number of 3447.34 $cm^{-1}$ (indicating -OH with its Hydrogen bond with C=O at C-4), 3012.32 $cm^{-1}$ (showing -C-H stretch of aromatic), 1621.78 $cm^{-1}$, 1650.83 $cm^{-1}$ (represents C=O ketones hydrogen bonding with -OH at C-5), 1498.12, 1580.37 $cm^{-1}$ (indicating C=C aromatic), 2910.92 $cm^{-1}$ (showing -C-H methyl and ethyl), and 3631.47, 3736.89 (indicating a phenol group).

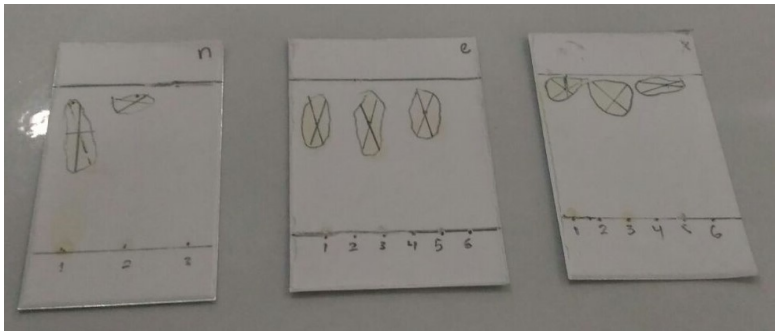

**Figure 1**. thin layer chromatography results

**Table 1** Result of Comparison Rf Value

| Eluen | Comparison (ml) | Solution | RF(cm) |
|---|---|---|---|
| Chloroform | 10 | Standar | 0.87 |
| | | Isolasi | 0.84 |
| Chloroform : n-heksan | 6:4 | Standar | 0.94 |
| | | Isolasi | 0.68 |
| Chloroform : Etil asetat | 7:3 | Standar | 0.78 |
| | | Isolasi | 0.72 |

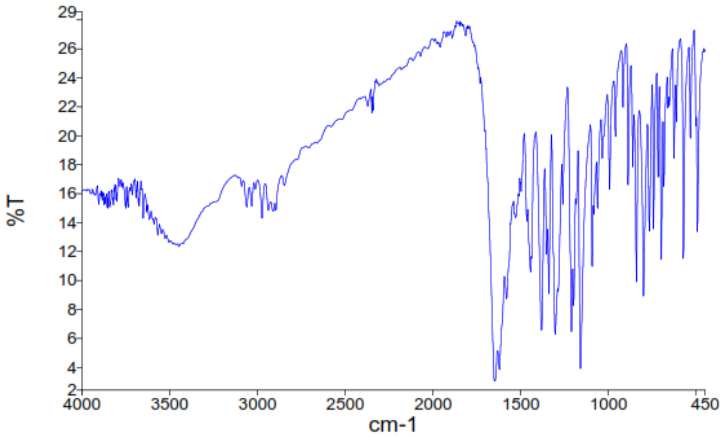


**Table.2** Result of FT-IR spectrophotometer

| No | Functional Group | Group of compounds | Frequency Range | Isolation results ($cm^{-1}$) |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| 1 | O-H | alcohol and phenol | 3200-3500 (hydrogen bond)<br>3500-3700(free) | 3447.34<br>3631.47 ; 3736.89 |
| 2 | C = O | ketone | 1650 – 1780 | 1621.78 ; 1650.83 |
| 3 | C - H | aromatic | 3000 – 3100 | 3012.32 |
| 4 | C - H | methyl and ethylene | 2850 – 3000 | 2910.92 |
| 5 | C = C | aromatic | 1450 – 1600 | 1498.12 ; 1580.37 |

## 4. CONCLUSIONS

Based on the purity test using the comparison of Rf values in Thin Layer Chromatography (TLC), the isolates obtained were identical to Pinostrobin compounds. It is confirmed by Infrared (IR) spectroscopy. The isolates obtained display a peak graph (Peak) identical to phinostobin compounds. Therefore, the isolates obtained are pinostrobin compounds from the temu kunci plant. Based on these results, further research is needed to ensure that the structure of the compounds in the isolates is 100% pinostrobin compounds.

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JURNAL ILMIAH BERKALA SAINS DAN TERAPAN KIMIA

**Jurnal Ilmiah Berkala: Sains dan Terapan Kimia**
*Vol. 17 No. 1, Januari 2023*

# Study of Formaldehyde Content in Different Types of Tofu Using Micro Scale Laboratory Based Visible Beam Spectrophotometry

# Studi Kandungan Formalin pada Berbagai Jenis Tahu Menggunakan Spektrofotometri Sinar Tampak Berbasis *Micro Scale Laboratory*

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## ABSTRACT

*Microscale laboratory is an analytical technique that requires a little reagent and produces a little waste. One application of microscale laboratory is the analysis of formaldehyde content in tofu. Formaldehyde is one of the chemicals that is harmful to the body but is often misused as a food preservative in tofu. This study aims at determining the content of formaldehyde in tofu using laboratory microscale-based Schiff reagents. The use of microscale laboratories is expected to reduce waste and support the implementation of green chemistry. The calibration curve is used to find the relationship between concentration and absorbance using a standard solution series of 10 ppm, 15 ppm, 20 ppm, 25 ppm, and 30 ppm measured at a wavelength of 561 nm. In the qualitative test, all samples were reactive and showed a purplish red color. In the quantitative test, the highest formaldehyde content in the tofu sample was obtained in the white tofu 3 with a content of 99.7 ppm.*

***Keywords***: *Formaldehyde, microscale, Schiff reagent.*

## ABSTRAK

*Microscale laboratory merupakan sebuah teknik analisis yang membutuhkan sedikit reagen dan menghasilkan sedikit limbah. Salah satu penerapan microscale laboratory adalah analisa kandungan formalin dalam tahu. Formalin merupakan salah satu bahan kimia yang berbahaya untuk tubuh namun seringkali disalahgunakan sebagai pengawet makanan pada tahu. Penelitian ini bertujuan untuk mengetahui kandungan formalin dalam tahu dengan menggunakan reagen Schiff berbasis microscale laboratory. Kurva kalibrasi digunakan untuk mencari hubungan antara konsentrasi dengan absorbansi dengan menggunakan deret larutan standar 10 ppm, 15 ppm, 20 ppm, 25 ppm, dan 30 ppm yang diukur pada panjang gelombang 561 nm. Pada uji kualitatif semua sampel tahu reaktif dan menunjukan warna merah keunguan. Pada uji kuantitatif didapatkan kadar formalin tertinggi dalam sampel tahu pada tahu putih 3 dengan kadar sebesar 99,7 ppm.*

**Keywords**: Formalin, *microscale*, pereaksi *Schiff*.

**Received**: July 20, 2022; **Accepted**: January 23, 2023: **Available online**: February 15, 2023

## 1. INTRODUCTION

Tofu is a food ingredient processed from soybeans that is very popular in the society. Various processed tofu have been known by the public, including white tofu, silk tofu, fried tofu (pong), bandung tofu, and milk tofu. The nutritional content contained in 100 grams of tofu includes 7.8 g of protein; 4.6 g of fat; 1.6 g of carbohydrates; 124 mg of calcium; 63 mg of phosphorus; and 68 kcal of energy(Sikanna, 2016). People often choose tofu as a substitute for animal protein because it has the best quality of vegetable protein and the most complete amino acid composition and has a high digestibility of 85 – 98%.

Tofu is a high water content foodstuff with a 70 – 90% percentage, so it cannot be stored for too long because it is easily damaged(Seftiono, 2017). The high water content leads to tofu as a suitable medium for microbial propagation. A tofu without preservatives only lasts for three days if stored in refrigerator with less than 15% temperature. Whereas boiled tofu can be stored in the refrigerator for seven days, if it is more than that, pink spots will appear on its surface.

As a perishable foodstuff, a good process of making tofu is absolutely necessary to keep the nutritional content of tofu intact when consumed. However, some manufacturers use harmful Food Additives (BTPs) to maintain the quality of the tofu until it reaches the consumer. Some preservatives in the form of additives or chemical substances commonly used by food manufacturers are formaldehyde, borax, and rhodamine (Nasution & Supriatna, 2019)B. Based on reports submitted by the Food and Drug Administration, 141 of the 401 food samples tested in 2020 in East Java were positive for formaldehyde. Formaldehyde is often chosen because it is very easy to get on the market and the price is more economical than BTP which is not prohibited so that it can generate as much profit as possible(Sikanna, 2016). However, producers ignore the negative impact will have on the consumers health who consume these foods.

Formalin is a formaldehyde compound in water with anaverage concentration percentage of 37%, methanol 15%, and the rest is water(Kholifah & Utomo, 2018). Formalin is strictly prohibited for its use as BTP. This is because formaldehyde is used as a germ killer, so it is commonly used for floor cleaners, warehouses, clothing, fly and other insect repellents, coloring agents, as well as explosives (Kholifah & Utomo, 2018). Formaldehyde is strongly discouraged from being added as BTP because this chemical will be a toxic in the body(Berlian, Pane, & Hartati, 2017). Long-term ingestion of formaldehyde will trigger the development of cancer cells, respiratory tract irritation, burns, and allergic reactions (Sikanna, 2016).

According to the Food and Drug Administration, tofu containing formaldehyde has very good shape characteristics, chewy, not easily crushed, lasts several days, and not easily rotten. Several studies have been conducted to test the formaldehyde content in tofu with various indicators. Research conducted by Sikanna (2016) used KMnO4 indicator showed that 6 out of 9 tofu samples in Palu City contained formaldehyde. Research conducted by Nasution&Supriatna (2019) used the dragon fruit peel indicator showed that there was a formaldehyde content in tofu sold at the Gede Bage market.

The research will be conducted using Schiff reagent based on microscale laboratory. Microscale laboratory is used because it is environmentally friendly and supports the green chemistry implementation. This is because during the research process, only very few chemicals are used, so it can reduce the waste of hazardous chemicals that are discharged into the environment. Over the past few years, microscale laboratory has become an

important part of developing chemical experiences, as they require little reagents and produce little waste (Botella & Ibanez., 2020). Microscale also increases interest and motivation in positive learning, fosters environmental awareness, and stimulates innovative thinking. Micro lab is not a simplification or miniaturization of normal experiments, but an innovation of chemical experiments that support green chemistry and quality education (Zhou, 2019). One application of microscale laboratory is to detect the presence of formaldehyde in tofu.

Considering the danger of formaldehyde if consumed for a long time, this study aims at testing qualitatively and quantitatively the formaldehyde content in tofu using Schiff reagent with Visible Spectrophotometry method. Through the test results, it is expected that people will be more careful in choosing the tofu which they will consume.

## 2. MATERIALS AND METHODS

### *2.1. Materials*

The tools used in this study were reaction tubes, drop pipettes, mortar and pestle, as well as a visible spectrophotometer (Genesys-20). The material used in this study was Schiff reagent, $H_2SO_4$ 96%, Formaldehyde 37%. The samples tested were ten tofu with three different types, namely white tofu, silk tofu, and fried tofu. Samples were obtained from four different markets in Malang.

### *2.2. Methods*

#### *2.2.1. The creation of calibration curves*

The calibration curve is a method that can be used to determine the concentration of formaldehyde in a tofu sample using a series of standard solutions whose concentration has been known. Calibration curves production used formaldehyde standard solutions at concentrations of 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm. The creation of the formaldehyde standard series was carried out by diluting 100 ppm with the help of a 5 mL volumetric flask, then adding Schiff's reaction amounting to 0.14 ml. Then the series of standard solutions were scanned using Visible Spectronics instrumentation at a wavelength of 561 nm.

#### *2.2.2. Determination of formaldehyde levels in tofu samples*

10 gr of tofu, each tofu sample is pounded until crushed. Add 10 mL of water then filter with filter paper until smooth. The filtrate was taken and then acidified with $H_2SO_4$ to pH 3, the purpose was to degrade the sample. After that, add 0.14 mL of Schiff reagent. Then it was scanned using a Visible Spectronics instrument at a wavelength of 561 nm.

## 3. RESULTS AND DISCUSSION

### *3.1. The Making of The Calibration Curve*

In the calibration curve scanning, the absorbance result was acquired as shown in the **Table 1**. The absorbance result would form a curve with a straight line (linear), which stated the relationship between substance's concentration in a standard solution and absorbance detected by the instrument.

**Table 1** Calibration Curve's Absorbance Data

| Concentration | Absorbance |
|---|---|
| 0 ppm | 0 |
| 10 ppm | 0,221 |
| 15 ppm | 0,276 |
| 20 ppm | 0,326 |
| 25 ppm | 0,415 |
| 30 ppm | 0,541 |

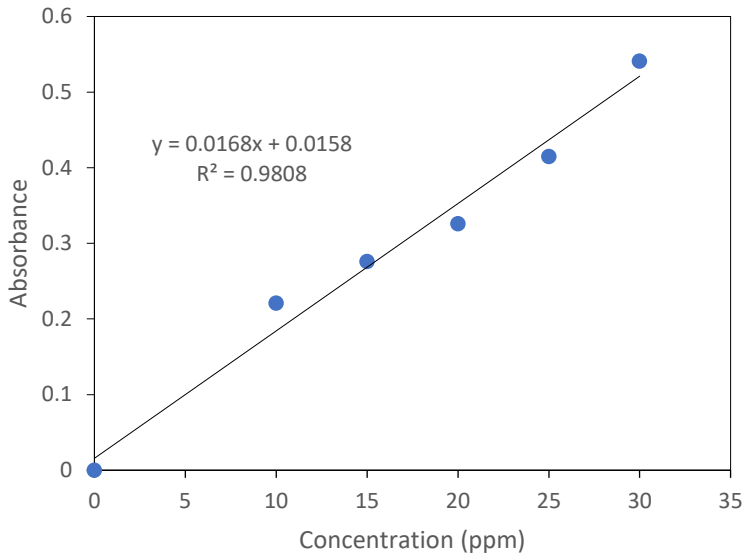


**Figure 1.** A straight line curve between concentration and absorbance

The linear relationship formed a straight line equation, as shown below:

$$Y = ax + b$$

Y = Absorbance
x = Analytic concentration
a = Slope

The relationship between the concentration and the absorbance is shown in **Figure 1.** From the curve, a straight line equation was acquired Y = 0,0168x + 0,0158 with the regression R = 0,9808.

### *3.2. The Qualitative Analysis of Formaldehyde Content in The Tofu*

The samples tested in this research were ten slices of tofu. All filtrated samples showed purplish red color when the Schiff reagent was dropped. It showed that the samples were positively proven to contain formaldehyde as shown in **Table 2**.

**Table 2.** The Qualitative Test Result of Tofu Samples

| Sample | Sample Source | Test Result |
|---|---|---|
| White Tofu 1 | Peddler | + |
| White Tofu 2 | Market A | + |
| White Tofu 3 | Market B | +++ |
| White Tofu 4 | Market C | ++ |
| White Tofu 5 | Market D | ++ |
| Silk Tofu 1 | Market A | ++ |
| Silk Tofu 2 | Market B | + |
| Silk Tofu 3 | Market D | ++ |
| Fried Tofu 1 | Market A | + |
| Fried Tofu 2 | Market B | + |

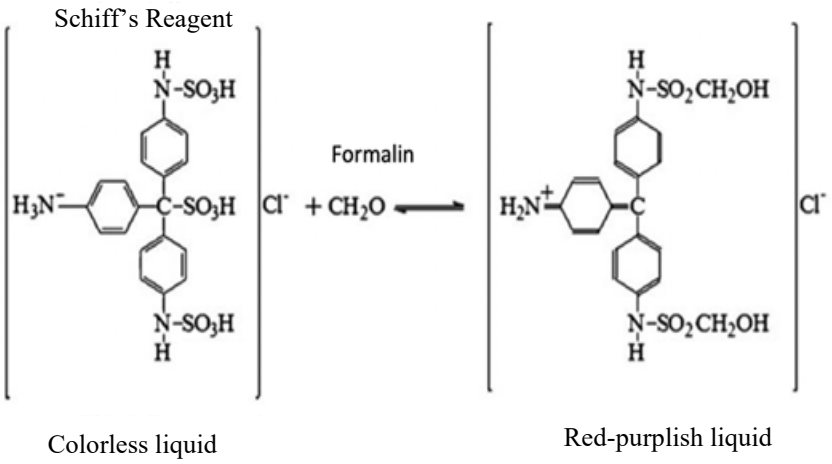


**Figure 2.** Reaction between Schiff reagent and Formaldehyde (Pielichowska K, 2012)

The more concentrated color showed a bigger formaldehyde concentration in a filtrate sample. The reaction between colorless Schiff reagent and colorless formaldehyde would result in a solution with pinkish color to purplish color as shown in the reaction in **Figure 2**.

*3.3. The Quantitative Analysis of Formaldehyde concentration in the Tofu.*

The level of formaldehyde in the samples which was proven qualitatively containing formaldehyde could be found out with regression analysis. The result of absorbance acquired from every sample was inserted in a straight line equation which had been gotten from the calibration curve. If the absorbance result was found out through the calibration curve, dilution was done and certain dilution factor score was gotten. The absorbance result of every sample with the dilution factor and the formaldehyde concentration is shown in **Table 3.** According to **Table 3**, it is known that the highest concentration of formaldehyde was found in the sample of white tofu 3 with 99,77 ppm concentration. Meanwhile, the lowest formaldehyde concentration was located in the sample of silk tofu 2 with 12,33 ppm concentration.

**Table 3.** The Result of Formaldehyde Concentration inside the tofu

| Sample | Sample's weight (gram) | Extract Volume (mL) | Absorbance | Dilution Factor | Concentration (ppm) |
|---|---|---|---|---|---|
| White Tofu 1 | 15,00 | 10,00 | 0,256 | 1 | 14,30 |
| White Tofu 2 | 15,00 | 10,00 | 0,253 | 1 | 14,12 |
| White Tofu 3 | 15,00 | 10,00 | 0,282 | 6 | 99,77 |
| White Tofu 4 | 15,00 | 10,00 | 0,234 | 2 | 26,92 |
| White Tofu 5 | 15,00 | 10,00 | 0,242 | 2,5 | 35,07 |
| Silk Tofu 1 | 15,00 | 10,00 | 0,281 | 1,5 | 24,15 |
| Silk Tofu 2 | 15,00 | 10,00 | 0,223 | 1 | 12,33 |
| Silk Tofu 3 | 15,00 | 10,00 | 0,261 | 1,5 | 22,36 |
| Fried Tofu 1 | 15,00 | 10,00 | 0,309 | 1 | 17,45 |
| Fried Tofu 2 | 15,00 | 10,00 | 0,234 | 1,5 | 19,95 |

*International Programme on Chemical Safety* (IPCS) mentioned that formaldehyde tolerance limit which could be received by the body in the form of liquid was 0,1 ppm. In one day; the allowed intake was 0,2 mg. Meanwhile, in the form of food, the limit of formaldehyde intake for an adult was 1,5 mg to 14 mg per day. According to the quantitative test result of formaldehyde, it showed that the concentration of formaldehyde in the samples had exceeded the limit which was set.

## 4. CONCLUSIONS

According to the result of the research, it can be concluded that the Schiff reagent can be used as *microscale laboratory,* because the Schiff reagent used in the research was only 0,14 ml. According to the test with the Schiff reagent, it was concluded that ten samples of tofu was proven positively containing formaldehyde. The highest formaldehyde concentration of the three kinds of tofu tested was found in the white tofu sold in one of the market in Malang, with 99,77 ppm concentration rate.

## ACKNOWLEDGMENT

We would like to thank the laboratory assistant of Chemical Analytic who has given the permission for our research, so we could finish the research on time; and also to the laboratory assistant of Organic Chemistry who has helped the usage of our research material.

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JURNAL ILMIAH BERKALA SAINS DAN TERAPAN KIMIA
**Jurnal Ilmiah Berkala: Sains dan Terapan Kimia**
*Vol. 17 No. 1, Januari 2023*

# Macro and Micro Mineral Composition of *Haruan* Fish (*Channa striata*) in Banjar District, South Kalimantan

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## ABSTRACT

Minerals have an essential role in the human body. Information about the mineral content of haruan fish in Banjar Regency is very limited. This research aimed to determine the mineral compound of haruan fish originally from ponds in Banjar Regency. Pond-sourced fish samples were processed until they became powders. The ash compound of haruan fish powder was calculated using the gravimetric method. The mineral compound was analyzed using an XRF spectrometer. Wild haruan fish contained 98.85% macro minerals, with the mineral composition of 14.4% P (10.2 mg/kg), 19.9% Ca (19,9 mg/kg), 60.6% K (42.5 mg/kg), and 0.85% S (0.6 mg/kg). Farmed haruan fish contained 94,76% macro minerals, consisting of 15.6% P (12.9 mg/kg), 17.2% Ca (14.3 mg/kg), 61.1% K (50.8 mg/kg), and 0.86% S (0.7 mg/kg). Wild haruan contained 3.51% micro minerals, consisting of 4.44% Fe (3.7 mg/kg), 0.10% Cu (0.08 mg/kg), and 0.22% Zn (0.18 mg/kg). Farmed haruan contained 4.76% micro minerals, consisting of 4.44% Fe (3.7 mg/kg), 0.10% Cu (0.08 mg/kg), and 0.22% Zn (0.18 mg/kg). Wild haruan fish contained 0.56% trace element, 0.46% Rb (0.32 mg/kg), and 0.1% Re (0.07 mg/kg). Farmed haruan fish contained 0.42% trace element, 0.34% Rb (0.28 mg/kg), and 0.08% Re (0.06 mg/kg).

**Keywords**: haruan fish, macro mineral; micro mineral

## ABSTRAK

*Mineral mempunyai peranan yang sangat vital bagi tubuh manusia. Informasi tentang kandungan mineral yang terdapat pada ikan haruan di Kabupaten Banjar sangat terbatas. Tujuan penelitian ini adalah untuk mengetahui kandungan mineral ikan haruan yang berasal dari Kabupaten Banjar. Sampel ikan yang diperoleh dari tambak dan sungai dipreparasi sampai menjadi serbuk. Serbuk haruan diabukan dan dihitung persentase kadar abu dengan menggunakan metode analisis gravimetri. Kandungan mineral dianalisis menggunakan spektrometer XRF. Daging ikan haruan alam mengandung mineral makro sebesar 98,85 % dengan komposisi mineral P 14,4 % (10,2 mg/kg), Ca 19,9 % (19,9 mg/kg) K 60,6 % (42,5 mg/kg) dan S 0,85 % (0,6 mg/kg). Daging ikan haruan budidaya mengandung mineral makro sebesar 94,76 % dengan komposisi mineral P 15,6 % (12,9 mg/kg) Ca 17,2 % (14,3 mg/kg) K 61,1 % (50,8 mg/kg) dan S 0,86 % (0,7 mg/kg). Daging ikan haruan alam mengandung mineral mikro sebesar 3,51 % dengan komposisi mineral Fe 3,20 % (2,24 mg/kg) Cu 0,089 % (0,06 mg/kg) dan Zn 0,22 % (0,15 mg/kg). Daging ikan haruan budidaya mengandung mineral mikro sebesar 4,76 % dengan komposisi mineral Fe 4,44 % (3,7 mg/kg) Cu 0,10 % (0,08 mg/kg) dan Zn 0,22 % (0,18 mg/kg). Daging ikan haruan alam mengandung unsur runut sebesar 0,56 % yaitu Rb 0,46 % (0,32 mg/kg) dan Re 0,1*

*% (0,07 mg/kg). Daging ikan haruan budidaya mengandung unsur runut sebesar 0,42 % yaitu Rb 0,34 % (0,28 mg/kg) dan Re 0,08 % (0,06 mg/kg).*

**Keywords**: ikan haruan; makro mineral; mikro mineral

**Received**: January 20, 2022; **Accepted**: February 2, 2023: **Available online**: February 15, 2023

## 1. INTRODUCTION

inerals are chemical elements other than hydrogen, oxygen, carbon, and nitrogen needed by the body. Minerals have an essential role in the human body. Calcium (Ca) and phosphorus (P) play important roles in bone and tooth formation. Zinc (Zn) and iodine (I) are enzyme cofactors that function in biological processes. Lack of calcium can cause osteoporosis. Zinc deficiency results in stunted growth, while a lack of iodine consumption can lead to goiter and mental retardation (Olson et al., 1988).

There are two categories of minerals: macro and micro. Macrominerals are essential minerals for the formation of various organ components. According to Winarno (1992), the macromineral content required by the human body is greater than 100 mg per day or greater than 0.01% of body weight. Some examples of macro minerals are calcium, phosphorus, sodium, and potassium. Microminerals are other types of minerals which total content in the body is less than 0.01% of body weight and is only needed in amounts less than 100 mg/day. Some examples of microminerals are chromium, iron, and iodine.

Meeting the needs of minerals in humans can be obtained by consuming food, both derived from plants or animals. The best source of minerals is animal products because the biological availability of minerals in plant product is lower. It is caused by the presence of mineral-binding substances, such as fiber, that inhibit mineral absorption (Santoso, 2009).

Fish, including fish captured in rivers and farmed fish, can be consumed as a source of animal nutrition. Banjar Regency residents frequently consume various fish, including haruan fish (Channa striata). This fish is popular since it is a commodity in Banjar Regency's freshwater fishing industry.

The mineral content of fish has been studied before. Chasannah et al. (2015) stated that the mineral nutrient content in haruan fish was in the form of Ca, K, and Fe, each of which was 12.15, 283.00, and 0.17 mg/100g sample. Santoso (2009) stated that the macro mineral content of gourami aged 7 months - 1 year for Ca, K, and Mg were 162.37, 128.85, and 9.63 mg/kg, respectively; Microminerals for Fe, Cu, Zn, and I were 78.26, 18.72, 22.45, and 0.08 µg/g, respectively. The macro mineral content of gourami aged 2.5 - 3 years for Ca, P, K, Mg, and Na were around 91.33, 610, 88.74, 7.65, and 59.85 mg/kg, respectively. Microminerals for Fe, Cu, Zn, and I were 46.18, 12.83, 14.25, and 0.08 µg/g, respectively. Nurhayati (2014) stated that the highest levels of microminerals were found in milkfish weighing ± 150 g, namely Zn 6.95 ± 0.16, Cu 0.55 ± 0.0, and Fe 12.14 ± 0.06 mg/ kg bk. The highest I content was found in milkfish weighing ± 102 g, which was 76.33 ± 0.01 µg/100 g body weight.

Animal sustenance can be obtained by consuming either wild-caught or farmed fish. According to the Statistics Indonesia (BPS) for Banjar Regency, aquaculture production in 2017, 2018, and 2019 increased by 58,041.77, 58,105.50, and 60,870.36 tonnes, respectively. There is currently a lack of data and information on the mineral content of haruan fish in Banjar Regency. Therefore, more research is needed. This study is to provide valuable information

about the mineral content of haruan fish, which is widely consumed in Banjar Regency.

## 2. MATERIALS AND METHODS

### *2.1. Materials*

The tools used were a food grinder, 30 mL porcelain cup, tongs, desiccator, stainless steel sample spoon, analytical balance (OHAUS Galaxy 400), oven (Thermologic), sieve no. 20 (mesh size 0331 inches, wire diameter 0.510 mm), ashing furnace, sample containers (plastic and glass), X-Ray Fluorescence (Jeol Element Analyzer type JSX-3211). The materials used wer0065 distilled water and Haruan fish samples.

### *2.2. Sampling*

Sampling was performed at haruan fish cultivation sites and rivers in the Banjar Regency area. Sampling was carried out in two places. Wild haruan fish was taken from the Riam Kanan Reservoir, Aranio District, Banjar Regency, South Kalimantan Province. The farmed haruan fish was harvested from ponds in Tungkaran Village, Banjar Regency, South Kalimantan. The largest fish was chosen so that the same size was obtained. The fish was placed in a refrigerated box and transported to the FMIPA ULM Banjarbaru Basic Laboratory for analysis.

### *2.3. Determination of Water Content in Fish Meat*

The ash content was determined by inserting an empty cup into the furnace. The temperature was then raised gradually until it reached 550°C and allowed to stand for 12 hours. The temperature was then lowered to around 40°C, and the empty cup was removed and cooled in a desiccator for 30 minutes and then weighed. A total of 5 grams of sample was put into an empty cup heated in an oven at 100°C for 24 hours. The crucible was transferred into the furnace, and the temperature was gradually increased until it reached 550°C ± 5C for 8 hours. The temperature was reduced to 40°C, and then the cup was removed using tongs and placed in a desiccator for 30 minutes. After cooling, the cup and its contents were weighed again.

### 2.4. Analysis using X-Ray Fluorescence (XRF)

A total of 5 grams of the sample that has been reduced to ash was placed in the sample container for XRF analysis. XRF analysis included reading the X-ray spectrum of the sample, identifying the peaks, and classifying each element as a major constituent based on the peak intensity. Parameter analysis consisted of qualitative analysis and quantitative analysis. The qualitative analysis included reading the spectrum of fluorescence light based on the size of the energy spectrum of the fluorescence light in kilo electron volt (keV) energy units. This value is unique to each type of material. Quantitative analysis was based on the intensity of the emitted energy. The information obtained from these two analyzes was the amount, composition, and type of material (Barone et al., 2003).

## 3. RESULTS AND DISCUSSION

### 3.1. Determination of Water Content in Fish Meat

Water content analysis was carried out to determine the amount of water contained in the haruan fish. Water content was obtained based on the weight lost after the sample was dried in the oven at 100°C. The results are presented in Table 1.

Based on Table 1, the meat of wild haruan fish contains a moisture content of 80.13%, while the meat of haruan fish produced by cultivation is 79.8%. It indicates that the water content contained is similar.

**Table 1. Water Content of Haruan Fish Meat**

| Sample | Water Content (%) | Average (%) |
|---|---|---|

| | | |
|---|---|---|
| Wild | 81.3 | 80.1 |
| | 78.4 | |
| | 80.7 | |
| Farmed | 79.8 | 79.9 |
| | 80 | |
| | 80 | |

Based on Table 1, the meat of wild haruan fish contains a moisture content of 80.13%, while the meat of haruan fish produced by cultivation is 79.8%. It indicates that the water content contained is similar. The results obtained are similar to Kelvin et al.'s research (2014), which is equal to 80.4%, and Ahmed et al. (2012), which is equal to 82.6%. Chasannah et al.'s (2015) study showed lower wild haruan meat by 78.8% and farmed haruan meat by 77%. The type of food and fish habitat may influence these disparities (Suwandi et al., 2014).

### *3.2. Determination of Ash Content in Fish Meat*

The determination of ash content aimed to determine the total minerals contained in fish and sediment samples. The results are presented in Table 2.

**Table 2. Ash content of Haruan Fish Meat**

| Sample | Ash Content (%) | Average (%) |
|---|---|---|
| Wild | 6.9 | 7 |
| | 6.8 | |
| | 7.2 | |
| Farmed | 8.02 | 8.3 |
| | 8.4 | |
| | 8.5 | |

Table 2 shows that the meat of wild haruan fish has an ash content of 7%, while the meat of farmed haruan fish is 8.3%. This result differs from Kelvin et al. (2014), which has a lower value of 1.5%, and Ahmed et al. (2012), which is equal to 0.4%. Ash content has a relationship with the mineral content of a material. The habitat of the haruan fish influences the difference in ash content. The ash content of the haruan fish depends on the habitat of the fish, which is related to the mineral content in the haruan fish's body (Sulthoniyah et al., 2013). High ash content implies that fish meat is rich in minerals.

The high value of this ash content may have resulted from the addition of fortification to the fish diet. This fortification aims to meet and increase the nutritional needs of fish (Harmain et al., 2017).

### *3.3. Composition of Macro and Micro Minerals in Haruan Fish Meat*

Table 3 shows that the mineral concentrations in natural and farmed haruan fish meat are different. It occurs because the body absorbs minerals as needed. The age of the fish has a significant impact on the accumulation of minerals in the body. The older the fish, the greater the mineral absorbed. According to Hafiludin (2016), the composition of vitamins, carbohydrates, fats, proteins, and minerals in each fish species varies according to age, metabolism, diet, environment, and reproductive mass. The meat of both wild and farmed haruan fish includes the same essential minerals, K, Ca, P, S, Fe, Zn, and Cu, but in different concentrations. Asfar (2014) found that haruan fish contains macro and micro minerals in the form of Na, K, Ca, Mg, Fe, Zn, Mn, Cu, and P, with the highest concentration being K of 2195 mg/kg and the smallest mineral being Cu of 1 .3 mg/kg.

**Table 3. Mineral Composition of Haruan Fish Meat**

| No | Mineral | mg/kg | |
|---|---|---|---|
| | | Wild | Farmed |
| 1 | (Ca) | 13.9 | 14.3 |
| 2 | (K) | 42.5 | 50.8 |
| 3 | (P) | 10.2 | 12.9 |
| 4 | (S) | 0.6 | 0.7 |
| 5 | (Fe) | 2.24 | 3.7 |
| 6 | (Zn) | 0.15 | 0.18 |
| 7 | (Cu) | 0.06 | 0.08 |

### *3.4. Trace Element in Haruan Fish Meat*

Trace elements are minerals with very small concentrations in soil, plants, and living organisms. Trace elements in nature are necessary to maintain the balance of other elements. The results of the trace element XRF spectrometer is presented in Table 4.

**Table 4. Trace Elements in Haruan Fish Meat**

| Sample | Elements (mg/kg) | |
|---|---|---|
| | Rb | Re |
| Wild | 0.32 | 0.07 |
| Farmed | 0.28 | 0.06 |

According to Tzu (2013), trace elements are found in waters with very small concentrations, namely 67.18-0.024 µg/L of water compared to other trace elements. Trace elements such as Rb and Re play an important role in various biological metabolic processes of organisms in an environment.

Wild haruan fish meat contained 0.32 mg/kg of Rb, while farmed haruan fish contained 0.28 mg/kg. Fahad et al. (2018) discovered that six fish samples collected from diverse locations in Bangladesh contained Rb. At 0.01%, tilapia (Oreochromis mossambicus) has the highest Rb concentration.

The Re element in the meat of wild haruan fish was 0.07 mg/kg, while in farmed haruan fish was 0.06 mg/kg. This value suggests that the Re concentration in haruan fish is safe. The Re element in fish is closely related to the environment in which the fish live. Chen et al. (2016) found that fish samples taken from the Canadian city of Ontario absorbed the radioactive element Re with the highest concentration of 0.06 mg/kg. This fish absorbs Re elements while seeking food in sediment deposits at the bottom of the sea. Therefore, radioactive elements in the sediments may accumulate in the fish's body.

## 4. CONCLUSIONS

The conclusions that can be drawn from this research are:

1. Minerals found in haruan fish meat in the Banjar Regency area include macro minerals such as K, Ca, P, and S, as well as micro minerals such as Fe, Zn, Cu, Rb, and Re.
2. Wild haruan fish meat contained macro minerals with a composition of 42.5 mg/kg K, 13.3 mg/kg Ca, 10.2 mg/kg P, and 0.6 mg/kg S; micro minerals with a composition of 2.24 mg/kg Fe, 0.15 mg/kg Zn, and 0.10 mg/kg Cu; and trace elements with a composition of 0.32 mg/kg Rb and 0.07 mg/kg Re.
3. Farmed haruan fish meat contained macro minerals with a composition of 50.8 mg/kg K, 14.3 mg/kg Ca, 12.9 mg/kg P, and 0.7 mg/kg S; micro minerals with a composition of 3.7 mg/kg Fe, 0.18 mg/kg Zn, and 0.08 mg/kg Cu; and trace elements with a composition of 0.28 mg/kg Rb and 0.06 mg/kg Re.

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