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GC-MS Analysis of Kabau (*Archidendron jiringoides*) Pod Peel Extract and Its Effect on Erythrocytes and Leukocytes in Male Mice Induced by Lead

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ABSTRACT

An increase in free radicals in the body can result from continuous exposure to heavy metals such as lead (Pb). This condition can result in oxidative stress due to damage to cellular structures, including the lipid bilayer membrane, cellular proteins, and DNA. One approach to reduce such damage is the administration of exogenous plant-derived antioxidants, including kabau. This study aimed to determine the chemical compound profile contained in kabau pod peel extract and to evaluate its effects on erythrocytes and leukocytes in lead-induced mice. GC-MS analysis was conducted to identify the types of compounds present in the extract. An *in vivo* experiment was conducted with six groups: three control and three treatment groups. The GC-MS results showed that the abundant antioxidant compounds were 2,3-butanediol and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl. The *in vivo* results indicated that administration of kabau pod peel extract did not significantly affect leukocyte and erythrocyte counts in the blood of mice exposed to lead acetate.

Keywords: antioxidant, archidendron, blood cell, GC-MS, kabau

INTRODUCTION

An increase in free radicals in the body can result from continuous exposure to heavy metals such as lead (Pb). Lead exposure may occur through inhalation or ingestion, resulting from vehicle emissions, smoke from burning waste, airborne chemicals, or food with poor hygiene (Umar *et al.* 2021). Such exposure can accumulate in body tissues, causing tissue damage (Riana *et al.* 2024). This condition may lead to oxidative stress due to damage to cellular structures, including the lipid bilayer membrane, cellular proteins, and even DNA (Rahman *et al.* 2012). Heavy metals increase ROS production, causing oxidative stress in blood cells, leading to changes in cell shape and cell death (Sharma *et al.* 2025).

Naturally, the body provides a mechanism to maintain a balance of free radical levels. However, when free radical levels in the body are elevated, exogenous antioxidants are needed to reduce them. Antioxidants are compounds that donate electrons to free radicals, preventing oxidative stress. Antioxidant compounds are widely found in plants, including the kabau plant. Kabau belongs to the family Fabaceae and grows naturally in tropical rainforests, such as those on Sumatra (Thao *et al.* 2016). This plant is known by various names in different regions, including Lampung Province, where it is called *julang-jaling*.

Kabau seeds exhibit antimicrobial, antifungal, and antioxidant activities due to their polyphenolic compounds (Teh *et al.* 2022). Riasari *et al.* (2019) reported that the outer peel of kabau from Lampung exhibits antioxidant activity, with an IC_{50} of 17.61 µg/mL. In addition, the ethyl acetate extract of the inner peel of kabau seeds exhibits antioxidant activity, with an IC_{50} of 273.57 ppm (Irawan *et al.* 2018). Ramawati *et al.* (2020) demonstrated that the ethanol extract of the inner peel of kabau seeds exhibits antioxidant activity with an IC_{50} value of 26.77 ppm. Antioxidants can change immunological and oxidative stress parameters and enhance the immune system (Okta *et al.* 2022). These antioxidant activities are attributed to the presence of flavonoids, tannins, saponins, phenols, and triterpenoids in the kabau plant (Rahmawati et al, 2019; Riana *et al.* 2024). GC-MS analysis is required to identify the compounds present in the kabau contains numerous volatile compounds. Antioxidant activity is presumed to help repair damage to blood cells caused by lead exposure. Thus, it is expected that the blood cell count will be maintained at a normal level.

METHODS

Sampling and Preparation of Kabau Simplicia

Kabau pod peel samples were obtained from South Lampung. The preparation of simplicia began with washing the kabau pod peels. A total of 2 kg of kabau

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pod peels were thoroughly washed and then air-dried at room temperature. The drying process aimed to reduce the moisture content of the kabau pod peels. In this study, drying was carried out at room temperature (±25–30°C) to prevent degradation of the active compounds. After drying, the kabau fruit peels were ground into a powder using a blender.

Preparation of Kabau Pod Peel Extract

The extraction of kabau pod peel was carried out by maceration in 2 L of 96% ethanol. The maceration process was conducted for two days, after which the extract was filtered using filter paper to obtain the filtrate. The resulting filtrate was then concentrated by rotary evaporation to remove the solvent, yielding a pure liquid extract free of residual ethanol.

GC-MS Analysis of Kabau Fruit Peel Extract

The GC-MS analysis was conducted to determine the chemical constituents of the kabau pod peel extract (*Archidendron jiringoides* Komariah). Gas Chromatography-Mass Spectrometry (GC-MS) is an analytical instrument used to identify compounds present in a sample. Chromatography is one of the most important chemical separation methods. Kabau fruit peel extract was injected into the GC instrument, and the analysis was carried out for 60 minutes with an injector temperature of 260°C, a detector temperature of 250°C, and a column temperature of 325°C. Helium gas was used as the carrier gas at a flow rate of 1 mL/min. Sample measurement was performed once. The GC-MS identification process detected several bioactive compounds, as indicated by chromatographic peaks and corresponding mass spectra.

***In Vivo* Study**

The mice were obtained from the Lampung Veterinary Center. The mice were randomly divided into six groups, each consisting of four animals based on the Federer formula. The normal control group (KP) received only standard food and water. The positive control group (K+) was administered lead acetate at 60 mg/kg body weight (BW) for 14 days, followed by oral vitamin C at 0.024 mg/kg BW for 21 days to ameliorate lead-induced liver cell damage. The negative control group (K−) received lead acetate at a dose of 60 mg/kg BW for 14 days and was subsequently left untreated for 21 days. The treatment groups P95, P190, and P380 were administered lead acetate at 60 mg/kg BW for 14 days, followed by kabau pod peel extract at 95, 190, and 380 mg/kg BW, respectively, for 21 days (Riana *et al.* 2025). The total treatment duration across all groups was 35 days.

Blood Collections and Enumeration of Erythrocytes and Leukocytes

Blood sample collection was carried out on day 36. The mice were euthanized using chloroform. Subsequently, blood was collected by heart puncture using a 1 cc syringe. The collected blood was then stored in EDTA tubes to prevent coagulation prior to leukocyte and erythrocyte counting. Blood samples were drawn using a leukocyte pipette up to the red-marked line corresponding to a volume of 0.5 in the pipette. The blood was then diluted 20-fold with Turk's solution for erythrocyte counting and 200-fold for leukocyte counting using Hayem's solution. The blood was drawn into a Thoma pipette up to the red line and homogenized for 15-30 seconds. The first 2–3 drops were discarded, and the diluted blood was then dropped onto the counting chamber. The numbers of leukocytes and erythrocytes were subsequently counted using a light microscope. Leukocytes were counted in four large squares, each consisting of 16 smaller squares, while erythrocytes were counted in the central square of the counting chamber. During counting, cells touching the left and upper borders were included, whereas cells touching the right and lower borders were excluded (Tambur 2006). The number of observed cells was calculated using the following formula:

$$\text{Number of cell}/\text{mm}^3 = \frac{N}{V} \times P$$

Notes:
N = Number of leukocytes in the counting chamber
V = Volume of the counting chamber
P = Dilution

Total volume of the leukocyte counting chamber = (1 mm × 1 mm × 0.1 mm) × 4 chambers = 0.4 mm³
Total volume of the erythrocyte counting chamber = (0.2 mm × 0.2 mm × 0.1 mm) × 5 chambers = 0.02 mm³.

RESULTS AND DISCUSSION

The pod peel extract was analyzed using GC–MS to identify the compounds present in the kabau pod peel extract based on their polarity. The analysis results showed the names of the compounds and their retention times. Based on the chromatogram data, there were 17 peaks, indicating the presence of 17 compounds in the kabau fruit peel extract. Of these 17 compounds, 10 were identified as having antioxidant activity, namely pyridine, 2,3-butanediol, 2-furanmethanol, butyrolactone, tetramethyl pyrazine, phenylethyl alcohol, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, hexadecenoic acid methyl ester, n-hexadecanoic acid, and 9-octadecenoic acid methyl ester (Table 1). The antioxidant compounds with the highest concentrations were 2,3-butanediol and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, with percentage area values of 88.5% and 100%, respectively.

2,3-Butanediol is a secondary metabolite that occurs naturally in plants and can also be synthesized

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Table 1 Compounds abundant in the kabau pod peel extract In this reshearch

Name	Retention time	Molecular weight (g/mol)	Molecule formula
Pyridine	4.303	79.1	C_5H_5N
2.3-Butanediol	5.796	90.122	$C_4H_{10}O_2$
2-Furanmethanol	7.075	98.1	$C_5H_6O_2$
Butyrolactone	8.508	86.09	$C_4H_6O_2$
Pyrazine, tetramethyl	13.557	168.19	$C_8H_{12}N_2$
Phenylethyl alcohol	14.416	122.16	$C_8H_{10}O$
4H-Pyran-4-one- 2.3-dyhydro-3.5-dyhidroxy-6-methyl	15.547	144.12	$C_4H_6O_2$
Hexadecenoic acid, methyl ester	34.359	268.4	$C_{17}H_{32}O_2$
n-Hexadecanoic acid	35.181	256.42	$C_{16}H_{32}O_2$
9-Octadecenoic acid, methyl ester	38.508	296.5	$C_{19}H_{36}O_2$

chemically (Bhuyar *et al.* 2021). This compound exhibits free-radical scavenging activity, indicating its potential as an antioxidant. In addition, 2,3-butanediol has been reported to possess anti-inflammatory and anti-aging properties, which contribute to its extensive application in cosmetic formulations (Hak *et al.* 2018). Another compound showing dominant antioxidant activity in kabau fruit extract is 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl**.** This compound belongs to the flavonoid class and contains phenolic functional groups, which are known to play a crucial role in antioxidant mechanisms. Previous studies have demonstrated that 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl exhibits significant antioxidant activity (Illing & Jelita 2018).

Leukocyte and erythrocyte counts were determined using a hemocytometer under a light microscope, and the results were expressed as the mean number of leukocytes and erythrocytes in mouse blood in units of cells/mm³. In the control group, the highest erythrocyte count was observed in KP mice (8.1×10^6 cells/mm³), while the lowest was found in K− mice (5.7×10^6 cells/mm³). In the kabau pod peel extract–treated group, P380 mice showed an erythrocyte count closest to that of KP mice (7.9×10^6 cells/mm³) (Figure 1).

Similarly, the highest leukocyte count in the control group was recorded in KP mice (10.6×10^6 cells/mm³), whereas the lowest was observed in K− mice (7.4×10^6 cells/mm³). In the treatment group, P380 mice exhibited the highest leukocyte count (9.6×10^6 cells/mm³), which was the closest to the KP group (Figure 2). Based on ANOVA analysis, the p-values for erythrocyte and leukocyte counts were 0.935 and 0.340, respectively ($p > 0.05$). These results indicate that administration of kabau pod peel extract had no significant effect on erythrocyte and leukocyte levels in the blood of mice previously exposed to lead. The low leukocyte count in K− mice is thought to result from reduced activity of myeloid precursors, which are derivatives of committed stem cells that produce leukocytes (Sugiharto *et al.* 2020). Lead exposure in mice can suppress blood cell formation in the bone marrow, resulting in decreased leukocyte production (Vakiti & Mewawalla 2023).

Administration of kabau pod peel extract for 21 days showed that the leukocyte count in P380 mice was within the normal range and most closely resembled that of KP mice. Observations also indicated that P95 and P190 mice exhibited leukocyte counts within the normal range, although not as optimal as those observed in P380 mice (Figure 2). Leukocytes play an essential role in the body's defense against infection and tissue damage. This finding demonstrates that administration of kabau fruit peel extract can maintain immune function by keeping blood leukocyte levels within the normal range (Ladokun *et al.* 2015). It is thought that the administration of lead has not caused too much damage and has not interfered with the formation of blood cells.

These results are consistent with the study by Riana *et al.* (2025), which showed that administration of kabau fruit peel from the species *Archidendron bubalinum* did not have a statistically significant effect on increasing erythrocyte and leukocyte counts, although an increase was observed.

CONCLUSION

The GC-MS analysis results showed that the dominant compounds present in the kabau fruit peel extract were 2,3-butanediol and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, which are strongly suspected to act as antioxidant agents that stabilize free radicals and thereby inhibit oxidative stress. Administration of kabau fruit peel extract (Archidendron jiringoides Komariah.) at doses of 95 g/kg BW, 190 g/kg BW, and 380 g/kg BW did not show a significant effect on the leukocyte and erythrocyte counts in the blood of mice previously exposed to lead acetate.

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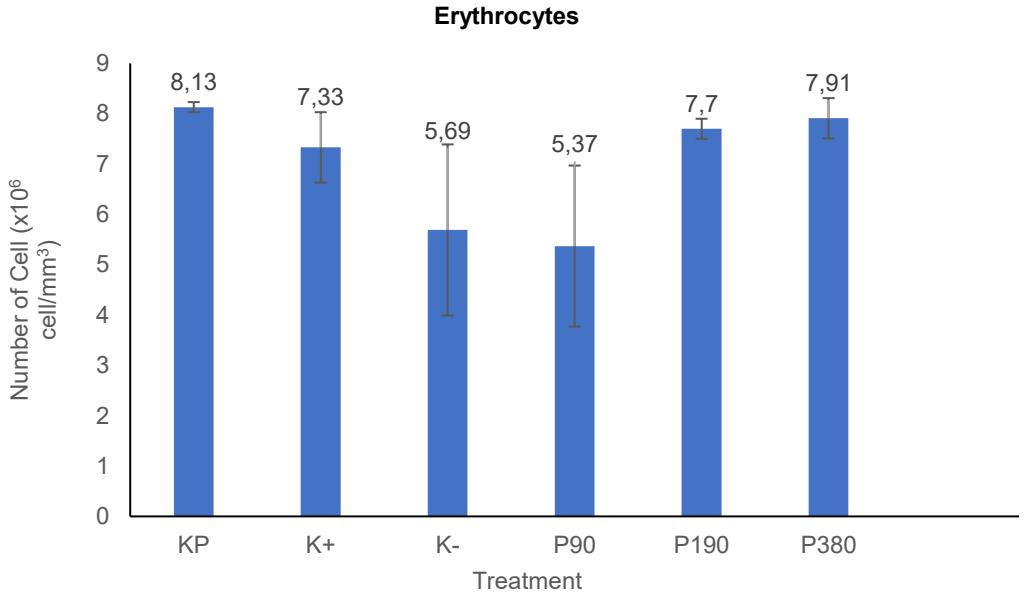

Figure 1 The effect of kabau pod peel extract on increasing erythrocytes count in lead-exposed mice.

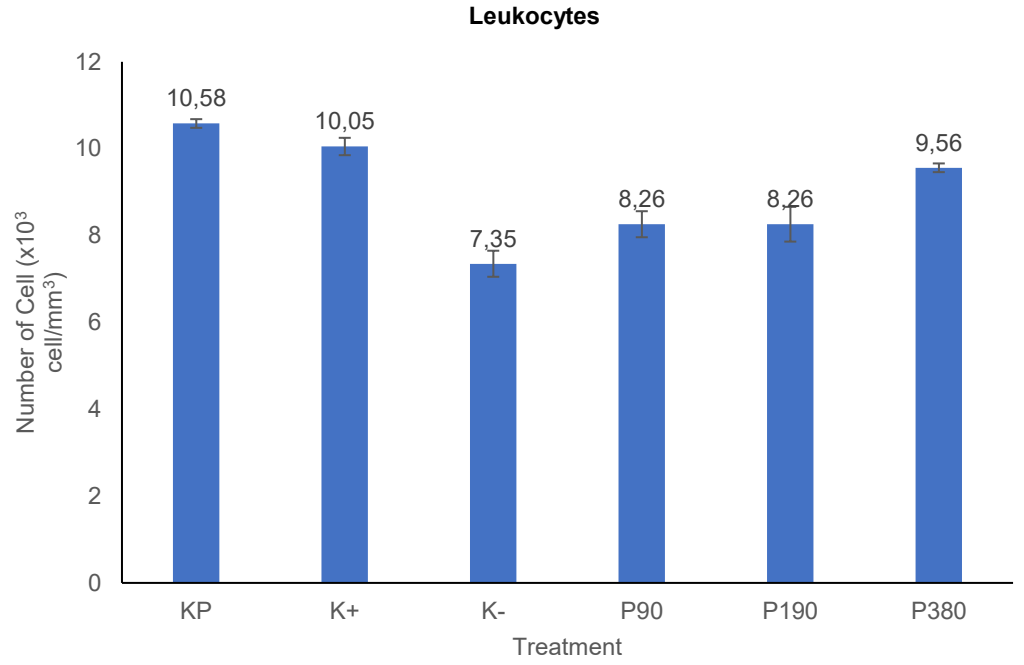

Figure 2 The effect of kabau pod peel extract on increasing leukocytes count in lead-exposed mice.

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