



## The effect of Ethanol Extract of Pirdot (*Saurauria vulcani*) on hematological profile and Keap1-Nrf2 inhibition of white rats induced Rhodamine B

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### ABSTRACT

The study was designed to evaluate the hematological effect of ethanol extract of Pirdot (*Saurauia vulcani*) (EES) in white rats induce rhodamine B and predict interaction of bioactive compound Pirdot to bind active site Keap1-Nrf2. Twenty- four Male Wistar rats (100-200 g) divided into four groups. Group P1 served as group control is administered with CMC 0.5%; group P2 is treated with Rhodamine B 750 mg/kg BW, Group KP1 administered EES 500 mg/kg BW; and Group KP2 is treated with EES 500 mg/kg BW+ Rhodamine B 750 mg/kg BW. Hematological parameters were assessed. The results revealed that red blood cell (RBC), white blood cell (WBC), thrombocyte count, and hemoglobin concentration (Hb) in rats induced Rhodamine B significantly lower than the control group. However, EES could improve the value of hematological profile. Our finding demonstrated that EES normalizes the value of hematological parameters in rats induce Rhodamine B. Moreover, beta-amyrin, pomolic acid and maslinic acid from Pirdot had good binding affinity to Keap1-Nrf2.

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### Introduction

The cases of chemical contaminant exposure in food (2016), for instance Rhodamine B, rose slightly at 66.7% in Indonesia (Ridjal et al., 2022). Rhodamine B contaminant is 1.02% found in food samples, for example chili sauce, red chocolate, cassava chips, red popcorn, and macaroni crackers (Sun et al., 2021). According to the International Agency for Research on Cancer, this synthetic dye is a triphenylmethane

compound that could be a radical compound identified as category 3 carcinogen (Zhu et al., 2022).

Hematological analysis is an important indicator to evaluate the effects of hazardous compounds on health, physiology and adaptation to environmental condition (Ben Taheur et al., 2022). The level of blood profiles, such as red blood cell (RBC), white blood cell (WBC), thrombocytes (TC) and hemoglobin concentration (Hb) were used to

assess health conditions and disease related to toxicant exposure (Powolny et al., 2023).

Because of their biological activities, ethnomedicine with bioactive compounds was used as preventive medicine, for example, natural antioxidants. *Saurauia vulcani* (EES) is a plant with potent antioxidant constituents, a family of Actinidiaceae (G Pasaribu et al., 2020). Additionally, some phytochemical are found in *Saurauia vulcani* or Pirdot, such as genistein, corosolic acid, ursolic acid, maslinic acid, oleanolic acid, stigmasterol and beta sitosterol, that have many biological activities consist of immunostimulant activity, wound healing activity and hepatoprotective properties (Gunawan Pasaribu et al., 2020; E Sinaga et al., 2022). However, its potential role against hematotoxicity of chemical compounds is unclear. Our study explored the potential of bioactive compound EES against Rhodamine B on hematological parameters.

## Materials and Methods

### Ethanol Extract of *Saurauia vulcani* (EES)

Fresh leaves of Pirdot (*S. vulcani*) were obtained from North Sumatera, Indonesia. The leaves were air-dried for seven days at room temperature of  $27 \pm 2^{\circ}$  C. Then the dried leaves were powdered and extracted in 95% ethanol by maceration method. The extract was macerated for 5 days and stirred once a day. After that, the filtrate was obtained by using Whatman filter paper, then the yield was concentrated by using a rotary evaporator.

### Experimental animal and design

Twenty- four male rats (100-200 g) were obtained from the Laboratory of Universitas Sumatera Utara (USU). They were acclimatized at room temperature for 7 days and allowed free access to feed and water ad-libitum. The animals were randomly divided into four groups, consist of group P1 served as group control is administered with CMC 0.5%; group P2 received Rhodamine B 750 mg/kg BW, Group KP1 is administered EES 500 mg/kg BW; and Group KP2 is treated with EES 500 mg/kg BW+ Rhodamine

B 750 mg/kg BW. The treatments were orally administered daily for 30 days. After the last administration, all animals were decapitated and blood samples were collected.

### Hematological parameters

In this study, the blood samples were collected through cardiac puncture and a total sample of 0.5 mL was put into a microcentrifuge tube with 10  $\mu$ L of the EDTA anticoagulant. Hematological parameters, such as red blood cells, white blood cells, and thrombocyte counts were calculated by hematology analyzer (ABX Micros 60) (JA et al., 2022).

### Statistical Analysis

The data was expressed as mean  $\pm$  SD using the statistical package for social science (SPSS) 26. The differences between groups were performed using one-way ANOVA with  $P < 0.05$  considered statistically significant.

### Molecular docking

To evaluate the potency of bioactive compound EES, we predict the interaction using molecular docking study AutoDock Vina. The 3D structure of Keap1 (PDB :4L7B) was obtained from RCSB (<https://www.rcsb.org/>) (Adelusi et al., 2021). Before docking, water molecules and ligands from structure Keap1 were removed, then polar hydrogen atoms were added. The grid box center was set at  $x=-2.4$ ,  $y=2.8$ , and  $z=-29.21$ . The visualization was performed by Discovery studio software.

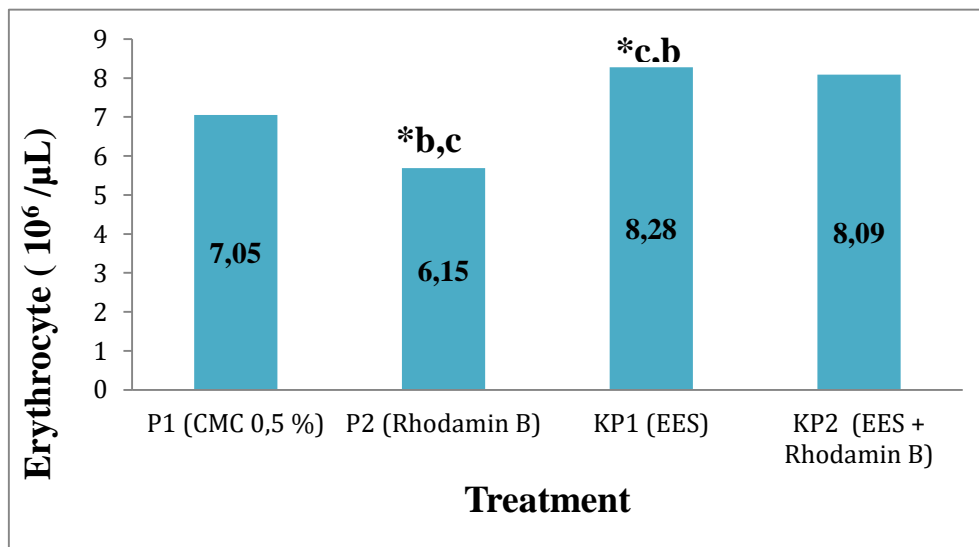
### Results and Discussion

Blood plays a key- component of animal physiology, which carry oxygen, nutrients, fight the infection and maintain homeostasis (Abdelmageed Marzook et al., 2019). Chemical contaminants in food have been a serious public health cause of stress response, for instance hematological disruption (Elcheva et al., 2019). Rhodamine B is a hazardous compound in food that contains weakly basic nitrogenous molecules and non-biodegradable colorful cation

(Sharma et al., 2022). Orally administered this toxic compound infected the gastrointestinal and absorbed it into the blood vessel (Katturajan & Evan Prince, 2021). Hematological profiles consist of RBCs, WBCs, thrombocyte, and hemoglobin concentration were also changed by Rhodamine B. Experimental data showed that Rhodamine B at dose 750 mg/kg BW significant decrease ( $p < 0.05$ ) hematological profile as shown in **Figure 1-4**. This result is similar to the previous observation which reported a decline value in hematological parameter induced  $CCl_4$ , while methanol extract *Dodonaea viscosa* significantly

improved the level of parameters (Tong et al., 2021).

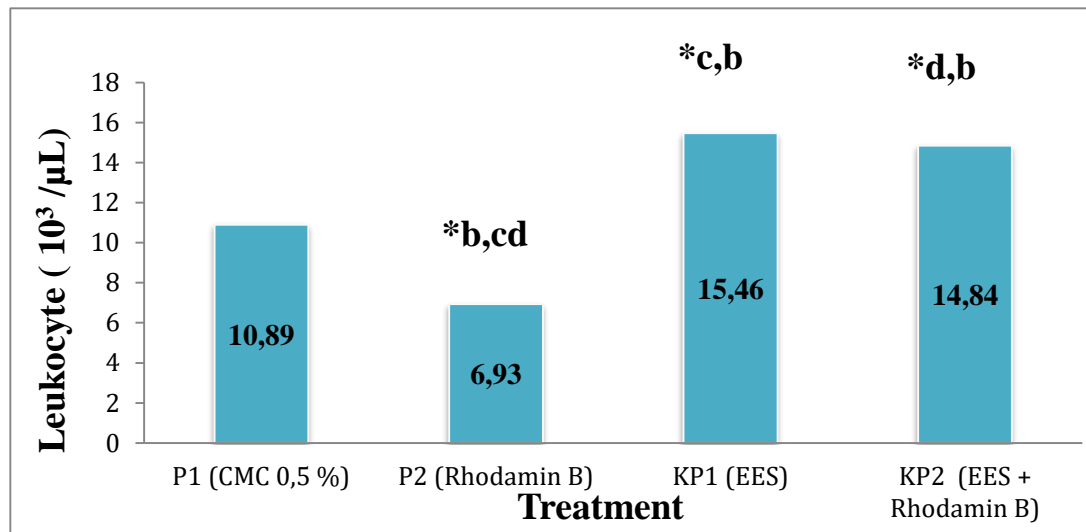
Our study also detected a significant difference in RBC value after Rhodamine B exposure compared to the KP1 group (Fig.1). Contaminated food ingestion induced reaction oxygen species (ROS) and oxidative damage in order to the abundance of oxygen within RBC (Tortora et al., 2019). The plant polyphenol with the high concentration of antioxidant activity reduces oxidative stress and oxygen consumption (Wang et al., 2023). The previous study reported that Pirdot has been a potential bioactive compound to elevate the value of erythrocyte (Erlintan Sinaga et al., 2021).



**Figure 1.** Effect of *S. vulcani* ethanol extracts on RBC value. \*a significant difference, a. P1 = (Control), b. P2 = (Rhodamine B), c. KP1 = (EES) dan d. KP2 = (EES + Rhodamine B)

We established that the reduction in WBC value in Rhodamine B is treated compared to across all groups (Fig. 2). The WBC components interpret the chemical compound exposure to stress situations that promote immunosuppression (Kar et al.,

2019) and development of abnormal cells (Silva et al., 2020). This is in line with previous research that doses of methylmercury reduced WBC value in chickens from the mining area (Abdulmalik et al., 2023).

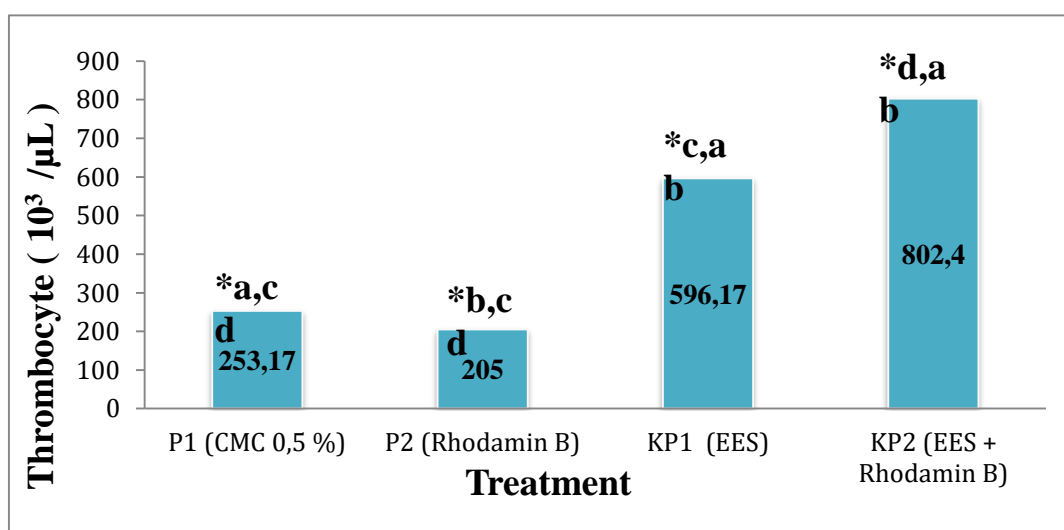


**Figure 2.** Effect of *S. vulcani* ethanol extracts on WBC value. \*a significant difference, a. P1 = (Control), b. P2 = (Rhodamine B), c. KP1 = (EES) dan d. KP2 = (EES + Rhodamine B)

When comparing the Rhodamine B treated group to the EES treated and KP2 group, we found a significant increase in thrombocyte count across all groups (Fig3). Blood coagulation and incorporation of platelets in order to form thrombotic conditions caused by exposure of toxic compounds in a daily intake from foods (GUAN et al., 2023). However, Pirdot as herbal medicine tends to prevent thrombosis by activated fibrinolysis and reduced plasma

viscosity in relation with previous study(Erlintan Sinaga et al., 2019).

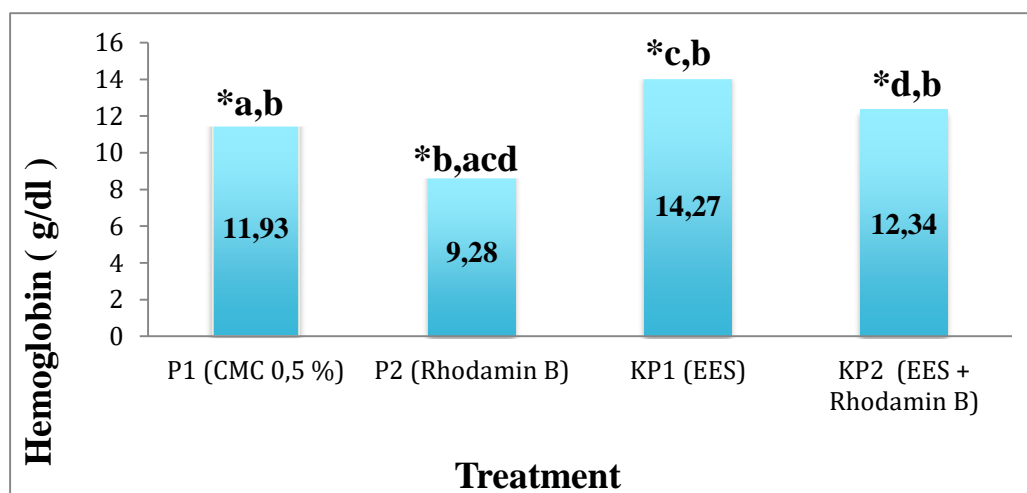
The experimental data also exhibited a significant drop in hemoglobin concentration in Rhodamine B groups than all the groups shown in Fig 4. Hemoglobin controlled the scavenging of RBC due to binding oxygen to form oxyhemoglobin, however the chemical compound could form hypoxic conditions by eliminating heme in reactions with oxyHb (Su et al., 2020).



**Figure 3.** Effect of *S. vulcani* ethanol extracts on Thrombocyte count. \*a significant difference, a. P1 = (Control), b. P2 = (Rhodamine B), c. KP1 = (EES) dan d. KP2 = (EES + Rhodamine B)

Nuclear factor erythroid 2 related factor 2 (Nrf2) is a prominent transcription factor to upregulate antioxidant response element (ARE) transcriptional detoxification enzymes and antioxidant proteins (Xu et al., 2023). The Keap1/ARE is an important signaling pathway to prevent cells from exogenous and endogenous oxidative stress

(Diniyah et al., 2023). Our study predicted a comprehensive docking study to reveal that beta-amyrin has the lowest binding -16.2 kcal/mol to active site Keap1-Nrf2 with hydrogen bond at residue Asn414 and hydrophobic interaction at Tyr334, Tyr572, Phe577 summarized at **Table 1**.



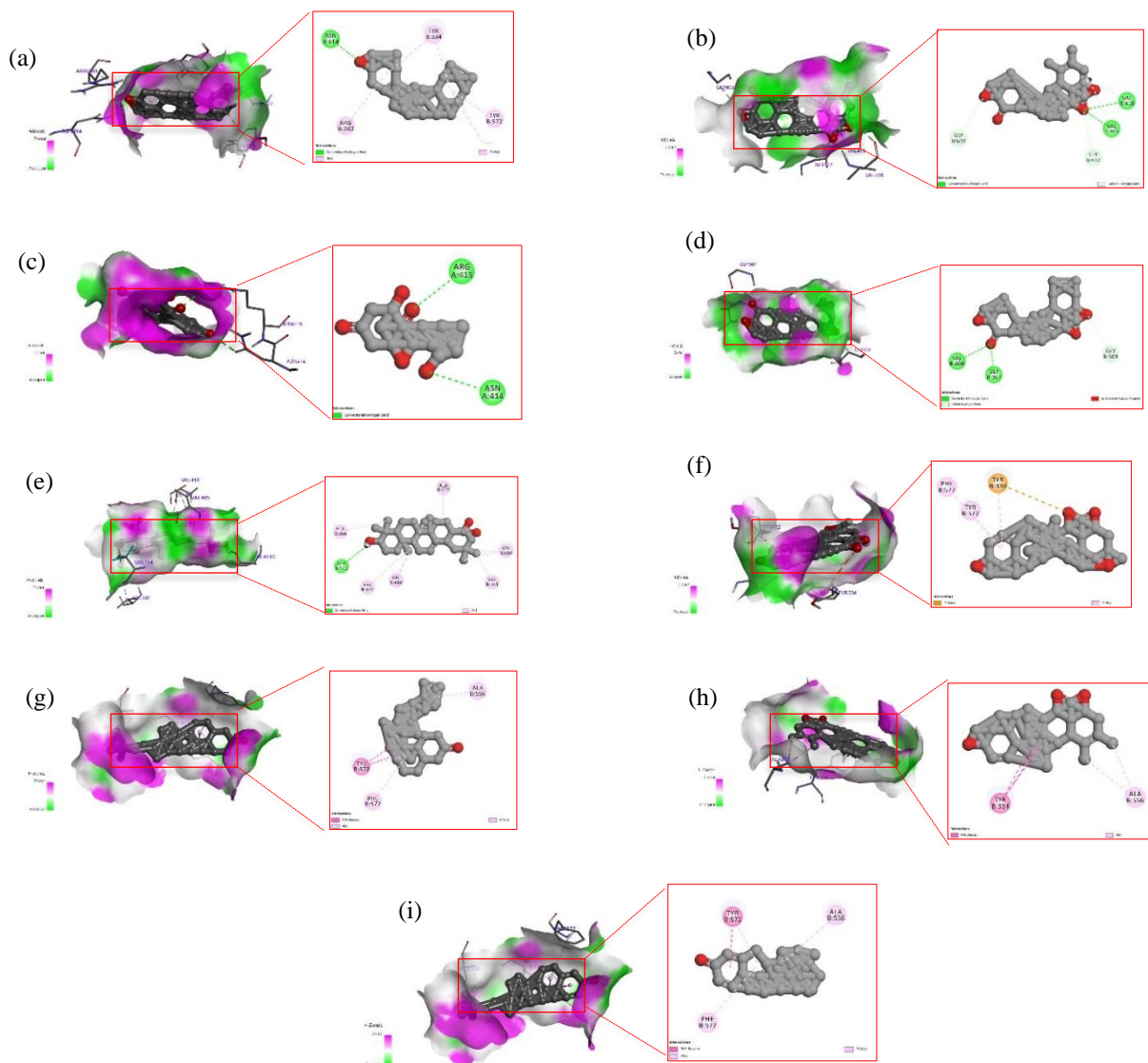
**Figure 4** Effect of *S. vulcani* ethanol extracts on Hemoglobin concentration. \*a significant difference, a. P1 = (Control), b. P2 = (Rhodamine B), c. KP1 = (EES) dan d. KP2 = (EES + Rhodamine B)

Molecular docking study demonstrated that some bioactive Pirdot, such as beta-amyrin, pomolic acid and maslinic acid (de la Torre et al., 2020; Prabhu et al., 2021; Ribeiro et al., 2020), could be the important compounds to elevate the value of

hematological parameters via bind to Keap1-Nrf2. This is in line with previous research that beta amyrin increases WBC count which is suggested to stimulate the immune system in response to chemical compound exposure (Henneh et al., 2020).

**Table 1.** Bioactive compound of Pirdot (EES) bound to Protein Keap1-Nerf2

Compound	Protein Keap1-Nerf2		
	Binding Affinity(kcal/mol)	Hydrogen Bond	Hydrophobic interaction
Beta-amyrin	-16.2	Asn414	Tyr334, Tyr572, Phe577
Corosolic acid	-15.4	Val418, Val465, Ala556	-
Genistein	-9.9	Tyr334, Ser363, Asn382, Ser383, Asn414, Arg415	-
Maslinic acid	-15.5	Gly367, Val606	Ala363, Ile559
Oleanolic acid	-10.1	Val369, Val420, Ala510	Val418, Ala607
Pomolic acid	-15.6	Ser363, Pro384	Tyr334, Ala556, Tyr572, Phe577
Stigmasterol	-11.3	Asn387	Tyr525, Ala556, Tyr572, Phe577w
Ursolic acid	-15.2	Ser363, Pro384	Tyr334, Ala556, Tyr572
Beta- Sitosterol	-11.3	-	Tyr334, Tyr525, Ala556, Tyr572, Phe577



**Figure 5.** (a) Beta amyrin; (b) Corosolic; (c) Genistein; (d) Maslinic acid; (e) Oleanolic acid; (f) Pomolic acid; (g) Stigmasterol; (h) Ursolic acid; (i) Sitosterol

## Conclusions

*S. vulcani* is an ethnomedicine plant that possesses the potential pharmacological. Our finding highlights in vivo the potential of leaves EES against Rhodamine B and predicts the interaction bioactive compound to Keap1/Nrf2 via in-silico approach. It demonstrated significant protection of EES in response to changing hematological parameters by Rhodamine B contaminants. Furthermore, the docking study revealed that some bioactive compounds EES, such as beta amyryn, pomolic acid, and maslinic acid, bind to Keap1-Nrf2 with good binding affinity in order to discover new Nrf2 activators.

## Declaration of competing interest

Authors declare no potential conflicts of interest.

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