Comparative analysis of herbs ability Bajakah wood (*Spatholobus littoralis*), red betel (*Piper crocatum*), and Papuan red fruit (*Pandanus conoideus*) in preventing free radicals due to rhodamine-B injection

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Abstract. Rhodamine B is a carcinogenic additive that can trigger free radicals. Bajakah wood (*Spatholobus littoralis*), red betel (*Piper crocatum*), and Papua red fruit (*Pandanus conoideus*) are herbs with high antioxidants to ward off free radicals. This study analyzed the ability of the three herbs to fight free radicals due to rhodamine B injection. Eighty-five mice (*Mus musculus*) were divided into 17 treatment groups. The groups consisted of mice without herbs and rhodamine B, only with rhodamine B, and a combination of rhodamine B with certain herbal doses. Free radical measurements were carried out using Electron Spin Resonance (ESR), and cell damage was observed through histopathology. The effective doses were 7.27 mg/ml (Bajakah), 3.95 mg/ml (red betel), and 8.83 mg/ml (Papua red fruit). Free radicals decreased to 0.6 A.u (Bajakah), 0.8 A.u (red betel), and 0.7 A.u (Papua red fruit). Histopathology showed a decrease in cell damage by 44.7% (Bajakah), 30.2% (red betel), and 26.6% (Papua red fruit). The results of free radicals correlate with cell damage. Bajakah wood is the most effective herb for preventing free radicals.

1 Introduction

Food safety is a serious problem faced throughout the world. The public is becoming increasingly concerned about the increasing need for food, which is not balanced with good supervision [1]. This incident was exploited by irresponsible parties to gain profits by carrying out illegal actions such as misusing additives and chemicals that are dangerous to health [2–4]. Rhodamine B is a dangerous synthetic dye prohibited in food products because it can damage the liver, kidneys, and spleen [5]. The main factor that makes Rhodamine B capable of causing various chronic diseases is free radical formation due to the interaction of Rhodamine B compounds with biomolecules in the body [6,7]. This research conducted several literature studies and identified three herbs that can be used as antioxidants to ward off free radicals caused by rhodamine B [8–10]. Herbal plants Bajakah wood (*Spatholobus*

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littoralis), red betel (*Piper crocatum*), and Papuan red fruit (*Pandanus conoideus*) were chosen because they have the best antioxidant content compared to other plants. Several previous studies, such as those conducted by Hamzah, showed that Bajakah wood (*Spatholobus littoralis*) is very effective as an antibiofilm for treating inflammation, diabetes mellitus (DM), and diabetic foot infections (DFI) [11]. Bajakah wood (*Spatholobus littoralis*) originates from Central Kalimantan and contains flavonoids, alkaloids, and carotenoids, which are anti-inflammatory, anti-allergic, anti-thrombotic, and anti-viral. Antioxidants in Bajakah wood (Spatholobus littoralis) very quickly ward off free radicals by transferring hydrogen atoms [12].

Red betel (*Piper crocatum*) is a climbing plant that grows in tropical plains and is often used as an ornamental plant because it has beautiful and beautiful colours. Over time, red betel leaves (*Piper crocatum*) have been used as anti-inflammatory, anti-mutagen, and anti-diabetic drugs [13]. Red betel (*Piper crocatum*) contains bioactive compounds of flavonoids, alkaloids, tannins, and essential oils, effectively preventing free radicals and oxidation of saturated fatty acids [9]. Previous research was conducted by Syarifah, which proved that red betel (*Piper crocatum*) was able to suppress the activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals by up to 74.90 ± 0.27% [14]. Another test carried out by Alfarabi showed the efficacy of red betel (*Piper crocatum*) in inhibiting fatty acid oxidation and the free radical cycle [15]. Papuan red fruit (*Pandanus conoideus*) is an endemic plant that grows wild in Papua forests. Papuan red fruit (*Pandanus conoideus*) contains tocopherol, cryptoxanthin, linoleic acid, oleic acid, α-carotene and β-carotene with 2 hydroaromatic rings, 3 ionic rings and pseudoionone [10]. The antioxidant content of Papuan red fruit (*Pandanus conoideus*) can act as an electron donor in warding off free radicals due to rhodamine B. Research conducted by Ning proves that Papuan red fruit (*Pandanus conoideus*) can stimulate nitric oxide production and reduce oxidative stress in endothelial cells [16]. Atmaja also proved that the influence of Papuan red fruit (*Pandanus conoideus*) is very significant in the healing process of incisor wounds, increasing the amount of angiogenesis and collagen density in the skin [17].

All three types of herbs can be used to ward off free radicals, but research has never directly analyzed and compared the most effective herbs to ward off free radicals caused by rhodamine B. This research differs from previous research because it focuses more on observing the type and relative levels of free radicals produced by rhodamine B and the effectiveness of the best herbs in warding off free radicals caused by rhodamine B. The implications of this research for food safety are very broad. In addition to providing an alternative to reduce the negative impacts of rhodamine B on the body, the results of this study can also encourage the development of herbal-based functional food products. Integrating these three herbs into the food system also has the potential to support sustainability because they come from abundant local natural resources and can be cultivated sustainably. It is hoped that the benefits of this research will be able to provide contributions and insight into alternative herbal medicines to control free radicals caused by rhodamine B.

2 Materials and methods

2.1 Preparation of experimental animals

This study used ninety standard laboratory male mice (*Mus musculus*) as research subjects. The mice's body weight (BW) was 28 ± 0.3 grams at three months of age. Acclimatization was carried out for seven days to avoid stress and enable mice to adapt to the cage environment. Mice undergoing the acclimatization process were divided into seventeen groups, as shown in Table 1.

Table 1. Distribution of treatment groups.

Treatment	Code	Amount	Rhodamine B (mg/gr BW)	Antioxidants (mg/ml)
Control	KK (+)	15 mice	-	-
	KK (-)	15 mice	3.5	-
Bajakah wood (*Spatholobus littoralis*)	KKB1	15 mice	3.5	3.63
	KKB2	15 mice		5.45
	KKB3	15 mice		7.27
	KKB4	15 mice		9.08
	KKB5	15 mice		10.90
Papuan red fruit (*Pandanus conoideus*)	KBM1	15 mice	3.5	4.41
	KBM2	15 mice		6.62
	KBM3	15 mice		8.83
	KBM4	15 mice		11.03
	KBM5	15 mice		13.24
Red betel leaves (*Piper crocatum*)	KSM1	15 mice	3.5	1.97
	KSM2	15 mice		2.96
	KSM3	15 mice		3.95
	KSM4	15 mice		4.94
	KSM5	15 mice		5.93

Rhoadamin B was given orally to experimental animals once a day (every 08.00 AM) using a gastric sonde. Bajakah herbal solution (*Spatholobus littoralis*), red betel (*Piper crocatum*), and Papuan red fruit (*Pandanus conoideus*) are given twice a day (every 01.00 PM and 07.00 PM). This treatment was carried out for three weeks. After three weeks of treatment, blood was taken from experimental animals to identify free radical types and their relative levels. The measurement results were also correlated with histopathological observations.

2.2 Relative levels measurement of free radicals

Free radical identification was carried out using electron spin resonance (ESR) with a constant current of 0.2 Ampere. Electron spin resonance (ESR) utilizes one radio frequency (RF) coil and two Helmholtz coils. Electron spin resonance (ESR) was calibrated using pure free radicals of type 2.2-diphenyl-1-picrylhydrazyl (DPPH). Blood tissue is measured after the instrument has been properly calibrated. The frequency variable will be applied to find the value of magnetic field (B) and lande factor (g-factor).

2.3 Histopathological observation

Histopathological observations were carried out using a light microscope integrated with a computer. The magnification was 400 times to see each treatment group's number and type of cell damage. The staining method and sample preparations were carried out using Giemsa according to previous observation procedures [18]. We analyzed the number of cells that experienced damage as a percentage calculated using Equation 1.

$$\text{Percentage of cell damage} = \frac{\Sigma \text{ damage cells}}{\Sigma \text{ observed cells}} \times 100\% \quad (1)$$

2.4 Statistical analysis

This study used analysis of Variance (ANOVA) and Pearson correlation tests as statistical analysis. Calculations were performed using Statistical Software 13.0 (10). The F value indicates a comparison of variability between groups. The greater the F value indicates the possibility of a significant difference. ANOVA test with a p-value <0.05 means significant. A correlation coefficient (r) approaching 1 means a very strong relationship.

2.5 Limitation

This study only measured free radicals using Electron Spin Resonance (ESR) and did not compare it with several other methods. Histopathology was only performed using a light microscope to observe the morphology of red blood cells (RBC), and we did not observe using a Scanning Electron Microscope (SEM) or Complete Blood Count (CBC). This study did not observe changes in body weight, take additional samples, or measure other physiological parameters.

3 Result

In this research, identification of free radicals using electron spin resonance (ESR) was carried out to see free radicals type produced by rhodamine B and to test the effectiveness of Bajakah wood herbs (*Spatholobus littoralis*), red betel (*Piper crocatum*), and Papuan red fruit (*Pandanus conoideus*) to counteract it. Free radical identification in the KK (+) group did not reveal any free radical, even though it had been tracked at the minimum to the maximum frequency that could be carried out by electron spin resonance (ESR). Free radicals appeared in all groups that received rhodamine B, as shown in Table 2. Based on the measurement results, it was found that two types of free radicals were produced by rhodamine-B, namely radical oxygen singlet ($^1O_2$) and radical superoxide anion (O_2^-) [19]. Different types of free radicals were found at different herbal doses, even in the same group. This event shows free radicals' instability when capturing and releasing electron donors provided by antioxidants [20]. Radical oxygen siglet ($^1O_2$) and radical superoxide anion (O_2^-) are very reactive to oxidase reactions that occur in the body. These two types of free radicals are types of oxidative radicals that easily cause oxidative stress [21]. Oxidative stress in the body causes metabolic dysregulation, which can damage glucose, lipid metabolism, and immune function to fight disease [22].

Table 2. Identification of free radical types

Treatment	Code	Frequency	Magnetic field	Lande factor	Free radicals type
Control	KK (+)	-	-	-	-
	KK (-)	21.3×10^5 Hz	8.5×10^{-5} T	1.74	O_2^-
Bajakah wood (*Spatholobus littoralis*)	KKB1	18.4×10^5 Hz	8.5×10^{-5} T	1.50	$^1O_2$
	KKB2	18.4×10^5 Hz	8.5×10^{-5} T	1.50	$^1O_2$
	KKB3	18.4×10^5 Hz	8.5×10^{-5} T	1.50	$^1O_2$
	KKB4	18.7×10^5 Hz	8.5×10^{-5} T	1.53	O_2^-
	KKB5	19.8×10^5 Hz	8.5×10^{-5} T	1.61	O_2^-
Papuan red fruit (*Pandanus conoideus*)	KBM1	18.4×10^5 Hz	8.5×10^{-5} T	1.50	$^1O_2$
	KBM2	18.4×10^5 Hz	8.5×10^{-5} T	1.50	$^1O_2$
	KBM3	18.4×10^5 Hz	8.5×10^{-5} T	1.50	$^1O_2$

Treatment	Code	Frequency	Magnetic field	Lande factor	Free radicals type
	KBM4	18.4×10^5 Hz	8.5×10^{-5} T	1.50	$^1O_2$
	KBM5	21.2×10^5 Hz	8.5×10^{-5} T	1.73	O_2^-
Red betel leaves (*Piper crocatum*)	KSM1	18.4×10^5 Hz	8.5×10^{-5} T	1.50	$^1O_2$
	KSM2	18.4×10^5 Hz	8.5×10^{-5} T	1.50	$^1O_2$
	KSM3	18.9×10^5 Hz	8.5×10^{-5} T	1.54	O_2^-
	KSM4	20.3×10^5 Hz	8.5×10^{-5} T	1.66	O_2^-
	KSM5	18.4×10^5 Hz	8.5×10^{-5} T	1.50	$^1O_2$

Rhodamine B compound does not directly contain free radicals but can disrupt the respiratory chain when it enters and interacts with biomolecules in the body [23]. Further analysis found that although free radicals were found in all treatment groups that received rhodamine B, relative levels of free radicals were successfully suppressed using herbs Bajakah wood (*Spatholobus littoralis*), red betel (*Piper crocatum*), and Papuan red fruit (*Pandanus conoideus*). Figure 1 shows that all types of herbs used in this study reduced relative levels of free radicals lower than the KK (-) group. The third dose is the most optimal dose to ward off free radicals caused by rhodamine-B. Herbs-type Bajakah wood (*Spatholobus littoralis*) is the most effective antioxidant for warding off free radicals caused by rhodamine B.

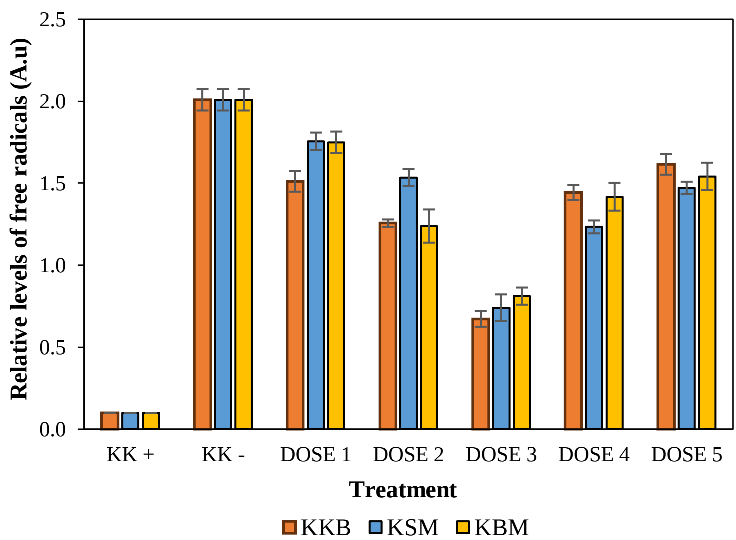

Fig. 1. Correlation of herbal doses to free radicals relative levels. Bajakah wood (KKB), red betel leaves (KSM), and Papuan red fruit (KBM).

Free radicals' relative levels increased again at the fourth dose. This shows that the herbal dose given has been overdosed and made free radicals unstable again. Giving herbal doses exceeding effective doses does not improve the situation; It worsens it [24]. The results of free radical identification were correlated with histopathological observations. The results of histopathological observations showed cell deformation in target cells, microcytic cells, macrocytic cells, hypochromic cells, and irregular shapes (Figure 2). The most cell damage was found in the KK (-) group, which only received rhodamine B without herbal treatment. The least damage to blood cells was found in the KK (+) group, which did not receive rhodamine B and herbs.

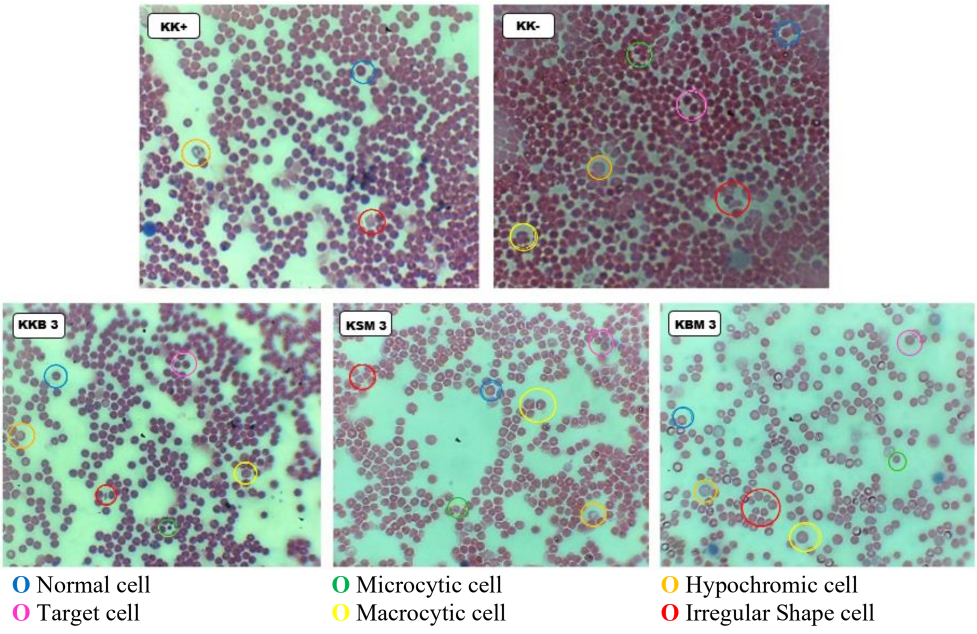

Fig. 2. Histopathology of blood cells that received Bajakah herbal 7.27 mg/ml (KKB 3), red betel leaf 3.95 mg/ml (KSM 3), and Papuan red fruit 8.83 mg/ml (KBM 3).

The KK (+) group showed round blood cells with visible cell wall lines. The negative control group showed blood cells that experienced cell wall degradation and shape changes. Herbal group of red betel (*Piper crocatum*) and Papuan red fruit (*Pandanus conoideus*) have made blood cells have a more regular shape, but the number of deformed cells is still large. Bajakah wood herb (*Spatholobus littoralis*) has the most significant ability to suppress cell damage, so many normal blood cells can still be found, and fewer cells experience deformation. The percentage of cell damage in each group is shown in Figure 3. Herbs red betel (*Piper crocatum*) and Papuan red fruit (*Pandanus conoideus*) were able to suppress cell damage that occurred, but the effect was less significant. Bajakah wood (*Spatholobus littoralis*) is the most significant antioxidant in warding off free radicals so that cell damage can be reduced to a minimum. The results of histopathological observations followed measurements of free radicals' relative levels. In the fourth dose, cell damage increased again when free radicals relative levels also increased.

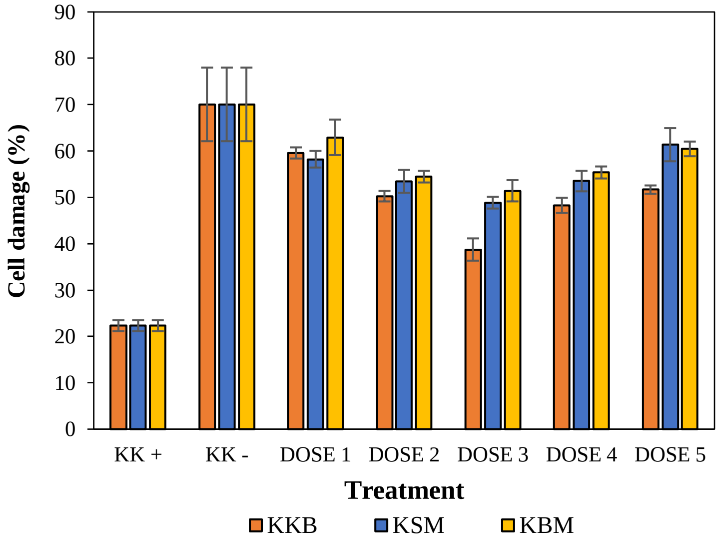

Fig. 3. Correlation of herbal dosage to cell damage percentage. Bajakah wood (KKB), red betel leaves (KSM), and Papuan red fruit (KBM).

Statistical analysis conducted on three types of antioxidants, namely Bajakah wood (*Spatholobus littoralis*), red betel (*Piper crocatum*), and Papua red fruit (*Pandanus conoideus*) showed significant differences and a close relationship between relative free radical levels and levels of cell damage in each treatment. The ANOVA test on the three types of antioxidants gave a very small p-value (<0.05), indicating that the difference between relative free radical levels and cell damage did not occur by chance. Papua red fruit (*Pandanus conoideus*) has an F value of 68.40 with a p-value of 2.67×10^{-6}, indicating a significant difference. Red betel (*Piper crocatum*) has a higher F value, which is 81.89, with a p-value of 1.04×10^{-6}, then Bajakah wood (*Spatholobus littoralis*) has the highest F value, which is 83.29 with a p-value of 9.53×10^{-7}. These results indicate that all antioxidants significantly affect both parameters tested.

Pearson correlation for the three types of antioxidants showed a very strong relationship between relative free radical levels and cell damage. Papua red fruit (*Pandanus considers*) showed a correlation coefficient of 0.962 (p-value 0.0005), followed by red betel (*Piper crocatum*) with a coefficient of 0.940 (p-value 0.0016), and Bajakah wood (*Spatholobus littoralis*) with the highest correlation coefficient, which was 0.965 (p-value 0.0004). These correlation results were statistically significant, indicating that increased free radicals were consistently associated with increased cell damage for all treatments. This confirms a direct relationship between the mechanism of action of antioxidants and the observed biological effects. Overall, the results of ANOVA and Pearson correlation provide a deeper understanding of the effectiveness of each antioxidant in reducing cell damage caused by free radicals. Although all three showed similar statistical significance, Bajakah wood (*Spatholobus littoralis*) showed the strongest correlation between free radicals and cell damage. Red betel (*Piper crocatum*) showed higher statistical values in the ANOVA test, indicating greater differences between the parameters tested. Papuan red fruit (*Pandanus conoideus*) had slightly lower correlation and ANOVA values but showed significant relationships and differences between variables, proving its potential as an antioxidant.

4 Discussion

Rhodamine B injected into the body has been proven to produce superoxide anion free radicals (O_2^-). Rhodamine B compound in the body will trigger an oxidation reaction, which causes electron donation to oxygen in the blood [25]. Oxygen molecule will bind to cytochrome P450, which contains flavine adenine nucleotide (FAD), flavin mononucleotide (FMN), and iron-sulfur (Fe-S), which facilitates the transfer of a single electron from NADPH to oxygen molecule resulting in the formation of radical superoxide anion (O_2^-) [26]. The Electron donor scheme is illustrated in Figure 4.

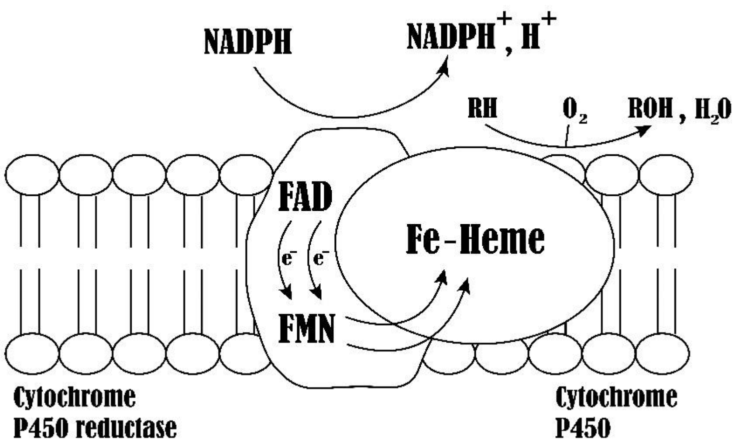

Fig. 4. Illustration of electron donors to oxygen.

Free radicals formed are molecules with unpaired electrons, making them highly reactive to biomolecules in the body, such as proteins, DNA, and chromosomes [27, 28]. Free radicals will not be stable until the electrons in the molecule are paired [29]. This instability encourages free radicals to seek electron pairs, which can damage the structure of the biomolecules they encounter. Excessive accumulation of free radicals can cause oxidative stress or a condition where the number of free radicals exceeds the body's antioxidant defense capacity. Prolonged oxidative stress can result in lipid peroxidation, cell damage, and mitochondrial dysfunction [30–32].

Rhodamine B is included in the group of xenobiotic substances that can be metabolized by the cytochrome P450 enzyme [33]. Cytochrome P450 is a group of enzymes that contain heme and play a role in the metabolism of various compounds. This enzyme can bind oxygen and carbon-based substrates and has redox components such as iron-sulfur (Fe-S). Cytochrome P450 is generally bound to phospholipid membranes and smooth endoplasmic reticulum in cells [34,35]. Cytochrome P450 catalyzes the transfer of single electrons to molecular oxygen through enzymatic activity and produces reactive oxygen species (ROS) such as superoxide anion radicals (O_2^-). Increased levels of rhodamine B in the body can stimulate the induction of the cytochrome P450 enzyme, thereby automatically triggering the emergence of free radicals [36]. The accumulation of free radicals can cause oxidative damage to biomolecules and cause various chronic degenerative diseases. The body has an endogenous antioxidant defense mechanism to protect itself from the damaging effects of free radicals. Still, excessive exposure to xenobiotic substances requires the body to have an additional defense system from exogenous antioxidants [21,37,38].

Our findings show that all herbs used can ward off free radicals caused by rhodamine B. Antioxidant content in Bajakah wood (*Spatholobus littoralis*), red betel (*Piper crocatum*), and Papuan red fruit (*Pandanus conoideus*) each has its character and ability to ward off free radicals. Bajakah wood (*Spatholobus littoralis*) is the most effective herbal in recovery and preventing cell damage caused by free radicals [8–10]. The antioxidant activity of Bajakah wood (*Spatholobus littoralis*) is very strong in stopping the oxidative stress cycle and forming new types of free radical derivatives. Mechanism of Bajakah wood (*Spatholobus littoralis*) in warding off free radicals caused by rhodamine B occurs in three ways, namely hydrogen atom transfer (HAT), single electron transfer (SET), and transition metal chelation (TMC) [39]. Hydrogen atom transfer (HAT) is a mechanism for donating one hydrogen atom to a free radical molecule to form a new, more stable, non-radical species. Single electron transfer (SET) is a mechanism for transferring one electron to a free radical to become a less reactive anion, then interacting again with hydrogen ions to form a new, more stable compound. Transition metal chelation (TMC) is a mechanism to prevent the oxidation process and free radicals formation by binding transfer metal ions which can be catalysts for formation of free radicals [40].

5 Conclusion

From the research that has been carried out, it can be concluded that results of measuring free radicals relative levels correlate with histopathological observations. Pearson correlation for the three types of antioxidants showed a very strong relationship between relative free radical levels and cell damage. Papua red fruit (*Pandanus conoideus*) showed a correlation coefficient of 0.962 (p-value 0.0005), followed by red betel (*Piper crocatum*) with a coefficient of 0.940 (p-value 0.0016) and Bajakah wood (*Spatholobus littoralis*) with the highest correlation coefficient, which was 0.965 (p-value 0.0004). The herb type that is most effective in preventing free radicals caused by rhodamine-B injection is Bajakah wood (*Spatholobus littoralis*) at a dose of 7.27 mg/ml. Free radicals relative levels can be suppressed with Bajakah wood herb (*Spatholobus littoralis*) to 0.6 ± 0.05 A.u and cell damage to 38.7%.

Ethics

Ethics Committee Approval: Brawijaya University Local Ethics Committee of the host approved the research (ethics approval number: 027–KEP–UB 2024 date: 28.02.2024).

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References

1. T. King, M. Cole, J.M. Farber, G. Eisenbrand, D. Zabaras, E.M. Fox, J.P. Hill, Food safety for food security: Relationship between global megatrends and developments in food safety. Trends Food Sci. Technol. **68,** 160–75 (2017).
2. T. Jansen, L. Claassen, I. van Kamp, D.R.M. Timmermans, All chemical substances are harmful.'public appraisal of uncertain risks of food additives and contaminants. Food Chem. Toxicol. **136,** 110959 (2020).
3. L. Mabuza, N. Sonnenberg, N. Marx-Pienaar, Natural versus synthetic dyes: Consumers' understanding of apparel coloration and their willingness to adopt sustainable alternatives. Resour. Conserv. Recycl. Adv. **18,** 200146 (2023).
4. S. Dey, B.H. Nagababu, Applications of food color and bio-preservatives in the food and its effect on the human health. Food Chem. Adv. **1,** 100019 (2022).

5. N. Devi, N. Winarni, I.P. Priyasana, G.A.D. Mayagita, V. Rahmadinha, K.M. Limba, A. Dewi, I.K.N. Sanjaya, N.P.L. Laksmiani, Penetapan Rhodamin B pada sampel lipstik dengan menggunakan Klt-Spektrofotodensitometri *J. Kim. (Journal Chem.)*. **14**, (2020).
6. C. Lops, A. Ancona, K. Di Cesare, B. Dumontel, N. Garino, G. Canavese, S. Hérnandez, V. Cauda, Sonophotocatalytic degradation mechanisms of Rhodamine B dye via radicals generation by micro-and nano-particles of ZnO. Appl. Catal. B Environ. **243**, 629–40 (2019).
7. V. Unsal, M. Cicek, İ Sabancilar, Toxicity of carbon tetrachloride, free radicals and role of antioxidants. Rev. Environ. Health. **36**, 279–95 (2021).
8. R.N.R. Sianipar, L. Suryanegara, W. Fatriasari, E.T. Arung, I.W. Kusuma, S.S. Achmadi, N.I.W. Azelee, Z.A.A. Hamid, The role of selected flavonoids from bajakah tampala (*Spatholobus littoralis* Hassk.) stem on cosmetic properties: A review. Saudi Pharm. J. **31**, 382–400 (2023).
9. H.X. Li, W. Widowati, R. Azis, S.Y. Yang, Y.H. Kim, W. Li, Chemical constituents of the Piper crocatum leaves and their chemotaxonomic significance. Biochem. Syst. Ecol. **86**, 103905 (2019).
10. I.A. Gunawan, R. Fujii, T. Maoka, Y. Shioi, K.M.B. Kameubun, L. Limantara, T.H.P. Brotosudarmo, Carotenoid composition in buah merah (*Pandanus conoideus* Lam.), an indigenous red fruit of the Papua Island. J. Food Compos. Anal. **96**, 103722 (2021).
11. H. Hamzah, S.U.T. Pratiwi, A. Jabbar, A.S. Hafifah, B.A. Al-Fajri, N. Nurhalisah, Bioactivity tracing of the ethanol extract of Bajakah Tampala (*Spatholobus littoralis* Hassk.) typical plant of Kalimantan Island as antibiofilm of *Staphylococcus aureus*. Open Access Maced. J. Med. Sci. **11**, 8–14 (2023).
12. D. Iskandar, N. Widodo, M. Warsito, Y.P.P.A. Rollando, Phenolic content, antioxidant, cytotoxic of fractions of *Spatholobus littoralis* Hassk from Kalimantan, Indonesia. J. Hunan Univ. Nat. Sci. **49** (2022).
13. I.N.E. Lister, C.N. Ginting, E. Girsang, E.D. Nataya, A.M. Azizah, W. Widowati, Hepatoprotective properties of red betel (*Piper crocatum* Ruiz and Pav) leaves extract towards H2O2-induced HepG2 cells via anti-inflammatory, antinecrotic, antioxidant potency. Saudi Pharm. J. **28**, 1182–9 (2020).
14. S.R.S. Kamaruzaman, K.F. Kasim, M.N. Jaafar, The effect of harvesting time on the antioxidant and anti-diabetic activity of *Piper crocatum* (sirih merah) extract. IOP Conference Series: Materials Science and Engineering. **864**, 12211 (2020).
15. M. Alfarabi, M. Bintang, M. Safithri, The comparative ability of antioxidant activity of Piper crocatum in inhibiting fatty acid oxidation and free radical scavenging. Hayati J. Biosci. **17**, 201–4 (2010).
16. N. Xia, C. Schirra, S. Hasselwander, U. Förstermann, H. Li, Red fruit (*Pandanus conoideus* Lam) oil stimulates nitric oxide production and reduces oxidative stress in endothelial cells. J. Funct. Foods. **51**, 65–74 (2018).
17. R.R.D. Atmaja, V.K. Annadiyah, M. Thoyyibah, S. Maimunah, B. Ma'arif, R. Mutiah, I.M. Budi, M. Amiruddin, F.R. Inayatilah, The effect of red fruit oil (*Pandanus conoideus* Lamk.) emulgel on angiogenesis and collagen density in incisive wound healing in mice (*Mus musculus*). J. Adv. Pharm. Technol. Res. **14**, 311–6 (2023).
18. H. Shokrani, H. Norouzian, O. Dezfoulian, Exo-erythrocytic stages of *Haemoproteus* sp. in common buzzard (*Buteo buteo*): A histopathological and molecular study. Int. J. Parasitol. Parasites Wildl. **16**, 64–9 (2021).
19. Z. Zhang, J. Fan, J. Du, X. Peng, Two-channel responsive luminescent chemosensors for dioxygen species: Molecular oxygen, singlet oxygen and superoxide anion. Coord. Chem. Rev. **427**, 213575 (2021).

20. X. Ruan, Y. Sun, W. Du, Y. Tang, Q. Liu, Z. Zhang, W. Doherty, R.L. Frost, G. Qian, D.C.W. Tsang, Formation, characteristics, and applications of environmentally persistent free radicals in biochars: a review. Bioresour. Technol. **281**, 457–68 (2019).
21. T.J. Costa, P.R. Barros, C. Arce, J.D. Santos, J. da Silva-Neto, G. Egea, A.P. Dantas, R.C. Tostes, F. Jimenez-Altayo, The homeostatic role of hydrogen peroxide, superoxide anion and nitric oxide in the vasculature. Free Radic. Biol. Med. **162**, 615–35 (2021).
22. J.E.B.F. Lima, N.C.S. Moreira, E.T. Sakamoto-Hojo, Mechanisms underlying the pathophysiology of type 2 diabetes: From risk factors to oxidative stress, metabolic dysfunction, and hyperglycemia. Mutat. Res. Toxicol. Environ. Mutagen. **874**, 503437 (2022).
23. H. Wang, Y. Zhang, P. Tan, L. Jia, Y. Chen, B. Zhou, Mitochondrial respiratory chain dysfunction mediated by ROS is a primary point of fluoride-induced damage in Hepa1-6 cells. Environ. Pollut. **255**, 113359 (2019).
24. N. Başaran, D. Paslı, A.A. Başaran, Unpredictable adverse effects of herbal products Food Chem. Toxicol. **159**, 112762 (2022).
25. L. Wolski, A. Walkowiak, M. Ziolek, Photo-assisted activation of H2O2 over Nb2O5– The role of active oxygen species on niobia surface in photocatalytic discoloration of Rhodamine B. Mater. Res. Bull. **118**, 110530 (2019).
26. C. Chen, J. Min, L. Zhang, Y. Yang, X. Yu, R. Guo, Advanced understanding of the electron transfer pathway of cytochrome P450s. ChemBioChem. **22**, 1317–28 (2021).
27. A.T. Jalil, M.A. Abdulhadi, L.R. Al-Ameer, O.F. Washeel, S.J. Abdulameer, M.S. Merza, M. Abosaooda, A.A. Mahdi, Free radical based nano cancer therapy. J. Drug Deliv. Sci. Technol. **87**, 104803 (2023).
28. S. Liu, G. Liu, L. Yang, D. Li, M. Zheng, Critical influences of metal compounds on the formation and stabilization of environmentally persistent free radicals. Chem. Eng. J. **427**, 131666 (2022).
29. L. Zhu, M. Luo, Y. Zhang, F. Fang, M. Li, F. An, D. Zhao, J. Zhang, Free radical as a double-edged sword in disease: Deriving strategic opportunities for nanotherapeutics. Coord. Chem. Rev. **475**, 214875 (2023).
30. S. Fei, Y. Xia Y, Z. Chen, C. Liu, H. Liu, D. Han, J. Jin, Y. Yang, X. Zhu, S. Xie, A high-fat diet alters lipid accumulation and oxidative stress and reduces the disease resistance of overwintering hybrid yellow catfish (*Pelteobagrus fulvidraco*♀× *P. vachelli*♂). Aquac. Reports. **23**, 101043 (2022).
31. J-X Ren, C. Li, X-L Yan, Y Qu, Y Yang, Z-N Guo, Crosstalk between oxidative stress and ferroptosis/oxytosis in ischemic stroke: possible targets and molecular mechanisms Oxid. Med. Cell. Longev. **2021**, 6643382 (2021).
32. S. Chen, Q. Li, H. Shi, F. Li, Y. Duan, Q. Guo, New insights into the role of mitochondrial dynamics in oxidative stress-induced diseases. Biomed. Pharmacother. **178**, 117084 (2024).
33. E. Sinaga, U. Hasanah, F.R.P. Sipahutar, Chemopreventive potential of *Saurauia vulcani* Korth in improving Rhodamine B induced hepato-renal carcinoma in rats. Pharmacol. Res. Chinese Med. **9**, 100336 (2023).
34. T. Zeng, G-L Lei, M-L Yu, T-Y Zhang, Z-B Wang, S-Z Wang, The role and mechanism of various trace elements in atherosclerosis. Int. Immunopharmacol. **142**, 113188 (2024).
35. K-K Chen, M. Minakuchi, K. Wuputra, C-C Ku, J-B Pan, K-K Kuo, Y-C Lin, S. Saito, C-S Lin, K.K. Yokoyama, Redox control in the pathophysiology of influenza virus infection. BMC Microbiol. **20**, 1–22 (2020).
36. Z. Jie, J. Liu, M. Shu, Y. Ying, H. Yang, Detection strategies for superoxide anion: A review. Talanta. **236**, 122892 (2022).

37. K. Jomova, R. Raptova, S.Y. Alomar, S.H. Alwasel, E. Nepovimova, K. Kuca, M. Valko, Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. Arch. Toxicol. **97**, 2499–574 (2023).
38. V.I. Lushchak, O. Lushchak, Interplay between reactive oxygen and nitrogen species in living organisms. Chem. Biol. Interact. **349**, 109680 (2021).
39. M.M. Kabanda, A theoretical study of the antioxidant properties of phenolic acid amides investigated through the radical-scavenging and metal chelation mechanisms. Eur. Food Res. Technol. **241**, 553–72 (2015).
40. S.G. Tumilaar, A. Hardianto, H. Dohi, D. Kurnia, A comprehensive review of free radicals, oxidative stress, and antioxidants: overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. *J. Chem.* **2024**, 5594386 (2024).