



Research article

Effect of bandotan leaf extract (*Ageratum conyzoides* L.) on oxidative stress, blood biochemistry and immunity in female quail (*Coturnix coturnix japonica* Temminck & Schlegel, 1848) reared under tropical conditions

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Abstract

Importance of the work: Heat stress causes physiological disorders, decreased immunity and increased mortality in quail in tropical regions. Bandotan leaf extract has the potential to address these issues through its antioxidant activity.

Objectives: To investigate the effects of bandotan leaf extract on oxidative stress, blood biochemistry and immune status in tropical quail (*Coturnix coturnix japonica* Temminck & Schlegel, 1848).

Materials and Methods: The study involved 200 female quail. Bandotan leaf extract was administered daily to birds through their drinking water at concentrations of 0% (as a control), 1.5%, 3% or 4.5%. The experiment was conducted in a completely randomized design. The parameters measured were: stress indicators (oxygen saturation, heterophil-to-lymphocyte ratio, superoxide dismutase activity, glutathione peroxidase and malondialdehyde (MDA) levels); blood biochemistry (cholesterol, glucose); and *ex vivo* bacterial clearance against *Salmonella pullorum*.

Results: The administration of bandotan leaf extract had a significant effect ($p < 0.05$) on MDA levels, blood cholesterol and the resistance of quails to *S. pullorum*. A 3% dose reduced MDA levels by 78.2%, indicating a decrease in oxidative stress and a lowered blood cholesterol level by 15.4%, reflecting improved lipid metabolism. In addition, the extract increased resistance to *S. pullorum* by 5.7%, indicating an enhanced immune response and a reduction in quail mortality by 71.4%.

Main finding: Administering 3% bandotan leaf extract in drinking water reduced oxidative stress, lowered cholesterol, enhanced *ex vivo* antibacterial clearance against *S. pullorum* and decreased mortality in female quail, indicating improved physiological resistance during heat exposure.

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Introduction

Climate change in recent decades has increased average air temperatures in various regions, including Indonesia, posing challenges to environmental sustainability, human wellbeing and livestock health (Nugrahaeningtyas et al., 2018). Specifically, Meteorology, Climatology and Geophysics Agency (2024) reported that air temperatures in Indonesia in 2023 were in the range 20–36°C, with relative humidity of 64.6–95.1%. These conditions exceed the ideal range for quail farming (20–24°C and relative humidity of 50–70%), according to Ulupi et al. (2016). Consequently, the quail population reportedly decreased by 9.07% compared to the previous year (Directorate of Livestock, Fisheries and Forestry Statistics, 2023).

Maheshwari et al. (2017) reported that exposure to high temperatures and humidity can trigger heat stress, which increases metabolic activity and free radical production in the body. This condition causes oxidative stress when the number of free radicals exceeds the capacity of the endogenous antioxidant system to neutralize them, resulting in cell damage, metabolic disorders, and decreased immune function. Furthermore, heat stress can trigger the accumulation of acetyl-coenzyme A, the main precursor of cholesterol biosynthesis (Xiao et al., 2023). Elevated blood cholesterol levels can reduce quail health and affect product quality (Khalifa and Noseer, 2019). Providing exogenous antioxidant supplements is one strategy to address oxidative stress. However, synthetic antioxidants have the potential to cause side effects if not used appropriately, including potential carcinogenicity, cytotoxicity and endocrine system disruption (Esazadeh et al., 2024). Furthermore, residues of these synthetic compounds can accumulate in products, such as eggs and meat, posing a health risk to consumers (Xu et al., 2021). Therefore, utilizing natural ingredients as antioxidant sources is a safer alternative.

Bandotan (*Ageratum conyzoides* L.) is a plant from the *Asteraceae* family that grows wild in tropical regions. Rumape et al. (2023) reported that bandotan leaf extracts from several regions in Indonesia consistently contained active compounds, including flavonoids, phenols, saponins, steroids, terpenoids and tannins. In general, flavonoids and phenols are known to have antioxidant and anti-inflammatory activities, saponins function as immunomodulators and cholesterol lowering agents, steroids have anti-inflammatory effects and regulate lipid metabolism, terpenoids exhibit antimicrobial activity, while tannins are known as astringent compounds with antibacterial and antioxidant properties (Kumar et al., 2021).

Several studies have demonstrated the pharmacological potential of bandotan leaf extract. For example, Buyung et al. (2024) reported that 2% bandotan leaf extract had strong antimicrobial activity against *Staphylococcus epidermidis*. Puspita et al. (2021) found that administering the extract at a dose of 0.01–0.04 g/20 g body weight to hyperlipidemic male rats (*Rattus norvegicus*) reduced total cholesterol and triglyceride levels. Supriyadi et al. (2014) showed that administering 5 mL/chicken/d of bandotan leaf extract reduced total blood cholesterol levels. Detering et al. (2017) reported that consuming 250 mg/d of bandotan leaf extract reduced prostate cancer symptom scores in humans. In addition, Adetuyi et al. (2018) reported that concentrations of 0.25–1.0 mg/mL had significant antioxidant activity against lipid peroxidation in the pancreas and penile tissue of rats induced by Fe²⁺. Based on these findings, bandotan leaf extract has the potential to be developed as a natural ingredient with broad biological activity for quail health.

The benefits of *bandotan* leaf extract have been studied extensively in mice, broiler chickens and humans (Puspita et al., 2021; Supriyadi et al., 2014; Detering et al., 2017); however, its effects have not been evaluated specifically on female quail raised in tropical environments. To date, no studies have examined its role in reducing oxidative stress, improving blood biochemical parameters or enhancing antibacterial clearance against *S. pullorum*. This gap exists because quail have distinct physiological characteristics and oxidative stress responses compared to broiler chickens and other animal models. Therefore, the current study has considerable scientific value by exploring the use of *bandotan* extract as a phytotherapeutic agent to enhance physiological resilience, immune function and overall performance in quail. Based on this rationale, it was hypothesized that *bandotan* extract supplementation may reduce oxidative stress, improve blood biochemical parameters and enhance the immune response to *S. pullorum* in female quail experiencing heat stress.

Materials and Methods

Ethics statements

The research method was reviewed and approved by the Ethics Committee for the Care and Use of Experimental Animals, School of Veterinary Medicine and Biomedical Sciences, IPB University, Indonesia (certificate number: 235/KEH/SKE/VII/2024).

Bandotan leaf extraction

Bandotan leaves were extracted using a maceration method with 96% ethanol as a solvent. The leaves were washed to remove dirt, then dried naturally in the shade for 1 wk, as a modification of the method of Ministry of Health of the Republic of Indonesia (2017) that used oven-drying at 60°C. Once dried, the leaves were ground into a powder. Then, the powder was macerated at a solid-to-solvent ratio of 1:10 (w/v) for 36 hr at room temperature. Next, the maceration results were evaporated using a rotary evaporator to obtain a thick bandotan leaf extract.

Phytochemical analysis

Flavonoids were tested by heating the extract in ethanol, adding 2 N HCl and magnesium powder and spectrophotometric quantification at 425 nm (Krishnaveni et al., 2016). Phenols were tested by adding 1% FeCl₃ and spectrophotometric quantification at 725 nm (Krishnaveni et al., 2016). Tannins were analyzed by heating the extract and adding 1% FeCl₃, with quantification using the trimetric method (Ryanata et al., 2015; Maulida et al., 2020). Triterpenoids and steroids were tested based on Khafid et al. (2023) by soaking the extract in glacial acetic acid and adding H₂SO₄, where triterpenoids (asiaticosides) were also quantitatively characterized using high performance liquid chromatography (Alfarra and Omar, 2014). Saponins were tested using the method of Khafid et al. (2023) based on the formation of stable foam after the addition of 2 N HCl.

Quail husbandry

In total, 200 female quails (*Coturnix coturnix japonica* Temminck & Schlegel, 1848) aged 4 wk were reared for 30 d. The paste extract was diluted with water (1:20), resulting in a stock solution containing 75 mg of extract per 1.5 mL. The bandotan leaf extract was administered daily to birds through their drinking water at concentrations of 0% (control), 1.5% (75 mg), 3% (150 mg) or 4.5% (225 mg), each mixed into 50 mL of drinking water per quail per day. Individual water intake was not measured; however, variation among groups was controlled by providing the same volume of water per cage and by maintaining uniformity in age, initial body weight and husbandry practices to ensure consistent consumption patterns. Feed was provided ad libitum using P-100 feed, containing a maximum of 13% moisture

and minimums of 20% protein, 7% fat, 7% crude fiber and 14% ash. The mineral content included 2.5–3.5% calcium and 0.6–1.0% total phosphorus. Total aflatoxin did not exceed 50 µg/kg and the feed contained at least 0.9% lysine, 0.4% methionine and 0.6% methionine plus cystine (PT. New Hope Indonesia, 2023).

Temperature-humidity index

Temperature-humidity index (THI) measurements were conducted based on a method adapted from Tao and Xin (2003), with the data obtained from dry-bulb and wet-bulb thermometers installed in each cage. Temperature measurements were taken three times daily at 0700 hours, 1200 hours and 1700 hours. The THI values were calculated using Equation 1:

$$\text{THI} = 0.85 \, t_{\text{db}} + 0.15 \, t_{\text{wb}} \quad (1)$$

where THI is the temperature-humidity index, t_{db} is the dry-bulb temperature and t_{wb} is the wet-bulb temperature.

Measurement of oxygen saturation

Oxygen saturation was measured using a pulse oximeter (Family DR FS101; Jakarta, Indonesia), following Jumadin et al. (2023). The device emits two wavelengths of light through the quail's feet and its sensors detect absorption differences between oxygenated and deoxygenated hemoglobin to calculate blood oxygen saturation.

Collection of blood and liver samples

One quail from each experimental unit was selected randomly. Blood was drawn via the jugular vein using a 3 mL syringe. To measure total leukocytes and the heterophil-to-lymphocyte (H/L) ratio, blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant (Ciepliński et al., 2019). Cholesterol levels were measured from serum without anticoagulant, while glucose levels were collected in tubes containing sodium fluoride (Turchiano et al., 2013). For the clearance test, blood was collected in tubes containing heparin anticoagulant (Ciepliński et al., 2019). Liver tissue was obtained after the quails had been euthanized. Liver tissue was removed carefully, rinsed with physiological NaCl solution and used for analysis of superoxide dismutase (SOD) activity and glutathione peroxidase (GPx) and malondialdehyde (MDA) levels.

Measurement of total leukocytes and heterophil-to-lymphocyte ratio

The total leukocyte count was determined by diluting the blood sample with Rees-Ecker solution using a leukocyte pipette, homogenizing the mixture and inserting it into a hemocytometer. Leukocytes were counted at 400× magnification. The number of heterophils and lymphocytes was assessed from Giemsa-stained blood smears examined at 100× magnification and the H/L ratio was calculated by dividing the number of heterophils by the number of lymphocytes (Maheshwari et al., 2017).

Measurement of cholesterol and glucose levels

Total cholesterol levels were measured using the cholesterol oxidase-phenol aminophenazone method (Thomas, 2012). Blood samples were left at room temperature for 30 min, centrifuged at 3,000 revolutions per minute (rpm) for 10 min to separate the serum, then 10 µL of serum was mixed with 1 mL of cholesterol reagent and incubated for 10 min until a pink color formed. The absorbance of the solution was measured using an ultraviolet-visible spectrum (UV-Vis) spectrophotometer at a wavelength of 500 nm.

Blood glucose levels were measured using the glucose oxidase-peroxidase method (Thomas, 2012). Blood samples were centrifuged at 3,000 rpm for 10 min to obtain plasma, which was then mixed with GOD-POD reagent and incubated at 37°C for 10 min. The resulting color intensity was measured using a UV-Vis spectrophotometer at a wavelength of 500 nm.

Measurement of endogenous antioxidants

The SOD enzyme activity was measured using a modified method from Maskar et al. (2015). A 0.5 g liver tissue sample was homogenized in 2.5 mL phosphate buffer and centrifuged at 3,000 rpm and 4°C for 10 min. The supernatant was mixed with 96% ethanol and chloroform, vortexed and centrifuged again to obtain the final supernatant. A 1 mL supernatant sample was reacted with 2.8 mL sodium carbonate buffer and 100 µL epinephrine. The SOD activity was measured using a UV-Vis spectrophotometer at 480 nm.

The GPx enzyme activity was measured using a modified method from Lefiana et al. (2021). A sample of 100 µL of liver homogenate was mixed with 200 µL of phosphate buffer (pH 7.0) and centrifuged to obtain the supernatant.

The supernatant was reacted with 0.1 M phosphate buffer (pH 7.0) containing EDTA, glutathione, glutathione reductase and nicotinamide adenine dinucleotide phosphate, then incubated before adding hydrogen peroxide (H₂O₂) as a substrate. The GPx activity was measured based on the decrease in absorbance at 340 nm using a UV-Vis spectrophotometer.

Measurement of malondialdehyde

The MDA levels were measured using a modified method from Ulhusna et al. (2019). A 0.5 g sample of liver tissue was homogenized in 5 mL of phosphate buffered saline (pH 10.2) and centrifuged to obtain the supernatant. A 1 mL sample of the supernatant was mixed with 4 mL of 0.25 M hydrochloric acid (HCl) containing 15% trichloroacetic acid, 0.38% thiobarbituric acid and 0.5% butylated hydroxytoluene, then heated at 80°C for 1 hr. After re-centrifugation, the MDA levels were determined by reading the absorbance at 532 nm using a UV-Vis spectrophotometer.

Ex vivo bacterial clearance assay

The *ex vivo* bacterial clearance assay against *S. pullorum* was conducted following the method of Jackson et al. (1998). Blood samples for this assay were collected into heparinized tubes. The *S. pullorum* strain used was a field isolate from chickens that had been stored in the Bacteriology Laboratory of the Faculty of Veterinary Medicine, Bogor Agricultural University (IPB), Indonesia since 2000. A suspension of *S. pullorum* was prepared at a concentration of 1×10^8 colony-forming units (CFU)/mL. Whole blood and bacterial suspension were mixed at a ratio of 1:1 (0.5 mL blood + 0.5 mL bacterial suspension; final volume, 1.0 mL) and incubated for 30 min at room temperature to allow interaction between blood immune components and bacteria. After incubation, 100 µL of the mixture was spread onto nutrient agar using the spread plate method (Cappuccino and Sherman, 2013) and incubated at 37°C for 24 hr. The number of colonies formed was then counted as an indicator of bacterial survival after incubation with quail blood. The bacterial control was prepared by mixing the bacterial suspension with sterile physiological saline (1:1, v/v) without the addition of blood. The control was treated under the same conditions as the test samples, including incubation and plating procedures. Bacterial clearance was expressed relative to the corresponding bacterial control using the following formula:

Clearance (%) = [(CFU in control – CFU after incubation with blood) / CFU in control] × 100.

Higher clearance values indicate greater antibacterial activity of quail blood.

Quail mortality

Mortality in each treatment was determined based on counting the number of birds that died during the rearing period.

Data analysis

A completely randomized design was used, with four treatments and five replications, with each replication consisting of 10 female quails aged 4 wk and reared for 30 d. Data were analyzed using the SPSS® version 22.0 software (IBM Corp.: Armonk, NY, USA). The Shapiro-Wilk test was used to assess data normality. When the data were normally distributed, they were subjected to analysis of variance (ANOVA). Where significant differences were observed ($p < 0.05$), Tukey's test was used for multiple comparisons. Results were presented as mean ± SD. The mortality data were analyzed descriptively. An individual quail was considered the experimental unit for variables derived from biological sample analyses, whereas the cage served as the experimental unit for mortality, as the treatment was administered through drinking water at the cage level.

Results and Discussion

Environmental conditions of the cage

The environmental conditions of the quail cages were measured at three times (morning, afternoon, evening) based on monitoring the dry air temperature, wet air temperature and the THI. The measurement results are presented in Table 1.

Table 1 Quail cage environmental temperature conditions.

Measurement time	t_{db} (°C)	t_{wb} (°C)	THI
0700 hours	25.50	23.64	25.22
1200 hours	33.00	28.72	32.35
1700 hours	28.00	24.84	27.52

t_{db} = dry-bulb temperature; t_{wb} = wet-bulb temperature; THI = temperature-humidity index

The daytime THI value reached 32.35, which according to Hahn et al. (2009) is classified as an emergency (≥ 30.0). This condition causes heat stress in quail due to difficulty in evaporating body heat. The accumulation of excess heat caused physiological and metabolic disorders, characterized by panting as a thermoregulatory mechanism. Exposure to extreme heat increases the production of free radicals that trigger oxidative stress, alter blood metabolites, such as cholesterol and glucose, and reduce immunity (Ulupi et al., 2016).

Phytochemical content of bandotan leaf extract

The results of the quantitative and qualitative phytochemical analyses of bandotan leaf extract are presented in Table 2. Flavonoids, phenols and tannins are polyphenolic compounds with antioxidant properties that can neutralize free radicals and prevent cellular oxidative damage (Agbafor et al., 2015). These compounds stabilize and inhibit free radicals through their hydroxyl groups and transition metal-chelating ability (Dai and Mumper, 2010). Tannins also help maintain redox balance and may reduce cholesterol levels by inhibiting HMG-CoA reductase activity (Fraga-Corral et al., 2021). Asiaticoside, a triterpenoid, has anti-inflammatory activity through suppression of pro-inflammatory mediators (Fernanda et al., 2022). Plant steroids, such as sitosterol and stigmasterol, may lower cholesterol levels and support lipid metabolism (Cheng et al., 2020). In addition, saponins exhibit antimicrobial, anti-inflammatory and hypocholesterolemic activities by disrupting bacterial membranes, inhibiting cyclooxygenase and binding bile acids for excretion (Tugiyanti et al., 2019).

Stress indicators

The administration of bandotan leaf extract significantly affected ($p < 0.05$) malondialdehyde (MDA) levels, an indicator of oxidative stress. The results of the analysis of various stress indicators are presented in Table 3.

Table 2 Phytochemical content of bandotan leaf extract.

Phytochemical compound	Content
Quantitative:	
Flavonoids (%)	3.33
Phenols (%)	6.57
Tannin (%)	0.06
Asiaticoside (mg/g)	5.67
Qualitative:	
Steroids	+
Saponins	+

Table 3 Stress-related indicators of female quail given bandotan leaf extract at different concentrations.

Parameter	Bandotan leaf extract concentrations			
	0%	1.5%	3%	4.5%
Oxygen saturation (%)	87.87±7.08	89.53±3.86	92.33±7.85	92.73±4.24
H/L	0.69±0.04	0.77±0.10	0.82±0.11	0.74±0.08
SOD (unit/mL)	56.41±3.87	56.52±2.30	62.04±6.64	61.00±7.78
GPx (unit/g)	30.75±5.19	30.65±5.63	34.21±1.91	30.56±2.56
MDA (nmol/mL)	1.01±0.29 ^a	0.43±0.27 ^b	0.22±0.15 ^b	0.32±0.16 ^b

H/L = heterophil-to-lymphocyte ratio; SOD = superoxide dismutase activity; GPx = glutathione peroxidase; MDA = malondialdehyde.

Mean±SD within each row followed by different lowercase superscript letters are significantly different ($p < 0.05$). Rows without superscript letters indicate no significant differences ($p \geq 0.05$) among means.

The administration of bandotan leaf extract at concentrations of 3% and 4.5% showed a tendency to maintain quail oxygen saturation within the normal range (92.33–92.73%). Oxygen saturation below 90% is categorized as hypoxemia (Abu et al., 2024). Although the differences in oxygen saturation among treatment groups were not statistically significant, quails receiving 3% and 4.5% bandotan leaf extract tended to show higher oxygen saturation values than those in the control and 1.5% groups. This trend may be related to the iron (Fe) content in bandotan leaves, which has been reported to contribute to hemoglobin synthesis and oxygen transport during heat stress exposure (Agbafor et al., 2015). This interpretation is in agreement with Salam et al. (2025), who reported that the Fe content of moringa leaves was involved in hemoglobin formation. Fe is an essential micronutrient required for hemoglobin synthesis, oxygen transport, electron transfer in cells and the synthesis of various enzymes in the body (Obeagu, 2025).

The administration of bandotan leaf extract had no significant effect on the H/L ratio, with values in the range 0.69–0.82. According to Cotter (2023), the normal H/L ratio range in poultry is 0.02–0.66, so the values in the current study were higher than the normal limit. This condition was consistent with the environmental measurements that showed a THI value of 32.35, which is classified as an emergency level (Table 1). This high H/L ratio was likely due to the relatively short treatment duration, which did not provide sufficient time for the quail to show significant changes.

The administration of bandotan leaf extract to quail did not significantly increase the activity of endogenous antioxidant enzymes, namely SOD and GPx, although a slight increase was observed at the 3% concentration. However, the MDA levels decreased significantly by 78.2% compared to the control, indicating a substantial reduction in oxidative

stress. This decrease in MDA could be related to the content of flavonoids, phenols and tannins, which acted as direct antioxidants through several mechanisms. Flavonoids and phenols can donate electrons or hydrogen to neutralize free radicals, thereby halting oxidative chain reactions (Platzer et al., 2022). Tannins can bind prooxidant metal ions, such as Fe^{2+} and Cu^{2+} , preventing the formation of hydroxyl radicals (Guan et al., 2025). Therefore, the bandotan leaf extract could reduce oxidative stress directly without relying on endogenous enzymatic pathways, in line with Özbilgin et al. (2022), who reported phenolic compounds reduced the MDA levels in quail meat. Although often considered a weed, bandotan leaves contain bioactive compounds capable of neutralizing free radicals. In the current study, the bandotan leaf extract reduced the MDA levels by 78.2% at a dose of 150 mg (equivalent to 3%), while moringa (*Moringa oleifera*) leaf powder at a concentration of 5% reduced MDA levels by 30.8% compared to the control (Salam et al., 2025).

The difference in effectiveness between the 3% and 4.5% concentrations of bandotan leaf extract on SOD and GPx enzyme activities and MDA levels could be explained by specific biological mechanisms. For example, increasing the antioxidant concentration does not always elicit a proportional response due to saturation, a phenomenon in which the enzymatic system reaches its maximum capacity (Vossen et al., 2011). At higher concentrations, flavonoids and phenols may also act as prooxidants or exhibit reduced bioavailability due to interactions among compounds (Galati and O'Brien, 2004). Research in chickens has shown that increasing doses of astaxanthin are effective only up to a certain point, without further significant increases in SOD, GPx, or reductions in MDA (Pertiwi et al., 2022). Therefore, a 3% concentration of bandotan leaf extract should be optimal for suppressing oxidative stress.

Blood biochemistry

The administration of the bandotan leaf extract significantly affected quail blood cholesterol levels; however, there was no significant effect on blood glucose levels. The results of the blood biochemistry analysis are presented in Table 4.

Based on the results, all treatment groups experienced a decrease in cholesterol levels compared to the control group. The greatest reductions occurred at doses of 1.5% and 3%, with cholesterol levels of 197.70 ± 5.33 mg/dL and 190.52 ± 5.92 mg/dL, respectively. At the 4.5% dose, the cholesterol levels were slightly higher than at the two lower doses but remained within the range of 180–220 mg/dL considered normal by Al-Tikriti (2019). In contrast, the control group had a cholesterol level of 225.29 ± 3.07 mg/dL, which was above the normal limit. The effectiveness of bandotan appeared to be higher than for other herbal interventions. Kurnia et al. (2024) reported that administering 10% noni juice (*Morinda citrifolia*) to quail was only able to reduce blood cholesterol by 14.72%, whereas the 3% dose of bandotan extract in the current study resulted in a greater reduction (15.4%) than in the control, despite using a much lower concentration than noni juice. This finding was consistent with the report by Supriyadi et al. (2014), which showed that administering 5 mL of bandotan leaf extract (equivalent to 2.08% in drinking water) was able to reduce blood cholesterol in broiler chickens.

Saponins lower cholesterol through a bile acid binding mechanism in the digestive tract, forming an insoluble complex that is excreted in the feces (Li et al., 2024). The loss of these bile acids encourages the liver to withdraw cholesterol from the blood circulation to synthesize new bile acids (Tugiyanti et al., 2019). Tannins contribute by inhibiting the HMG-CoA reductase enzyme, a key enzyme in the cholesterol biosynthesis

pathway (Fraga-Corral et al., 2021). The increase in cholesterol levels at a dose of 4.5% was associated with the accumulation of active compounds that are difficult to absorb by the digestive tract. Nakamura et al. (2024) explained that bandotan contains at least 15 phytochemicals, including the dominant compound 5,7,2',4'-tetrahydroxy-6,3'-diprenylisoflavone 5-O-(4''-rhamnosylrhamnoside) which has a very high molecular mass (714.29 Da). Based on the principles of Lipinski et al. (2001), molecules with a mass >500 Da have low bioavailability, have difficulty crossing the intestinal membrane and tend to be retained in the mucosa, increasing the metabolic burden and elimination process. The accumulation of these high-mass compounds can lead to saturation of detoxification and metabolic pathways, triggering a hormetic response and a shift from beneficial effects to physiological stress at high doses. This condition disrupts lipid metabolism regulation and re-escalates cholesterol levels (Jodynis-Liebert and Kujawska, 2020).

The administration of bandotan leaf extract at various concentrations did not significantly affect blood glucose levels, with all groups remaining within the normal range of 135.00–383.40 mg/dL (Scholtz et al., 2009). This indicated that bandotan leaf extract did not have a clear hypoglycemic effect, with glucose homeostasis in quail being maintained through the balance of insulin and glucagon hormones (Braun and Sweazea, 2008).

Immunity

The administration of bandotan leaf extract had no significant effect on total leukocyte count; however, it significantly affected the *ex vivo* clearance of *S. pullorum*. The results of the immunity test are presented in Table 5.

Table 4 Blood biochemistry of quail given bandotan leaf extract

Parameter	Bandotan leaf extract			
	0%	1.5%	3%	4.5%
Cholesterol (mg/dL)	225.29±3.07 ^a	197.70±5.33 ^c	190.52±5.92 ^c	208.83±4.79 ^b
Glucose (mg/dL)	164.66±29.86	172.45±30.49	178.56±76.92	154.34±82.08

Mean±SD within the same row followed by different lowercase superscript letters are significantly different ($p < 0.05$). A row without superscript letters indicate no significant differences ($p \geq 0.05$) among means.

Table 5 Leukocyte count and *ex vivo* bacterial clearance of *Salmonella pullorum* in female quail given bandotan leaf extract at different concentrations.

Parameter	Bandotan leaf extract			
	0%	1.5%	3%	4.5%
Leukocytes ($10^3/\text{mm}^3$)	20.27±2.88	23.50±7.93	24.87±10.94	16.32±3.35
<i>S. pullorum</i> clearance (%)	93.26±1.84 ^a	98.54±1.71 ^b	98.54±1.71 ^b	99.99±0.00 ^b

Mean±SD within the same row followed by different lowercase superscript letters are significantly different ($p < 0.05$). A row without superscript letters indicate no significant differences ($p \geq 0.05$) among means.

The leukocyte values in the quail were in the range 16.32×10^3 – 24.87×10^3 cells/mm³ and within the normal range of 13.00×10^3 – 26.00×10^3 cells/mm³, according to Wardiny et al. (2012). Although not statistically significant, there was considerable inter-individual variation, indicating differences in physiological responses to the bioactive compounds in the bandotan leaf extract. This was related to the hormetic properties of the phytochemical compounds, which are stimulative at low to moderate concentrations but can be neutral or reduced at high concentrations (Jodynys-Liebert and Kujawska, 2020).

Based on the results of the *ex vivo* bacterial clearance assay (Table 5), *S. pullorum* exhibited lower survival after incubation with blood from quails treated with bandotan leaf extract than after incubation with blood from the control group, as reflected by the higher bacterial clearance values. This finding suggests that bandotan leaf extract enhanced the antibacterial activity of quail blood. In the present study, bandotan leaf extract increased bacterial clearance to 99.99%, which was comparable to the value of approximately 99.96% reported for moringa leaf extract by Salam et al. (2025).

This effect may be associated with the bioactive compounds present in bandotan leaf extract, particularly phenolic compounds, flavonoids and tannins, which have been reported to contribute to antibacterial activity. Mechanistically, phenolic and flavonoid compounds may disrupt the integrity of bacterial cell membranes through protein denaturation and increased membrane permeability, thereby accelerating bacterial cell lysis. Tannins may also contribute by inhibiting essential enzymes required for bacterial metabolism and replication, ultimately leading to bacterial death. The increase in bacterial clearance without a significant change in leukocyte count suggests that bandotan leaf extract may have improved the functional antibacterial capacity of quail blood rather than leukocyte number.

Quail mortality

The administration of the bandotan leaf extract at concentrations of 3% and 4.5% reduced quail mortality by 71.4% compared to the control group. Mortality data for quail given bandotan leaf extract is presented in Fig. 1.

The decrease in quail mortality was related to the pharmacological activity of bioactive compounds in the bandotan leaf extract, which have antioxidant and antibacterial properties (Agbafor et al., 2015). Based on the results of the

ex vivo bacterial clearance assay (Table 5), quail in the treatment groups showed greater clearance against *S. pullorum* than those in the control group. Furthermore, lower liver MDA levels in the treatment groups (Table 3) indicated reduced oxidative stress. The combined antioxidant and antibacterial effects may have worked synergistically to suppress infection, reduce oxidative damage and lower mortality in female quail.

Conclusion

Administering bandotan leaf extract through drinking water, especially at a dose of 3%, was effective in reducing oxidative stress, as indicated by a 78.2% reduction in MDA, lowering cholesterol by 15.4%, enhancing *ex vivo* antibacterial clearance against *S. pullorum*, and reducing mortality by 71.4% compared to the control. These findings indicate that the extract improved physiological resistance during heat exposure and that a 3% dose could be practically applied to reduce mortality associated with heat stress or infection. Further research is needed to evaluate the long-term effects of administering bandotan leaf extract on egg production performance and egg quality in quail.

Conflict of Interest

The authors declare that there are no conflicts of interest.

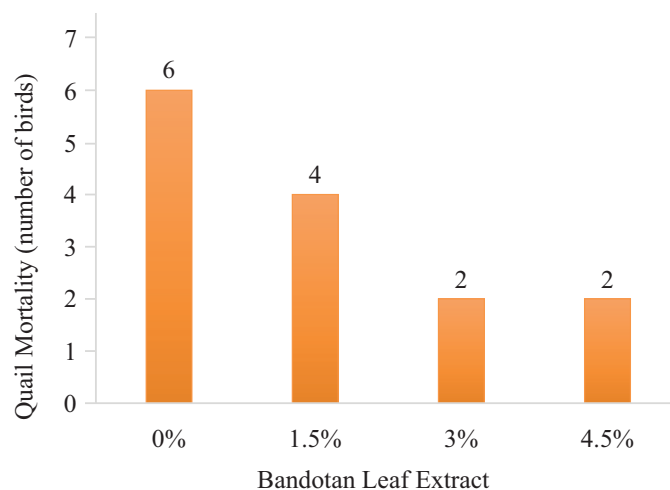


Fig. 1 Mortality of female quail given bandotan leaf extract at different concentrations during the experimental period.

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References

- Abu, K., Khraiche, M.L., Amatoury, J. 2024. Obstructive sleep apnea diagnosis and beyond using portable monitors. *Sleep Med.* 113: 260–274. doi.org/10.1016/j.sleep.2023.11.034
- Adetuyi, F.O., Karigidi, K.O., Akintimehin, E.S., Adeyemo, O.N. 2018. Antioxidant properties of *Ageratum conyzoides* L. (*Asteraceae*) leaves. *Bangladesh J. Sci. Ind. Res.* 53: 265–276. doi.org/10.3329/bjsir.v53i4.39190
- Agbafor, K.N., Engwa, G.A., Obiudu, I.K. 2015. Analysis of chemical composition of leaves and roots of *Ageratum conyzoides*. *Int. J. Curr. Res. Aca. Rev.* 3: 60–65
- Alfarra, H.Y., Omar, M.N. 2014. HPLC separation and isolation of asiaticoside from *Centella asiatica* and its biotransformation by *A. Niger*. *Int. J. Pharm. Med. & Bio. Sc.* 3: 1–8
- Al-Tikriti, S.S.A. 2019. The genetic selection for four generations and its effect on the blood biochemical parameters in the white quail. *Adv. Anim. Vet. Sci.* 7: 151–156. doi.org/10.17582/journal.aavs/2019/7.3.151.156
- Braun, E.J., Sweazea, K.L. 2008. Glucose regulation in birds. *Biochem Mol Biol.* 151: 1–9. doi.org/10.1016/j.cbpb.2008.05.007
- Buyung, E., Edy, H.J., Mansauda, K.L. 2024. Uji aktivitas antijerawat ekstrak daun bandotan (*Ageratum conyzoides* L) terhadap bakteri penyebab jerawat. *Pharmacon.* 13: 403–408. doi.org/10.35799/pha.13.2024.49088. [in Indonesian]
- Cappuccino, J.G., Sherman, N. 2013. *Microbiology: A Laboratory Manual*. 8th ed. Pearson Education, Boston, MA, USA.
- Cheng, Y., Chen, Y., Li, J., Qu, H., Zhao, Y., Wen, C., Zhou, Y. 2020. Dietary β -sitosterol regulates serum lipid level and improves immune function, antioxidant status and intestinal morphology in broilers. *Poult. Sci.* 99: 1400–1408. doi.org/10.1016/j.psj.2019.10.025
- Ciepliński, M., Kasprzak, M., Grandtke, M., Steliga, A. 2019. The effect of dipotassium EDTA and lithium heparin on hematologic values of farmed brown trout *Salmo trutta* (L.) spawners. *Aquac. Int.* 27: 227–239. doi.org/10.1007/s10499-018-0308-5
- Cotter, P.F. 2023. Aviary caged hens with unusual lymphocytes and normal H/L ratios. *Asian J. Res. Anim. Vet. Sci.* 6: 115–122. doi.org/10.9734/AJRAVS/2023/v6i2238
- Dai, J., Mumper, R.J. 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* 15: 7313–7352. doi.org/10.3390/molecules15107313
- Detering, M., Steels, E., Koyyalamudi, S.R., Alliffranchini, E., Bocchietto, E., Vitetta, L. 2017. *Ageratum conyzoides* L. inhibits 5-alpha-reductase gene expression in human prostate cells and reduces symptoms of benign prostatic hypertrophy in otherwise healthy men in a double blind randomized placebo controlled clinical study. *Biofactors* 43: 789–800. doi.org/10.1002/biof.1389
- Directorate of Livestock, Fisheries and Forestry Statistics. 2023. *Livestock in figures 2023*. https://www.bps.go.id/en/publication/2023/12/22/5927b06e1dcde219f76ccc59/livestock-in-figures-2023.html, 22 December 2023
- Esazadeh, K., Ezzati Nazhad Dolatabadi, J., Andishmand, H., Mohammadzadeh-Aghdash, H., Mahmoudpour, M., Naemi Kermanshahi, M., Roosta, Y. 2024. Cytotoxic and genotoxic effects of tert-butylhydroquinone, butylated hydroxyanisole and propyl gallate as synthetic food antioxidants. *Food Sci. Nutr.* 12: 7004–7016. doi.org/10.1002/fsn3.4373
- Fernenda, L., Ramadhani, A.P., Syukri, Y. 2022. Review: Aktivitas pegagan (*Centella asiatica* (L.) urban) pada kulit. *J. Sains Farm. Klin.* 9: 237–244. doi.org/10.25077/jsfk.9.3.237-244.2022. [in Indonesian]
- Fraga-Corral, M., Otero, P., Cassani, L., et al. 2021. Traditional applications of tannin rich extracts supported by scientific data: Chemical composition, bioavailability and bioaccessibility. *Foods* 10: 1–33. doi.org/10.3390/foods10020251
- Galati, G., O'Brien, P.J. 2004. Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. *Free Radic. Biol. Med.* 37: 287–303. doi.org/10.1016/j.freeradbiomed.2004.04.034
- Guan, Q., Cao, Y., Zhang, Y., Zhou, B., Wang, J., Baranenko, D., Cifuentes, A., Ibañez, E., Yuan, C., Lu, W. 2025. Dual roles of tannins as polyphenols and metal complexing agents in the prevention and treatment of Alzheimer's disease: Exploring the potential of dietary interventions. *Trends Food Sci. Technol.* 163: 105177. doi.org/10.1016/j.tifs.2025.105177
- Hahn, G.L., Gaughan, J.B., Mader, T.L., Eigenberg, R.A. 2009. Thermal indices and their applications for livestock environments. In: DeShazer, J.A. (Ed.). *Livestock Energetics and Thermal Environmental Management*. American Society of Agricultural and Biological Engineers. New York, NY, USA. pp. 113–130.
- Jackson, G.J., Madden, J.M., Hill, W.E., Klontz, K.C. 1998. Investigation of food implicated in illness. https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam, 13 October 2025
- Jodynys-Liebert, J., Kujawska, M. 2020. Biphasic dose-response induced by phytochemicals: Experimental evidence. *J. Clin. Med.* 9: 718. doi.org/10.3390/jcm9030718
- Jumadin, L., Satyaningtjas, A.S., Manalu, W., Ekastuti, D.R., Nisa, C., Hendrawan, M.Y., Yuvensius, Milna, R. 2023. Kadar glukosa, kolesterol dan kadar asam urat darah puyuh pada fase starter, grower, dan layer. *J. Veteriner.* 24: 494–504. doi.org/10.19087/jveteriner.2023.24.4.494. [in Indonesian]
- Khafid, A., Wiraputra, M.D., Putra, A.C., Khoirunnisa, N., Putri, A.A.K., Suedy, S.W.A., Nurchayati, Y. 2023. Uji kualitatif metabolit sekunder pada beberapa tanaman yang berkhasiat sebagai obat tradisional. *Bull. Anat. Fisiol.* 8: 61–70. doi.org/10.14710/baf.8.1.2023.61-70. [in Indonesian]
- Khalifa, M.I., Noseer, E.A. 2019. Cholesterol quality of edible eggs produced by quail fed diets containing probiotic and/or ginger (*Zingiber officinale*). *Livest. Res. Rural Dev.* 31: 165. http://www.lrrd.org/lrrd31/10/mrwa31165.html
- Krishnaveni, G., Sailaja, O., Kumar, K.K. 2016. Phytochemical screening and quantitative analysis of hexane, acetone, methanol and water extracts of *Salicornia virginica* by UV-spectrophotometry. *Pharm. Lett.* 8: 52–56

- Kumar, M., Pratap, V., Nigam, A.K., Sinha, B.K., Singh, M.K., Gour, J.K. 2021. Plants as a source of potential antioxidants and their effective nanoformulations. *J. Sci. Res.* 65: 57–72. doi.org/10.37398/JSR.2021.650308
- Kurnia, S.D., Sholikhati, A., Dewi, O.Y., Islamiyah, N. 2024. Low density lipoprotein dan high density lipoprotein darah puyuh yang telah diberi suplementasi pada air minum. *J. Med. Indones.* 5: 47–52. [in Indonesian]
- Lefiana, D., Dasrul, D., Sugito, S., Ardyes, R. 2021. Pengaruh ekstrak tomat dan wortel terhadap kadar kolesterol dan enzim glutation peroksidase (GPx) hati tikus putih hiperkolesterolemik. *JLAH.* 4: 27–32. doi.org/10.32530/jlah.v4i1.359. [in Indonesian]
- Li, M.-Z., Wang, L., Chen, G.-J., Chen, Z.-X. 2024. Biopolymer-based sequestrants for lowering cholesterol: Structures, in vitro bile acid anion binding effects and interaction mechanisms. *J. Funct. Foods.* 113: 106002. doi.org/10.1016/j.jff.2024.106002
- Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J. 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 46: 3–26. doi.org/10.1016/S0169-409X(00)00129-0
- Maheshwari, H., Sasmita, A.N., Farajallah, A., Achmadi, P., Santoso, K. 2017. Pengaruh suhu terhadap diferensial leukosit serta kadar malondialdehid (MDA) burung puyuh (*Coturnix coturnix japonica*). *Bioma.* 13: 22–30. doi.org/10.21009/bioma13(2).4. [in Indonesian]
- Maskar, D.H., Hardinsyah, Damayanti, E., Astawan, M., Wresdiyati, T., Hermanianto, J., Winandita, T. 2015. Pengaruh kedelai produk rekayasa genetik terhadap kadar malonaldehid, aktivitas superoksida dismutase dan profil darah pada tikus percobaan. *J. Nutr. Food Res.* 38: 41–50. doi.org/10.22435/pgm.v38i1.4421.41-48. [in Indonesian]
- Maulida, S., Hakim, A.R., Mohtar, M.S. 2020. Analisis kadar tanin ekstrak etanol kulit batang kemiri (*Aleurites moluccana* (L.) willd) dengan metode titrimetri. *J. Pharm. Care Sci.* 1: 85–93. [in Indonesian]
- Meteorology, Climatology and Geophysics Agency. 2024. Monthly air temperature anomalies 2023. <https://www.bmkg.go.id/iklim/anomali-suhu-udara-rata-rata-tahun-2024>, 22 December 2023
- Ministry of Health of the Republic of Indonesia. 2017. Farmakope Herbal Indonesia Edisi II. Jakarta, Indonesia. [in Indonesian].
- Nugrahaeningtyas, E., Baek, C.-Y., Jeon, J.-H., Jo, H.-J., Park, K.-H. 2018. Greenhouse gas emission intensities for the livestock sector in Indonesia, based on the national specific data. *Sustainability.* 10: 1912. doi.org/10.3390/su10061912
- Obeagu, E.I. 2025. Iron homeostasis and health: understanding its role beyond blood health - a narrative review. *Ann. Med. Surg.* 87: 3362–3371. doi.org/10.1097/MS9.00000000000003100
- Özbilgin, A., Kara, K., Urçar Gelen, S. 2022. Effect of citrus flavonoid on storage time and meat quality of pharaoh quail (*Coturnix pharaoh*). *Braz. J. Poult. Sci.* 24: 1–10. doi.org/10.1590/1806-9061-2022-1645
- Pertiwi, H., Nur Mahendra, M.Y., Kamaludeen, J. 2022. Astaxanthin as a potential antioxidant to improve health and production performance of broiler chicken. *Vet. Med. Int.* 14: 1–9. doi.org/10.1155/2022/4919442
- Platzer, M., Kiese, S., Tybussek, T., Herfellner, T., Schneider, F., Schweiggert-Weisz, U., Eisner, P. 2022. Radical scavenging mechanisms of phenolic compounds: A quantitative structure-property relationship (QSPR) study. *Front. Nutr.* 9: 882458. doi.org/10.3389/fnut.2022.882458
- PT. New Hope Indonesia. 2023. Nutritional content of P-100 laying quail feed. <https://h5.lazada.co.id/products/karungan-pakan-p100-50kg-makanan-burung-puyuh-petelur-new-hope-indonesia-i7596620161.html>, 13 July 2024
- Puspita, I., Fakhruddin, F., Irawan, Y. 2021. Pengaruh pemberian ekstrak etanol herba bandotan (*Ageratum conyzoides*) terhadap penurunan kadar kolesterol total dan trigliserida pada mencit (*Mus musculus*) hiperlipidemia. *J. Borneo Cendekia.* 5: 85–95. doi.org/10.54411/jbc.v5i1.228. [in Indonesian]
- Rumape, O., Ischak, N.I., Ishak, S.A. 2023. Toksisitas ekstrak daun bandotan (*Ageratum conyzoides* L.) sebagai insektisida nabati terhadap mortalitas hama ulat *Spodoptera frugiperda*. *Jamb. J. Chem.* 5: 31–45. [in Indonesian]
- Ryanata, E., Palupi, S., Azminah, A. 2015. Penentuan jenis tanin dan penetapan kadar tanin dari kulit buah pisang masak (*Musa paradisiaca* L.) secara spektrofotometri dan permanganometri. *Calyptra.* 4: 1–16. [in Indonesian]
- Salam, A., Ulupi, N., Maheshwari, H. 2025. Stress indicators, immunity and performance of quail during growth period with the supplementation of moringa leaf meal (*Moringa oleifera* L.) in feed. *Int. J. Vet. Sci.* 14: 737–743. doi.org/10.47278/journal.ijvs/2025.008
- Scholtz, N., Halle, I., Flachowsky, G., Sauerwein, H. 2009. Serum chemistry reference values in adult japanese quail (*Coturnix coturnix japonica*) including sex-related differences. *Poult. Sci.* 88: 1186–1190. doi.org/10.3382/ps.2008-00546
- Supriyadi, E., Roslizawaty, Zuhrawati, D. 2014. Pengaruh pemberian ekstrak daun bandotan (*Ageratum conyzoides* L.) terhadap kadar total kolesterol darah ayam broiler. *J. Med. Vet.* 8: 108–109. [in Indonesian]
- Tao, X., Xin, H. 2003. Acute synergistic effects of air temperature, humidity and velocity on homeostasis of market-size broilers. *Trans. ASAE.* 46: 491–497. doi.org/10.13031/2013.12971
- Thomas, L. 2012. Labor und Diagnose, 8th ed. TH-Books Publishing Company Ltd. Frankfurt, Germany. [in German]
- Tugiyanti, E., Iriyanti, N., Apriyanto, Y.S. 2019. The effect of avocado seed powder (*Persea americana* Mill.) on the liver and kidney functions and meat quality of culled female quail (*Coturnix coturnix japonica*). *Vet. World.* 12: 1608–1615. doi.org/10.14202/vetworld.2019.1608–1615
- Turchiano, M., Nguyen, C., Fierman, A., Lifshitz, M., Convit, A. 2013. Impact of blood sample collection and processing methods on glucose levels in community outreach studies. *J. Environ. Public Health.* 256151: 1–4. doi.org/10.1155/2013/256151
- Ulhusna, F.A., Winarto, A., Wresdiyati, T. 2019. The profile of superoxide dismutase and malondialdehyde level in the liver tissue of hypercholesterolemic rats treated with *Holothuria nobilis* polysaccharide. *J. Kedokt. Hewan.* 13: 37–44. doi.org/10.21157/j.ked.hewan.v13i2.13189
- Ulupi, N., Afnan, R., Rukmiasih, R. 2016. Level of ammonia, dust, production performance and egg quality of laying hens on cage and litter system in tropical area. *IJSBAR.* 3: 339–348
- Vossen, E., Ntawubizi, M., Raes, K., Smet, K., Huyghebaert, G., Arnouts, S., De Smet, S. 2011. Effect of dietary antioxidant supplementation on the oxidative status of plasma in broilers. *J. Anim. Physiol. Anim. Nutr.* 95: 613–620. doi.org/10.1111/j.1439-0396.2010.01041.x
- Wardiny, T. M., Retnani, Y., Taryati. 2012. Pengaruh ekstrak daun mengkudu terhadap profil darah puyuh starter. *J. Ilm. Teknol. Peternak.* 2: 110–120. [in Indonesian]
- Xiao, M., Xu, J., Wang, W., et al. 2023. Functional significance of cholesterol metabolism in cancer: from threat to treatment. *Exp. Mol. Med.* 55: 1982–1995. doi.org/10.1038/s12276-023-01079-w
- Xu, X., Liu, A., Hu, S., et al. 2021. Synthetic phenolic antioxidants: metabolism, hazards and mechanism of action. *Food Chem.* 353: 129488. doi.org/10.1016/j.foodchem.2021.129488