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Antibacterial activity of asam gelugur (*Garcinia atroviridis*) fruit extract and productive performance of Japanese quails (*Coturnix coturnix japonica*) fed with *Garcinia atroviridis* powder

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Abstract The HPLC analysis detected 435.14±6.67 mg/g (43.51%) of hydroxycitric acid (HCA) in the crude extract. Acetone extracts at the greatest concentration (200,000 µg/ml) were found to inhibit the growth of *E. coli* and *S. enterica*, with inhibition zones of 35.53 and 33.08 millimeters (mm), respectively. The MIC against *E. coli* and *S. enterica* was 12,500 µg/mL. The MBC values required to kill *E. coli* and *S. enterica* were 25,000 and 12,500 µg/mL, respectively. The results demonstrated a statistically significant increase in the final body weight of quail chicks supplemented with 0.2% commercial organic acids and those receiving 10 and 20 g/kg of *G. atroviridis* powder, relative to the control group, at 7–8, 8–9, and 3–9 weeks of age ($P < 0.05$). Furthermore, these findings supported the use of *G. atroviridis* as a feed additive for Japanese quails. Specifically, dietary supplementation with 10–20 g/kg of *G. atroviridis* powder significantly improves the productive performance of Japanese quails at 7–9 weeks of age.

Keywords: Antibacterial activity, *Garcinia atroviridis*, Productive performance, *Coturnix coturnix japonica*

Introduction

The Japanese quail has long been a popular poultry species for farming due to its meat and eggs being a good source of protein. This ongoing demand has driven the development of more efficient production processes to response consumer needs. The Japanese quail is considered an economically important animal due to the consumption of its meat and eggs. Quails are easy to raise, resilient, disease-resistant, and adaptable to different environments. Furthermore, the ongoing population growth has resulted in an increased need for animal

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protein sources such as chicken and quail meat, which are easily digestible, inexpensive, and ideal for customers. As a result, demand for quail meat has risen. In the past, the use of antibiotics or harmful chemicals in animal feed and drinking water was common in quail farming to increase the production performance, promote gut health, and improve carcass quality to meet consumer demand. However, the inappropriate use of these substances can lead to residues in animal products and antibiotic resistance in consumers. Consequently, medicinal plants like *G. atroviridis*, which are rich in diverse organic acids possessing significant pharmacological and biological activities, represent a promising feed additive to enhance the productive performance, carcass characteristics, and meat quality of Japanese quails.

Garcinia atroviridis Griff. ex T. Anders (asam gelugur), a medium-sized fruit tree, is a member of the Guttiferae family. This species is naturally distributed across Peninsular Malaysia, India, Myanmar, and Thailand (Mackeen *et al*., 2002; Tan *et al*., 2014). The genus Garcinia comprises approximately 180 known species globally, with 29 species reported specifically in Thailand (Ritthiwigrom *et al*., 2013). Of these, five species (*G. atroviridis, G. dulcis, G. mangostana, G. nigrolineata*, and *G. scortechinii*) are endemic to Southern Thailand (Phongpaichit *et al*., 2006; Kerddonfaek, 2010). The plant is highly prevalent in Southern Thailand, with abundant growth reported specifically in the Sai Khao sub-district of Khok Pho district, Pattani province (Niyomdecha *et al*., 2021). Ecologically, *G. atroviridis* flourishes in lowland areas, demonstrating adaptability to both clay loam and sandy clay loam soil types. The flowering period typically occurs between January and February, with subsequent fruit production commencing in February. The main harvest season generally extends from July to September (Niyomdecha *et al*., 2021). *G. atroviridis* is widely employed in culinary applications as a flavoring agent (Lim, 2012). Furthermore, it is recognized as a medicinal plant due to its rich chemical profile. Research indicates that the plant contains a range of organic acids, including citric acid, tartaric acid, malic acid, ascorbic acid, pentadecanoic acid, octadecanoic acid, and dodecanoic acid (Sebola *et al*., 2011). Research by Niyomdecha *et al*. (2022a) further established that hydroxycitric acid (HCA) is the crucial organic acid in *G. atroviridis*, quantified at 495.01±2.13 mg/g (49.50%). This HCA exhibited significant antibacterial properties, with a minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 12,500 and 25,000 µg/mL, respectively, against *E. coli* and *S. enterica*. Several studies have reported that the fruit of *G. atroviridis*, due to its high HCA and diverse organic acid content, possesses a wide array of beneficial bioactivities. These properties include antibacterial, antitumour, antifungal, anti-inflammatory and management of high blood pressure. Furthermore, the plant is known to inhibit

fatty acid synthesis, promote animal health, and prevent diseases in animals (Pangsuban *et al*., 2009; Hamidon *et al*., 2017), stimulate appetite, balance gut microbiota, and promote intestinal health. Organic acids act as acidifiers, inhibiting the growth of pathogenic bacteria while increasing beneficial bacteria, resulting in a healthier digestive system and improved animal growth. The antibacterial activity of *G. atroviridis* fruit extract was assessed, and the productive performance of Japanese quails fed *G. atroviridis* powder was investigated.

Materials and methods

Antibacterial activity assay

Plant material

Fresh fruits of *G. atroviridis* were collected during July and August 2023 from the Sai Khao sub-district, Khok Pho district, Pattani province, Thailand. The *G. atroviridis* plant material was formally identified by Assoc. Prof. Dr. Charan Leeratiwong at the Prince of Songkla University Herbarium. A voucher specimen (accession number: A. Niyomdecha 001) has been deposited at the Prince of Songkla University Herbarium, located within the Department of Biology, Faculty of Science, Prince of Songkla University.

Microorganisms

The test bacterial strains employed for the antibacterial activity assay were *Escherichia coli* (*E. coli*; gi: CP033762.1) and *Salmonella enterica* (*S. enterica*; gi: KX355299.1). Both strains were obtained from the Faculty of Veterinary Science, Prince of Songkla University, Songkhla, Thailand.

Bacterial culture

The bacterial stock culture was initially prepared by culturing on Mueller Hinton Agar (MHA) plates, followed by incubation at 37°C for 18 to 24 hours. Subsequently, a single bacterial colony was inoculated into 100 mL of Mueller Hinton Broth (MHB) and incubated at 37°C for 4 hours. The final bacterial culture density required for the assay was standardized to 1.0x108 colony forming units per mL (cfu/mL). This standardization was achieved by diluting the fresh liquid culture and comparing its turbidity to a 0.5 McFarland standard density (Niyomdecha *et al*., 2022a).

G. atroviridis fruit extraction

Fresh *G. atroviridis* fruits were initially prepared by slicing them into small pieces and then drying them in a hot air oven at a temperature range of 55–60°C for three days. The resulting dried-sliced fruit was subsequently ground into a powder using an electric blender. This powder was then subjected to maceration with acetone at a 2:4 L (w/v) ratio at room temperature for a duration of thirty days. Following maceration, the mixtures were filtered, and the filtrate was concentrated by removing the solvent (acetone) using a rotary evaporator at a temperature of 50–55°C under reduced pressure. This protocol, adapted from the method described by Plodpai *et al*. (2013), yielded the crude *G. atroviridis* fruit extract, which was then stored in a refrigerator at 4°C until required for further analysis.

Quantification of hydroxycitric acid (HCA) in *G. atroviridis* fruit extract

The quantification of hydroxycitric acid (HCA) within the *G. atroviridis* fruit extract was performed using High-Performance Liquid Chromatography (HPLC). The analysis employed a Hewlett-Packard 1100 Series (HPLC-1) system equipped with a Diode Array Detector (DAD). HCA was detected at a wavelength of 210 nm. A precise amount of the acetone extract (10.26 mg) was accurately weighed and dissolved in 10 mL of 0.1% phosphoric acid (H_3PO_4). The mixture was thoroughly mixed using a vortex mixer for 1 minute. Prior to injection into the HPLC system, the sample was meticulously filtered through a 0.22 µm nylon membrane to remove particulates. 95% Potassium hydroxycitrate tribasic monohydrate (Sigma, USA) was used as the external standard for calibration and accurate HCA measurement. The acetone extract was separated using a ZORBAX Eclipse XDB-C8 column with dimensions of 150×4.6 mm id. and 5 µm particle size. The mobile phase comprised 0.1% H_3PO_4 in deionised water. The sample injection volume was set at 20 µl, and the flow rate was kept at 0.5 ml/min. The sample was run for ten minutes at 25°C column temperature. After that, HCA was detected at a wavelength of 210 nm, and the HCA concentration in the extract was measured by comparing the extract's peak regions to those of the standard. The HPLC analysis was finally performed (Gogoi *et al*., 2014).

Disc diffusion method

The antibacterial activity of the crude *G. atroviridis* fruit extract was investigated using the disc diffusion method, as established by Bauer *et al*. (1996). Bacterial cultures were standardized to achieve a turbidity equivalent to the 0.5 McFarland standard. The standardized bacterial suspension was

uniformly spread across the surface of Mueller Hinton agar plates using a sterile cotton swab, creating an even microbial lawn. Paper discs were impregnated with the *G. atroviridis* fruit extract, using a volume of 10 µl per disc, and subsequently placed onto the inoculated agar surface. Notably, the acetone extract of *G. atroviridis* was dissolved in dimethyl sulfoxide (DMSO) for this testing, which served as the vehicle for the extract delivery. The *G. atroviridis* extract was tested at five serial concentrations: 25,000, 50,000, 100,000, 150,000, and 200,000 µg per mL. DMSO was used as a negative control. Ciprofloxacin at a concentration of 100 µg per mL per disc served as the positive control. For the acetone-extracted samples, four treated discs were placed on the agar surface, ensuring an equidistant distribution. The inoculated plates were incubated at 37°C for a period of 18–24 hours. Antibacterial activity was determined by measuring the diameter of the zone of inhibition (in mm) surrounding each disc. Measurements were precisely taken using callipers. To ensure the reliability of the data, the entire experiment was performed in triplicate for every concentration of the *G. atroviridis* extract against each bacterial strain tested.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The Minimum Inhibitory Concentration (MIC) of the *G. atroviridis* acetone fruit extract was assessed using the broth microdilution method. The extract was prepared for testing in 96-well microtiter plates. An aliquot of the acetone extract (100 µl) was diluted to create a concentration range spanning from a minimum of 781.25 µl/mL up to a maximum of 100,000 µl/mL. Test bacterial strains were first cultured on Mueller Hinton Agar (MHA) and incubated overnight at 37°C. An aliquot of the culture was then transferred and suspended in 50 mL of Mueller Hinton Broth (MHB), followed by incubation at 37°C for 3–5 hours to reach the exponential growth phase. The resulting bacterial suspension was standardized using a 0.85% NaCl solution to match the turbidity of the 0.5 McFarland standard. This standardization corresponds to a final concentration of approximately $1.5x10^8$ cfu/mL. In the 96 well microtiter plates, which already contained the serially diluted *G. atroviridis* extract, 100 µl of the standardized culture was added to each well containing 100 µl of MHB supplemented with the extract. This inoculation step resulted in a final bacterial concentration of approximately 106 cfu/mL in each test well. The plates were then incubated at 37°C for 16–18 hours. 1% DMSO was used as a negative control. Ciprofloxacin (100 µl) was used as a positive control. It was diluted to final concentrations ranging from 1.95 to 250 µg/mL and incubated under identical circumstances. The MIC was defined as the lowest concentration of *G. atroviridis* crude extract that did not cause noticeable turbidity in the tested

bacteria. A 10 µl aliquot from the clear wells with the MIC value was distributed on fresh MHA plates and incubated overnight at 37°C. The MBC value was established as the lowest concentration of crude extract required to totally kill the bacterium. The experiment was carried out in triplicate. (CLSI, 2008).

Effects of G. atroviridis powder supplementation in diet on the productive performance of Japanese quails

The current investigation was reviewed by the Institutional Animal Care and Committee, Princess of Naradhiwas University, Thailand No PNU.AE-2024/01.008.

***G. atroviridis* powder preparation**

The *G. atroviridis* fresh fruits were collected from the Sai Khao sub-district of Khok Pho district, Pattani province, Thailand. The collected fruits were uniformly sectioned into small pieces, measuring 2–3 centimeters. These slices were subjected to sun-drying for a duration of 72 hours. Following sun-drying, the slices were transferred to a hot air oven and dried at a controlled temperature range of 60–65°C for an additional three days. The dried slices were subsequently comminuted into a fine powder using an electric grinder. The resulting *G. atroviridis* powder was meticulously sieved through a 1-millimeter mesh. The final powdered material was hermetically sealed in glass bottles and maintained under refrigerated conditions at 4°C until required for subsequent experimentation or analysis.

Animal and experimental design and treatments

The effects of asam gelugur (*G. atroviridis*) powder supplementation in diet on productive performance of Japanese quail were studied. The experiment was conducted in a Completely Randomized Design (CRD). A total of 192 male Japanese quails, aged 3 weeks (average body weight is 78.63±5.05 grams), were weighed and randomly devided into four treatment groups with four replicates containing 12 chicks each. Four dietary treatments i.e., control diet (T1), control diet supplemented with 0.2% commercial organic acids (T2), control diet supplemented with 10 g/kg and 20 g/kg of *G. atroviridis* powder (T3 and T4, respectively) were used. The quails were fed *ad libitum* with a basic commercial quail's starter mash during the rearing period. The experimental feed was a commercial ready-made feed for quail chicks during the starter phase (1 day to 4 weeks of age), containing no less than 22% protein, no less than 3% fat, and no more than 13% moisture. The feed was analyzed on a dry matter basis. The quails were kept in an open housing system with proper cleanliness and good

ventilation. The environmental conditions were the same for all groups. The cage size used is 52 centimeters in width, 73 centimeters in length, and 43 centimeters in height.

Productive performance

This study was undertaken on Japanese quails to evaluate their productive performance. The following performance parameters were monitored and recorded: final body weight (LBW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and survival rate. To track daily environmental fluctuations, the internal housing temperature and relative humidity (RH) were systematically recorded three times per day: at 9:00 a.m., 12:00 p.m., and 3:00 p.m.

Statistical analysis

The collected data were subjected to statistical analysis using a one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was used for the analysis of comparisons of means. Descriptive statistics were compared with the UNIVARIATE procedure of SAS software (SAS, 2017).

Results

Antibacterial activity assay

The crucial active ingredient of *G. atroviridis* fruit

The crude extract of acetone extract was brownish gum. The main active ingredient found in an aceton extract was HCA, identified by HPLC analysis at 435.14±6.67 mg/g (43.51%), with a retention time of 2.942 minutes.

Antibacterial susceptibility testing

The antibacterial efficacy of various concentrations of the *G. atroviridis* fruit acetone extract was determined using the disc diffusion assay. The results found that the acetone extract demonstrated effective inhibition against both *E. coli* and *S. enterica* across all concentrations evaluated. A statistically significant increase ($P<0.01$) in the diameter of the inhibition zones was observed for both bacteria as the extract concentration increased, relative to the negative control. The maximum concentration tested (200,000 µg/ml) of acetone extracts inhibited the largest inhibition zones for both strains, measuring 35.53 mm against *E. coli* and 33.08 mm against *S. enterica*. The inhibition zones for 150,000, 100,000, 50,000, and 25,000 µg/mL acetone extracts of *E. coli* were 31.39, 28.08, 25.67,

and 18.36 mm, respectively. The inhibition zones of *S. enterica* measured 33.08, 28.86, 24.92, 22.53, and 16.44 mm, respectively (Table 1 and Figures 1 and 2).

Table 1. The effects of acetone extract of *G. atroviridis* fruit on clear inhibition zones around the discs of *E. coli* and *S. enterica*

	The inhibition zones (mm) ± SEM[3/]	
	E. coli	***S. enterica***
1%DMSO[1/]	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}
Acetone extracted 25,000 µg/mL	18.36 ± 0.63^{f}	16.44 ± 0.85^{f}
Acetone extracted 50,000 µg/mL	25.67 ± 0.61^{e}	22.53 ± 0.23^{e}
Acetone extracted 100,000 µg/mL	28.08 ± 0.52^{d}	24.92 ± 0.38^{d}
Acetone extracted 150,000 µg/mL	31.39 ± 0.46^{c}	28.86 ± 0.27^{c}
Acetone extracted 200,000 µg/mL	35.53 ± 0.31^{b}	33.08 ± 0.40^{b}
Ciprofloxacin[2/]	42.81 ± 0.37^{a}	41.94 ± 0.43^{a}
P-value	P<0.0001	P<0.0001

[1/]Plate inoculated without acetone extract as negative control, [2/]Ciprofloxacin 100 µg/mL (Sigma, USA) as a positive control, [3/]SEM: Standard error of the mean. At the 0.01 level, there is no significant difference between the mean values in the same column followed by the same letter. Each value is the mean of three replicates, followed by ± the standard error of the mean (n = 3).

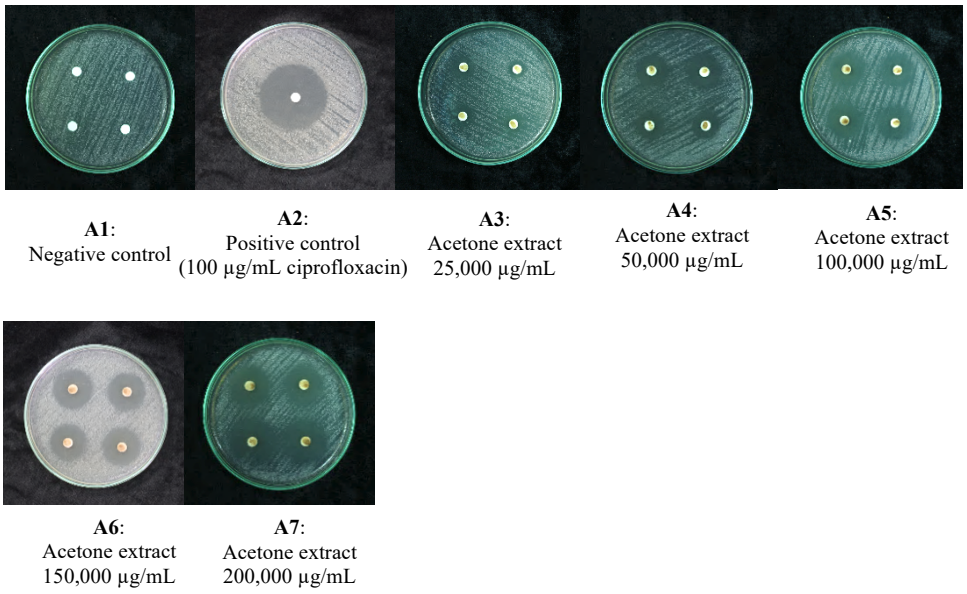

Figure 1. The inhibitory zones (mm) of various doses of acetone extract from *G. atroviridis* fruit against *E. coli* (A1–A7)

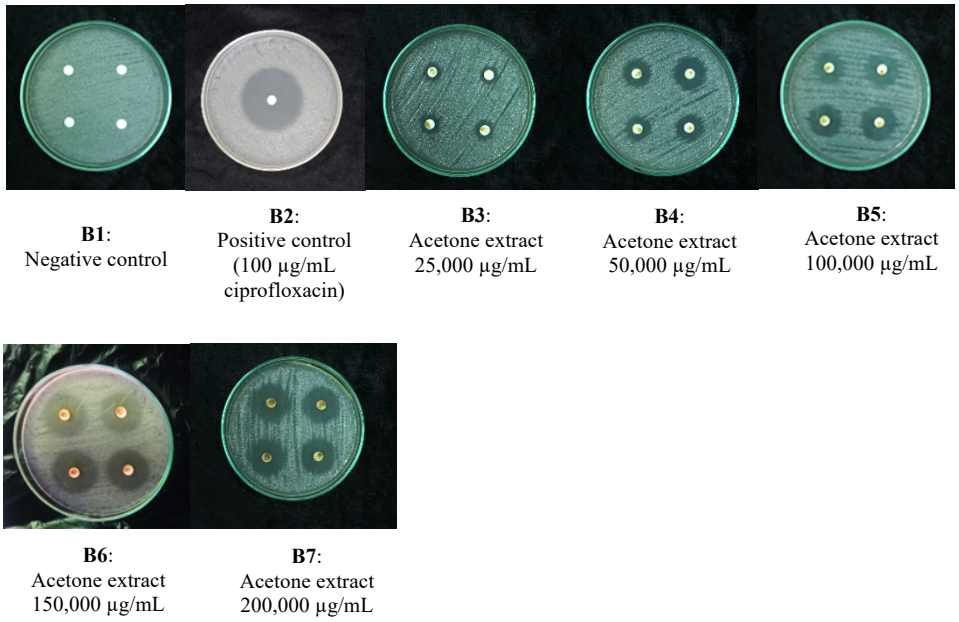

Figure 2. The inhibitory zones (mm) of various doses of acetone extract from *G. atroviridis* fruit against *S. enterica* (B1–B7)

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays

The antibacterial efficacy of the acetone extract derived from *G. atroviridis* fruit was quantitatively assessed by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values using the broth microdilution method. The extract was tested against two gram-negative bacterial strains at concentrations spanning from 781.25 µg/mL to 100,000 µg/mL. A uniform MIC value of 12,500 µg/mL was found to be effective in inhibiting the growth of both *E. coli* and *S. enterica*. The MBC values required for complete eradication of the strains were differentiated: 25,000 µg/mL for *E. coli* and 12,500 µg/mL for *S. enterica* (see Table 2 for a detailed breakdown of results).

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of acetone extract of *G. atroviridis* fruit against *E. coli* and *S. enterica*

	MIC (µg/mL)		MBC (µg/mL)	
	E. coli	*S. enterica*	*E. coli*	*S. enterica*
Acetone extracted	12,500	12,500	25,000	12,500
Ciprofloxacin[1/]	1.95	1.95	1.95	1.95

[1/]Ciprofloxacin (Sigma, USA) as a positive control
MIC (µg/mL) = minimum inhibitory concentration
MBC (µg/mL) = minimum bactericidal concentration

Productive performance

The effects of *G. atroviridis* powder supplementation in diet on the productive performance of Japanese quails were investigated. The findings showed that quails supplemented with 0.2% commercial organic acids, and those supplemented with 10 and 20 g/kg of *G. atroviridis* powder, showed significantly higher final body weight in compared to the control group ($P<0.05$) at 7–8, 8–9, and 3–9 weeks of age. However, this study found that all of the treatment groups did not affect on feed intake, final body weight, average daily gain, feed conversion ratio, and survival rate of Japanese quails at 3–6 weeks of age, as presented in Table 3.

Table 3. Effects of *G. atroviridis* powder supplementation in diet on productive performance of Japanese quails at 3–9 weeks of age

Age of bird (weeks)	Experimental groups	Feed intake (g/b)	Final body weight (g/b)	Average daily gain (g/b)	Feed conversion ratio	Survival rate (%)
3–4	T1	15.11	94.75	15.89	1.01	100.00
	T2	15.31	95.63	16.98	1.05	100.00
	T3	15.85	95.65	16.79	1.09	100.00
	T4	15.74	94.39	16.23	1.08	100.00
	SEM	0.27	1.00	1.69	0.10	-
	P-value	0.7472	0.9592	0.9954	0.9906	-
4–5	T1	17.83	120.94	26.19	0.76	100.00
	T2	18.69	121.29	25.67	0.73	100.00
	T3	18.32	122.96	27.31	0.69	100.00
	T4	18.62	122.23	27.83	0.68	100.00
	SEM	0.30	1.09	1.36	0.05	-
	P-value	0.7420	0.9095	0.9376	0.9415	-
5–6	T1	19.07	133.04	12.10	2.09	100.00
	T2	20.79	135.31	14.02	2.03	100.00
	T3	20.78	136.71	13.75	1.99	100.00
	T4	20.87	135.99	13.77	1.59	100.00

Age of bird (weeks)	Experimental groups	Feed intake (g/b)	Final body weight (g/b)	Average daily gain (g/b)	Feed conversion ratio	Survival rate (%)
	SEM	0.65	1.37	1.77	0.29	-
	P-value	0.7184	0.8012	0.9792	0.9253	-
6–7	T1	20.43	144.73	11.69	2.02	100.00
	T2	21.96	149.26	13.95	1.98	100.00
	T3	21.94	147.75	11.04	2.18	100.00
	T4	22.49	148.85	12.86	2.04	100.00
	SEM	0.45	1.65	1.29	0.26	-
	P-value	0.4349	0.7666	0.8604	0.9932	-
7–8	T1	21.38	153.41[b]	8.68	2.99	100.00
	T2	22.66	158.33[a]	9.07	2.87	100.00
	T3	22.82	158.67[a]	10.92	2.86	97.92
	T4	23.24	158.58[a]	9.73	2.54	100.00
	SEM	0.74	0.71	1.25	0.34	0.52
	P-value	0.8307	0.0582	0.9244	0.9707	0.4262
8–9	T1	22.42	159.48[b]	6.07	3.72	100.00
	T2	23.21	167.92[a]	9.58	2.95	100.00
	T3	22.64	169.48[a]	10.80	2.31	97.92
	T4	22.92	167.55[a]	8.97	2.93	100.00
	SEM	0.63	1.02	0.99	0.24	0.52
	P-value	0.9728	0.0200	0.4149	0.3053	0.4262
3–9	T1	116.24	159.48[b]	80.63	1.45	100.00
	T2	122.63	167.92[a]	89.27	1.39	100.00
	T3	122.33	169.48[a]	90.62	1.36	97.92
	T4	123.88	167.55[a]	89.38	1.39	100.00
	SEM	1.23	1.02	1.96	0.03	0.52
	P-value	0.1800	0.0200	0.2994	0.8218	0.4262

T1 (treatment 1): Control group (commercial feed without *G. atroviridis* powder), T2 (Treatment 2): Commercial feed supplemented with 0.2% commercial organic acid, T3 (Treatment 3): Commercial feed supplemented with *G. atroviridis* powder at 10 g/kg, and T4 (Treatment 4): Commercial feed supplemented with *G. atroviridis* powder at 20 g/kg.

[ab]Means with different letters in the same column show significant differences ($P<0.05$).

[1/]SEM: Standard error of the mean.

Discussion

Antibacterial activity assay

The crude extract of *G. atroviridis* demonstrated effective inhibition of pathogenic bacterial growth. This antimicrobial activity is likely attributable to the presence of organic acids and phenolic compounds within the fruit. Analysis of the acetone extract further identified hydroxycitric acid (HCA) as the main active ingredient, constituting a significant 43.51% of the extract's composition. This finding suggests HCA is a key contributor to the observed antibacterial

efficacy. In fact, a previous study reported that the crude extract of *G. atroviridis* from ethanol extraction yielded 38.09% and contained a higher concentration of HCA (49.50%). (Niyomdecha *et al*., 2022a). Meanwhile, Susanti *et al.* (2020) declared that *G. atroviridis* extract contained 11.35 ± 0.55% (w/w) HCA. Furthermore, the HCA concentration of *G. cambogia* fruit has been determined to be between 42% and 44% (Edirisinghe *et al*., 2015). Additionally, another researcher claimed that *G. cambogia* fruit contained 20–30% HCA (Lewis and Neelakantan, 1965).

For antibacterial susceptibility testing, the results of current study demonstrated that acetone extract of *G. atroviridis* fruit exhibited potential antibacterial properties against both *E. coli* and *S. enterica*. Similarly, a previous study by Niyomdecha *et al*. (2022a) illustrated that ethanol extract of *G. atroviridis* fruit as an antibacterial agent which dominantly effective against *E. coli* and *S. enterica*., In addition, the inhibition zones of 200,000, 150,000, 100,000, 50,000, and 25,000 µg/mL ethanol extracts of *E. coli* were 33.11, 27.19, 23.11, 20.25, and 16.03 mm, respectively. The diameters of the inhibition zones of *S. enterica* were 31.58, 25.39, 21.17, 18.28, and 15.56 mm, respectively. The results agree with Bacayo *et al.* (2018) also confirmed that 100% concentration of *G. atroviridis* extract could have potential against the growth of *Klebsiella pneumoniae* and Methicillin-resistant *Staphylococcus aureus* (MRSA), with diameters of inhibition zones of *Klebsiella pneumoniae* and MRSA at 15.33 and 10.76 mm, respectively. The inhibitory effects observed are consistent with findings by Al-Askalany (2018), who reported that a 10% water extract (both cold and hot) of *G. cambogia* was highly effective against both gram-positive (*B. cereus*) and gram-negative (*E. coli*) bacteria. Furthermore, other researchers have also indicated the significant antibacterial activity of a methanol extract from *G. atroviridis* fruit (Mackeen *et al*., 2000). This extract demonstrated high potential against a panel of bacteria, including *S. aureus*, *B. subtilis B28* (mutant) and *B29* (wild type), and *E. coli*. The effective concentration for these strains was reported as a minimum inhibitory dose (MID) of 500 µg/disc. The current results are compared with other studies on *G. atroviridis* extracts: Niyomdecha *et al*. (2022a) reported that an extract had an MIC value of 12,500 µg/mL, effective against both *E. coli* and *S. enterica*. The MBC value required to completely kill both bacteria was found to be 25,000 µg/mL. Meanwhile, Tongboon (2013) also found that the ethanol extract of *G. atroviridis* fruit reported an MIC value of 1 mg/ml for inhibiting *S. aureus* and *E. coli*. Another researcher declared the MIC value against *Pseudomonas aeruginosa*, *Bacillus cereus, E. coli* and *Cryptococcus neoformans* were 100, 400, 800, and 800 µg/mL. respectively. Additionally, the minimum lethal concentration (MLC) against *Pseudomonas aeruginosa*, *Bacillus cereus* and *Cryptococcus neoformans* were 400, 800, and

800 µg/mL, and more than 800 µg/ml against *E. coli*. (Mackeen *et al*., 1997). Moreover, Hart and Cock (2016) demonstrated that the crude extract derived from *G. cambogia* fruit exhibited significant broad-spectrum antibacterial activity. This efficacy extended to the inhibition of growth in both gram-positive and gram-negative bacterial strains. It is well-established that the fruit of *G. atroviridis* is a rich source of various organic acids. Based on findings from multiple studies (Amran *et al*., 2009; Al-Askalany, 2018), the compounds identified in the fruit include citric acid, acetic acid, lactic acid, malic acid, tartaric acid, ascorbic acid (vitamin C), and hydroxycitric acid (HCA), which are the natural agents that could have several advantages, such as pharmaceutical properties (antibacterial activity), as well as lack of toxicity. Moreover, Hamidon *et al*. (2017) also reported that *G. atroviridis* fruit contained other active ingredients such as flavonoids, isoflavonoids, and alkaloids, which showed high potential against pathogenic bacteria.

Makras and Vuyst (2006) reported that several mechanisms have been suggested for the antibacterial activities of organic acids in inhibiting the growth of pathogenic bacteria. Firstly, a decrease in pH level due to the production of organic acids. Secondly, the undissociated form of organic acids can enter bacterial cells and then dissociate within the cytoplasm. This dissociation releases hydrogen ions (H^+), leading to an accumulation of H^+ and a reduction in the pH value inside the bacterial cell. According to Carpenter and Broadbent (2009) confirmed that the intracellular accumulation of anions is a key factor contributing to the inhibition of bacterial growth. Moreover, a hugh number of H+ ions within the bacterial cell stimulate the activation of H^+ ATPase pumps, which actively transport H^+ ions out of the cell. This process results in energy loss for the bacteria, leading to cell death. Additionally, other active ingredients, such as flavonoids, isoflavonoids which found in *G. atroviridis* fruit, also damage the bacterial cell membrane, further contributing to bacterial death.

The findings of this investigation also demonstrated that *G. atroviridis* fruit contained HCA, as a key active ingredient and orther organic acids which showed potential as an antibacterial agent with antibacterial properties.

Productive performance of Japanese quails

Our findings of the current study revealed that quail chicks fed diets supplemented with commercial organic acids and *G. atroviridis* powder showed significantly increased final body weight. These results may be dued to varios important substances present in *G. atroviridis* fruit, such as hydroxycitric acid (HCA), other organic acids, and phenolic compounds (Lewis and Neelakantan, 1965). These compounds effectively stimulate feed appetite, leading to increased

feed intake in the animals. As a result, the quails received sufficient nutrients to meet their body's requirements, promoting increased final body weight.

According to a previous study by Niyomdecha *et al*. (2022b) investigated the effect of supplementing broiler chicken feed with *G. atroviridis* powder, linking its benefits to the presence of key organic acids and other bioactive compounds. Dietary inclusion of 32 g/kg of *G. atroviridis* powder. This supplementation led to a significant increase in the feed intake, final body weight, and body weight gain of broiler chickens during the 4–5-week -old period. The observed positive effects on productive performance are attributed to the fruit's rich phytochemical profile, which includes the key active ingredient (hydroxycitric acid, HCA), other organic acids (citric acid, acetic acid, malic acid, and tartaric acid), and phenolic compounds. Jantan *et al.* (2011) quantified the total phenolic content in *G. atroviridis* fruit at 4.4 mg gallic acid equivalent per gram. Besides another researcher declared that there are numerous bioactive compounds contain in the *G. atroviridis* fruit such as alkaloids, flavonoid, saponins, and tannins which were highly effective against the growth of pathogenic bacteria (*S. aureus*) (Djarot *et al*., 2020). Furthuremore, some researchers also reported that *G. atroviridis* fruits contained beta-carophyllene, beta-caryophyllene alcohol, β-caryolanol, and alpha-humulene. These active ingredients possess high antioxidant levels, antioxidant capacities and free radical scavenging activities (Tan *et al.*, 2012). A comprehensive review of the literature indicates that the fruits of *G. atroviridis* offer a wide range of potential health and therapeutic benefits (Hamidon *et al*., 2017), including antioxidant, antimicrobial, antifungal, antiobesity, cytotoxicity, antimalarial, anti-inflammatory and antinicotine stress activities. Furthermore, several independent studies have specifically confirmed that *G. atroviridis* fruit extracts demonstrate strong antimicrobial, antioxidant, and antitumor-promoting activities (Mackeen *et al*., 1997; Mackeen *et al*., 2000), as well as antifungal and antibacterial properties (Al-Askalany, 2018) that possess effective benefits for animal health. Additionally, *G. cambogia* fruit extract has been shown to efficiently improve glucose metabolism in animals (Hayamizu *et al*., 2003). According to Suwanmanee *et al*. (2014), they were found that *G. atroviridis* fruit extracts have high potential against fungi growth and showed good property for reducing inflammatory cytokines. Moreover, *G. atroviridis* fruit possesses antioxidant properties and exhibits strong antimicrobial activity. Meanwhile, Sripradha and Magadi (2015) investigated the therapeutic effects of the crude extract from *G. atroviridis* fruit, demonstrating significant positive impacts on metabolic health and inflammation in an animal. A dose of 400 mg/kg body weight of the crude extract effectively reduced both glucose intolerance and leptin levels. The crude extract also caused a significant decrease in tumor necrosis factor-alpha (TNF-

α) levels, a cytokine that induces inflammation in animals. As a result, this led to a reduction in inflammation and improved animal health. However, research by Kardaya *et al*. (2022) indicated that supplementing the diets of spent layer ducks with 2, 4, and 6% of *G. atroviridis* leaf meal significantly improved meat quality by reducing both cooking loss and crude fat content. This finding is supported by Dihansih *et al*. (2020), who specifically reported that supplementation with 2% of *G. atroviridis* leaf meal decreased the fat content in both the meat and the meat with skin of ducks. Conversely, when supplementing broiler chickens' diets with *G. atroviridis* leaf meal, Sebola *et al*. (2011) observed no significant difference in key production indices, including feed intake, final body weight, body weight gain, and feed conversion ratio of broiler chickens.

G. atroviridis fruits are rich in various organic acids that efficiently mediate a reduction in gastrointestinal pH level, thereby suppressing the growth of bacterial pathogens. This mechanism is associated with a reported improvement in the digestive system, augmented nutrient utilization, and enhanced gut integrity in Japanese quails. Consequently, the evidence supports the conclusion that incorporating *G. atroviridis* powder into the diet serves to boost the productive performance of Japanese quails.

HPLC confirmed a high HCA content, measuring 435.14±6.67 mg/g (43.51%). Evaluation of the acetone extract's antimicrobial activity showed that the maximum concentration (200,000 µg/mL) produced significant inhibition zones against *E. coli* (35.53 mm) and *S. enterica* (33.08 mm). The extract's potency was further defined by an MIC of 12,500 µg/mL for both pathogens. The MBC for complete eradication was 25,000 µg/mL for *E. coli* and 12,500 µg/mL for *S. enterica*. Crucially, quails receiving a dietary supplement of 0.2% commercial organic acids, or *G. atroviridis* powder at 10 g/kg and 20 g/kg, exhibited a statistically significant ($P<0.05$) improvement in final body weight compared to the control, evidenced across the 7–8, 8–9, and 3–9 weeks of age.

The finding can be concluded that the acetone extract of *G. atroviridis* fruit contained HCA, as a crucial active ingredient showing potential as an antibacterial agent with antibacterial properties. Therefore, *G. atroviridis* could be used as a feed additive for Japanese quails. In addition, supplementing the diet with 10–20 g/kg of *G. atroviridis* powder could improve the productive performance of quail chicks at 7–9 weeks of age. For further study, it should investigate the *G. atroviridis*'s powder supplementation in diet to evaluate gut health, carcass quality, and meat quality of Japaneses quails.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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