

The Characterization of *Ficus lyrata* Warb Fruit Extract and the Effect on Toxicity, Physicochemical, and Microbiology Properties of Chicken Carcass

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Abstract— The use of plant extraction as natural and safe preservatives, have been highly recommended for the improvement of the microbiological and sensory quality of food. In the present investigation, the dried fruit of *Ficus lyrata* Warb was subjected to bio preservative to chicken carcass. This study aimed to characterize the ethyl acetate extract on the phytochemical compound, antioxidant activity, antibacterial activity, to determine the toxicity of the extraction with lethal dose (LD₅₀) value and to study the application of the extract as chicken carcass preservative. The result showed that the fruit extract has phenolic compounds, flavonoids and tannins. Gas Chromatography Analysis resulted that the extract contains catechin, luteolin-6.8-C-diglucoside and Orientin. DPPH and clear zone analysis showed that the extract has a high of antioxidant and antibacterial activity. The acute toxicity analyzes showed that the extract of *Ficus lyrata* Warb fruit has not the LD₅₀ below the concentration of 15000 mg/kg weight, that means the extract was practically non-toxic material. The application of extract to chicken carcass preservation had resulted the pH of carcass in the range 5.51—5.98 and decreased the temperature from 26 to 24°C and effected to inhibit of chicken carcass decay for 2 hours. The extract of *F. lyrata* Warb fruit also inhibited the bacterial growth until 2.4×10^6 CFU/g within the 12 hours of storage and increased the tenderness of carcass to 0.6 N. Based on the observed characteristics, it was obtained that the use fruit extract as a natural preservative can extend the shelf life of fresh chicken carcasses.

Keywords— antibacterial activity; chicken carcass; ficus lyrata Warb fruit; LD50 and natural preservative.

I. INTRODUCTION

Ficus constitutes one of the largest generations of medicinal plants with about 750 species[1]. In Indonesia, the most important species of *Ficus* are *F. Lyrata warb*, *F. elastica*, and *Ficus carica*. Various parts of the plant like bark, leaves, stem, fruits, seeds, and fruit are medicinally important[1]. The dried fruits are a good source of flavonoids and polyphenols and some bioactive. The chemical constituent of the fruit contains b-sitosterol, glycol, lupeol, lupeol acetate, friedelin, higher hydrocarbons and other phytoesters. This fruit has a cooling effect, usually used for mouthwash and throat pain. Traditionally, in some countries in Africa, fruit, roots and leaves of *F. lyrata* have been used in the treatment system for different digestive disorders such as (colic, loss of appetite and diarrhea), respiration (sore throat, coughing and bronchial problems), as an anti-inflammatory and interference cardiovascular. The plant *F. lyrata* has been reported to have many bioactive

compounds such as arabinose, β -amyrins, β -carotene, glycosides, β -setosterols and xanthoxol, alkaloids, flavonoids, coumarin, saponins, phenolic compounds and terpenes. Phenolic compounds are important classes of phytochemicals that have a variety of biological functions such as astringents, antioxidants, anticancer, anti-inflammatory, and antibacterial [2]–[4].

The increasing of using chemical preservative is dangerous to human health. Literature reports and ethnobotanical records suggest that plants are potential of the pharmaceutical industry. They may provide a natural source of antimicrobial agents that will/or provide novel or lead compounds that may be employed in controlling some infections globally [1].

The fruit extract of *F. lyrata* Warb contain the bioactive compounds, especially phenolic compounds, flavonoids, triterpenoids, and tannins[5]. Extraction process using ethyl acetate solvent chosen as the optimal solvent. The effects of antimicrobial activity against *E. coli* and *P. aeruginosa* has a

clear zone and the biggest inhibition compared to ethanol and water is equal to 4.70 mm and 2.97 mm. Clear zone in bacteria *B. subtilis* gives effect to the antimicrobial activity of 5.98 mm [6].

The mindset and pattern of life, the people starting with changes in unhealthy diet that cause toxins in the food. The consumption model can lead the variety of diseases, such as high blood pressure, coronary artery disease, and metabolic disorders, having an unhealthy diet will form free radicals continuously [7]. Free radicals are atoms or molecules that contain one or more unpaired electrons in their outer orbitals, which are unstable and highly reactive. Free radicals will react to the surrounding molecules to get electron pairs to reach the stable atoms or molecules [8], [9]. If it occurs continuously and cannot be stopped in the body, it will increase the free radicals in the body that trigger pathological effects, such as cancer and atherosclerosis [10]. Antioxidants can be found in various types of plants and fruits. Plants that can act as antioxidants are mostly plants from the genus *Ficus*. One of them is *Ficus lyrata* Warb. Some *Ficus* species can be used for medicine, which is already known to have antioxidant activity and phytochemical composition [11], [12]. DPPH is one of the antioxidant activity testing methods used. This method is chosen because it is easy, fast, and has sensitive levels that can analyze many samples. DPPH is the most stable free radical compound compared to other radical compounds. The commonly used free radicals in the study of antioxidants or free radicals are DPPH [13], [14].

It is necessary to evaluate, in a scientific base, the potential use of the extract of *F. lyrata* Warb to preserve the food products, one of them is chicken carcasses. The specific purposes of this research are to study the effect of fruit extract on characteristics of extract include phytochemical compound, antimicrobial activity, antioxidant activity and toxicity), and application of its extract to preserve chicken carcass on physicochemical and microbiology characteristic of chicken carcass.

II. MATERIAL AND METHODS

A. Preparation of Fruit of *Ficus lyrata* Warb

The fruit was collected from around Padjadjaran University campus, Indonesia. Before used, the fruit of *Ficus lyrata* Warb was sun-dried, cleaned, oven-dried at 105°C overnight and ground. Only the fruit of small size, max. 60 mesh was used.

B. Preparation of extracts

100 g of the powdered fruit was soaked in 800 mL of ethyl acetate as a solvent and kept in a room temperature incubator for about 48h. The extracts were then filtered using Whatman no.1 filter paper. The resulting filtrates were then concentrated by evaporation using rotavapor and oven vacuum at 40°C. The dried extract was stored at 4°C for further analysis. [15].

C. Analysis of Extract; Phytochemical, Antibacterial Activity and Antioxidant Analysis

1) *Phytochemical screening Analysis*: The extracts were screened for phytochemicals like alkaloids, flavonoids,

triterpenoids, phenolic compounds and tannins, following the procedure of Harborne [15]–[17].

2) *Gas Chromatography Analysis*: The condition of the machine used is as follows:

- Machine Type: UPLC-QToF-MS / MS, Acquity UPLC BEH C18 column 1.7µm, 2.1x50 mm. Program temperature of 40°C/2' increases 10°C per minute to 290°C / 5' with flowrate 0.3 mL / min.
- Eluent (mobile phase) consist of H₂O + 0.1% formic acid and MeOH + 0.1% formic acid.
- Sample Preparation, the sample to be tested is vortexed for 30 minutes with a rotation speed of 15,000 rpm
- Injection, the chromatography tool is turned on and the sample injection temperature is set. The sample was injected as much as 0.5 mL.
- Data processing, peak - peak (peak) will appear on the chromatogram. The spectra contained in the chromatogram will be processed using Masslynx 4.1 software to determine whether there are any desired compounds.

3) *Antibacterial activity*: Antibacterial activity for the fruit and leaf extracts was evaluated by disc diffusion method Kirby Bauer⁵⁾. The bacteria used in the study include *Salmonella sp*, *E. coli*, and *B. subtilis* were obtained from the Pharmacy Faculty, Padjadjaran University, Indonesia. The bacterial isolates were first sub cultured in a nutrient broth and incubated at 37°C for 36 h. Amoxicillin (250 mg) was used as a standard antibiotic for comparison of the results. Muller-Hinton agar medium was loaded into Petri dish, stored until freezing and then inoculated tested bacterial to medium. The diameter of the zone of inhibition was measured in mm. The experiment was repeated in triplicates and the average values were calculated.

4) *Antioxidant Activity*: The antioxidant activity used to determine antioxidant activities is IC₅₀ and carried out with 517 nm wavelength [13] [18]. IC₅₀ is a parameter used to determine antioxidant activities and IC₅₀ value is defined as the antioxidant compound concentration, which causes a 50% loss of DPPH activity. The antioxidant activity of *Ficus lyrata* fruit with duration variations of maceration uses DPPH radicals.

D. Toxicity Analysis

Acute toxicity test of fruit extract is a test that is performed to measure the degree of the effect of an extract in an animal (mice), and observations made in the first 24 hours on one occasion. Acute toxicity testing parameters using the LD50. LD50 is essentially the value of a dose that causes the death of about 50%, more precisely according to the OECD single dose of a substance that can cause death in 50% of test mice were determined [19] [20]. In this experiment, 42 of Swiss Webster mice divide into 7 groups to determine the variation of extract concentration per body weight (mg/ kg BW).

E. Preservation of chicken carcass using fruit extract

The chest of the chicken carcass was divided into 100-g samples, placed on *styrofoam* and storage at room

temperature. The immersion of chicken carcass using the extract of *F. lyrata* fruit was conducted with a variation of extract concentration, including 0, 5, 10 and 15% of the extract. The analysis was conducted to evaluate the effect of the extract on physicochemical and microbiology characteristics of the chicken carcass.

III. RESULTS AND DISCUSSION

A. Phytochemical Compound of Ethyl Acetate Extract

The results of the *F. lyrata* fruit extract phytochemicals analysis by spectrophotometer and GC are shown in Table 1 and 2.

TABLE I
THE PHYTOCHEMICAL COMPOUND OF FRUIT EXTRACT

Phytochemical compound	Value (mg/L)
Phenolic	28.5± 3.45
Flavonoid	13.6± 1.70
Tanin	27.5± 2.30

TABLE II
THE PHYTOCHEMICAL COMPOUND OF FRUIT EXTRACT

Compound	Present (+) or absence (-)
Apigenin 6.8-di-C-glucoside	-
Acid of 5-O-caffeoylquinic	-
epiafzelechin-epicatechin	-
Catechin	+
luteolin-6.8-C-diglukoside	+
Orientin	+

The fruit extract has several types of secondary metabolites, namely phenolic compounds, flavonoids, tannins for ethyl acetate solvents. The Table 2 shows that the bioactive content present in this extract are Catechin, luteolin-6.8-C-diglukoside and Orientin. Flavonoids and tannins are included in phenolic compounds which have an important role in biochemical processes that can cause major disruptions because of their ability to form protein complexes through hydrogen bonds. When plant cell content mixed with membranes becomes damaged during the process of isolation, phenol compounds react very quickly to form complexes with proteins. As a result, there are often obstacles to the action of enzymes in plant extracts [18] [24]. This is what causes *F. lyrata* fruit extract to have antimicrobial activity.

B. Analysis of Antibacterial Activity of Ethyl Acetate Extract

In the evaluation of the antibacterial activity of the fruit, the zone of inhibition was observed in the extracts of ethyl acetate. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract. The antibacterial activity of the extract against 3 bacteria is shown in Table 3.

TABLE III
ANTIBACTERIAL ACTIVITY OF FRUIT EXTRACT

Bacteria	Clear Zone (mm)
<i>E. coli</i>	4.70± 0.67
<i>P. aeruginosa</i>	2.97± 0.05
<i>B. subtilis</i>	5.98± 0.16

The clear zone formed in the Table 3 shows that *F. lyrata* fruit extract has antimicrobial activity. *B. subtilis* shows the highest inhibitory effect compared to other microbes. This is caused by *B. subtilis* is due to the nature of the bacteria itself which is a gram-positive bacterium that is more sensitive to antimicrobials and has a low lipid content (1-4%) in the cell wall composition. Whereas in *E. coli* and *P. aeruginosa* the cell has a lower clearer zone because the bacteria are gram negative bacteria which are more resistant to antimicrobials and have high lipid content (11-12%) in cell wall composition [1].

C. Analysis of Antioxidant Activity of Extract

The antioxidant activity used to determine antioxidant activity is IC₅₀ and carried out with a wavelength of 517 nm [13]. IC₅₀ is a parameter used to determine antioxidant activity and IC₅₀ value is defined as the concentration of antioxidant compounds which causes a 50% loss of DPPH activity. Evaluation of the antioxidant activity of *F. lyrata* fruit extract with variations in the duration of maceration using DPPH radicals (2.2 Difenyyl-1-Pikrihidrazil). The antioxidant compound reaction that will be seen is by DPPH color decay from purple to yellow. Measurement of antioxidant activity can be tested using the principle of Spectrophotometry [13] [22]. The results of the observation of the antioxidant activity of *F. lyrata* fruit extract are in Table 4. The results showed that the extraction time had a significant effect on the antioxidant activity of *F. lyrata* fruit extract.

TABLE IV
ANTIOXIDANT ACTIVITY OF FRUIT EXTRACT

Maceration Time (h)	IC ₅₀ (ppm)
6	144.67 ± 2.67
12	104.72 ± 0.80
18	90.35 ± 7.88
24	87.25 ± 3.19
30	89.14 ± 12.96

Ficus lyrata fruits extracted using ethyl acetate solvents for 6 hours, 12 hours, 18 hours, 24 hours, and 30 hours has moderate antioxidant activity. The antioxidant activity obtained from *Ficus lyrata* fruits showed in the 6-hour extraction duration with IC₅₀ of 144.67 ppm, and the highest was obtained in 24-hour extraction duration which showed IC₅₀ of 87.253 ppm. The extraction time treatment was carried out for 6 hours to 30 hours in *F. Lyrata fruit* using ethyl acetate solvent (87.25 ppm - 144.67 ppm). The IC₅₀ value of less than 50 ppm shows the intensity of antioxidant activity is very strong, while the IC₅₀ value in the range of 50 ppm - 100 ppm shows the intensity of strong antioxidant activity. For IC₅₀ values in the range of 101 ppm - 150 ppm shows the value moderate intensity of antioxidant activity, while IC₅₀ values greater than 150 ppm indicates that the intensity of the value of antioxidant activity is weak [13]. *F. Lyrata* fruit extracted for 6 hours, 12 hours, 18 hours, 24 hours, and 30 hours has moderate antioxidant activity.

D. Acute Toxicity of Fruit Extract Using Ethyl Acetate

Toxicity testing was conducted with the observations the behavior of mice for 24 hours and the percentage mortality observation for 14 days. The observation of animal mortality

for 14 days after the feed of the extract varied of extract concentration/body weight (mg/kg BW) can be seen in Table 5 below.

TABLE V
THE PERCENTAGE OF MICE CUMULATIVE MORTALITY IN 14 D FEEDING THE EXTRACT

Extract (mg/kg BW)	Mice Cumulative Mortality (%)							
	0h	2 h	4 h	24 h	48h	72 h	7d	14d
Control (0)	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0
500	0	0	0	0	0	0	0	0
5000	0	0	0	0	0	0	0	0
7500	0	0	0	0	0	16.67	16.67	16.67
10000	0	0	0	0	0	16.67	16.67	16.67
15000	0	0	0	0	0	16.67	16.67	16.67
	0	0	0	0	0	16.67	33.33	33.33

Table 5 shows that the death mice occurred start at a dose of 5000 mg / kg BW, 7500 mg / kg BW, 10000 mg / kg BW, 15000 mg / kg BW with the percentage of deaths in sequence: 16.67%, 16.67 % and 33.33%. The mortality began to occur in the 72 h or three days. Whereas in the control, a dose of 50 mg/kg, a dose of 500 mg / kg BW there are no deaths until day 14. The percentage of mortality in this toxicity test was not reached 50%.

Based on the results of the toxicity tests and compared with the toxicity classification by Loomis, 1996 [20] the extract of *F. Lyrata* using ethyl acetate solvent practically categorized as non-toxic, with LD50 greater than 15,000 mg / kg BW and included in the group practically non-toxic.

E. The Effect of Fruit Extract on Physicochemical of Chicken Carcass

1) pH

The degree of acidity (pH) is an important factor for the final quality of chicken meat which is influenced and determined by the rate of postmortem glycolysis and muscle glycogen reserves and ultimate meat [23]. Control is a chicken carcass was placed at room temperature for given extract preservation without the fruit extract. Overall data the effect of fruit extract on the pH of chicken carcass represented in Table 6.

During the 12 h observation in the control and samples occur instability pH of chicken even though the pH of the samples is still at the normal pH of 5.61- 5.87. Instability of pH allows happening because they relate to rigor mortis. Rigor mortis occurs 24-48 hours after slaughter biochemical events that involve complex because it involves the loss of creatine phosphate (CP) and adenosine triphosphate (ATP) of the muscle. Rigor Mortis development speed is influenced by several factors, namely glycogen levels at the time of death and the carcass temperature [23].

Table 6 shows that the control at the 4h until the end of the observation (12 h) has increased the pH is about 6.2 to 6.4, It has entered a period of not fresh (decay phase). Meanwhile, the chicken carcass by adding the fruit extract to be able to maintain a pH of about 5.4 to 5.8, especially for higher concentrations of 10 and 15%. Based on this data it appears that the higher fruit extract concentration influenced

the chicken's ability to maintain the pH in the range of freshie 5.4 to 5.8. These phenomena represent the addition of fruit extract effective to inhibit the decay process of chicken meat. The increase in pH of chicken meat is one indicator of decay [24].

TABLE VI
THE EFFECT OF FRUIT EXTRACT CONCENTRATION ON THE PH OF THE CHICKEN CARCASS DURING THE STORAGE TIME

Concentration (%)	Time (hour)						
	0	2	4	6	8	10	12
Control	5.61	5.65	6.36	6.22	6.20	6.22	6.35
5	5.85	5.85	5.99	5.81	5.83	5.77	5.87
10	5.70	5.70	5.89	5.78	5.69	5.51	5.59
15	5.63	5.84	5.85	5.76	5.52	5.84	5.62

The addition of fruit extract effective to maintain the pH of chicken carcasses approaching isoelectric pH is 5.4 to 5.5 so that the acidic pH can inhibit and suppress the growth of spoilage bacteria. The process of change in pH occurs by speeding up the process of glycolysis in muscle.

2) Temperature

Temperature greatly affects the quality of meat determine the growth rate and the number of microorganisms on the meat. The overall of the effect of the fruit extract on chicken carcasses temperature represented in Table 7.

TABLE VII
THE EFFECT OF FRUIT EXTRACT CONCENTRATION ON THE TEMPERATURE OF CHICKEN CARCASS

Concentration (%)	Time (hour)						
	0	2	4	6	8	10	12
Control (0)	25.4	25.1	25.2	24.5	24.8	24.4	24.4
5	25.9	25.6	25.3	24.5	24.5	24.3	24.3
10	26.2	25.4	25.2	24.4	24.5	24.3	24.3
15	26.1	24.4	24.4	24.4	24.4	24.3	24.3

Based on Table 7 above shows that the longer the period of storage, the temperature decreased and the value is in the range 24-26°C. Based on the data can be seen in the samples of 5%, 10%, and 15%, in the consecutive drop in temperature is 25.9 to 24.3 °C, 26.2 to 24.3 °C and 26.1 to 24.3 °C. The same thing also happened to control, namely the decrease in temperature from 0 h to 12 his 25.4 °C to 24.4 °C.

In this study, chicken carcasses were given extracts of the largest concentration of 15% has the lowest temperature. But one by one, the influence of the treatment did not look at changes in temperature, as well as when compared with controls. Temperature is also highly correlated with pH. High temperatures can accelerate the decline in muscle pH postmortem and can increase muscle protein denaturation and movement of water into the extracellular space [25] [26]. Besides temperature affect the speed of rigor mortis, where the high speed of development of rigor, comparable to high temperatures, which accelerates the loss of CP and muscle ATP[23] , For that temperature is an important indicator of the quality of chicken meat.

3) Initial Decay Analysis

Initial decay analysis using Pb- Acetate method is a qualitative method to detect decay rapidly. The result of Pb-acetate analysis as shown in Table 8.

TABLE VIII
INITIAL DECAY ANALYSIS USING PB- ACETATE METHOD OF THE EXTRACT

Extract	Initial decay at storage time (h)						
	0	2	4	6	8	10	12
Control (0%)	-	-	-	-	-	V	V
5%	-	-	-	-	-	-	-
10%	-	-	-	-	-	-	-
15%	-	-	-	-	-	-	-

Note : (-) : not detected, (v) : detected

Table 8 shows that control at 10 and 12 hours of storage has detected the decay. This phenomenon shows that adding the fruit extract of *F. lyrata* effects to extend the fresh time of chicken carcass. In the treatment of 5%, 10%, 15%, generally there is no change in the color of the meat. This result can be said that the extract can inhibit the decay process so that it can prolong the shelf life of chicken meat up to 12 hours. Meat decay can be caused by enzyme activity meat (autolysis), chemistry (oxidation and microorganisms, the mechanism of decomposition occurs in a complex. Decay can occur aerobically or anaerobically. Aerobic decay is caused by bacteria and can cause surface slime, changes in flesh color, odor and taste changes, whereas Anaerobic decomposition is caused by facultative anaerobic microbes that can cause acidic odors, and protein decomposition

4) Tenderness

Tenderness analysis is one indicator of physical quality in meat products. The tenderness in this analysis was carried out using an analyzer texture measuring device. the results of the overall effect of the addition of *F. lyrata* Warb extract to chicken carcass tenderness during the observation period can be seen on Table 9.

TABLE IX
THE EFFECT OF FRUIT EXTRACT CONCENTRATION ON THE TENDERNES OF CHICKEN CARCASS

Concentration (%)	Time (hour)				
	0	2	4	6	8
Control (0%)	0.96	0.77	0.72	0.70	0.79
5	0.76	0.77	0.76	0.75	0.74
10	0.83	0.75	0.69	0.66	0.77
15	0.88	0.70	0.72	0.65	0.63

Table 9 above shows that the addition of the extract of *F. lyrata* Warb extract can compress meat or carcass. This can be seen in the 15% addition extract can decrease the tenderness value is 0.63N. Whereas in control chickens the tenderness value was 0.79 N. The smaller the tenderness value of the meat the more tender the meat. Tenderness related to the composition of the meat itself, which is in the form of a binding cloth (collagen), meat fibers, and fat cells that are between the meat fiber cells. The changes in texture due to the rapid glycolysis in chicken meat will produce a

rapid rigor mortis process. That causes are there is no time for protein degradation, so the meat becomes hard. Protein damage will reduce the binding power of water in meat and will give a moist texture. It is also caused by components such as connective tissue constituents and filamentous threads in broiler chicken carcasses as damaged as a result of biochemical changes and microorganism activity, so there is no more power to sustain the structure of broiler chicken carcasses in a compact manner resulting in changes in flexibility on the chicken carcass [27] [21].

F. The Effect of Fruit Extract on Microbiology Characteristic of Chicken Carcass

Analysis of the number of microorganisms found on chicken carcasses during the observation period and how the fruit extract influence on the growth of microbes on the carcass of the chicken using TPC (Total Plate Count) methods. The results of the overall effect of the fruit extract on the TPC of chicken carcasses during the period of observation can be seen in Fig 1.

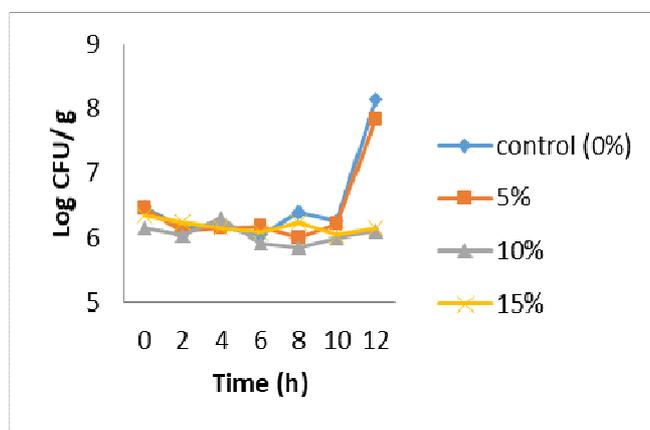


Fig. 1 The Effect of Extract Concentration on Microbial Growth

Figure 1 shows that the samples chicken carcass added 5% and 10, on the sample 15% occurred at the 12 hours is 1.4×10^6 CFU / g. While the increased control of the highest TPC mainly on 8-12 h is 2.5×10^6 - 1.8×10^8 CFU /g. When compared between the control and samples, in the samples of the fruit extract more capable to inhibit microbial growth rate, especially in the adding of fruit extract of 10% and 15%. It can be concluded that the addition of fruit extract effect to potential inhibits the growth of microbes.

IV. CONCLUSION

The characterization of *F. lyrata* fruit extract was obtained in this research. The extract has the phytochemical compound that effect to antimicrobial and antioxidant activity. The result showed that toxicity test LD50 value the fruit extract of *F. lyrata* Warb extract was not toxic up to the concentration of 15000 mg/kg BW. It represents that the extract safe for use. Applications of fruit extract to preserve of chicken carcasses affect increase shelf life up to 12 hours with the characteristics of decreasing the pH to 5.59, decreasing the temperature of meat up to 24.3 °C, the initial decay was not detected until the 12 h, and contain TPC lower than control.

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