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RESEARCH ARTICLE

INHIBITION OF HISTAMINE FORMATION ON THE FRIGATE TUNA (*Auxis thazard thazard*, L) USING LEAF EXTRACT OF *Jatropha curcas*

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Abstract

The study of the inhibition of histamine formation on the frigate tuna using *Jatropha* leaf extract has been done. *Jatropha* leaf macerated in stages ranging from hexane, chloroform, ethyl acetate and methanol. Results of preliminary research note that the ethyl acetate extract and methanol with a concentration of 1% (w / v) is the best extract that provides antibacterial activity against several of histamine-forming bacteria. In this experiment, frigate tuna were divided into 3 groups, group 1 was soaked for 30 minutes in water, group 2 soaked for 30 minutes in a solution of ethyl acetate extract of leaf *Jatropha* with a concentration of 1%, and group 3 was soaked for 30 minutes in a solution of methanol extract of leaf *Jatropha* with a concentration of 1%. After that, frigate tuna stored at room temperature for 5 hours, then analyzed the number of bacterial colony with total plate count method (TPC), number of histamine-forming bacteria and histamine levels. The results showed that the value of TPC the frigate tuna its soaked in water are log 6.68 (cfu / g), soaked in a solution of ethyl acetate extract are log 4.94 (cfu / g) and soaked in a solution of methanol extract are log 5.61 (cfu / g). The number of histamine-forming bacteria in the frigate tuna its soaked in water are log 5.62 (cfu / g), soaked in a solution of ethyl acetate extract are log 4.40 (cfu / g) and soaked in a solution of methanol extract are log 5.54 (cfu / g). histamine levels of the frigate tuna its soaked in water are 17.73 mg / kg, soaked in a solution of ethyl acetate extract are 11.07 mg / kg and soaked in a solution of methanol extract are 14.02 mg / kg. Thus, the ethyl acetate extract of leaves of *Jatropha* is more effective to inhibiting the growth of histamine-forming bacteria and inhibit the formation of histamine in fresh frigate tuna that stored for 5 hours at room temperature.

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Introduction

Histamine poisoning is an issue that is always warm associated with food safety and public health. Histamine is known as scrombrotoxin because generally caused by eating fish from the family Scombridae, such as tuna, mahi-mahi, and mackerel (Murray *et al.* 1981). Beside, eating fish, vegetables, fruit, fermented products containing biogenic amines such as putrescine and kadaverin can also increase the risk of histamine poisoning (Shalaby, 1996; Hungerford, 2010). Histamine poisoning is not only caused by eating fish with high histamine, but also influenced by human factors, such as suffering from histamine intolerance (Hungerford, 2010)

Formation of histamine in fish is directly related to the concentration of histidine, the number and type of bacteria that produce the enzyme histidine decarboxylase (HDC) or histamine-forming bacteria, the position of the meat and the environmental conditions (Lehane and Olley, 1999; Barceloux, 2008). Histidine is an amino acid that can undergo the process of decarboxylation by HDC enzyme produced by fish and bacteria become histamine compound (Keer, *et al.* 2002). Some types of Scombroid contain high free histidine in the flesh, as an example of

big eye tuna as much as 491 mg/100 g, mahi-mahi as much as 344 mg/100 g, skipjack tuna as much as 1.192 mg/100 g, yellow fin tuna as much as 740 mg/100 g, mackerel as much as 600 mg/100 g, and albacore tuna up to 2 g/100 g (Perez-Martin, *et al.*, 1988; Antoine, *et al.*, 1999).

Some types of bacteria are known produce histamine, among others, *Morganella morganii*, *Enterobacter aerogenes*, *Raoultella planticola*, *Raoultella ornithinolytica*, and *Photobacterium damsela* that can produce more than 1000 ppm of histamine. *Hafnia alvei*, *Citrobacter freundii*, *Vibrio alginolyticus* and *Escherichia coli* can produce histamine at concentrations less than 500 ppm (Taylor and Speckhard, 1983; Butler, *et al.* 2010). In addition, the histamine-forming bacteria found in marine fish is *Hafnia alvei*, *Klebsiella pneumoniae*, *Escherichia coli*, *Clostridium perfringens*, *Lactobacillus* sp., *Enterobacter aerogenes*, and *Proteus morganii* (Eitenmiller, *et al.* 1982).

Environmental conditions, especially temperature, are also strongly associated with the formation of histamine. Various studies have report that histamine levels tend to increas during storage and handling that not well (Silva, *et al.* 1998). The results of research about the optimum temperature and the lowest temperature limit for the formation of histamine varies greatly. The optimum temperature of histamine formation by *Morganella morganii* is 25 °C and *Proteus vulgaris*, but at a temperature of 15 °C histamine still produced. in fish meat (Kim, *et al.* 2001). Production of histamine at a temperature of 0 °C as much as 0.61 mg / kg on day 17, and the production of histamine at a temperature of 8 °C increase rapidly as many as 15 mg/100 g on day 8 (Guizani, *et al.*, 2005). Histamine formation occurs very rapidly at a temperature of 20 °C, which is up to 10 times after being stored for 24 hours, increased up to 11:14 mg/100 g of tuna. Food and Drug Administration set a limit on the critical temperature for the formation of histamine in the center of the fish are 4.4 °C (FDA, 2011), Indonesia also set a limit on the critical temperature for tuna are 4.4 °C (BSN, 2006), while the EU determines the temperature of the center of tuna fish, which is the melting temperature of ice, about 0-2 °C (Dalgaard, *et al.*, 2008).

Inhibition of HDC enzyme activity is one way to control histamine formation, because if histamine has been formed, it will be difficult to eliminate existing histamine in fish by heating or freezing (Wendakoon and Sakaguchi, 1995). Natural inhibitor to the enzyme HDC include extracts of various types of spices such as black pepper, cinnamon, and saga (4% v / v) (Wendakoon and Sakaguchi, 1995) dan 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) (Lane and Snell, 1976). The epigallocatechin-3-galic compound from green tea reported can also inhibit the enzyme HDC (Rodríguez-Caso *et al.*, 2003). The combination of cinnamon and liquid smoke gives the best in minimizing the effects of histamine on female mackerel during storage (Mahendradatta dan Tawali, 2006). The combination of cloves and cinnamon in powder form is more effective to minimize the content of histamine in mackerel compared with combination of condiment in the form of extracts (Mahendradatta dan Adiansyah, 2008). Fishing communities in Maluku, Indonesia, often adding a few leaves of *J. curcas* on the boiling process of the frigate tuna (*Auxis thazard thazard*, L) that its quality has begun to decline. The purpose of added the leaves of *Jatropha* is to prevent histamine poisoning when the fish is consumed.

Results of previous studies was reported that *jatropha* leaf extract at a concentration of 1% (w / v) gave inhibitory effects on three types of histamine-forming bacteria are *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Clostridium perfringens*. In addition, *C. perfringens* are very sensitive to ethyl acetate extracts and methanol extracts of the leaves of *Jatropha*, with a MIC of 0.10% (w / v). Thus, the two extracts were chosen to inhibit the formation of histamine in fresh frigate tuna that stored for 5 hours at room temperature.

MATERIALS AND METHOD

1. Material

Jatropha curcas leaves were collected from Waitatiri village, Ambon, Maluku Province, Indonesia, where the plant grows under natural condition. *J. curcas* leaves are used in this study is that older leaves (dark green). Fresh fish, namely the frigate tuna (*Auxis thazard thazard*, L) with a total body length of 23-24 cm, the organic solvent used is hexane, chloroform, ethyl acetate and methanol, distilled water, filter paper, nutrient agar (NA), nutrient broth (NB), plate count agar media, Niven media (0.1% tryptone, 0.3% yeast extract, 1.8% L-histidine monohydrochloride monohydrate, 0.1% CaCO₃, 0.5% NaCl, 2.5% agar, and 0.003% phenol red), glasswool, NaOH 1 N, HCl 0.1 N, orto-ptalatdikarbosilhid 0.1 % in methanol, H₃PO₄ 3.57 N, resin (Dowex 1-x8 50-100 mesh), standard solution of histamine (1 mg/ml atau 1000 ppm) and histamine forming bacteria culture namely, *Klebsiella pneumonia*, *Enterobacter aerogenes* and *Clostridium perfringens* were obtain from the Microbiology laboratory, Faculty of Medicine, University of Indonesia. The microorganisms were maintained at 4°C on Nutrient Agar slant in the Microbiology laboratory, Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia and fresh subcultures were made before use.

2. Equipment

The equipment used was an analytical balance, bottles, funnel, rotary evaporator vacuum, petri dish, erlenmeyer, flask, beaker glass, whatman paper number 42, water bath, incubators, autoclave, Bunsen, micro pipette, blender jar, stirrer-plate, pH meters, spektrofourometer and a set of glassware.

3. Research Methods

a. Extraction

The powdered of leaves of *J. curcas* macerated with hexane, then filtered. The residue was macerated again with different solvents, namely chloroform, ethyl acetate and methanol. The length of time for each maceration solvent is 3 x 24 hours. Furthermore, each filtrate evaporated with a rotary evaporator vacuum. Thus, the entire amount of extract obtained 4 types, but which will be used to soak the frigate tuna is ethyl acetate and methanol extracts.

b. Research Procedures

Body length of the frigate tuna used in this study ranges from 23-25 cm. Quality of the frigate tuna observed using score sheet organoleptic. In addition, the frigate tuna to be used should be free of formaldehyde (using test equipment Quantofix formaldehyd). Then, the frigate tuna brought from the laboratory to the way packed in styrofoam with ice in accordance with the requirements of handling fish.

Before being given treatment, the frigate tuna was analyzed the number of bacterial colonies with total plate count method (Fardiaz, 1989), number of histamine-forming bacteria (Niven, *et al.* 1981) and histamine levels (SNI 2354.10: 2009). Frigate tuna were divided into 3 groups, group 1 was soaked for 30 minutes in water, group 2 soaked for 30 minutes in a solution of ethyl acetate extract of leaf jatropha with a concentration of 1%, and group 3 was soaked for 30 minutes in a solution of methanol extract of leaf jatropha with a concentration of 1%. After that, drained, then stored at room temperature for 5 hours and then the frigate tuna was analyzed the number of bacterial colonies with total plate count method, number of histamine-forming bacteria and histamine levels. This experiment was conducted with 2 replications.

4. Time and Research Location

This study was conducted from June to November 2013. This research was conducted in the laboratory of phytochemical and Biofarmaka, Faculty of Pharmacy, University of Hasanuddin, Makassar, Indonesia and laboratory BPPMHP, Department of Marine and Fisheries, Makassar, Indonesia.

RESULTS AND DISCUSSION

1. Analysis of the quality of fresh frigate tuna

Formalin test results showed that fresh frigate tuna used in this study declared free of formaldehyde. The average of total plate count (TPC) of fresh frigate tuna at 2.2×10^4 cfu / g, number of histamine-forming bacteria are 2.0×10^4 cfu / g and histamine content are 9.03 mg / kg (Table 1). These results indicate that the number of histamine-forming bacteria is lower than the TPC. Based on analysis of TPC, the frigate tuna used in this study are still relatively fresh. Fresh fish quality requirements according to SNI 01-2729-1992, TPC is lower than 5×10^5 cfu / g (BSN, 1992).

Table 1. Data Microbiological test and histamine levels of fresh frigate tuna (*Auxis thazard thazard*, L)

Analysis	Replication		Average
	1	2	
Total Plate Count	2.6×10^4 cfu/g	1.9×10^4 cfu/g	2.2×10^4 cfu/g
Number of histamine-forming bacteria	1.4×10^4 cfu/g	2.6×10^4 cfu/g	2.0×10^4 cfu/g
Histamine levels	10.30 mg/kg	7.75 mg/kg	9.03 mg/kg

2. Total plate count of frigate tuna after storage 5 hours

TPC value of the frigate tuna that treated soaking in ethyl acetate extract, methanol extract, and water ranges between 8.5×10^4 cfu / g to 5.1×10^6 cfu / g. The average of TPC value of the frigate tuna its soaked in

water are log 6.68 (cfu / g), soaked in a solution of ethyl acetate extract are log 4.94 (cfu / g) and soaked in a solution of methanol extract are log 5.61 (cfu / g) (Figure 1).

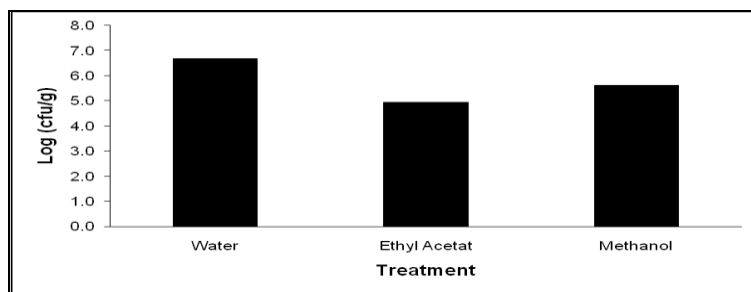


Figure 1. Total plate count of frigate tuna after stored at room temperature for 5 hours

Are naturally, there are a lot of bacteria on the skin of fish, gills and in the stomach. Stomach and gills of fish are part of the body that is highly susceptible to bacterial growth (Sumner, *et al.* 2004). Additionally, the ambient temperature of fish is one of the factors that can support the growth of bacteria. The lower the storage temperature, the slower the growth of bacteria. Based on the research results, the value of log TPC barramundi fish (*Lates calclifer*) ranged between 3-3.2 that stored at 0 ° C for 1-3 days (Yassouralipour, *et al.* 2010), TPC log value tuna (*Thunnus albacares*) is 4.11 cfu / g that stored at 4 ° C for 24 hours (Zapata, *et al.* 2011).

TPC value the frigate tuna that is immersed in water is higher, this is because there is no antibacterial compounds in the water. Whereas the ethyl acetate extract and methanol extracts contain secondary metabolites which have antibacterial properties. Based on the results of the preliminary study, it was reported that the ethyl acetate extract contains compounds alkaloids, phenolics, saponins, tannins and terpenoids, while the methanol extract containing flavonoids, phenolics, tannins, terpenoids and steroids. The this secondary metabolites that serve as antibacterial to inhibit bacterial growth rate in fish. The results showed that, the tuna that soaked in a solution of ethyl acetate extract and methanol extract has a TPC log value is lower and according to SNI 01-2729-1992, not rejected or is expressed the quality is still fresh. On the other hand, the tuna that soaked in water has been rejected or is expressed not suitable for consumption.

3. Number of histamine-forming bacteria on frigate tuna after Storage 5 Hours

The results showed that the number of histamine-forming bacteria in frigate tuna ranged between 2.3×10^4 cfu / g to 4.6×10^5 cfu / g. The average number of histamine-forming bacteria in frigate tuna that soaked in water (control) are log 5.62 (cfu / g), soaked in a solution of ethyl acetate extract are log 4.40 (cfu / g) and soaked in a solution of methanol extract are log 5.54 (cfu / g) (Figure 2).

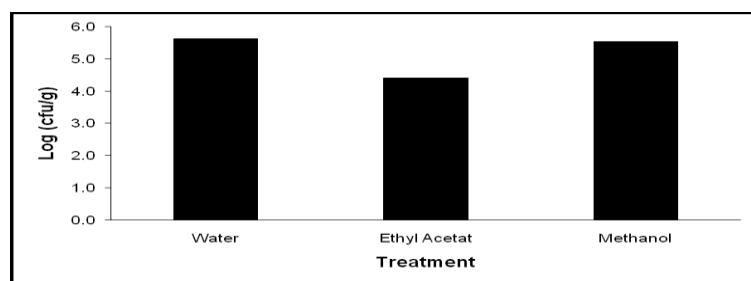


Figure 2. Number of histamine-forming bacteria on frigate tuna after stored at room temperature for 5 hours

Figure 2 shows that the value of the log of histamine-forming bacteria have a similar pattern to the value of the log TPC. Log value of histamine-forming bacteria was highest in the treatment of immersion in water and lowest in immersion in a solution of ethyl acetate extract. The process of formation of histamine in fish is influenced by the activity of the enzyme Histidine Decarboxylase (Mangunwardoyo, *et al.* 2007). Histamine-forming bacteria normally present in the water, settling on the gills, in the intestines of fish and not harmful to the fish itself (Ko, 2006). Various types of bacteria are able to produce the enzyme HDC included in the family Enterobacteriaceae, eg *Enterobacter agglomerans*, *Enterobacter cloacae*, *Enterobacter intermedium*, *Hafnia alvei*, *Klebsiella pneumoniae*,

and *Morganella morganii* (Allen, 2004). Histamine-forming bacteria is generally a group of Gram-negative bacteria mesophilic enterik (Butler, *et al.* 2010), and life at a temperature range of 20-45 °C (Tiwari, *et al.* 2009). Histamine-forming bacteria are often found at room temperature (mesophilic) is *Proteus* spp. and *Morganella morganii* (Kim, *et al.* 2003), whereas which can grow at cold temperatures (psikrofilik) is *Photobacterium phosphoreum* and *Photobacterium histaminum* (Ishimoto, *et al.* 1995).

Histamine-producing bacteria are not always part of the normal bacteria on the skin or the inside (gills and meat) fish. The presence of histamine-forming bacteria in high amounts are not necessarily directly related to high levels of histamine in the sample. This is because the bacteria in the sample did not have same ability to produce histamine. The presence of histamine-forming bacteria can also be caused by cross-contamination of equipment used (Gingerich, *et al.* 2001; Lehane and Olley, 1999)

4. Histamine content on frigate tuna after Storage 5 Hours

Histamine content of frigate tuna that soaked in water (control) was 17.73 mg / kg, soaked in a solution of ethyl acetate extract are 11:07 mg / kg and soaked in a solution of methanol extract are 14:02 mg / kg (Figure 3).

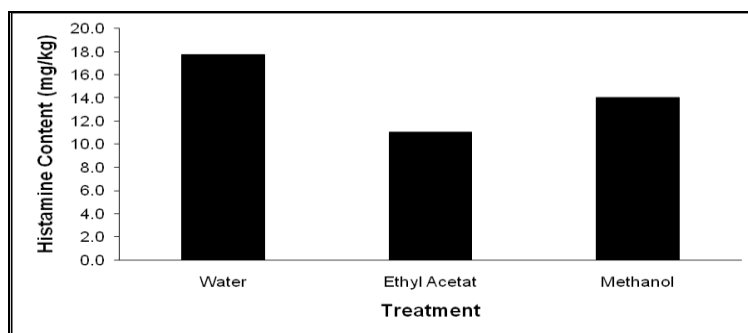


Figure 3. Histamine content on frigate tuna after stored at room temperature for 5 hours

Figure 3 shows the value of the histamine content of tuna fish has the same pattern with TPC value and the value of the amount of histamine-forming bacteria. Histamine content on frigate tuna that soaked in water is higher than that soaked in a solution of ethyl acetate extract and methanol extract. Food and Drug Administration establishes that, tuna, mahi-mahi and a type of fish like that have histamine content at 5 mg / 100 g is the level to watch out for as an indicator of decomposition, while the level of 50 mg/100g is dangerous or can cause poisoning. Therefore, if found fish containing histamine at 5 mg/100 g in one unit, then the possibility of the other units, the level of histamine can reach more than 50 mg/100 g (FDA, 2001).

CONCLUSION

Ethyl acetate extract of leaves of *Jatropha curcas* more effective at inhibiting the growth of histamine-forming bacteria and inhibit the formation of histamine on fresh tuna (*Auxis thazard thazard*, L) stored for 5 hours at room temperature.

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