# THE BIOACTIVITY OF HEXADECANOIC ACID COMPOUND ISOLATED FROM HYDROID Aglaophenia cupressina LAMOUREOUX AS ANTIBACTERIAL AGENT AGAINST Salmonella typhi

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Original Article

# THE BIOACTIVITY OF HEXADECANOIC ACID COMPOUND ISOLATED FROM HYDROID *Aglaophenia cupressina* LAMOUREOUX AS ANTIBACTERIAL AGENT AGAINST *Salmonella typhi*

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**ABSTRACT**

Background & Objectives: The new, more effective antibiotic sources are urgently needed now to overcome the multi-drug resistance (MDR) in the treatment of infectious diseases caused by pathogenic microorganisms. This study was aimed to find out the bioactivity of the hexadecanoic acid isolated from hydroid *Aglaopheniacupressina* Lamoureoux with inhibition mechanisms or lethal to Salmonella typhi that frequently contaminate food materials. Methods: This study was an experimental study with the following treatment stage: isolation and characterization of the compounds from hydroid *A. cupressinaLamoureoux* by chromatography. Hexadecanoic acids and the isolates from hydroid *A. cupressinaLamoureoux* were then tested against *S. typhi* at various concentrations (10 ppm, 20 ppm, and 30 ppm). Result: Study findings indicated that: (1) hexadecanoic acid at the concentration of 30 ppm is bactericidal against *S. syphi.* Interpretation & Conclusion: From the study findings, it can be concluded that hexadecanoic acid (30 ppm) has antibacterial activity by damaging the cells wall of *S. typhi.*



## 1. Introduction

The main problem in the treatment of infectious diseases caused by pathogenic microorganisms is the emergence of the multi-drug resistant (MDR) pathogens. This issue has become an important focus of researches on new bioactive compounds. According to Murniasih (2003), the development of new medicines derived from marine biota is attracting researchers attention due to the very high marine biodiversity and the uniqueness of the produced secondary metabolites structure1,3. This can be achieved by isolating bioactive compounds from marine natural materials, which are safe for health while capable of inactivating bacteria and fungi that contaminate food materials. These active substances accumulate in high number in some marine invertebrates including sponges, tunicates, mollusks, and cnidaria3.

The hydroid *Aglaophenia cupressina* Lamoureoux is an invertebrate from cnidaria phylum that lives by attaching itself to sponges. In addition to alkaloid, diterpene, tridentatol A, and prostaglandin, it also contains histamines, protein and histamine liberator in its nematocyst4 also confirmed that rough extract of *Aglaophenia sp* (0.05%) is capable of inhibiting the growth of *Fusariumoxysporum* & *Sp. Vanilas.* It is assumed that there are remaining many other bioactive compounds from secondary metabolites of hydroid *A. cupressina*L. that can be utilized as pesticide, antifungal and antibacterial. According to(5), secondary metabolite compounds can be treated as lead compound in agricultural and medicinal industries.

This study was a continuation of a previous study on isolation and characterization of secondary metabolites from hydroid *A. cupressina* Lamoureoux as antimicrobial precursor6. From the discovered compounds, one of them was hexadecanoic acid with antibacterial activity in *Escherichia coli* and have been published7. This study was intended to know the ability of hexadecanoic acid and its mechanism in inhibiting or inactivating the Gram-negative bacteria growth such as *Salmonella typhi* which is a pathogenic bacterium that frequently contaminate food materials and result in intoxication. According Schneider et al. (2008), *S. typhi* is not inactivated by cells after consumption, but multiplying itself in cells, and then out of the cells to blood circulation, spreading to entire body that result in systemic infections.

### Material and Methods

This study was conducted in Microbiological Laboratory of Hasanuddin University from May to September 2014 with experimental method.

### Material

Material used in this research such as hydroid Aglaophenia cupressina Lamoureoux, hexadecanoic acid compound, pure culture (*S.typhi*), dimethyl sulphoxide (DMSO) (E Merck), chloramphenicol (P4alpharma), physiological NaCl 0.9%, glucose nutrient agar (GNA) medium, nutrient agar (NA) medium, nutrient broth (NB) medium, Muller Hinton agar (MHA) medium (oxoid).

### Procedure

### Extraction, Partition and Isolation

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The collected samples were cleaned from their substrate, washed, and macerated with methanol for 24 hours three times, filtered and evaporated to obtain thick macerates. The macerates was then partitioned (liquid-liquid 1:1) with n-hexane. Filtrates or n-hexane layer was evaporated to obtain thick extract of n-hexane and continued with vacuum chromatography column, and pressured chromatography column to obtain pure compounds. The obtained pure compounds were then analyzed by thin layer chromatography and melting point measurement, using the reference hexadecanoic acid(7).

Antibacterial Activity Test using Agar Diffusion Method

20 mL of Sterile Muller Hinton agar (MHA) was poured aseptically into petri disc and left to solidify as base layer. After that, the suspension of test bacteria was added 1 mL each into 10 mL of medium above the base layer and left to semi-solid as seeding layer. Six partswith inner diameter of 5 mm, outer diameter of 8 mm, height of 10 mm, were put aseptically with sterile pincer on medium surface at interval of 2-3 cm from petri disc edges, and then stored at room temperature. Each of the parts was added with 0.25 mL hexadecanoic acid and β-cytosterol isolated from hydroid A. cupressinaLamoureoux at concentrations of 10 ppm, 20 ppm, and 30 ppm. Chloramphenicol as positive control and DMSO as negative control, 0.25 mL each, and then incubated at 37oC for 24 hours and 48 hours. Observations were performed by measuring the inhibitory diameter of bacterial growth around the parts by using calliper to observe the ability of the compound in inhibiting the test bacterial growth. The inhibitory ability measurement results at 24 hours and 48 hours were tabulated and analysed.

**Results And Discussion**

**Analysis of Hexadecanoic Acid Structure**

Yellowish white crystal weighting 50 mg with melting point of 430 – 440 C. The compound was fluorescent under UV and blue in colour, but it was not visible by TLC colour test. UV spectrum (CH3OH) indicated maximum absorption at λmax of 212 (164 9m; IR spectrum (KBr) νmax of 3472 cm-1 (OH), 2921, 1855 cm-1 (aliphatic C-H), 2672 cm-1 (aliphatic C-H), 1707 cm-1 (C-O), 1466 cm-1 and 1415 cm-1 (CH2 and CH3), 1299 cm-1 (C-O). NMR spectrophotometer analysis includes 1H-NMR , 13C-NMR, and HMBC as indicated in Figure (1) and Table (1).

Hexadecanoic acid compound had 16 carbons, which were identified by signals appeared as :δC 180.64 signals with the most distant chemical shift indicated carbon at carboxyl group, signals at δC34.30 and 32.12 indicated C-2 and C-14. There are 6 carbons in symmetrical position, namely: C-7, C-8, C-9, C-10, and C-11. It provided one signal with high intensity, namely δC 29.89. In addition, there were very near signals at δC 29, 89;29,79;29,56;29,44;29,25, each of them represented C-4; C-5; C-13; C-6 and C-12. Signals at δC24.85 ; 22.88 and 14.31 represented C-13, C-15 and C-16, respectively.

Analysis of 1H-NMR spectrum indicated the following signals: δH 2,33 (2H,t,J= 7.35) representing 2 proton at C-2, signal at δH 1.62 (2H,m,J=7.35) representing 2 protons C-3, signal at δH1.24-1.28 (20H,m) indicated 20 hydrogen at C-4; C-5; C-6; C-7; C-8;C-9;C-10; C-11; C-12 and C-13, signal δH 1,28-1,29 (2H, m) represented 2 protons at C-14, signal at δH = 1,31-1,33 (2H, m) representing 2 protons at C-15, signal with δH = 0,87 (3H, t, J=6.70) represented 3 protons at C-19, signal at 3.75 (1H, s ) represented proton at hydroxyl group. The number of hydrogen in the compound were 32.

The compound structure elucidation was supported by HMBC spectrum that indicated distant correlation between proton signals and carbon signals as follow; proton signal δH 2,33 (H-2) correlated distantly to carbon signals δC180,64 (C-1) and 24,85 (C-3), proton signal δH 1,28-1,29 (H-14) correlated to signal δC 22,88 (C-15). Proton signals δH 1,31-1,33 (H-15) correlated to carbon signals δC 32,12 (C-14) and δC 14,31 (C=16) and the correlation between proton signal δH 0,87 (H-16) and carbon signals δC 32,12 (C-14) and δC 32,12 (C-14) and δC 22, 88 (C-15).

**Antibacterial Activity by Inhibitory Diameter Measurement**

Inhibitory diameter measurement in *Salmonella typhi*

The results of inhibitory diameter measurement indicated that antibacterial bioactivity of hexadecanoic acid against *S. typhi* at the concentrations of 10ppm and 20ppm for incubation at 24 hours did not subject to inhibitory zone change compared to incubation at 48 hours. In contrast, at concentration of 30ppm the inhibitory zone at 24 hours was 16.00 ppm, but at incubation period of 48 hours the inhibitory zone increased to 17.00 ppm. This indicated that the hexadecanoic acid at concentration of 10 ppm and 20 ppm was bacteriostatic, whereas at the concentration of 30 ppm it was bactericidal. This means that at concentration of 30ppm, hexadecanoic acid not only inhibit the S. typhigrowth but also lethal.

According to Madigam et al. (2012), the bacteriostatic properties can change into bactericidal when the concentration of the compound gets bigger. The mechanism of bactericidal compound affects the bacterial growth by damaging and breaking the cells wall so the bacteria cannot stand with external effect, or disturbing the bacterial cell membrane wholeness, that the active substances or metabolites exchange in and out of the cells will be disturbed10.

Chloramphenicol used as positive control was an aminoglycoside antibiotic, which was bacteriostatic that did not kill the bacteria but inhibit the synthesis of the proteins that are urgently needed in multiplication and division of bacterial cells11. Whereas the DMSO used as negative control did not have antibacterial properties, used to dissolve and stabilize the intracellular extract to be tested12.

**Mechanisms of Bacterial Cells Wall Chemical Reaction to Hexadecanoic Acid**

Figure 6 shows the esterification reaction between hexadecanoic acid and hydroxyl group of lipopolysaccharide that compose the cells wall of gram-negative bacteria. The reaction resulted in alteration in lipopolysaccharide membrane structure into asymmetric. This disturbs the balance in the membrane lipid structure, so the integration and flexibility of the cells membrane are perturbed, which according to Kim et.al. (1995) the reaction between membrane active components and antibacterial compound results in changes in membrane compositions followed by cells swelling and then cytoplasm membrane damage, distended and lysed. According to Radiati (2002), the OH group of hexadecanoic acid can be toxic to cell protoplasm, infiltrating and damaging the cells wall and denaturize the protein in the cytoplasm and form hydrogen bond on the active site of enzyme.

**Table 1. $^{1}$H-NMR and $^{13}$C-NMR Data of Hexadecanoic Acid Compound[7]**

| No. | $^{13}$C-NMR δc (ppm) | $^{1}$H-NMR $\delta_H$ : ppm (multiplicity, *J* in Hz) | HMBC |
|---|---|---|---|
| 1 | 180,64 | - | - |
| 2 | 34,3 | 2.33 (2H, *t J* = 7.35) | 1,3 |
| 3 | 24,85 | 1.62 (2H, m, *J* = 7.35) | 1,2 |
| 4-13 | 29,89; 29,79; 29,56; 29,44; 29,25 | 1.24 – 1.28 (20 H, *m*) | - |
| 14 | 32,12 | 1.28 – 1.29 (2H, *m*) | 15 |
| 15 | 22,88 | 1.31 – 1.33 (2H, *m*) | 14,16 |
| 16 | 14,31 | 0.87 (3H, *t*, *J* = 6.70) | 14,15 |
| OH | - | 3.75 (1H, s) | - |

**Table 2. Antibacterial bioactivity of hexadecanoic acid against Salmonella typhi at the concentrations of 10ppm and 20ppm for incubation at 24 and 48 hours**

| Treatment | Inhibitory Zone Diameter (mm) 24 hour | 48 hour |
|---|---|---|
| A = Hexadecanoic acid at 10ppm | 18,00 | 18,00 |
| B = Hexadecanoic acid at 20ppm | 18,50 | 18,50 |
| C = Hexadecanoic acid at 30ppm | 16,00 | 17,00 |
| D = Negative Control (DMSO) | 0,00 | 0,00 |
| E=Positive Control (Chloramphenicol) | 12,00 | 15,00 |

Source; processed data 2014

**Fig 1. NMR Spectrometer Analysis**

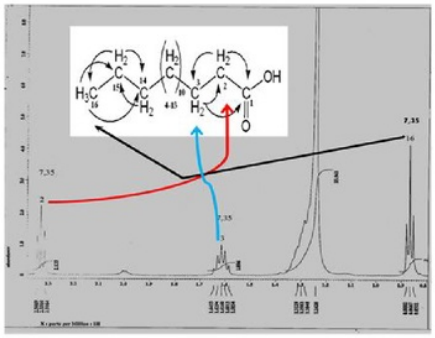


**Fig. 2. Salmonella typhi, incubation at 24 hours**

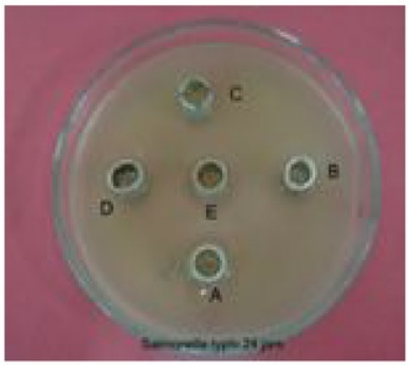



**Fig 3. Salmonella typhi,incubation at 48 hours**

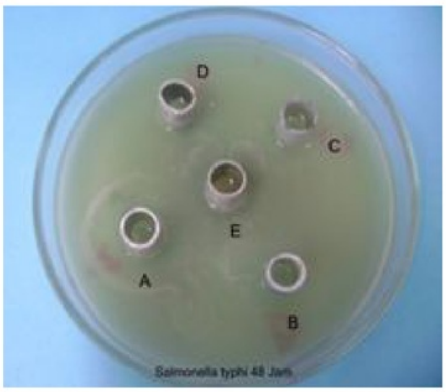
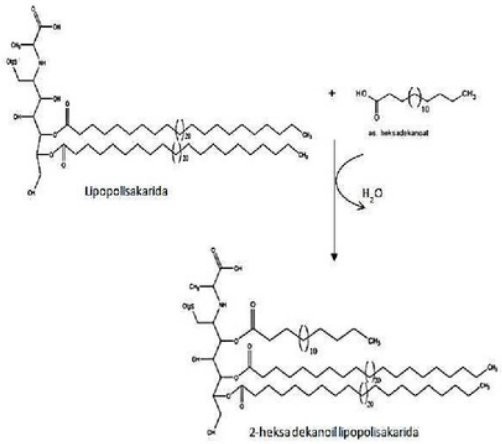



**Fig 4. Bacterial Cells Wall Reaction to Hexadecanoic Acid**




## Conclusion

Hexadecanoic acid at concentration of 30 ppm is bactericidal against Salmonella typhi by damaging its cell membrane structure.

## Acknowledgement

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