



## Antibacterial Activity of Scopoletin from Stem Bark of *Aleurites moluccana* Against *Salmonella typhi*

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### Abstract

In antibacterial screening by diffusion test, methanol extract of stem bark *A.moluccana* showed excellent growth inhibition of *Salmonella Typhimurium* cause of typhoid fever. Some coumarin metabolites are very good as an anti-bacterial against *Salmonella Typhimurium*. Method: Simplicia of *A.moluccana* stem bark was extracted by maceration using methanol. The dry extract is extracted liquid using water-organic solvent (hexane and ethyl acetate) in a separating funnel. The hexane and ethyl acetate fraction was monitored for active spots with TLC bioautography. The active compound is separated using vacuum liquid chromatography and radial chromatography. Results: The fraction J of KCV and J2 subfraction results from radial chromatography showing the antibacterial activity of *S. thpimurium*. From the J2 subfraction obtained pure isolates in the form of yellowish needle crystals. The isolate was tested for antibacterial *S.Typhimurium* using the microdilution method with a value of MIC is 250 µg/ml. Based on spectroscopic data and comparing the published spectra of the compound, the elucidation of the isolate is Scopoletin (7-hydroxy-6-methoxycoumarin).

**Keywords:** *Aleurites moluccana* stem bark, KLT Bioautography, *Salmonella Typhimurium*, Scopoletin

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**Submitted:** 07 September 2019

**Accepted:** 17 February 2020

**DOI:** <https://doi.org/10.25026/jtpc.v5i1.218>

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### ■ Introduction

WHO noted, total of 216,510 deaths in 2000, caused by 21,650,974 diseases from typhoid fever included Southern, Central and Southeast Asia. [1].

It is estimated that in 2011, between 600,000-1.3 million typhoid cases each year, causing more than 20,000 deaths in Indonesia [2]. *Salmonella Typhimurium* is the cause of typhoid and

paratyphoid fever, and a large number of serotypes are responsible for the severe gastrointestinal disease. The serotypes can be distinguished by agglutination and staining. *Aleurites moluccana* bark is used by the people of East Kalimantan Indonesia for antibacterial drugs for typhoid fever. [3-4].

Previous studies have shown the antibacterial activity against some Isolates clinical isolates from *A. moluccana* extracts which may be caused by the composition of polyphenols, including as a coumarin group [5]. Some coumarin metabolites such as scopoletin are very good as antibacterial. [6-8]. This study wanted to show the antibacteria activity of the content of the scopoletin from *A.moluccana* against *Salmonella Typhimurium* that causes typhoid fever.

## ■ Materials and Methods

### Samples

The plants used in the study were *A.moluccana* stem bark. Sample used in the study was obtained from Topeng village, Klaten district, Central Java. Determination of plants is done at the Herbarium of the The School of Life Sciences and Technology, Bandung Institute of Technology. Sample preparation includes, selection of healthy skin (wet sorting), washing, drying, grinding, sifting to obtain dry powder ready for extraction.

### Materials

Materials, distilled water, dimethyl sulfoxide (DMSO), bacterial media (Nutrient Agar 70148, Muller Hilton Agar (MHA), and Muller Hilton Broth (MHB) (Sigma-Aldrich)), organic solvents (methanol, n-hexane, ethyl acetate), 10% KOH reagent in ethanol, silica gel (Meck®) GF254, silica gel (Meck®) plate GF254.

### Microbial Suspensions

*Salmonella Typhimurium* culture was bred to get starter bacteria. Test bacterial cultures using the standard McFarland no. 0.5 on Abs 0.132 at  $\lambda$  600-625 nm with UV visible spectrophotometer (Hewlett Packard® 8453). The number of suspensions produced  $1.5 \times 10^6$  CFU / unit [9-10].

### Antibacterial with Bioautography Test

In the test sample antibacterial activity using TLC was carried out using the silica gel GF254 plate (Meck®). MHA media are mixed with bacterial test suspension. The chromatographic results were placed on the medium for 30 minutes then released. Incubated (Wise Cube® WIG-105) at 37 °C for 24 hours. Antibacterial activity is indicated if there is a clear zone in the media. The distance of RF is confirmed by the results of Thin Layer Chromatography (Camag®) to localize stains that have activity [11].

### Microdilution Test

Microdilution test was carried out with test hole microplate (iwaki®). The concentration used starts from 1000, 500, 250, 125, 62.50 and 31.25  $\mu\text{g/ml}$ . The test sample was dissolved with NaCl sterile with the help of DMSO. All holes in the microplate are filled with 100  $\mu\text{L}$  MHB media. The test hole was filled with 100  $\mu\text{L}$  bacterial suspension. The negative control (DMSO) and bacterial control was also carried out at different holes. Incubated at 37 °C for 24 hours. The experiment was repeated three times. Minimum Inhibition Concentration is determined by looking at turbidity and sediment [11-12].

## ■ Results and Discussion

The skin of *A.moluccana* stem bark obtained carried out by drying and grinding to obtain simplicia powder. Some stages of chemical separation of *A.moluccana* bark include maceration using methanol, fractionation using hexan and ethyl acetate, vacuum chromatography, and radial chromatography [13]. The initial profile of activity against *S.typhimurium* bacteria was used TLC bioautography. Antibacterial activity in the J fraction and sub J2 fraction has antibacterial activity. The compound on the TLC plate diffuses into the media so that direct contact with bacteria occurs. The active J fraction inhibits the *S.Typhimurium* bacteria in the form of a clear area which has the same RF as the chromatogram profile on blue fluorescent spots under UV 254 or 366 nm shown in Figure 1.

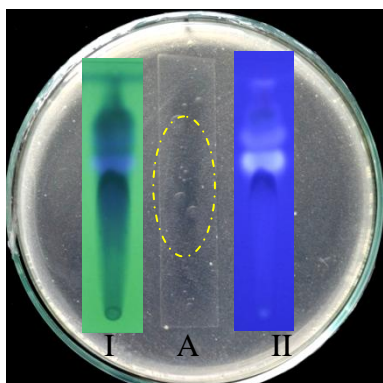


Figure 1. (A) bioautogram inhibiting the activity of *S. Typhimurium* bacteria growth (MHA, 37°C, 24 hours) KCV ethyl acetate fraction compared to TLC chromatogram (Mobile phase hexane: ethyl acetate 4: 6) monitored under UV 254 nm (I) and UV 366 nm (II)

In Figure 2 there is a good J2 subfraction activity. The chromatogram profile was detected in the form of blue fluorescent spots under UV 254 and 366 nm at Rf 0.6.

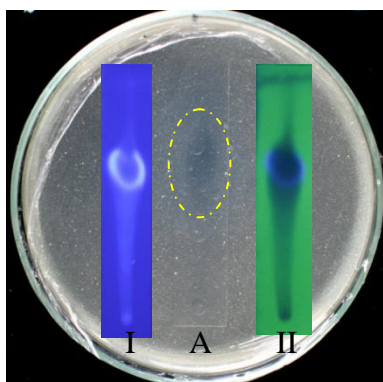


Figure 2. (A) bioautogram of fraction activity (J2) in the growth of *S. Typhimurium* bacteria. Mobile phase Hexane: ethyl acetate (4: 6). Monitored under (I) UV 254 nm (II) UV 366 nm. (MHA, 37°C, 24 hours)

In the J2 fraction, recrystallization was carried out, obtained by the pure compound in the form of yellowish-white needle crystals. Previous isolates were obtained and characterized using chemical tests, UV-Vis spectrophotometers and nuclear magnetic resonance (1D-NMR and 2D-NMR), as well as literature. The isolate is a scopoletin (7-

hydroxy-6-methoxycoumarin), which has been identified in previous studies. [13].

Quantitative testing of minimum inhibitory concentration (MIC) of isolates by microdilution method using a well microplate test. The profile of antibacterial activity in MHA media is seen as a clear zone.



Figure 3. MIC of microdilution test results on isolates is 250 µg/ml, and DMSO control □. Left-right hole number concentration No. 1-9 (1000, 500, 250, 125, 62.50, 31.25 µg/ml). *S. Typhimurium* bacteria, incubation on 37°C 24 hours, Muller Hilton Broth (MHB) media.

Scopoletin MIC against *S. Typhimurium* bacteria (Figure 3) was found at a concentration of 250 µg/ml which occurred in all three replications. Antibacterial activity is indicated by the absence of sediment and turbidity. MIC values have been confirmed by scraping samples of 250 µg/ml and 125 µg/ml on MHA media. Bacterial growth is only found at a concentration of 125 µg/ml.

Coumarin is composed of heterocyclic form of benzene and pyrone rings substituted for oxygen and their derivatives with strong biological activity. Coumarin is considered a heterocyclic bioactive mixture which promises various anti-microbial activities because it can damage the cell wall permeability [7,14,15].

Scopoletin compound has been known to have activity against the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus* with MICs +/- 1000 µg/ml [16]. In testing the bacteria *S. Typhimurium* (U937) which is resistant to human intracellular macrophages invitro also shows good results

[17]. In addition scopoletin has other activities, such as antitumor, prostate cancer cells, antidiabetic, antihyperlipidemic and others [18-20]

## ■ Conclusion

The Scopoletin (7-hydroxy-6-methoxycoumarin) compound obtained from the stem bark of *Aleurites moluccana* to have antibacterial activity against *Salmonella Typhimurium* with a MIC concentration of 250 µg/ml.

## ■ Acknowledgements

This research is was supported by Research Laboratory and Development of Pharmacy Tropical, Pharmacy Mulawarman University and Research Division of Organic Chemistry, Institute of Technology Bandung.

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