



## Potency of Mangosteen Pericarp Extract to Inhibit 38-kDa and Ag85 Protein Secretion by *Mycobacterium tuberculosis* H37Rv

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### Abstract

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. The  $\alpha$ -mangostin in mangosteen pericarp extract can inhibit *M. tuberculosis* growth. This study examined the potency of  $\alpha$ -mangostin in mangosteen pericarp extract to inhibit 38-kDa and Ag85 protein secretion from *M. tuberculosis* H37Rv. The samples used in this study were divided into three independent variable groups (3.125  $\mu$ g/ml; 6.25  $\mu$ g/ml; and 12.5  $\mu$ g/ml  $\alpha$ -mangostin in the mangosteen pericarp extract), a positive control group (rifampin), a negative control group (*M. tuberculosis* H37Rv), and the Garcia® group (trademark of the mangosteen peel extract capsule). HPLC-MS/MS detected that the mangosteen pericarp extract contained 5,984.55  $\mu$ g/g  $\alpha$ -mangostin. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and dot blot analysis were conducted to analyze the profile and specificity of both proteins. This study revealed that 6.25  $\mu$ g/ml of mangosteen pericarp extract had the greatest potency for inhibiting 38-kDa protein secretion from *M. tuberculosis* H37Rv, and 12.5  $\mu$ g/ml mangosteen pericarp extract had greater potency for inhibiting Ag85 protein secretion than did the other doses. Thus,  $\alpha$ -mangosteen from mangosteen pericarp extract can inhibit Ag38 and Ag85 secretion.

**Keywords:**  $\alpha$ -mangostin, Ag38 kDa, Ag85, *Mycobacterium tuberculosis*.

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### 1. Introduction

Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis*. Most *M. tuberculosis* attack the lungs but can also attack other organs [1]. Based on WHO 2012 global tuberculosis report, Indonesia was the fourth largest contributor to tuberculosis

worldwide after India, China and South Africa. The treatment duration for new tuberculosis cases lasts at least 6 months and includes 4 first-line antituberculosis drugs: isoniazid, rifampicin, ethambutol and pyrazinamide. In 2011, the World Health Organization (WHO) estimated that 500,000 tuberculosis cases were resistant to isoniazid and rifampicin annually. In Indonesia, the number of TB-MDR (tuberculosis-multidrug-resistant) cases is approximately 2% for new cases and 20% for retreatment cases [1]. The high prevalence rates of tuberculosis as well as TB-MDR cases demonstrate the need to seek natural-based therapies. Mangosteen (*Garcinia mangostana* Linn.) is a tropical plant with a biological compound that has potential therapeutic applications. Prenylated xanthenes from mangosteen pericarp containing  $\alpha$ - and  $\beta$ -mangostin and garcinone B have potential as antituberculosis drugs by inhibiting *Mycobacterium tuberculosis* growth [2,3].

The Ag85 protein plays a role in bacterial cell wall development through its mycolyl-transferase activity, which can increase trehalose dimycolate (TDM) and arabinogalactan binding with mycolic acid in the *M. tuberculosis* bacterial cell wall, thus potentially becoming a target of anti-TB drugs [4]. In this study, we examined the antituberculosis activity of mangosteen pericarp extract on *M. tuberculosis* through its ability to inhibit secretion of the 38-kDa and Ag85 virulence proteins.

## 2. Materials and Methods

This research was conducted in the Pharmacognosy and Phytotherapy Laboratory and Biomedical Laboratory, Faculty of Medicine, Brawijaya University, and the Instrument Analysis Laboratory, Chemical Engineering, State Polytechnic of Malang; and Balai Besar Laboratorium Kesehatan (BBLK) Surabaya.

### 2.1. Design

This research was an in vitro experimental study with a post-test-only design. Mangosteen pericarp powder (UPT Materia Medika, Batu) was extracted by maceration using a 96% ethanol solvent. The phytochemical contents (alkaloids, triterpenoids, tannins, polyphenols, saponins, flavonoids) of the extracts were determined, and  $\alpha$ -mangostin was identified using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [5, 6]. The mangosteen pericarp extract concentrations used were 3.215  $\mu\text{g/ml}$ , 6.25  $\mu\text{g/ml}$ , and 12.5  $\mu\text{g/ml}$ .

The 38-kDa and Ag85 protein profiles were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using gel electrophoresis with silver staining (4% separating gel - 4% stacking gel) and Coomassie blue staining (12% separation gel - 4% stacking gel) [7]. Specificity of the 38-kDa and Ag85 proteins was tested by the dot blot method. The results were read using ImageQuant LAS 500, from GE Healthcare Bio-Sciences, Sweden.

### 3. Results and Discussion

#### 3.1. Determination of Protein Levels in Culture Filtrates of *M. tuberculosis* H37Rv

*M. tuberculosis* H37Rv isolates (BBLK Surabaya) were grown in Lowenstein-Jensen medium and incubated at 37°C for 4 weeks, then cultured in BACTEC MGIT liquid medium for 7 days. Protein levels of the 5 treatments (i.e., 3.125 µg/ml, 6.25 µg/ml and 12.5 µg/ml mangosteen pericarp extract; negative control; and Garcia® (trademark of the mangosteen 2.12 mg/ml peel extract capsule) were then measured. The protein levels in the bacterial culture filtrate were determined by a NanoDrop spectrophotometer. Table 1 shows that the highest protein content

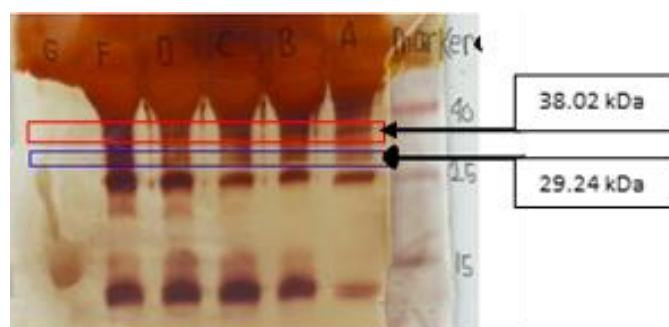
(2.65 mg/ml) was in tube 4, which contained the negative control.

#### 3.2. Analysis of the 38-kDa and Ag85 Protein Profiles using SDS-PAGE Silver Staining and Coomassie Blue Staining

SDS-PAGE silver staining showed poorly separated protein bands (Figure 1). The molecular weight of the protein target was calculated using interpolation, which showed a protein with a molecular weight of 38.02 kDa as was expected for the 38-kDa protein. Then the same formula was used to obtain a molecular weight of 29.24 kDa, which was likely the Ag85 protein with a molecular weight of 30 kDa.

**Table 1.** Protein Level Determination Results from the *M. tuberculosis* H37Rv Culture Filtrate Using the NanoDrop

Samples	mg/ml	Absorbance
Tube 1 (3.125 µg/ml extract)	1.62	1.622
Tube 2 (6.25 µg/ml extract)	2.64	2.643
Tube 3 (12.5 µg/ml extract)	2.64	2.642
Tube 4 (negative control)	2.65	2.653
Tube 6 (Garcia®)	2.12	2.119



**Figure 1.** Results of the 38-kDa and Ag58 protein profile analysis using SDS-PAGE silver staining. Description: A: mangosteen pericarp extract 1 (3.125 µg/ml); B: mangosteen pericarp extract 2 (6.25 µg/ml); C: mangosteen pericarp extract 3 (12.5 µg/ml); D: negative control (*M. tuberculosis* H37Rv bacterial growth); F: Garcia®; G: Medium BACTEC-MGIT.

Figure 2 shows the SDS-PAGE Coomassie blue staining protein band profiles. The interpolation results revealed a protein with a molecular weight equal to 37.15 kDa, which was expected for the 38-kDa protein, and a protein with a molecular weight of 29.58, kDa which was presumed to be the Ag85 protein (30 kDa).

### 3.3. Specificity Test Using the Dot Blot Method.

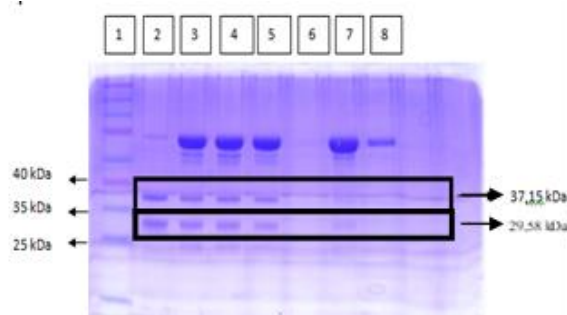
The results of the dot blot test reading using the ImageQuant LAS 500 on the 38-kDa protein showed the smallest signal value for treatment B ( $\alpha$ -mangostin 6.25  $\mu$ g/ml),

followed by treatments F (Garcia®), H (blanko solution), G (MGIT), A ( $\alpha$ -mangostin 3.125  $\mu$ g/ml), D (negative control), C ( $\alpha$ -mangostin 12.5  $\mu$ g/ml), and E (positive control, rifampin). This suggested that 6.25  $\mu$ g/ml  $\alpha$ -mangostin could effectively inhibit 38-kDa protein secretion. The dot blot test reading on the Ag58 protein showed that 12.5  $\mu$ g/ml  $\alpha$ -mangostin inhibited Ag58 protein secretion the most. Table 2 shows these results for the 38-kDa protein.

The antituberculosis mechanism of the flavonoid resulted from its inhibiting fatty acid synthase II (FAS II), thus inhibiting the formation of mycolic acid and Ag85 protein

**Table 2.** Results of the Dot Blot Test Reading on the 38-kDa Protein Using ImageQuant LAS 500

Treatment	Signal reading value
A (3.125 $\mu$ g/ml)	135873.76
B (6.25 $\mu$ g/ml)	19069.41
C (12.5 $\mu$ g/ml)	219134.53
D (negative control)	196052.53
E (positive control)	296210.78
F (Garcia®)	78072.51
G (MGIT medium)	107014.54
H (blanko solution)	10383737



**Figure 2.** Results of the 38-kDa & Ag85 protein profile analysis with SDS-PAGE Coomassie blue staining. Description: 1: marker; 2: mangosteen pericarp extract 1 (3.125  $\mu$ g/ml); 3: mangosteen pericarp extract 2 (6.25  $\mu$ g/ml); 4: mangosteen pericarp extract 3 (12.5  $\mu$ g/ml); 5: negative control (*M. tuberculosis* H37Rv bacterial growth); 6: positive control (Rifampin 1.0  $\mu$ g/ml); 7: Garcia®; 8: BACTEC-MGIT medium

secretion [8]. The antibacterial mechanism of terpenoid group compounds is caused by a disruption in the lipid membrane of the bacterial plasma membrane, which results in intracellular leakage and alters the membrane permeability [9].

The protein profile analysis of the *M. tuberculosis* H37Rv bacterial culture filtrate sample with SDS-PAGE silver staining required a pure sample because this method has a high sensitivity that is 30–100 times more sensitive than Coomassie blue staining. The silver staining method can detect very small protein amounts (in nanograms) [10]. The target protein's position can be estimated by calculating the molecular weight using the interpolation formula. Based on this calculation, proteins with molecular weights of 38.02 kDa and 29.24 kDa were detected and located between the 40-kDa and 25-kDa protein markers, which were suspected to be the 38-kDa protein and the Ag85 protein (30 kDa).

No protein band profile was seen based on the SDS-PAGE Coomassie blue staining results, as seen in Figure 2, line 6 (rifampin as the positive control). This was because when the samples were concentrated with 45% 4'-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid (AMS), no precipitate was found on the positive control sample, which could affect the SDS-PAGE results. Immunoblotting was required to react antigens and specific antibodies. Therefore, the LAS ImageQuant 500 was used to measure the specificity of the antigen-antibody bond to

confirm that the protein with the molecular weight of 37.15 kDa was the 38-kDa antigen and the 29.58-kDa protein was the Ag58 protein and to determine a dose that could effectively inhibit secretion of both proteins.

The immunoblotting technique used in this research was the dot blot method. The LAS reading results showed that the 6.25 µg/ml mangosteen pericarp extract had the smallest value, thus indicating that the 38-kDa protein content was also low in treatment B. The protein profiles with the Coomassie blue staining showed that a 37.15-kDa protein had a thinner protein band at the 6.25 µg/ml treatment dose compared with the 3.125 µg/ml dose. Therefore, the mangosteen pericarp extract (*Garcinia mangostana* L.) dose that effectively inhibited the 38-kDa antigenic protein secretion was 6.25 µg/ml.

The comparison treatment with Garcia® showed thin protein bands that were inconsistent with the LAS ImageQuant 500 reading result, which indicated that the Garcia® had a larger signal than the 6.25 µg/ml dose, but Garcia® should have had a lower signal. In addition, the positive controls showed no visible protein bands, which were inconsistent with the LAS ImageQuant 500 reading results, thus indicating that the positive control treatment (rifampin) had the greatest signal value.

Dot blot analysis on the Ag85 protein strengthened the protein profile analysis results by the SDS-PAGE Coomassie blue staining, in which Garcia® inhibited Ag85 protein

secretion more than the other treatments. Comparing the three mangosteen pericarp extract doses, the 12.5 µg/ml dose inhibited most of the Ag85 secretion. These results showed the thinnest 29.58-kDa protein band compared with the mangosteen pericarp extract with doses of 3.125 µg/ml and 6.25 µg/ml α-mangostin. This was consistent with the dot blot test reading on the Ag58 protein, which showed that 12.5 µg/ml α-mangostin inhibited Ag58 protein secretion the most. Ag85 proteins play roles in bacterial cell wall development via mycolyl-transferase activity, which can synthesize trehalose dimycolate (TDM), a primary lipid found on the exterior of virulent *M. tuberculosis* cells, necessitating large doses to inhibit Ag85 protein secretion [4].

Based on the results of this study, several points must be noted: the length of the bacterial inoculation must be optimized to obtain the optimal protein secretion level in the culture filtrate; the immunoblotting procedure using the dot blot method should also be considered relative to the blocking and washing techniques, and the blocking solutions should be adjusted to the detection procedures to avoid an overreaction affecting the results.

#### 4. Conclusion

This study showed that α-mangosteen from mangosteen pericarp extract can inhibit Ag38 and Ag85 secretion.

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