

# Immunomodulator Potential of Miana Leaves (*Coleus scutellarioides* (L) Benth) in Prevention of Tuberculosis Infection

Sesilia Rante Pakadang<sup>1</sup>, Chatarina Umbul Wahjuni<sup>2</sup>, Hari Basuki Notobroto<sup>2</sup>, Dwi Winarni<sup>3</sup>, Ressay Dwiyanti<sup>4,6</sup>,  
Yadi<sup>5,6</sup>, Muhammad Sabir<sup>4,6</sup>, Mochammad Hatta<sup>6,\*</sup>

<sup>1</sup>Health Technology Department, Ministry of Health, Makassar, Indonesia

<sup>2</sup>Faculty of Public Health, Airlangga University, Surabaya, Indonesia

<sup>3</sup>Faculty of Life Sciences and Technology, Airlangga University, Surabaya, Indonesia

<sup>4</sup>Department of Microbiology, Faculty of Medicine, Tadulako University, Palu, Indonesia

<sup>5</sup>Department of Microbiology and Immunology, Faculty of Medicine, Mulawarman University, Samarinda, Indonesia

<sup>6</sup>Molecular Biology and Immunology Laboratory for Infectious Diseases, Microbiology Department, Faculty of Medicine,  
Hasanuddin University, Makassar, Indonesia

\*Corresponding author: hattaram@indosat.net.id

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**Abstract Aim**, The aim of this study to investigate the immunomodulator effects of miana leaves (*Coleus scutellarioides* (L) Benth) on prevention of tuberculosis in wistar rats, **Method**, Samples of white male Wistar rats were divided into 4 groups, the samples were treated with miana leaves extract (EDM) and then infected by intra tracheal *M. tuberculosis* H37Rv strain and subsequently given a placebo, EDM and GAB (combined Rifampicin and EDM) and healthy control animals were treated with EDM. In this study we measure the level of Immunomodulatory parameter; the number of T-lymphocytes and CD4 T-cells measured by flowcytometry method, the levels of IFN- $\gamma$  and TNF- $\alpha$  were measured by ELISA and the number of *M. tuberculosis* colonies derived from the rat lung in LJ media. **Result**, Results showed the increasing of T-lymphocytes, CD4 T-Cells, the levels of IFN- $\gamma$ , TNF- $\alpha$  and decreasing in the number of *M. tuberculosis* colonies after EDM treatment. **Conclusion**, Miana leaves extract (*Coleus scutellarioides*, (L) Benth.) increased the number of T-lymphocytes, CD4 T-cell counts, levels of IFN- $\gamma$  and TNF- $\alpha$  and decreased in the number of *M. tuberculosis* colony in infected wistar lung.

**Keywords:** immunomodulator, miana leaves (*Coleus scutellarioides* (L) Benth), mycobacterium tuberculosis, CD4, IFN- $\gamma$ , TNF- $\alpha$

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## 1. Introduction

Tuberculosis (TB) remains one of the world's deadliest communicable diseases. In 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease, 360 000 of whom were HIV-positive. The majority of cases worldwide in 2012 were in the South-East Asia (29%), African (27%) and Western Pacific (19%) regions. Indonesia is the fifth countries with the largest number of incident cases in 2012 after India, China, South Africa, with incident cases 0.4 million–0.5 million. The detection of new cases of pulmonary TB in Indonesia in 2013 was 196.310 cases. South Sulawesi province becomes seventh ranks with 10.970 new cases of pulmonary tuberculosis patients [1]. Tuberculosis is slowly declining each year and it is estimated that 37 million lives were saved between 2000 and 2013 through

effective diagnosis and treatment. However, the mortality from the disease is still unacceptably high, therefore efforts to combat it must be accelerated within the context of the Millennium Development Goals (MDGs) [2].

Tuberculosis can infect anyone who lives in the patient environment. People who live and interact with the patient can be infected with *M. tuberculosis* by patients droplets inhaled through the air. A person with a healthy condition without immunity impairment will be spared from tuberculosis, although it has been exposed. Activation of macrophages serves to increase phagocytosis, increase the destruction of microorganisms, increase chemotaxis, act as antigen presenting cells, increases the secretion of enzymes and increase the important biological substance. Macrophages are important effector cells involved in phagocytosis, microbial killing and antitumour activity. Macrophages also display accessory cell function, in that they can present antigen to foster the development of T-lymphocyte-mediated immunity. These Properties may be

influenced by cytokines such as IFN- $\gamma$  and TNF- $\alpha$  released from macrophages, lymphocytes and other tissue cells [3,4,5].

Macrophage-*Mycobacterium* interactions and the role of macrophage in host response can be summarized under the following conditions: surface binding of *M. tuberculosis* to macrophages; phagosome-lysosome fusion; mycobacterial growth inhibition and killing; recruitment of accessory immune cells for local inflammatory response and presentation of antigens to T-Cells for development of acquired immunity. The specific acquired immune response mainly responsible for protective Th1 cytokines and through CD8 cells bringing about cytotoxicity. The complexity of the host-pathogen interaction and underlines the importance of identifying the mechanisms involved in protection [6].

It is important to improve immunity status of patients during tuberculosis chemotherapy. Immunostimulant supplementation may represent a novel approach for fast recovery in tuberculosis patients. Immunomodulatory drugs can be chemical or natural products such as herbal remedies derived from plants. The tendency back to nature today, causing the utilization of herbal medicine became very popular in the community. Medicinal plants now used as a primary or complementary therapy to boost immunity or maintain health and fitness. One of the medicinal plants used in Toraja ethnic communities (in the province of South Sulawesi, Indonesia) is miana (*Coleus scutellarioides*, (L) Benth). Preliminary study in 2013 on practitioners of traditional healers in Tana Toraja Toraja concluded that tribal communities have been using the leaves of miana for TB treatment. The survey found that 74% of TB patients using traditional medicine as complementary so that miana leaves as become indigenous in the treatment of TB in the Toraja. Although it has been used empirically by the Torajanese for TB treatment but there is no scientific demonstration, then the miana leaves potential to be researched and developed considering tuberculosis cases in Indonesia is still high.

## 2. Materials and Methods

### 2.1. Miana Leaves Preparation

The powdered plant material was macerated with alcohol 70% for about 48 hours with occasional shaking. Macerate was decanted and filtered through cloth and then through filter paper to obtain a clear extract. This process was repeated with the same volume of hydro-alcoholic mixture. Macerates were pooled and collected in trays and evaporated to dryness at 30-35°C [7]. EDM dose used was 510mg/kg rat suspended with Sodium CMC 1% w/v.

### 2.2. Bacterial Strains

We using *Mycobacterium tuberculosis* H37Rv strain obtained from Tuberculosis Laboratory, Institute of Tropical Disease, Airlangga University. 50  $\mu$ L of  $5 \times 10^8$  cells/ml of bacteria was used [8,9].

### 2.3. Rats

Healthy adult male wistar rats (150 – 200g) were obtained from the Medical Faculty, Airlangga University,

Indonesia. The animals were housed in groups of 4 in solid bottom cages. The animals were acclimatized for a period of one week and were kept under pathogen free conditions. The animals had free access to filtered water and standard pellet phokphand CP593 (PT. Charoen Phokphand Indonesia). The freshly prepared aqueous solution of EDM was administered to animals orally in the same dosage (510mg/kg). K1 is a healthy control animal group were not infected with *M. tuberculosis* but only with a placebo (on days 1-30) followed by EDM for 21 days (on days 31-51). K2 is a group of animals treated with EDM orally once daily for 21 days then on day 22 were infected with *M. tuberculosis*. On day 22-51, EDM replaced with placebo (Na CMC). K3 is a group of animals treated with EDM orally once daily for 51days then on day 22 were infected with *M. tuberculosis*. K4 is a group of animals treated with EDM orally once daily for 21days then on day 22 were infected with *M. tuberculosis*, followed by combined EDM and rifampicin on day 22-51. K2, K3 and K4 are groups of animals administered with preventive EDM for 21 days and later infected with *M. tuberculosis* H37Rv strain (day 22) in intratracheal. We infected with 50 $\mu$ l of  $5 \times 10^8$  unit/ml in anaesthetized wistar with ketamin (100mg/ml); diazepam (5mg/ml) intramuskular [8,10,11,12,13].

### 2.4. Immunity parameter

Immune parameters tested in this study including the number of T-lymphocytes and CD4 T-Cells from blood samples rats, using flowcytometri methods; IFN $\gamma$  and TNF- $\alpha$  level from rat's blood samples, using ELISA method and the number of *M. tuberculosis* colonies from rat's lung samples in Lowenstein Jansens medium.

### 2.5. Ethics Statement

All animals were maintained in accordance with protocols approved by the institutional animal ethical committee of Animal Care and Use Committee of Veterinary Faculty, Airlangga University with ethical clearance number: 364-KE.

## 3. Results

### 3.1. In Vivo Effects of EDM on T-lymphocyte and CD4T-Cells Number in Animal Groups

The K1 as a control group of healthy test animals were given EDM. In this K1 group shown that the mean of T-lymphocyte and CD4 T-Cells is 54.67 and 22.16 while K2 as a control group of sick animals that were 83.63 and 42.05. The mean of T-lymphocyte and CD4 *T-cell* in the K3 as group were 79.96 and 38.54, while in the K4 group were 58.98 and 28.25. There were significantly different among all groups (Figure 1).

### 3.2. In Vivo Effects of EDM on IFN-gamma and TNF-alpha Levels in Animal Groups

The K1 as a control group of healthy animals were given EDM was shown that the mean level of IFN gamma and TNF alpha were 0.72 and 1.22 while K2 group as a control group of sick animals that were given preventive

EDM and placebo was shown that the mean level of IFN-gamma and TNF-alpha were 6.37 and 140.77. The mean level of IFN gamma and TNF alpha in K3 group were

2.30 and 73.25 while in K4 group were 0.77 and 9.55. There was significantly different among all groups (Figure 2).

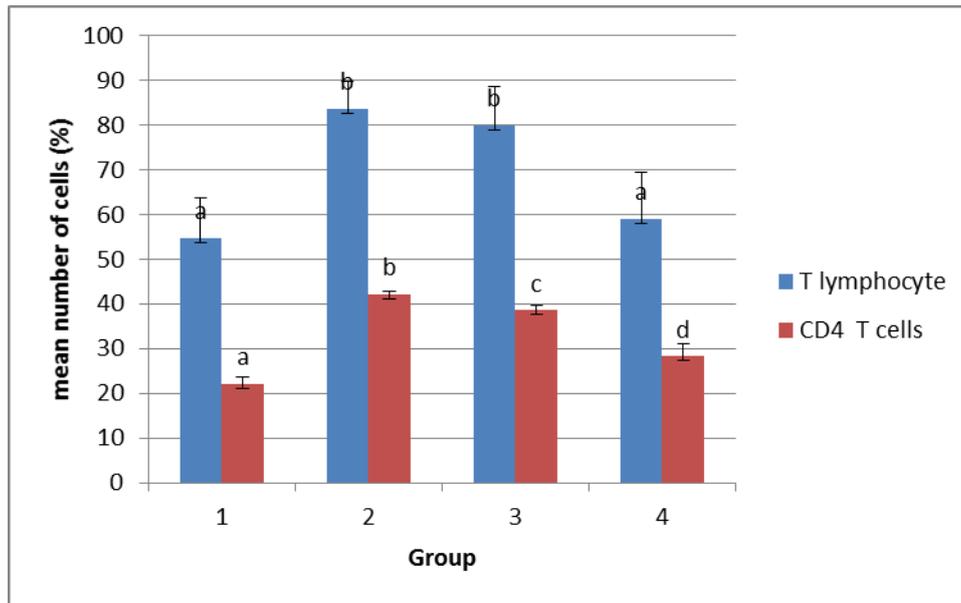


Figure 1. Mean of number cells of T-lymphocyte and CD4 T-Cells among animal groups.

a, b, c, d, The same superscript showed no difference between groups of each variable  
 K1: a control group of healthy test animals were given EDM  
 K2: a control group of sick animals that were given preventive EDM and placebo  
 K3: group of animals were given preventive EDM and  
 K4: group of animals were given preventive EDM and GAB.

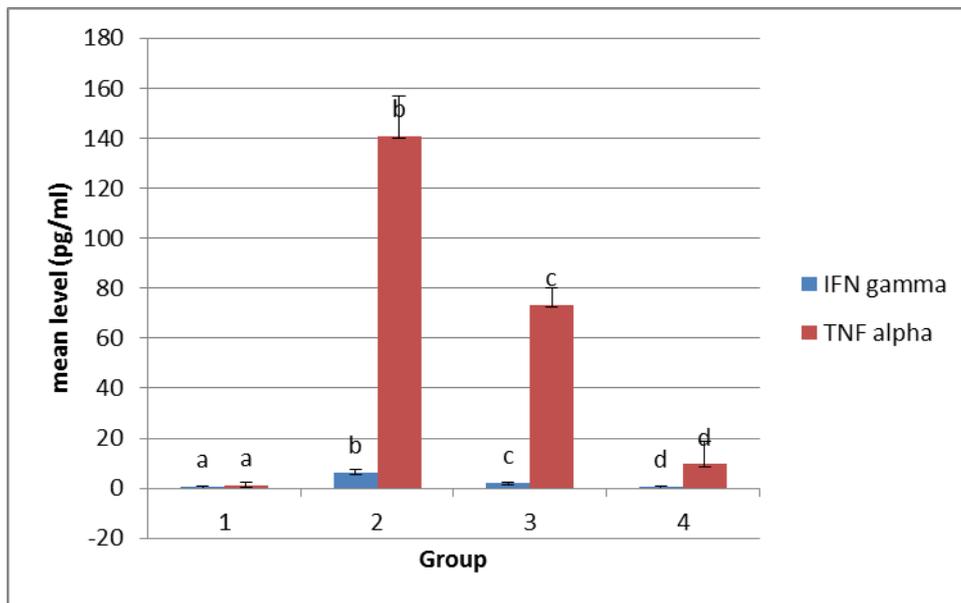


Figure 2. Mean of IFN gamma and TNF alpha level in animal groups.

a, b, c, d, The same superscript showed no difference between groups of each variable  
 K1: a control group of healthy test animals were given EDM  
 K2: a control group of sick animals that were given preventive EDM and placebo  
 K3: group of animals were given preventive EDM and  
 K4: group of animals were given preventive EDM and GAB.

### 3.3. In Vivo Effects of EDM on *M. tuberculosis*

The K1 as a control group of healthy animals were given EDM was shown that the mean of *M. tuberculosis* colony was 0 while K2 as a control group of sick animals that were given preventive EDM and placebo was shown

that the mean of *M. tuberculosis* colony was 321.88. Furthermore, In the K3 as group of animals were given preventive EDM was shown that the mean of *M. tuberculosis* colony was 129 while in the K4 as groups of animals were given preventive EDM and GAB was shown that the mean of *M. tuberculosis* colony was 0. There significantly different among all groups (Figure 3).

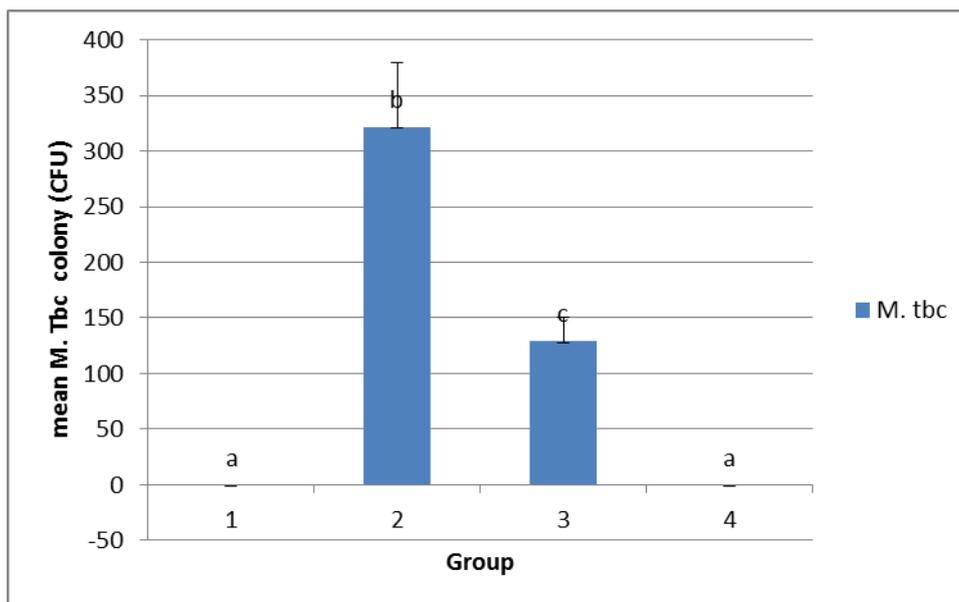


Figure 3. Mean of *M. tuberculosis* colony among animal groups.

a, b, c, d, The same superscript showed no difference between groups of each variable

K1: a control group of healthy test animals were given EDM

K2: a control group of sick animals that were given preventive EDM and placebo

K3: group of animals were given preventive EDM and

K4: group of animals were given preventive EDM and GAB.

The relationship among all variable was shown that significantly different except the relationship between number of CD4 *T-cell* and *M. tuberculosis* colony;

number T-lymphocyte and *M. tuberculosis* colony and level of TNF alpha and *M. tuberculosis* colony (Table 1).

Table 1. Results Analysis of the Relationship among Variables

Independent Variable	→	Dependent Variable	Coeffisien (standardized)	P
T-lymphocyte	→	CD4 T-Cells	0.3973	0.0304
CD4 T-Cells	→	<i>M. tuberculosis</i>	-0.0876	<b>0.6008</b>
CD4 T-Cells	→	TNF- $\alpha$	0.9003	<0.001
CD4 T-Cells	→	IFN- $\gamma$	0.6970	<0.001
T-lymphocyte	→	<i>M. tuberculosis</i>	0.0142	<b>0.8439</b>
TNF- $\alpha$	→	<i>M. tuberculosis</i>	0.0710	<b>0.6403</b>
IFN- $\gamma$	→	<i>M. tuberculosis</i>	0.9557	<0.001

Description → Related  
→ Not related

Results of Kruskal-Wallis test showed that there was significantly difference in the number of T-lymphocytes, CD4 *T-cell* counts, levels of IFN $\gamma$ , TNF- $\alpha$  levels and the number of *M. tuberculosis* colonies from rat's lung samples.

## 4. Discussion

EDM affected on T-lymphocyte proliferation in rats. This results based on differences in the number of T-lymphocytes of treatment group. This study suggested that EDM makes significant changes in the experimental groups when compared with control group, so that oral administration of 510mg/kg of EDM to male rats caused a significant increase in T-lymphocyte proliferation. EDM acts on T-lymphocyte proliferation as an immunomodulator to increase immunity (immunostimulant). Medicinal plants

are plants or parts of plants can strengthen the function of organs, can get rid of toxins or disease and can build up the immune system [14]. This medicinal plants serve as complementary and alternative medicine (CAM) in improving immunity or modulate the immune response to pathogens or regulate of T-Cells [15]. Miana leaves as one of the medicinal plants have been used empirically.

Cytokines has primary function to control the initial infection, maintain T-cell responses in mediating immune host [16] and involves in all competent immune cells; CD3 (white blood cells), CD4 (helper T-Cells) and CD8 (cytotoxic T-Cells) [17]. IFN- $\gamma$  cytokines produced by lymphocytes particularly T-Cells and natural killer cells (NK) [4] acting on T-lymphocytes to promote the differentiation of naive T-Cells to CD4 and Th1 cells. Th1 cells are involved in the elimination of pathogens in intracellular vesicular compartments [18]. The results of this study indicate that EDM has an effect on the number

of CD4T-Cells. This result determined based on differences in the number of CD4T-Cells of treatment group. Another mechanism of herbal CAM in improving immunity is to modify the level and quality of the immune response of T-Cells, B cells, and cytokines [15]. Treatment with EDM alone or combined with EDM (GAB) has effects on the increased production of T-Cells that improve immunity of the host. Immunity to tuberculosis infection dominated by Th1 CD4 cells. Th1 CD4 cell secrete IFN- $\gamma$  and other cytokines and then activates macrophages as bacteriostatic at the site of infection [19].

In this study for groups of infected animals that received the GAB shows the low number of CD4 T-Cells. The use of EDM as a preventive has influence on increasing immunity before infection and increased CD4 T-cell as early host defense. The main function of CD4 T-Cells is producing IFN- $\gamma$  and other cytokines to activate macrophages. The importance of EDM as a preventive is to improve host immunity before exposure to the infection. This research Consistent with previous study, showed that at the beginning of the infection the number of MHC Class II or CD4 decline rapidly making the levels of IFN- $\gamma$  is also reduced. Reducing in CD4 T-Cells is caused by the rapid reactivation of infection. Apoptosis of infected cells by CD4 T-Cells also play a role in controlling infection.

All EDM treated animals showed increasing in IFN- $\gamma$  level compared to healthy control animals. This reveals that the EDM serve as an immunomodulator to improve immunity (immunostimulant). Herbal plant can boost immunity by the mechanisms on altering the balance between the inflammatory and anti-inflammatory cytokines [15]. Immunomodulatory function of medicinal plants is determined by the active component contained in the plant T-Cells. Miana plant as one of the family Lamiaceae containing essential oils, flavonoids, tannins and other alkaloids potentially as an immunomodulator and proven to increase the production of cytokines such as IFN- $\gamma$  [20]. The main function of IFN- $\gamma$  is to participate in the fight against tuberculosis [21]. IFN- $\gamma$  from T-Cells inhibits the intracellular replicating *M.tuberculosis* in macrophages. This shows the IFN- $\gamma$  is necessary for intracellular bactericidal activity. IFN- $\gamma$  is required by humans and rats to control *M.tuberculosis*. CD4 T-Cells are a significant source of IFN- $\gamma$  during acute infection in rat and are required to control bacterial growth and survival of the host and enhance the function of CD8 T-Cells during *M.tuberculosis* infection [22].

At the end of the study found a group of infected animals treated with EDM and GAB, showing the lowest level of IFN- $\gamma$  compared to other infected groups. This means that at the beginning of the infection the cytokines IFN- $\gamma$  production is adequate to eliminate *M. tuberculosis*. At the end of the infections, cytokine levels are very low compared to other groups of infected animals. IFN- $\gamma$  is a key cytokine involved in the immune response to mycobacteria with the main function to activates the macrophages [23].

The results showed the EDM increased the level of TNF $\alpha$ . This reveals that the EDM as an immunomodulator can improve immunity and prove EDM can be used as a complement in the treatment of tuberculosis. Levels of TNF- $\alpha$  found to be very low, especially in the group of infected animals with GAB compared to infected animals' control. TNF- $\alpha$  synergize with IFN- $\gamma$  to stimulates the

production of reactive nitrogen intermediates (RNIS), thereby inhibit *M. tuberculosis* in macrophages, stimulate the migration of immune cells to the site of infection and contributes in the formation of granulomas that control disease progression [23]. Elevated levels of TNF- $\alpha$  that is substantially in CD4 T-Cells in subjects infected with *M. tuberculosis* is the strongest predictor of diagnosis of active disease compared latent infection [22].

The results showed that EDM decreased the number of *M. tuberculosis* colonies. In this study demonstrate that administration of EDM as complement of rifampicin was not differing from healthy controls. *Mycobacterium tuberculosis* that infect the host actually be engulfed by macrophages as the natural defenses are then formed granulomas to kill and prevent the spread of this bacteria. The process of granuloma formation and destruction of bacteria involves the immune mechanisms such as the proliferation of T-lymphocytes, CD4 T-Cells and the production of cytokines such as TNF- $\alpha$  and IFN- $\gamma$  [24].

Amos analysis showed that the increased proliferation of T-lymphocytes has effects on the increase in the number of CD4 T-Cells, but has no effect on the decrease in the number of *M. tuberculosis* colonies. Although the provision of EDM significantly affect the increase in T-lymphocyte proliferation, but an increase in T-lymphocytes do not have a direct influence on the reduction in the number of *M. tuberculosis* colonies. The antibacterial properties of the herbs plant derived from the chemical content in cells, but the mechanism of bacterial killing in the host influenced by mechanisms of host immunity. EDM has been shown to increase proliferation of T-lymphocytes but did not exhibit significantly in reducing the number of *M. tuberculosis* colonies directly. CD4 T-Cells, cytokines IL-12, IFN- $\gamma$  and TNF are essential in *M. tuberculosis* infection control, but a host of factors that determine why some individuals are protected from infection while the other hosts continue to develop the disease. Statistical analysis also found that the increase in the number of CD4 T-Cells affect the increased levels of IFN- $\gamma$  and TNF- $\alpha$ . EDM increased host immunity such as T-lymphocytes, CD4 T-Cells, cytokines IFN- $\gamma$  and TNF- $\alpha$ . Host immune response to *M. tuberculosis* mediated by cellular immunity, in which cytokines and Th1 cells play an important role [23]. This suggests that the mechanism of immunity that occurs in the the host is complex.

## 5. Conclusion

Miana leaves extract (*Coleus scutellarioides*, (L) Benth.) increased the number of T-lymphocytes, CD4 T-cell counts, levels of IFN- $\gamma$  and TNF- $\alpha$  and decreased in the number of *M. tuberculosis* colony in infected Wistar lung.

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