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ARTICLE

ANTI-TUBERCULOSIS ASSAY OF NANOHERBAL AND ETHANOLIC EXTRACT OF *LANTANA CAMARA* LINN FLOS IN VITRO AND IN VIVO

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ABSTRACT

Background: *M. tuberculosis has been resistant to the synthetically anti-tuberculosis drugs. Traditionally Lantana camara* Linn-*flos (tembelekan in Indonesia) has been used to treat cough and bloody sputum. This study was conducted to investigate the potency of nanoherbal and extract of tembelekan flos as anti-tuberculosis in vitro and in vivo.* ***Method:*** *Anti-tuberculosis in vitro assay of nanoherbal and extract were performed at the concentration of 50 mg/ml and 25 mg/ml by Lowenstein-Jensen (LJ) method. Anti-tuberculosis in vivo assay was conducted in guinea pigs infected with M.* Tuberculosis *H37Rv into the bronchi using nebulizer. The nanoherbal and the extract were administered once a day at the dosage of 50 mg/kg BW and 25mg/kg BW. Anti-tuberculosis assessment was conducted by examining M.* Tuberculosis *on bronchial fluid specimens that were taken every week and assayed with LJ method.* ***Result:*** *In vitro assay showed that nanoherbal and extract could inhibit the growth of M.* Tuberculosis*: the 50 mg/ml of nanoherbal showed M.* Tuberculosis *negative for 5 weeks, while the 50 mg/ml extract showed positive +1 of M.* Tuberculosis *for 6 weeks. In vivo assay showed that both the nanoherbal and the extract performed anti-*tuberculosis *activity in guinea pigs infected with M.* Tuberculosis*: single daily dose of 50 mg/kg BW nano herbal for 4 weeks or extracts for 5 weeks decreased the bacterium from +3 to negative.* ***Conclusions:*** *In vitro nanoherbal and ethanol extracts of Lantana camara* Linn *flowers have strong activity to M. tuberculosis growth retardation. In vivo nanohebal and ethanol extracts of flowers tembelekan concentration of 50mg / Kg BW has a strong activity as anti-*Tuberculosis *in animal experiments.*

KEY WORDS
Lantana camara Linn, flos, nano herbal, ethanolic extract, anti tuberculosis acti

INTRODUCTION

Tuberculosis (TC) is one of the leading causes of death diseases, due to infection by *M. tuberculosis* that can infect latently or progressive, and the majority (80%) attacks respiratory tract and lungs. In general, in the world there are 2 billion people are infected and 2-3 million people die of Tuberculosis every year. Indonesia ranks third in the number of Tuberculosis infected people after India and China. TC is a disease threat in Indonesian, especially for the productive age between 15-55 years, and it is the third cause of death after heart disease and acute respiratory disease in all ages.

Increasing the number of TC cases is caused by a variety of factors, namely the lack of patient compliance rate for treatment, because treatment of this disease requires a long time; approximately 6 months, also the incidence of dual resistance, lack of endurance host against mycobacterium, and reduced power bactericidal drugs. People with TC can be treated and hospitals may provide optimal therapy with medicines such as rifampicin, ethambutol, isoniazid, pyrazinamide, and streptomycin or a combination of these drugs that are known as OAT KDT. The possibility of microbial resistance makes it is necessary to look for an alternative medicine; for example from natural ingredients that can help the treatment of patients of infected Tuberculosis.

To supply the medicines of TC, this paper discusses the experimental research result on *Lantana camara* Linn (in Indonesia is called as *tembelekan*). Indonesia is very rich with a diverse range of useful plants as medicine such as *Lantana camara* Linn., family of *Verbenaceae*. According to the *Ensiklopedia Tanaman Obat Tradisonal* [1, 2], *tembelekan* traditionally been used for various treatments, including for the stop of the bleeding, cough and bloody phlegm and asthma. The way of usage: taken 6 - 10g dried flowers boiled in 3 cups water until it is remaining 2 cups. Cold taxable income, filtered boiling water, drink 3 times a day.

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Several previous studies, including the methanol extract of leaves of *tembelekan* in vitro to inhibit the growth of *M. tuberculosis H37Rv* [3],the results of the test structure elucidation of leaves *tembelekan* obtained derived *flavonoids* can inhibit the growth of *M. tuberculosis* in vitro [4], *tembelekan* plants in vitro inhibit the growth of bacteria *Escherichia coli, Pseudomonas vulgaris, Pseudomonas aeroginosa, Streptococcus aureus*, the combination of garlic and leaf of *tembelekan* inhibits the growth of *M. tuberculosis* [5].

Referring to the efficacy of *tembelekan* that has been traditionally used to treat cough and bloody phlegm [6, 1], and the methanol extract of leaves *tembelekan* in vitro to inhibit the growth of *M. tuberculosis* [3], it is likely that flowers of *tembelekan* have been potential for the treatment of Tuberculosis. Due to large volume, it is difficult in the storage and transportation of *tembelekan* leaves, so it has to be developed in a more practical way; such as nanoherbal or extract form. Niño herbal has a very small particle size of the nanometer measurement. It is found that the effect of drug particle size on the rate of dissolution and bioavailability and comprehensively is demonstrated by drugs that absorbing the gastrointestinal tract. Particle size reduction of it may increase the rate of absorption and bioavailability, and one of particle size reduction efforts is to create the nano-scale shapes [7].

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In practical way, natural materials are made in the form of nano herbal likely to have advantages over the form of extracts for chemical compounds that might be still relatively intact, more soluble and easily

absorbed so that the possibility of onset of the drug will be faster and smaller doses [7]. On the other hand, the production of nano herbal requires less time and low costs because it does not use filters.

Based on these processes, the authors thus made the nanoherbal and ethanol extracts of *tembelekan*'s flowers and observed its potential in vitro of anti-tuberculosis with Lowenstein-Jensen method in LJ media, and in vivo with the infected Guinea pigs with *Mycobac-terium* Tuberculosis H37Rv. The results were expected to prove the efficacy of anti-tuberculosis of *tembelekan*'s flowers, and found the potential anti-tuberculosis difference between nano herbal and extracts of *tembelekan* flowers. So the flowers of *tembelekan* have a high possibility to be developed into anti-Tuberculosis medicines from natural materials with a rational way, cheap budget, and easily available.

Types of Anti-Tuberculosis Drugs

Tuberculosis drugs are commonly divided into two groups; namely, primary and secondary.

a. Primary drugs: *Isoniazid, rifampicin, pyrazinamide,* and *streptomycin Etam-butol* (*canamycin, amicacin*). These medications are most effective and have low toxicity, but causing any resistance quickly when used as a single agent. So, given the combination of 3-4 drugs, for sensitive TB germs are widely used in a combination of *isoniazid, rifampicin* and *pirazinamuida*.

b. Secondary drugs: *clofazimine, flourcinolon, cycloserine, rifabutin*, and *p-aminosalicylic* acid (PAS). These drugs have weaker activity and typically only used when there is resistance or intolerance facing primary drugs, also against infection with *M. avium intrasellulare* in HIV patients

Category of Tuberculosis

Tuberculosis has several categories namely:

The first category is that have the characteristics such as: a) new patients with positive pulmonary Tuberculosis acid-resistant bacteria (AFB). b) New patients with negative pulmonary Tuberculosis acid-resistant bacteria (AFB) and positive chest radiograph.c) Patients with extra pulmonary Tuberculosis

The second category has characteristics such as:

a. Relapse patients have been treated and recovered, but relapsed again

b. Failure patients, have been treated but less disciplined

c. Patient treatment is interrupted, has been treated but stops before recovering

Tuberculosis Treatment Measures

Tuberculosis therapy actions are performed by category of:

1. The first category treated through two phases is known with the code: 2 HRZE/4H3R3

a. Intensive phase; every day is given to the combination drug *isoniazid, pyrazinamide,* and *ethambutol* for two months.

b. Advanced phase: After the completion of the continuation phase is given by the combination of drugs *isoniazid* and *rifampicin* for four (4) months and 3 times a week

2. The second category treated through two phases namely known with the code: 2 HRZES/ HRZE

a. Intensive phase; every day is given to the combination of drug *isoniazid, rifampicin, pyrazinamide,* and *ethambutol,* and *streptomisin* for two months.

b. Advanced phase: Once completed given the drug combination of *isoniazid* and *ethambutol* for 5 months, with provision 3 times a week

If after two months of smear is still positive, plus 1 month intensive phase as inserts with HRZE

Lantana camara Linn (*Tembelekan*) Plants

Lantana camara Linn (*Tembelekan*) is an herbaceous plant, erect or slightly climbing, height 0.5 to 4 m, a characteristic odor. Woody stems, branched, twigs quadrangular, prickly, hairy, single leaf, face, round eggs, pointed tip, Compound interest grain shape, the inside of the crown-haired, white color, pink, orange, yellow, and so forth [6].

Determination of *Lantana camara* Linn (Tembelekan)

Division	: Spermatophyta
Sub Division	: Angiosperms
Class	: Dicotyledoneae
Sub Class	: Dialypetalae
Ordo	: Solanales
Family	: Verbenaceae
Genus	: Lantana
Species	: *Lantana camara* Linn

Synonym : *Lantana aculenta* Linn = *Lantana aculeata* = *Lantana antillana* Rafin = *Lantana mutabilis* Salisb = *Lantana polyacanthus* SCH. = *Lantana scabrida* Soland = *Lantana viburnoides* Blanco. The name of the region: Sumateran: Interest fence, Singapore wood, Tahi ayam (Malayan). Java: Kembang satek, saliyara, tai hayam, tai kotok, cente (Sundanese), *Kembang telek, oblo, punyengan, pucengan, tembelek, tembelekan, teterapan, wauna, wileran, kamanco, mainco, tamanjho (Madura*)

Fig. 1: *Lantana camara* Linn (*Tembelekan*) Plants

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Chemical contents of *Tembelekan* Flos

Based on the result of previous research, *tembelekan* contains the chemical compounds namely *alkaloid, cardiac glycosid, steroid, saponin, flavonoid, tannin,* essential oil, *triterpenoid*. Some chemical compounds namely *lantadene, lantadene* A, B, *lantanolic acid, humulene,* β- *Caryophyllene,* γ-*terpinene,* α-*pinene*, and p-cymene *triterpenoids, camarin, lantacin, camarinin, lantadienone, camaradienone, lantanoic acid, camaranoic acid, camarolic acid and lantrigloylic, lantadene, lantadene A, B, lantanolic acid, lantic acid, humulene* (4).

Some benefits

Traditionally, the root of *Lantana camara* Linn is as a reliever fever (*antipyretics*), detoxifying (*antibiotics*). Pain relievers (*analgesics*), rheumatism, stop bleeding (*hemostatic*), influenza, Tuberculosis glands, and vaginal discharge. The leaves are slightly poisonous (toxic), nutritious eliminate itching (*anti pruritic*), antitoxic, eliminate swelling, stimulating vomiting, skin diseases, bruises, sores, swelling, itching, fever, and arthritis. The flowers stop bleeding, cough and bloody phlegm, and asthma [6, 1, 2].

Several studies have been conducted to prove the efficacy of *tembelekan* plant based on the methanol extract of leaves in vitro to inhibit the growth of *M. tuberculosis* H37Rv [3]. The test results of the leaf structure elucidation *tembelekan* of obtained *flavonoid* derivatives inhibit the growth of *M. tuberculosis* in vitro [4]. *Tembelekan* plants in vitro retarded the growth of bacteria *E coli* ATTC 25 922, ATTC 13315 *Pseudomonas vulgaris, Pseudomonas aeroginosa* ATTC 15 442, 15 748 *V.chlareae* ATTC, *Streptococcus aureus* ATTC 12692. A combination of garlic and *tembelekan* obstructed *M. tuberculosis* [5]. *Tembelekan* leaf extract can heal the wounds of the artificial skin of mice [8]. *Tembelekan* leaf ethanol extract has antibacterial activity [9]. *Tembelekan* has anti-inflammatory effects [10]. The leaf of *tembelekan* has the effect of anti motility and indigestion in the intestines [11]. The leaf of *tembelekan* has the effect of anti-fever and potentially as an anti-malarial [12].

Nano Herbal

In this research, nano material has a particle size 10^{-9} meters. Various form materials are: nano materials, nano particles, nano composites, nano magnets, nano energy, nano medicine, and nano herbs. Some of the uniqueness of the nano particles such as:

a. The particle size is very small.

b. Contact surface area in nano material becomes greater cause physical, chemical, and biological changes such as its reaction kinetics, reaction rate, a bond is formed.

c. There were changes in the optical properties and magnetic properties. A change of this nature provides a variety of benefits including more soluble.

d. Easy to get into the cell organ, easily absorbed so that the possibility can increase the rate of absorption, onset of drug bioavailability will be faster the better, with smaller doses.

e. Particles that having a size smaller than 200 m are more easily sterilized by filtration with a sieve size of 0.22 µm.

Therefore, the particle size of the ingredients has given smaller size than the cells in the human body, then the ingredients are easy to enter cells and reaction bioavailability is better, the case against bacterial cells, with ingredients having a particle size smaller compared the bacterial cell will cause the ingredients easily penetrate bacterial cells, thereby the retardation the growth of bacteria is faster and stronger. Some particle size of some cells can be seen in [Table 1] below:

Tabel 1: Particle size of some cells

The Object	Size (nm)
Carbon Atoms	0.1
DNA double helix	3
The ribosome	10
The Virus	100
Bacteria	1000
Red blood cells	5000

The production of Nano Herbal

Top down approach:

The production size of nano from the great slab to the nano material got through in the process of grinding, cutting (crushing), grafting, until formed the size of nano. Such production of the flour; it is made from the mashed rice first, so also with the top down approach.

Bottom up approach:

This method is commonly used in the industry, because it can be constructed structure nano-sized objects from below. This method produces a uniform size so that no longer needs a separation process. Therefore, it can be designed conditionally for the properties that will be generated from these nanostructures

After completion of the production of nano particles, some observations on the particle size characteristics and a test were done by using SEM (Scanning Electron Microscopy), XRD (X-ray Diffraction), DTA (Differential Thermal Analyzer), and PSD (Particle Size Distribution).

MATERIALS AND METHODS

Plants Extraction

Tembelekan flos was dried and powdered, and then *phytochemistry* screening and quality assay of simplicy was done. Extraction by percolation then conducted using 80% ethanol.

Materials

Percolator, rotary evaporator, freeze dryer), a tool determination of water content, scales, HEM (high energy milling), SEM (Scanning Electron Microscopy), XRD (X-Ray Diffraction), DTA (Differential Thermal Analyzer), PSD (Particle Size Distribution), autoclave, oven, hot plate, incubator, refrigerator, inspisator, homogenizer glass, microscope, thermometer. The animal experiment that used were male guinea pigs weighing 300 to 400 grams

Anti-tuberculosis test in Vitro

Potential or effectiveness nano herbal and flower extracts tests of *tembelekan* were done with ability test and potential inhibition of the growth of bacteria *M. tuberculosis* H37Rv that has been included in the media LJ used in concentrations of each nano herbal and extracts around 25 mg / ml and 50 mg / ml, *rifampicin* comparison 40µg / ml, and *ethambutol* 10µg / ml, also conducted on blank media with the phases of work as follows:

The Production of bacterial suspension

One drop of sterile distilled water is dripped into a mixing glass; wire transferred one loopful of the colony from the culture medium into a mixing glass. Then it destroyed by rotating the tool until homogeneous and poured 7 ml sterile distilled water. Then take 0.1 ml diluted with 9.9 ml of sterile distilled water obtained bacterial suspension concentration of approximately 0.01 mg / ml [13].

The Inoculated bacteria suspension

It is inoculated 0.1 ml bacterial suspension concentration of 0.01 mg / ml into three tubes containing LJ medium containing each test material and the comparative material of various concentrations. Then, razed the entire surface of the medium and incubated at a temperature of 370C for 6 weeks was observed growth of bacteria in every week with readability criteria:

(-) : no growth

(+1) : seen there are little yellow colony 1-200 colonies

(+2) : ½ from media covered by the yellow color (200-500 colonies)

(+3) : ¾ from media covered by the yellow color (500-2000 colonies)

(+4) : media fully covered by the yellow colony (more than 2000 colonies)

Anti-tuberculosis in Vivo Test

Anti-tuberculosis activity of *ethanolic* extract of *mimba* cortex was determined using infected Guinea-Pigs. Guinea-Pigs were spread using 3 ml suspension of *M. tuberculosis H37RV* (from *CV. Varka Bayak Medan*) directly on bronchus by using nebulizer every 24 hours during 7 days respectively, continued with once every 2 days during 7 days and once every 3 days during 7 days. The spread of 20 ml *aquadest* was taken from esophagus. Identification and cultivation of *M. tuberculosis* was done on LJ medium. The positive Tuberculosis Guinea-Pigs then divided in 5 groups. 1st group: Extract 50 mg/kg BW, 2nd group: Extract 25 mg/kg BW, 3rd group: Nano herbal 50 mg/kg BW, 4th group: Nano herbal 25 mg/kg BB. 5th group: OAD KDT one times a day. After administrated with each treatment, specimen sampling was done each week for 4 – 5 samplings. Specimens were placed in assay tube then homogenized with phosphate buffer pH 7 and inoculated into two assay tubes contain with LJ medium. Inoculums were incubated at 37°C for 6 – 8 weeks and the growths were observed with criteria:

(-) : no growth

(+1) : medium covered with slight colony, 1 – 200 colonies

(+2) : ½ of medium surface covered with yellow colony, 200 – 500 colonies

(+3) : ¾ of medium surface covered with yellow colony, 500 – 2000 colonies (13).

RESULT AND DISCUSSION

Table 2. Results of in vitro test

Material Test	Concentration	Colony Growth on x week					
		1st	2nd	3rd	4th	5th	6th
Control	0 µg/ml	-	-	1+	2+	3+	4+
		-	-	1+	2+	3+	4+
Rifampicin	40 µg/ml	-	-	-	-	-	-
		-	-	-	-	-	-
Ethambutol	10 µg/ml	-	-	-	-	-	-
		-	-	-	-	-	-
Tembelekan flos Nano herbal	50 mg/ml	-	-	-	-	-	-
		-	-	-	-	-	-
	25 mg/ml	-	-	-	-	1	1
				-	-	1	1
Tembelekan flos Extract	50 mg/ml	-	-	-	-		1+
				-	-		1+
	25 mg/ml	-	-	-	-	2+	2+
				-	-	2+	2+

Table 3. Result of In Vivo Test

Material Test	Concentration	Colony Growth on x week					
		1st	2nd	3rd	4th	5th	6th
OAT KDT	¼ of tablet	3+	2+	1+	-	-	-
		3+	2+	1+	-	-	-
Tembelekan flos Nanoherbal	50 µg/ml	3+	2+	1+	-	-	-
		3+	2+	1+	-	-	-
	25 µg/ml	3+	2+	2+	1+	1+	-
		3+	2+	2+	1+	1+	-
Tembelekan flos Extract	50 mg/ml	3+	-	-	1+	-	-
		3+	-	-	1+	-	-
	25 mg/ml	3+	3+	2+	2+	1+	1+
		3+	3+	2+	2+	1+	1+

The result of [Table 2] shows Colony growth on sample week

a. On the media that given by *Rifampicin* 40 µg/ml and *Ethambutol* 10 µg/ml, look no growth of Tuberculosis began the first week until the sixth week.

b. In nano herbal, the concentration of 50 mg / ml, look no growth of Tuberculosis began the first week to the sixth week, and a concentration of 25 mg / ml at week starts to look their V-harbor Tuberculosis per-tum category +1

c. In the extract with a concentration of 50 mg / ml, look no growth Tuberculosis began the first week until week-V, and a concentration of 25 mg / ml at week IV began their growth categories Tuberculosis +

The result of [Table 3] shows that:

a. All of test animals were infected with positive *M. tuberculosis* bacteria Tuberculosis infected with categories +3.

b. In granting OAT KDT, seen to have negative Tuberculosis at the third week.

c. In the production of nanoherbal dose of 25 mg / Kg BW seen to have negative Tuberculosis at week 5, and at doses of 50 mg / Kg BW, was negative at the third week.

d. At a dose of 25 mg extract / Kg BW seen to have negative Tuberculosis at sixth week, and at doses of 50 mg / Kg BW, was negative at the fourth week.

In vivo test results demonstrate the potential of anti-tuberculosis nanoherbal stronger than the extract, but still lower when compared with the OAT KDT so, nanoherbal *tembelekan* flowers can be used as a companion drug OAT KDT in the treatment of Tuberculosis, especially in coping resistance.

CONCLUSION

(i) *In vitro* nanoherbal and ethanol extracts of *Lantana camara* Linn have strong activity to *M. tuberculosis* growth retardation, with a concentration of 50 mg / ml; nanoherbal gave a negative result until week 5, and extracts 1+ at sixth week. (ii) *In vivo* nanohebal and ethanol extracts of flowers *tembelekan* concentration of 50mg / Kg BW has a strong activity as anti-Tuberculosis in animal experiments. (iii) Anti-tuberculosis nanoherbal strongly cures the Tuberculosis in animals of 3+ becomes negative for 4 weeks compared to extract for 5 weeks.

This research suggested to the medical staff providing the extracts of nanoherbal made of *tembelekan* as a companion drug OAT KDT dealing with the treatment of Tuberculosis patients to accelerate the destruction of *M. tuberculosis* . It is very vulnerable to resistance. For the pharmaceutical industry is expected to produce the nanoherbal *tembelekan* capsule; it is a companion to the anti-tuberculosis drugs.

CONFLICT OF INTEREST

There is no conflict of interest.

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