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Evaluation of Anti-Mycobacterium Activity of *Lantana camara* Flos Extract Against Mycobacterium Tuberculosis.

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ABSTRACT

Tuberculosis (TB) is an airborne disease caused by M*ycobacterium tuberculosis.* In 2015, there were an estimated 10.4 million new (incident) TB cases worldwide and caused 1.4 million deaths. The main challenge of TB treatment is the resistance of *Mycobacterium tuberculosis.* Therefore, there is a need to develop new drugs to combat resistant *M. tuberculosis.* Recently, the exploration of bioactive compounds produced by natural resources is gaining its significance. *Lantana camara* is a plant used as traditional medicine. In Muna district, province of Southeast Sulawesi, Indonesia, the flower (flos) of *Lantara camara* have been used as TB treatment. The aim of this research is to determine anti *M. tuberculosis* Strain H37Rv and MDR activities of *Lantara camara* flos extract. *Lantara camara* flos were macerated with methanol and evaporated with rotary evaporator. Anti *Mycobacterium tuberculosis* assay was done with Microscopic Observation Drug Suspectibility (MODS). The results showed that the extract had antimycobacterium activity againts *M. tuberculosis* strain H37Rv dan MDR at 1000 ppm. Chemical screening indicated that the extract contains alkaloids, terpenes, saponins, flavonoids and sterols.

Keywords: anti-mycobacterium, *Lantana Camara,* tuberculosis

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INTRODUCTION

Tuberculosis (TB) is an airborne disease caused by *Mycobacterium tuberculosis* (MTB) that usually affects the lungs leading to severe coughing, fever, and chest pains. Although current researches in the past four years have provided valuable insight into TB transmission, diagnosis, and treatment, much remains to be discovered to effectively decrease the incidence of and eventually eradicate TB. The disease still puts a special attention to the public, being only second to HIV/AIDS in causing high mortality rates [1]. In 2015, there were an estimated 10.4 million new (incident) TB cases worldwide and caused 1.4 million TB deaths, and an additional 0.4 million deaths resulting from TB disease among people living with HIV [2].

The main challenge of TB treatment is the resistance of *Mycobacterium tuberculosis* (MTB) which is multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB). In 2015, there were an estimated 480.000 new cases of multidrug-resistant TB (MDR-TB) and an additional 100.000 people with rifampicin-resistant TB (RR-TB) who were also newly eligible for MDR-TB treatment [2]. Therefore, there is a need to develop new drugs to combat those resistant *M. tuberculosis*, especially multi-drug resistant (MDR) strain. Continuous efforts are underway in the search for novel bioactive compounds to develop new anti-tuberculosis drugs. To this end, bioactive compounds of natural origin, particularly from plants, are gaining significance [2].

Lantana camara is one of the plants used as traditional medicine. *Lantana camara* have been shown to have several pharmacological effects such as antiinflammatory, antibacterial, antifungal, hepatoprotective, antidiabetic, cytotoxic, and wound healing activity [4]. In Muna district of South-east Sulawesi Province, Indonesia, the flower (Flos) of lantara camara have been used as TB treatment.

MATERIAL AND METHOD

Sample preparation and Extraction

Lantara camara flos were collected from Muna District, South-east Sulawesi, Indonesia. The plant was determined by Biology Department of Haluoleo University. The flos were dried and macerated with methanol and evaporated with rotary evaporator.

Bacterial strains and inoculum preparation

M. tuberculosis strains H37Rv and MDR were supplied by Microbiology Laboratory, medical faculty of Hasanuddin University. All cultures were grown in Middle brook 7H9 liquid medium fortified with oleic acid complex of bovine serum albumin-dextrose-catalase (OADC) at 37°C and agitated once a day for 2 weeks. The inoculum suspension was made in Phosphate Buffer Solution in turbidity Standard of No.0.5 McFarland.

Anti Mycobacterium assay

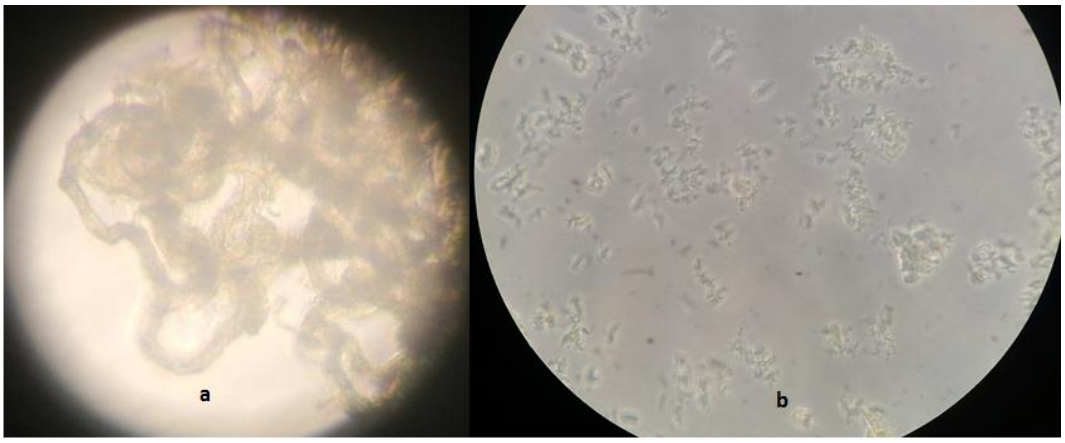

Fig.1 Microscopic observation of *M.tuberculosis* Cord Formation (a) 40 x magnification (b) 10x magnification

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Anti Mycobacterium Assay was conducted using MODS (Microscopic Observation Drug Suspectibility) method according Isrul et all (2017) [3] .The MODS media were prepared in 24-well tissue-culture plates. Each well contained 950 µl of *M.tuberculosis* inoculum, Middlebrook 7H9 broth, oxalic acid, albumin, dextrose, and catalase (OADC), and polymyxin, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA). Fifty microliters extract stock solutions were added to give the final extract concentrations to be 1000 ppm, 500 ppm, 250 ppm, 125 ppm, and 62,5 ppm. The negative control was DMSO and positive control were INH and Ofloxacin. The rest of the well is used to growth control containing bacteria and media only. The cultures were examined under an inverted light microscope at a magnification of 10× every day, from day 7 to day 15. To minimize cross-contamination and occupational exposure, plates were permanently sealed inside plastic zip lock bags after inoculation and were subsequently xamined within the bag. The growth of *M.tuberculosis* was identified by cord formation (Figure 1)

Phytochemical Screening.

The presence of various phytochemical constituents such as alkaloids, saponins, flavonoids, sterols, and terpenoids were screened qualitatively by using standard procedures.

RESULT AND DISSCUSSION

Antimycobacerium assay with MODS method was observed on the 7th day to determine the *M. tuberculosis* growth. The growths were identified by the cord formation of *M. tuberculosis*. Table 1 shows that *Lantara camara* flos extracts at 1000 ppm have the antimycobacterium activity against *M. tuberculosis* strain H37Rv and MDR. Figure 2 shows the microscopic observation of the assay.

Table 1. Antimycobacerium assay of Lantara camara extract againt M.Tuberculosis strain H37Rv and MDR

Sampel	Replication	*M.Tuberculosis*	
		H37Rv*	MDR*
Control	1	+	+
	2	+	+
	3	+	+
Negative control	1	+	+
	2	+	+
	3	+	+
Positive control	1	-	-
	2	-	-
	3	-	-
Extract 1000 ppm	1	-	-
	2	-	-
	3	-	-
Extract 500 ppm	1	+	+
	2	+	+
	3	+	+
Extract 250 ppm	1	+	+
	2	+	+
	3	+	+
Extract 125 ppm	1	+	+
	2	+	+
	3	+	+
Extract 62.5 ppm	1	+	+
	2	+	+
	3	+	+

* + = positive of M.tuberculosis growth, - = negative of M.tuberculosis growth

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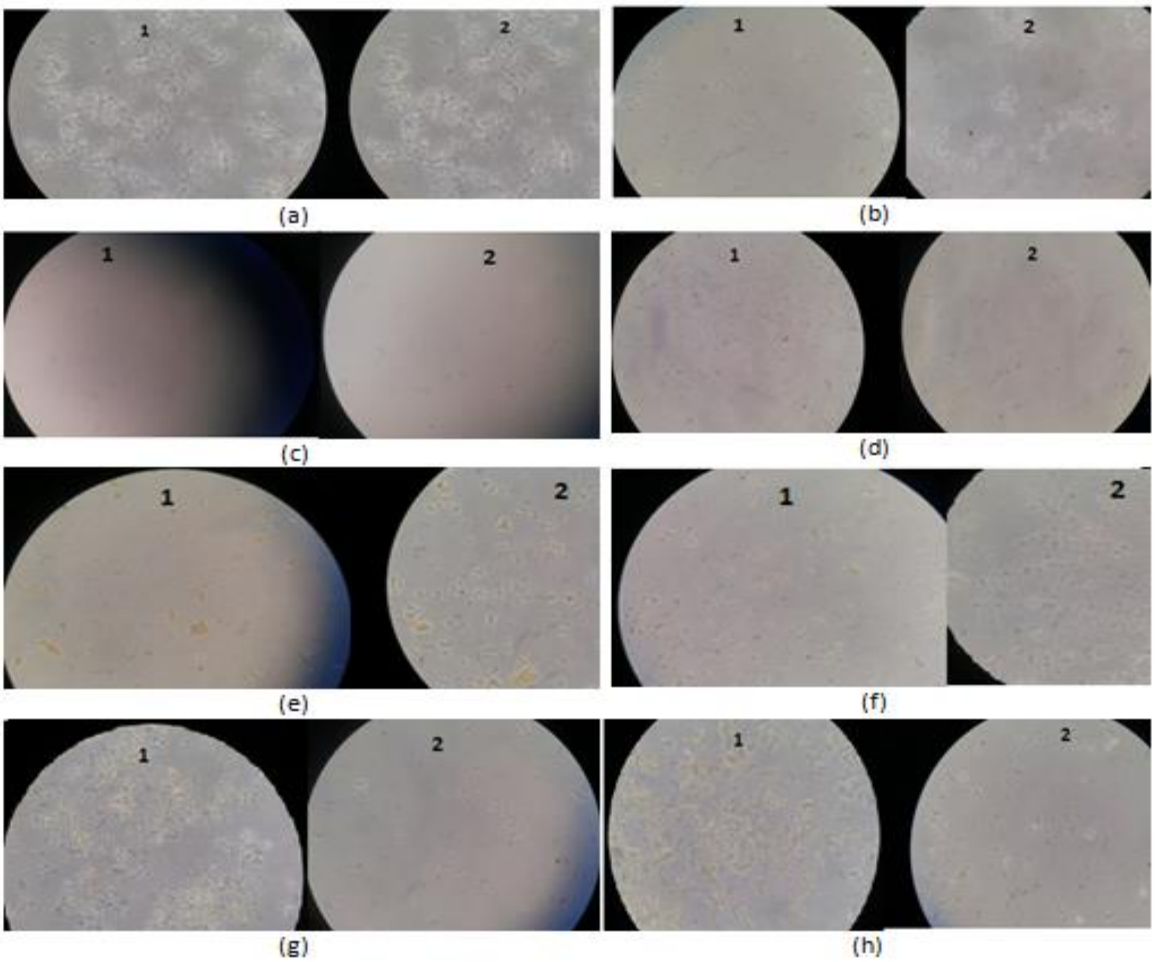

Fig.2 Microscopic observation of antimycobacterium assay (1) *M.tuberculosis* strain H37Rv (2) *M.tuberculosis* strain MDR (a) control, (b) negative control, (c) positive control, (d) extract 1000 ppm, (e) extract 500 ppm, (f) extract 250 ppm , (g) extract 125 ppm, (h) extract 62,5 ppm

Table 2 shows the result of phytochemical screening of lantara camara flos extract. The Screening indicated that the extract contains alkaloid, terpenes, saponin, flavonoids and sterol.

Table 2. Phytochemical Screening of *Lantara camara* flos extract

Compound	Result
Alkaloid	(+)
Flavonoid	(+)
Terpene	(+)
Saponin	(+)
Sterol	(+)

Several studies have reported alkaloids, flavonoids terpenes compounds as anti *M. tuberculosis*. Alkaloids that are reported like vasicine acetate and 2-acetyl benzylamine which inhibited both the sensitive and MDR strains of M. tuberculosis at minimum inhibitory concentrations (MIC) of 50 and 200 µg/mL, respectively [6,7]. Jha *et al*. reported six quinazoline alkaloids from *Justicia adhatoda* L plant have significant antimycobacterial activity, and in silico analysis confirmed that those alkaloids inhibit β-ketoacyl-acyl-carrier protein synthase III (FabH), an enzyme involved in the initial step of fatty acid biosynthesis, leading to poor cell wall development and affecting survival of bacilli [7,8]. Trans-trans isomer of 1-piperonyl-piperidine, is an antimycobacterial agent which at 128 µg/mL completely inhibits the efflux pump of M. smegmatis mc^2 155. The root extract of *Tabernaemontana elegans* Stapf., *Apocynaceae*, has an MIC of around 128–256 µg/mL against *Mycobacterium sp* due to the presence of indole alkaloids; voacangine and dregamine [7,9].

Few plant extracts contain a high amount of antimycobacterial flavonoids and most of them belong to the classes of flavones and flavonones. Flavonoid sulphates, quercetin 3,7 di-O methyl 3-sulphate and kaempferol 7-Omethyl3-sulphate, were reported with an MIC of 25 µg/mL, which is also synergistic with the usual antimycobacterial agents. Two new flavonones, pinocembrin and cryptocaryone , from the leaves of Cryptocarya chinensis Hemsl., Lauraceae, were effective against M. tuberculosis H37Rv. Sharma et al. reported that epigallocate-chin gallate/epigallocatechin-3-gallate directly inhibits fatty acid synthase (IC50 17.4 µM) by interacting with the residues near the NADH binding site. Fisetin, from *Cotinus coggygria* syn Rhus continus Scop., Anacardiaceae, is an inhibitor of an unknown mycobacterial dehydratase (Rv0636) at MIC 63 µg/mL and is involved in mycolic acid synthesis [7].

Terpenes are reported to act as anti *M. tuberculosis*. Labdan diterpene isolated from the *Curcuma amadarhizome*. Dihydro-β-agarofuran, a sesquiterpene, isolated from *Celastrus vulcancola* were found to have activity against *M.tuberculosis* MDR. Other terpenes were caniojane, ent-trachylobane-3-one, ent-trachylobane-17-al, and leubethanol [3,7].

Alkaloids, flavonoids, and terpenes have the possibilities as antimycobacterium compound. Further studies are needed to isolate those active compounds.

CONCLUSION

Lantara camara flos extract have antimycobacterium activity againts *M. tuberculosis* strain H37Rv dan MDR at 1000 ppm. Chemical screening indicated that the extract contains alkoloids, terpenes, saponin, flavonoids and sterols.

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