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In-vitro Anti-tuberculosis Activity and Phytochemical Screening of Lantana (*Lantana camara* L.) Flower

Pengujian Aktivitas Antituberkulosis secara *In-vitro* dan Skrining Fitokimia Bunga Tembelekan (*Lantana camara* L.)

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ABSTRACT

This study aimed to determine the quality of raw materials, screen the phytochemicals, and evaluate the anti-tuberculosis activity of Lantana (*Lantana camara* L.) flower extract. The method used was direct observation in the laboratory. Lantana flower were processed into crude drugs and then extracted using 70% ethanol solvent. The extract obtained was evaluated for phytochemical screening and anti-tuberculosis activity assay. The in-vitro anti-tuberculosis activity assay was carried out using the Lowenstein-Jensen method. The results showed that the ethanolic extract of the Lantana flower was thick, dark brown with an aromatic odour and tasteless. The ethanolic extract of the Lantana flower showed a specific gravity of 1.0028 g/ml, the ethanol-soluble extractable of 1.15%, water-soluble extractable of 1.187%, the loss on drying of 8.21%, and total ash content of 9.40%. It contained chemical compounds such as alkaloids, flavonoids, tannins, saponins, steroids, and glycosides. The *in-vitro* results showed that extract at concentrations of 25, 50, and 100 µg/ml showed no colonies on the media from the 1st to the 6th week. Hence can be reported as susceptible. It can be concluded that the ethanol extract of the Lantana flower contains chemical compounds that potentially as anti-tuberculosis.

Keywords: *Lantana camara* L. flowers, phytochemical screening, standardization, tuberculosis

ABSTRAK

Penelitian ini bertujuan untuk menetapkan standar mutu bahan baku, skrining fitokimia, dan aktivitas antituberkulosis ekstrak bunga tembelekan (Lantana camara L.*). Metode yang digunakan adalah observasi langsung di laboratorium. Bunga tembelekan*

diolah menjadi simplisia kemudian diekstraksi dengan menggunakan pelarut etanol 70% dan ekstrak yang diperoleh kemudian dilakukan skrining fitokimia dan uji aktivitas antituberkulosis dilakukan secara in-vitro dengan menggunakan metode Lowenstein-Jensen. Hasil penelitian menunjukkan bahwa ekstrak etanol bunga tembelekan berbentuk kental, berwarna coklat tua dengan bau aromatik serta tidak berasa, berat jenis sebesar 1,0028 g/ml, kadar sari larut etanol sebesar 1,15%, kadar sari larut air sebesar 1,187%, susut pengeringan sebesar 8,21%, kadar abu total sebesar 9,40%, serta mengandung alkaloid, flavonoid, tanin, saponin, steroid, dan glikosida. Pada minggu ke-1 sampai ke-6, ekstrak dengan konsentrasi 25, 50, dan 100 ppm secara in-vitro tidak menunjukkan adanya koloni pada tabung media sehingga dapat dikatakan susceptible. Sehingga, dapat disimpulkan bahwa ekstrak etanol bunga tembelakan mengandung senyawa kimia yang berpotensi sebagai antituberkulosis.

***Kata kunci**: bunga tembelekan, skrining fitokimia, standardisasi, tuberkulosis*

Introduction

According to the World Health Organization (WHO), Indonesia is the country the second highest prevalence of tuberculosis after India with a death rate of 140,000 per year and new cases of 262,000 per year. The number of these cases will continue to increase based on socioeconomic status due to intake nutrition consumed, lack of healthy living behaviour and the spread of HIV-AIDS (WHO, 2020).

Meanwhile, PERMENKES RI No. 67 of 2016 concerning tuberculosis control (Kemenkes, 2017) targeted the tuberculosis elimination by 2035 and Indonesia free of tuberculosis by 2050 (Yanti, 2012). Elimination of tuberculosis is the achievement of the number of tuberculosis cases 1 per 1,000,000 population. In 2017 the number of cases is 254 per 100,000 population or 25.40 per 1 million population (Kemenkes, 2018).

The number of confirmed pulmonary tuberculosis new cases in South Sulawesi province increased from the previous year of 2015. There was 130 per 100,000 population confirmed. South Sulawesi province was placed the seventh rank with a CNR value of 153 after North Sulawesi (Irianti, 2018).

Tuberculosis is a disease caused by *Mycobacterium tuberculosis* that infect and attack the lungs (Depkes, 2005). The increasing number of patients diagnosed with tuberculosis is caused by several risk factors. One of them is the patient's non-compliance in taking the medication regularly due to the length of treatment which is less than 6 months so that resistance often occurs (Yanti, 2012).

Significant drug resistance occurs if a patient is received rifampin for less than 5 months (Lew, 2018). The use of synthetic drugs for tuberculosis until now has been of concern because of the large number of resistance to its treatment over a long period. Therefore alternative treatment is needed, such as

the use of Lantana (*Lantana camara* L.) flower (Irianti, 2018).

Lantana plant was a shrub-like habitus, a wild plant with a taproot with a young green and hairy rectangular stem and an old whitish brown stem with thorns attached to the stem. One part of the Lantana plant that can be used as traditional medicine is the flower (Dibua, 2010). Lantana flower has been used for generations as a traditional medicine to treat internal diseases and coughs. It is used by taking five handfuls of fresh Lantana flowers, then washed and then dried. Then the dried sample is boiled in three glasses of water, filtered, the filtrate is cooled and ready to drink (Fatimah, 2018). According to Gautam et al (2012), the *in-vitro* methanol extract of the Lantana leaves inhibited the growth of the *M. tuberculosis*.

Methods

Materials

M. tuberculosis H37RV isolate was obtained from the Laboratory of Microbiology, Medical Faculty of the Hasanuddin University, Lowenstein Jensen (Difco ™) base media was added with eggs and glycerol was used as growth medium of *M. tuberculosis* H37RV bacteria, Rifampicin and Isoniazid (INH) were used as control positive, blanko as control negative, ethanol 70% was used as sample solvents, chemical reagents (Mayer, Wagner, Dragendroff, Lieberman-Bouchard), concentrated sulfuric acid (H_2SO_4 P), anhydrous acetic acid, iron (III) chloride ($FeCl_3$) solution, magnesium powder was used in phytochemical screening, distilled water and 0.9% NaCl used as a diluent.

Plant materials

The Lantana flower was obtained from the Moncongloe District of Maros Regency in September 2020. The identification and authentication from the Secretariat of Indonesian Flora Diversity with a voucher specimen number 006/KKS-PEN/2020.

Experiments

1. Extraction

   The Lantana flower was processed into the crude drugs. Furthermore, the crude drugs (250 g) were extracted using 70% ethanol by maceration for three days The filtrate was evaporated used a rotary evaporator to obtain a thick extract (Depkes, 1995)
2. Phytochemical screening

   The phytochemical screening is performed to analyze the classes of secondary metabolite compounds in extracts of Lantana flower (Depkes, 1995) as follow.

   Alkaloid assay was conducted by weighing 1 gram of extract, put in a porcelain altogether with 5 ml of chloroform and 3 drops of ammonia solution. The chloroform fraction was separated and acidified with 2 drops of sulfuric acid (H_2SO_4), the acid fraction was divided into three parts then put into each test tube and labelled. In the first tube that the fraction was added with Mayer

adhesive if to be positive if there was white or yellow sediment. The second tube was with Wagner's reagent if there is brown sediment which means it contains alkaloid. The third tube with Dragendroff reagent if it contains alkaloid then a red to brown precipitate was formed.

Flavonoids assay was conducted by weighing 1 gram of extract and added with methanol, heated and filtered. The filtrate was added with sulfuric acid. If the filtrate changes colour to red then the positive extract contains flavonoids

Saponins assay was conducted by weighing 1 gram of the extract and added with 5 ml of aquadest and heated to five minutes. Thereafter, the extract was filtered using filter paper and the filtrate was shaken if the extract foam forms contain saponins for 10 minutes.

Tannins assay was conducted by weighing 1 gram of extract, put into a test tube then added with 5 ml of aqua dest, heated to a few minutes, filtered used filter paper, and the filtrate was added with 1% FeCl3. If the filtrate contains tannins was dark blue or blackish green.

Terpenoids/ steroids assay was conducted by weighing 1 gram of extract and added 0.5 ml of chloroform and Lieberman reagent. If there was a violet colour it means that it was positive for terpenoids, and if green it contained steroids.

Glycoside assay was conducted by weighing 1 gram of extract in a test tube, added with heated aquadest, and filtered. The filtrate was added with 5 ml of acetic acid and 10 drops of sulfuric acid, if it contains glycosides will form a blue or green colour.

3. Determination of standard parameter

Standardization experiments were conducted in the Makassar Health Laboratory Center. There were two treatments for standardization of parameters (specific and non-specific). The specific assay was to described shape, colour, smell and taste using the five senses. Non-specific assays included determination of water content, drying losses, total ash, water-soluble extract, ethanolic soluble extract (Depkes, 2000).

4. *In-vitro* anti-tuberculosis activity assay

Microbial observation was conducted at the Department of Microbiology Laboratory of the Hasanuddin University of Makassar. The sterile macCartney tube was inserted as 5 ml of sterile aqua dest and inserted one loop of the colony from the culture media. Thereafter, stirred slowly ad turbidity was obtained to 0.5 mc Farland standard. Five scrub tubes were prepared and labelled, and then on the first tube were taken 1 ml of the initial suspension diluted (10^{-1}) with 4 ml of distilled water, stirred ad homogeneous. The second tube was taken 1 ml of the first suspension diluted (10^{-2}) with 4 ml of sterile aqua dest stirred ad homogeneously. The

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third tube was taken 1 ml of the second suspension (10^{-3}) diluted with 4 ml of sterile aqua dest, stirred ad homogeneous. The fourth tube was taken 1 ml of the third suspension diluted (10^{-4}) with 4 ml of sterile water, stirred ad homogeneous. In the fifth tube, 1 ml of the fourth suspension was diluted (10^{-5}) with 4 ml of distilled water, stirred ad homogeneous (Stockholm, 2018).

The *in-vitro*, an anti-tuberculosis activity assay was performed using *M. tuberculosis* reference strain H37RF. The suspension was taken in 0.1 ml from the scrub tubes containing *M. tuberculosis* H37RF. Then, it was taken in tubes containing LJ media positive control, negative control and extract of Lantana flower with various concentrations. Aliquots were serially diluted in was tube and then incubated at 37°C and observed bacterial growth for four to six weeks. Results are read four weeks (early reading) and six weeks (final reading) after inoculation. If after four weeks of incubation the proportion of resistant colonies is higher than the critical proportion, the strain can be reported as resistant. Moreover, if the reading on four weeks shows that there are no colonies on the drug containing media and the colonies on the control tubes are mature, the strain can be reported as susceptible. With the exception of these two instances, all other results should be reported after the reading on six weeks (Stockolm, 2018).

Results and Discussion

The identification and authentication result of the Lantana flower plant taken from Moncongloe District of Maros Regency. The Lantana flower was a compound flower arising from the axillary of the leaf, the length of the flower stalk is 2.3-2.8 cm. Attached flower crowns are tubular/bell-shaped, and often alternating orange, pink, red or white colour, green flower petals (Figure 1). The plant of the Lantana was a member Verbenaceae family.

Figure 1. Lantana flower

Phytochemical screening assays were performed to analyze the classes of secondary metabolites in the extract of Lantana flower. Table 1 showed chemical compounds in ethanolic extract of Lantana flower such as alkaloid flavonoids, tannins, saponins, steroids, glycosides. Secondary metabolites in ethanolic extracts of Lantana flower can have the potential to be anti-tuberculosis or can inhibits the growth of *M. tuberculosis* bacteria. Alkaloid scaffolds have an important role in the

tuberculosis drugs development pipeline, as they are always parts of the anti-tuberculosis agents of the molecules in clinical trials. The alkaloid scaffolds in the anti-tuberculosis drug were quinoline, imidazole, quinolinone, and oxazolidinone (Kittakoop P et al, 2014).

Flavonoid were found to be potential inhibitors of *M. tuberculosis* showed moderate level activity against all *M. tuberculosis* cells (Villaume SA *et al*, 2017). Phytochemical compounds alkaloid, phenol, saponin, steroid, tannin, and terpenoid extracted can inhibit *M. tuberculosis* through different molecules mechanism (Kerry RG *et al*, 2018). Phytochemical analysis shows the compounds. The secondary metabolites contained in the extract are flavonoid, polyphenol, tannin, monoterpenoids and sesquiterpenes, quinine, and saponins. The results of the anti-tuberculosis test above showed active activity natural anti-tuberculosis (Fitri Kusuma SA *et al*, 2018).

Table 1. The phytochemical screening assay of Lantana flower ethanolic extract

Class of secondary metabolite	Result
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Steroids	+
Glycosides	+

Note: + = present

Standardization test performed to ensure the quality of the ethanolic extract of the Lantana flower and constantly values of certain parameters requirements (Depkes, 2000). The standardization consist of specific parameters included organoleptic assay (consistency, colour, smell and taste) while non-specific parameters included determination of water content, loss on drying, total ash, water-soluble extractive content, ethanolic soluble extract (Kemenkes, 2017).

In the organoleptic assay of extract, It was found that the extract of Lantana had a thick shape, dark brown colour and an aromatic smell and a separate taste (Table 2). In the specific parameters which the fifth sense was used to aiming for initial recognition in a subjective and simple.

Table 2. Specific parameters of Lantana flower extract

Parameters	Result
Consistency	Thick
Colour	Dark brown
Smell	Aromatic
Taste	Tasteless

The result of the determination of the specific gravity was 1.0028 g / ml. The showed the specific gravity provides a limit on the mass of the volume unit which was a special parameter of liquid extract until which can still be poured the extract was concentrated (thick). The result of determining the ethanol-soluble extractable content of 1.15% (Table 3).

The extract was more soluble in water because the water-attracting polar can compound and the water content of the crude drugs was 1.187%, that

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showed the extract of flowers have presence of polar compounds, this indicated that the water content level was optimal and the absorption of water into the extract during storage appropriated so as not to cause contamination of microbial growth (Kemenkes, 2017).

Table 3. Non-specific parameters of Lantana flower extract

Parameters	Value
Specific gravity	1.0028 g/ml
Ethanol-soluble extractable	1.15%
Water-soluble extractable	1.19%
Loss on drying	8.21%
Total ash	9.40%

The results of loss on drying determination were 8.21% also these values showed the amount of the compounds that evaporates during heating where the drying loss is the level of the vaporized process part of a during substance the heating. Measurement of total ash content showed the compounds of inorganic material or minerals obtained (9.40%), this indicated that the ethanolic extract of Lantana flower contains minerals such as magnesium, calcium and sodium.

The *in-vitro* anti-tuberculosis activity assay performed with concentration extract of Lantana flower (25, 50, and 100 µg/ml) and used rifampicin and isoniazid as the positive control.

The results of the *in-vitro* anti-tuberculosis activity test showed that the ethanolic extract of the Lantana flower at concentrations of 25, 50 and 100 µg/ml effectively inhibits the growth of *M. tuberculosis* H37RV from the first week to the sixth week and thereafter were no bacilli growth, this can be said to be the same as the controlled used were isoniazid with a concentration of 0.2 µg/ml and rifampin 40 µg/ml (Table 4, Figure 2).

Table 4. The *in-vitro* anti-tuberculosis activity assay

Concentration	Colony growth in the sample					
	I	II	III	IV	V	VI
Control	-	-	-	+	++	+++
Isoniazid 20 µg/ml	-	-	-	-	-	-
Rifampicin 40 µg/ml	-	-	-	-	-	-
Extract 25 µg/ml	-	-	-	-	-	-
Extract 50 µg/ml	-	-	-	-	-	-
Extract 100 µg/ml	-	-	-	-	-	-

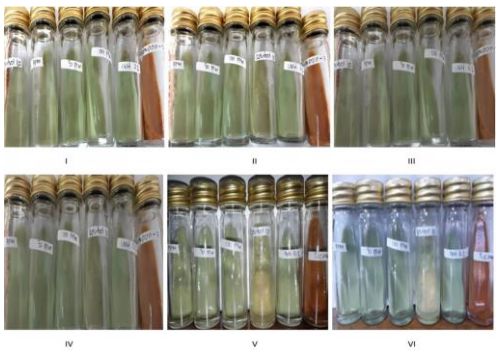

Figure 2. The bacterial growth profile during 6 weeks of observation

Observation during 6 weeks showed concentrations at 25, 50 and 100 µg/ml the ethanolic extract of the Lantana flower gave the same results as

the positive controlled (rifampin and isoniazid) which was negative or the absence of colony growth while the blank concentration showed positive results in week 4 to week 6 this was because on the surface of the blank media there was a yellow colony growth. The presence of *M. tuberculosis* colonies if the surface of the media was coloured yellow or orange (Wahyuningrum R *et al*, 2017). According to nano herbal and ethanolic extract of Lantana flower have the potential as anti-tuberculosis (Fatimah C, 2018).

Conclusion

The ethanolic extract of the Lantana flower contains alkaloid, flavonoid saponin, tannin, steroid and glycoside. While the standardization test of specific and non-specific parameters obtain indicate flower had been the qualified standard requirements for the quality of the raw material for the drug.

The ethanol extractable of the Lantana flower contains chemical compounds that potentially as anti-tuberculosis. This exploration is important for a work to track down new reasonable wellsprings of medications for tuberculosis disease in beating drug opposition and to inspect the viability of customary prescriptions utilized by the Indonesian public, particularly in the Maros region.

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