ORIGINAL ARTICLE

Antimycobacterial activity of *Gynura procumbens* leaves extract against *Mycobacterium tuberculosis*

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Abstract

Introduction: Tuberculosis (TB) has existed for millennia and remains a major global health problem. The main challenge of TB treatment is the resistance of *Mycobacterium tuberculosis*. Therefore, there is a need to develop new drugs to combat those resistant *M. tuberculosis*. Continuous efforts are underway in the search for novel bioactive compounds and bioactive compounds of natural origin, particularly from plants, are gaining significance. *Gynura procumbens* is one of the plants used as traditional medicine. In Konawe district of Southeast Sulawesi, Indonesia, *G. procumbens* leave had been used as TB treatment. The present study was designed to investigate the antimycobacterial activity of *G. procumbens* leaves extract and its phytochemical composition. **Materials and Methods:** *G. procumbens* Leaves were extracted by maceration, and antimycobacterial evaluation was conducted using microscopic observation drug susceptibility method. **Results:** *G. procumbens* extract at 500 ppm has antimycobacterial activity against *M. tuberculosis* Strain H37Rv and multidrug-resistant. Phytochemical screening of the extract revealed the presence of flavonoids and terpenes. **Conclusion:** The study revealed *G. procumbens* extract potentially to be developed as a traditional medicine formulation for TB treatment and further studies on isolation of active principles from extracts, and their bioactive determinations are to be carried out.

Key words: *Gynura procumbens*, microscopic observation drug susceptibility, *Mycobacterium tuberculosis*, tuberculosis

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It typically affects the lungs (pulmonary TB) but can also affect other sites (extrapulmonary TB). TB has existed for millennia and remains a major global health problem. It was one of the top 10 causes of death worldwide, ranking above HIV/AIDS as one of the leading causes of death from an infectious disease. 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.[1]

The main challenge of TB treatment is the resistance of *M. tuberculosis* which is the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB.[2,3] In 2015, there were an estimated 480.000 new cases of MDR-TB and 7.500 new cases XDR TB.[1] Therefore, there is a need to develop new drugs to combat those resistant *M. tuberculosis*. Continuous efforts are underway in the search for novel bioactive compounds to develop new anti-TB drugs. To this end, bioactive compounds of natural origin, particularly from plants, are gaining significance.[2]

Medicinal plants have been a valuable source of therapeutic agents, and still many of today's drugs are plant-derived natural products or their derivatives.[4] Medical plant contains compounds are known as a secondary plant, the

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compounds are classified based on their chemical structure, their classification is alkaloids, terpenes, flavonoids, steroids, phenolics, flavonoids, etc.[5] From plant extracts, antimycobacterial compounds with a mechanism of activity have been reported according to their classifications.[6]

Gynura procumbens (family: Asteraceae) is one of the plants used as traditional medicine. Several studies have reported the pharmacological activity of *G. procumbens* leave such as anti-inflammatory, antidiabetic, antihyperlipidemia, antiulcerogenic, and antihypertensive.[7-11] In Konawe district of Southeast Sulawesi, Indonesia, *G. procumbens* leaves have been used empirically as TB treatment.

To the best of our knowledge, there is no scientific investigation of antimycobacterial activity of *G. procumbens* leaves. Therefore, the present study was designed to investigate the phytochemical composition and antimycobacterial activity of *G. procumbens* leaves extract.

MATERIALS AND METHODS

Plant Materials

G. procumbens Leaves were collected from Konawe District, Southeast Sulawesi, Indonesia. Plant material was washed under running tap water and shade dried at ambient temperature. Thereafter, dry up sample was then powdered and conserved sheltered from humid conditions until use. The plants were taxonomically authenticated by Biology Department of Haluoleo University Indonesia.

Preparation of the Extracts

The powdered *G. procumbens* leaves (100 g) were extracted by maceration with technical grade methanol at room temperature for 48 h. A rotary evaporator was used to evaporate the extract to dryness at 40°C to produce dried crude extract with a yield of 17.8 g (17.8%). The extract was stored in a desiccator.

Phytochemical Screening

Phytochemical screening to detect the presence of alkaloids, saponins, flavonoids, sterols, and terpenes was performed by standard procedures.[12]

Bacterial Strains and Inoculum Preparation

Two strains of *M. tuberculosis* were used in this research. *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 an 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.

Selection and Preparation of Dose

The extract was prepared by serial dilution. The dilution was done 1/2 of the initial dose. 1000 ppm was chosen, because it is the maximum limit of detection on microscopic observations on microscopic observation drug susceptibility (MODS) method. The equivalent dose for 1000 ppm is calculated and dissolved with DMSO.

Antimycobacterial Activity

Antimycobacterial evaluation was conducted using MODS method according to Widarini *et al*.[2] The MODS media were prepared in 24-well tissue culture plates. Each well contained 950 µl of *M. tuberculosis* inoculum, Middlebrook 7H9 broth, OADC, and polymyxin, amphotericin B, nalidixic acid, trimethoprim, and azlocillin. 50 µl 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 was INH (0.1 ppm) and ofloxacin (1 ppm). The rest of the well is used to control the growth 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 examined within the bag. The antimycobacterial activity was determined by the presence of *M. tuberculosis* growth. The growth of *M. tuberculosis* was identified by cord formation [Figure 1].

RESULTS

Phytochemical Screening

Phytochemical screening results revealed that the extract contains terpenes and flavonoids [Table 1].

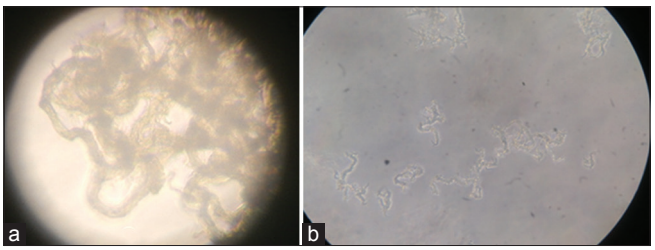

Figure 1: Microscopic observation of *Mycobacterium tuberculosis* cord formation (a) ×40 magnification (b) ×10 magnification

Antimycobacterial Activity

Antimycobacterial evaluation with MODS method was observed on the 7th day to determine the *M. tuberculosis* growth. The growths of each well were identified by the cord formation of *M. tuberculosis* [Figure 1]. The evaluation shows that *G. procumbens* leaves extract has antimycobacterial activity against *M. tuberculosis* Strain H37Rv and MDR at 1000 ppm and 500 ppm [Table 2, Figures 2 and 3].

DISCUSSION

Antimycobacterial activities of various plants have been reported. Plant-derived compound has been attracting much attention as potent alternatives for infectious diseases. Plants have secondary metabolites that providing an excellent opportunity for expansion of modern chemotherapies against a microorganism.[13] In Konawe district of Southeast Sulawesi, Indonesia, *G. procumbens* leaves have been applied as a treatment for TB disease. Therefore, *G. procumbens* needs to be investigated to observe its potential as an antimycobacterial agent.

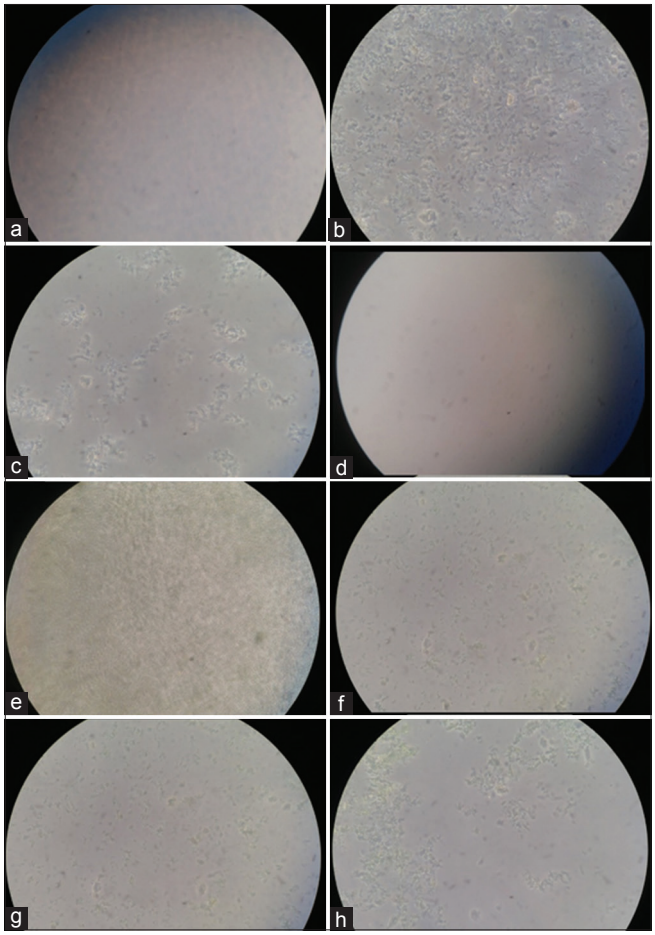

Figure 2: Microscopic observation of anti mycobacterium assay on *Mycobacterium tuberculosis* strain H37Rv (a) control, (b) negative control, (c) positive control, (d) extract 1000 ppm, (e) extract 500 ppm, (f) extract 250 ppm, (g) extract 125 ppm, and (h) extract 62.5 ppm

Table 1: Phytochemical screening of *Gynura procumbens* extract

Compound	Result
Alkaloids	(−)
Sterols	(−)
Terpenoids	(+)
Saponins	(−)
Flavonoids	(+)
Triterpenoids	(−)

Table 2: Antimycobacterial evaluation of *Gynura procumbens* leaves extract against *M. Tuberculosis* strain H37Rv and MDR

Sample	Replication	*M. Tuberculosis*	
		H37Rv*	MDR*
Control	1	+	+
	2	+	+
	3	+	+
Negative control (DMSO)	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. *M. tuberculosis*: *Mycobacterium tuberculosis,* MDR: Multidrug-resistant

MODS Method is a current method in TB culture. It is cheaper and has shorter turn-around time than conventional

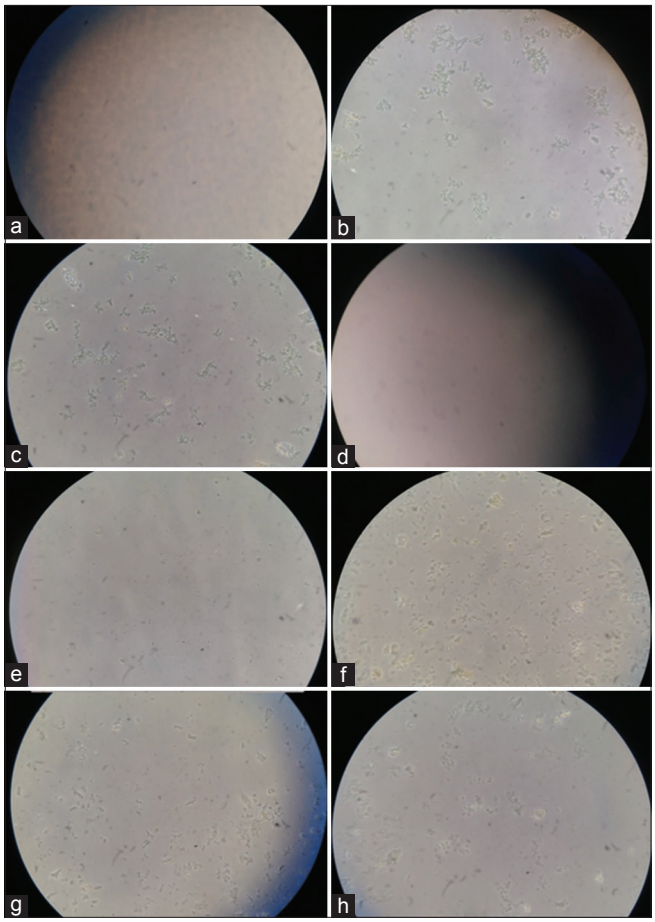

Figure 3: Microscopic observation of antimycobacterium assay on *Mycobacterium tuberculosis* strain multidrug-resistant (a) control, (b) negative control, (c) positive control, (d) extract 1000 ppm, (e) extract 500 ppm, (f) extract 250 ppm, (g) extract 125 ppm, and (h) extract 62.5 ppm

gold standard methods.[14] MODS assay has been validated, and the previous studies revealed that MODS assay had 92%–97.5% sensitivity compare to Löwenstein-Jensen, the microplate Alamar Blue assay, and the tetrazolium microplate assay.[15] The MODS assay is a liquid culture method based on microscopic detection of characteristic *M. tuberculosis* morphology (Cord formation). *M. tuberculosis* cell wall contains trehalose 6.6'-dimycolate which provides the form of a mycobacterium like cord formation.[16]

Antimycobacterial evaluation of *G. procumbens* extract performs that minimum inhibitory concentration (MIC) of the extract is 500 ppm for both H37Rv and MDR strain. Observations on the 7th day revealed a similar result (microscopic appearance) to the positive control (INH 0.1 ppm and ofloxacin 1 ppm). The extract was considered active if it gave MIC ≤500 ppm.[13] The large difference in the concentration of inhibition between the extract (500 ppm) and the positive control (1 ppm) is understandable because the extract still contains many compounds, the difference will not be much different if the sample is isolated compound.

Phytochemical screening results of *G. procumbens* extract reveal the presence of flavonoids and terpenes. Several studies have reported flavonoids and terpenes compounds as anti *M. tuberculosis*. There was positive correlation between the flavonoid and terpenes content and antimycobacterial activity.[6]

Several research has proved flavonoids as antimycobacterial. Habbu *et al*. reported the antimicrobial activity of the ethyl acetate extract of *Argyreia speciosa* (Burm. F) Boj. with MIC of 50 µg/mL due to flavonoids.[13] The methanolic extract of Bromelia balansae Mez. was identified to have various flavonoid glycosides and showed moderate activity with a MIC of 128 µg/mL.[17] Pinocembrin and cryptocaryone, two new flavonoids from *Cryptocarya chinensis* Hemsl. leaves were effective against *M. tuberculosis*.[18] Sharma *et al*., 2008, reported that epigallocatechin gallate/epigallocatechin-3-gallate (flavonoid) directly inhibits fatty acid synthase by interacting with the residues near the NADH binding. Fatty acid synthase is the enzyme that essential to form mycobacterium cell wall.[19]

Terpenes have been reported to have anti *M. tuberculosis* activity. A new trachybalone diterpene derivatives, ent-trachylobane-3-one and ent-trachylobane-17-al from Jungermannia exsertifolia ssp. cordifolia Steph. revealed moderate activity against M. tuberculosis. Abietane and its derivative isolated from *Plectranthus grandidentatus* Gurke. were reported to have activity against MDR forms, which were better than the usual antimycobacterial agents.[3,6]

CONCLUSION

G. procumbens extract has antimycobacterial against *M. tuberculosis* strain H37Rv and MDR. Phytochemical screening of the extract revealed the presence of flavonoids and terpenes. The study revealed that *G. procumbens* extract is potential to be developed as a traditional medicine formulation for TB treatment and further studies on isolation of active principles from extracts and their bioactivity determinations are to be carried out.

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