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New Compound Anti *Mycobacterium tuberculosis* from Methanolic Fraction of Bangle Rhizome (*Zingiber cassumunar* Roxb.)

Muhammad Isrul*[,1], Gemini Alam[2], Sartini[2], Nasrum Massi[3]

[1]Pharmacy Department, STIKES Mandala Waluya, Kendari-Indonesia,
[2]Pharmacy Faculty, Hasanuddin University, Makassar-Indonesia,
[3]Medical Faculty, Hasanuddin University, Makassar-Indonesia

Abstract : Tuberculosis (TB) remains a major global health problem, responsible for ill health among millions of people each year.One of the main problem of TB treatment is the resistance of *Mycobacterium tuberculosis.* Continuous efforts to develop new drugs to combat *M. Tuberculosis*are under way and bioactive compounds of natural origin, particularly from plants, are gaining significance. Bangle Rhizome (*Zingiber cassumuna r*Roxb.) has been used empirically as anti *Mycobacterium tuberculosis* drug. The aim of researchto determineanti *M.tuberculosis* Strain H37Rv and MDR activity of methanol extract fraction of bangle rhizome (*Zingiber cassumunar* roxb.). powdered Bangle Rhizome were macerated with hexane and methanol, respectively. Methanol extract then was fractionated by vacuum liquid chromatography. Anti *Mycobacterium tuberculosis* assay was done with Microscopic ObservationDrugSuspectibility (MODS) method and at final concentration at 100 ppm of fractions. Methanolextract of bangle rhizome resulting fivefactions (Fraction A,B,C,D and E). *M. tuberculosis* assay results show that the most active fraction is B. Chemical screening of fraction B using TLC visualization reagents indicated that fraction B contains flavonoids and terpenes.
Keyword : *Zingiber cassumunar* Roxb., *Mycobacterium tuberculosis, MODS.*

Introduction

Tuberculosis (TB) remains a major global health problem, responsible for ill health among millions of people each year. TB ranks as the second leading cause of death from an infectious disease worldwide. In 2013, there were an estimated 9.0 million incident cases of TB and 1.5 million people died from the disease (1.1 million deaths among people who were HIV-negative and 360 000 among people who were HIV-positive). Among these deaths there were an estimated 210 000 from MDR-TB, a relatively high total compared with 480 000 incident cases of MDR-TB[1]. therefore, there is a need to develop new drugs to combat *M. tuberculosis*, especially multi-drug resistant (MDR) strain, and continuous efforts are under way 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].

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Bangle (*Zingiber Cassumunar* Roxb.) is one of the plants used as traditional medicine. Bangle rhizome have been shown to have several pharmacological effects such as aanti-inflammatory, antibacterial, and analgetic[3,4,5]. Previousstudies have been conducted to test the activity ofthe methanol dan hexane extract of bangle rhizomeand The Result show that hexane and methanol extract haveM. Tuberculosis strains H37Rv and MDR inhibitory activity and methanol extractis the the most active extract[6].

The present study was aimed to assay the anti *M.tuberculosis* Strain H37Rv and MDR activity of methanol extract fraction of bangle rhizome. (*Zingiber cassumunar* roxb.)

Materials and Methods

Plant material

Bangle Rhizome (*Zingiber cassumunar* Roxb.) were obtained from sungguminasa garden, Gowa, South Sulawesi, Indonesia. The Plant Material was determination by Botany Laboratory, Department of Biology, Matematics and Science Faculty Hasanuddin University.

Extraction and Fraction

Bangle that have been dried and powdered were macerated with technical grade of hexane and methanol, respectively. Methanol extract then was fractionated by vacuum liquid chromatography with hexane, hexane-ethyl acetate, ethyl acetate, and methanol as eluent.

Bacterial strains and inoculum preparation

M. tuberculosis strains H37RvandMDR were supplied by Microbiology Laboratory, medical faculty of Hasanuddin University. Allcultures 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.0.5McFarland.

Anti Mycobacterium assay

Anti Mycobacterium Assay was conducted using MODS(Microscopic Observation Drug Suspectibility) method according Anitaetal[7] with modification.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 100 ppm. The negative control was DMSO. 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 examined within the bag. The growth of *M.tuberculosis* was identified by cord formation (Figure 1).

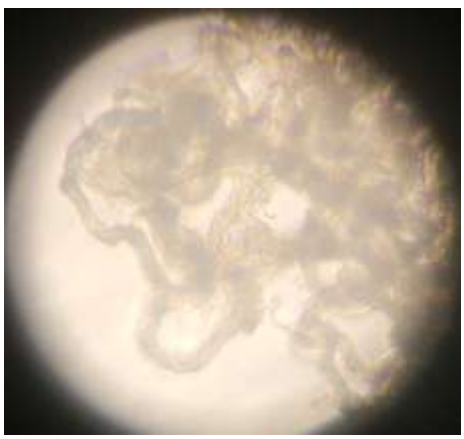

Figure1. Cord formation of *M.tuberculosis*

Result and Discussion

Fractionation Result of methanol extract of bangle rhizome produced 20 fractions that group into five factions (Fraction A, B, C,D and E) based on thin layer chromatography(TLC) profile. The fractions were then tested the activity of anti*M. Tuberculosis* with MODS method.

Observations of anti *M. Tuberculosis* assay with MODS method was begun on day 7 to determine the active fraction. Observations carried out by formations cord of *M. tuberculosis*. On the 14th day of observation (Table 1) shows the fraction B as the most active fraction.

Table 1 : Anti mycobacterium assay of methanol extract fraction of Bangle Rhizome.

Sample	**Replication**	***M.tuberculosis***	
		H37Rv	**MDR**
Fraction A 100 ppm	1	-	-
	2	+	+
	3	-	+
Fraction B 100 ppm	1	-	-
	2	-	-
	3	-	-
Fraction C 100 ppm	1	-	+
	2	-	-
	3	-	-
Fraction D 100 ppm	1	+	+
	2	+	+
	3	+	+
Fraction E 100 ppm	1	+	+
	2	+	+
	3	+	+
Negative control (DMSO)	1	+	+
	2	+	+
	3	+	+
Control (*M.tuberculosis*)	1	+	+
	2	+	+
	3	+	+

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

The Result of chemical screening compound of fraction B was done by using TLC visualization reagents (Table 2) to determine the group of compounds which contained in fraction B. The Results showed Fraction B positive in $FeCl_3$, Sitoborat, and the Lieberman Burchard reagent showed blue green color spot. The results indicated that fraction B contains flavonoids and terpenes.

Several studies have reported flavonoids and terpenes compounds as anti *M. tuberculosis*. One of Terpenes are reported to be as anti *M.tuberculosis*is labdan diterpene compound isolated from the *Curcuma amada*rhizome[8]. Sesquiterpene compounds Dihydro-β-agarofuran from*Celastrus vulcancola* active against *M.tuberculosis* MDR[9]. The Others, terpene compounds are caniojane, ent-trachylobane-3-one, ent-trachylobane-17-al, and leubethanol[10,11,12].

Table 2 :Chemical screening compound of fraction B

Reagents	**Result**
Dragendorf	(-)
FeCl3	(+)
Sitoborat	(+)
Liberman Burchard	(+)

+ = positive reaction, - = negative reaction

Flavonoid Compounds that are reported as anti *M.tuberculosis* such as Pinocembrin and cryptocaryone, isolated from the leaf *Cryptocarya chinensis* Hemsl., which had been proven effective to *M.tuberculosis* strain H37Rv[13]. Other flavonoid compounds are Linaroside and lantanoside isolated from leaf *Cryptocarya chinensis*, which as bacteriostatic compound to *M.tuberculosis* H37Rv[14]. Sherma et al. Reported that the flavonoid compound epigallocatechin gallate / epigallocatechin-3-gallate, can inhibit enoyl-acyl carrier protein reductase an enzyme that plays an important role in the synthesis of fatty acids in bacteria which is generally used as a cell wall constituent, particularly *M. tuberculosis* which contain high fatty acid in its cell wall[15,16].

Test results showed the content of some of the compounds that allow the compounds to act as anti *M.tuberculosis* strains H37Rv and MDR. Therefore, further research is needed to isolate the active compound.

Conclusion

Methanolic Fraction of Bangle Rhizome (*Zingiber Cassumunar* Roxb.) show anti Mycobacterium activity against H37Rv and MDR Strain.The Result of chemical screening compound of fractionindicated that fraction B contains flavonoids and terpenes. Literature investigation shows that flavonoid and terpenes have been proved as anti *Mycobacterium tuberculosis* agent. Further research to isolate the compound required to discover new drugs to combat *M. Tuberculosis*.

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