Arch Vet Sci Med 2023; 6 (4): 17-27 *DOI: 10.26502/avsm.038*

Research Article

In-Vitro Antimycobacterial Activities of *Thymus serrulatus* ("Tosigne") and *Trigonella foenum gracium* ("Abish") Against *Mycobacterium tuberculosis* and *Mycobacterium bovis*

Fikir Gete[1] and Temesgen Kassa[2*]

[1]Addis Ababa University, King George VI St, Addis Ababa, Ethiopia

[2]Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia

[*]**Corresponding Author:** Temesgen Kassa, Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia.

Received: 08 August 2023; **Accepted:** 21 August 2023; **Published:** 15 December 2023

Citation: Fikir Gete, Temesgen Kassa. *In-Vitro* Antimycobacterial Activities of *Thymus serrulatus* ("Tosigne") and *Trigonella foenum gracium* ("Abish") Against *Mycobacterium tuberculosis* and *Mycobacterium bovis*. Archives of Veterinary Science and Medicine 6 (2023): 17-27.

Abstract

Background: Tuberculosis (TB) is a serious infectious disease affecting many people across the world, particularly sub-Saharan Africans. Ethiopia is ranked 7th among the 22 high-TB burden countries in the world. Conventional chemotherapeutic control approaches have faced serious, flourishing drug resistance strains. Traditional herbal remedies have endeavored to supplement or replace ineffective drugs.

Methods: This study was conducted using an experimental study design to determine the antimycobacterial activity of leaves of *Thymus serrulatus* ("Tosign") and seeds of *Trigonella foenum gracium* ("Abish"). The ethanol and methanol crude extracts of the plants were tested against the Mycobacterium tuberculosis H37Rv reference strain, SIT149, and M. bovis (BCG and SB1176).

Results: Methanol and ethanol crude extracts of *Thymus serrulatus* and *Trigonella foenum gracium* have demonstrated promising activity against all the isolates of Mycobacterium species tested. Antimycobacterial activity was documented within an inclusive minimum inhibitory concentration (MIC) range of 0.78–50µg/ml for the extracts of two plant species. The highest antimycobacterial effect with a MIC of 0.78µg/ml was obtained in methanol extracts of *Thymus serrulatus* against most strains of M. tuberculosis and M. bovis isolates except M. tuberculosis H37RV which showed a MIC of 12.5µg/m. For *Trigonella foenum gracium*, the antimycobacterial effect with MIC was observed in the range of

0.78-12.5µg/ml against M. tuberculosis and M. bovis isolates with ethanol extraction.

Conclusions: This finding could serve as baseline information for further antimycobacterial agent study of these plants, and future studies ought to identify the exact bioactive chemicals involved in the antimycobacterial effect.

Keywords: Antimycobacterial activity; *Mycobacterium bovis*; *Mycobacterium tuberculosis*; Plant extract

Abbreviations: ALIPB: Aklilu Lemma Institute of Pathobiology; BMM: Micro Broth Dilution Method; DMS: Dimethyl Sulphoxide; MDRTB: Multiple Drug Resistance Tuberculosis; NCCLS: National Committee For Clinical Laboratory Standards; OADC: Oleic Acid, Albumin, Dextrose, and Catalase; REMA: Resazurine Microplate Assay; SIT149: Spoligo Type; XDRTB: Extensive Drug Resistance Tuberculosis

1. Introduction

Medical plants offer a great deal of hope to fulfill these needs and have been used for curing diseases for many centuries. These have been used extensively as pure compounds or as crude materials. Only a few plant species have been thoroughly investigated for their medicinal properties [1]. Ethiopia is one of the few countries in the world that has a unique wealth of medicinal plants and vast traditional knowledge of the use of herbal medicine for the cure of various diseases [2,3]. So far, few plants have been tested against Mycobacterium species. The rising prevalence of multidrug-resistant tuberculosis and widespread drug resistance in tuberculosis highlights the critical need for newer anti-tuberculosis compounds and drugs.

Tuberculosis (TB) is one of the leading infectious diseases and health burdens in the world. It is a common and deadly infectious disease caused by various strains of Mycobacterium tuberculosis in humans. It has been estimated that one third of the world's population is infected with TB, with more than nine million new cases diagnosed and approximately two million people killed annually [4]. Tuberculosis (TB) is still the leading cause of morbidity and mortality in developing countries, particularly in Asia and Sub-Saharan Africa [5]. According to the WHO [6] report on the epidemiological burden of TB, Ethiopia was one of the top-ranking countries among the 22 high-TB burden countries in the world, with an incidence rate of 224 per 100,000 populations.

For over a half-century, the conventional control approach has focused primarily on chemotherapy. For this purpose, a number of efficacious agents originally intended for TB treatment were introduced to the market starting in the late 1940s and halted with the introduction of rifampicine in the 1960s. These agents had reasonable efficacy when introduced. The use, or often misuse, of drugs over the years has led to the flourishing of drug-resistant strains. Globally, the emerging and re-emerging of deadly drug resistant strains of Mycobacterium tuberculosis (MDR-TB and XDR-TB), coupled with significant drug hepatotoxicity and lengthy therapy, paved the way toward a global TB therapeutic crisis [7].

In Ethiopia, the problem of MDR-TB has been recognized, and currently, according to the WHO [6] report, the MDR-TB burden in the country is estimated to be 1.6% in new TB cases and 12% in retreatment cases, indicating the growing significance of the MDR-TB problem. As a result, evaluating the antimycobacterial activity of traditional medicinal plants is a very dominant event in many parts of the world in search of new efficacious agents. Long-used Ethiopian traditional

medicinal plants for tuberculosis and respiratory ailments have either not yet been evaluated for antimycobacterial activity or are inaccessible to the scientific community. Antimycobacterial activities of *thymus serrulatus* and *Trigonella foenum* against *Mycobacterium tuberculosis* (SIT149, HRv37) and *Mycobacterium bovis* (SB1176, BCG) have yet to be demonstrated in the pool of scientific data. The plant extracts have anti-mycobacterium activities, pointing to scientific ground for the ethnomedical use of the plants against TB. This finding could serve as baseline information for further antimycobacterial agent studies of these plants. Future studies ought to assess the exact chemicals involved and identify any toxicity. There will also be ways to encourage the traditional use of the plant against TB after further research.

Most of the time, traditional plants usually lack scientific proof of efficacy, which doesn't mean that they aren't valuable; only more scientific work is needed to investigate their validity. Low cost and accessibility are not only advantages of using traditional plants, but also years of ethnomedicinal practice by the community, which show evidence of the efficacy of these traditional medicinal plants against tuberculosis and other respiratory diseases and suggest a need for scientific-based research. Hence, it is with this view and understanding that the present study was initiated with the following specific objectives:

- To investigate the *in-vitro* growth inhibition effect of traditionally used medicinal plants of *Thymus serrulatus* and *Trigonella foenum gracium* on growth of *Mycobacterium tuberculosis* and *Mycobacterium bovis* isolates and to see.
- To determine the minimum inhibitory concentration (MIC) of ethanol and methanol extracts of *Thymus serrulatus* and *Trigonella foenum gracium* against the susceptible *Mycobacterium species.*

2. Materials and Methods

2.1. Medicinal plant collection sites

The selected medicinal plants, *Thymus serrulatus* "Tossigne" and *Trigonella foenum gracium* "Abishe," were collected from two sites: the Debre Sina area (South Wollo zone of Amhara Region) and Bishoftu market (East Shewa Zone of Oromia Region).

Debre Sina (Amharic, "Mount Sinai") is one of the districtsin then Amhara Region of Ethiopia. The town is 180 km north of Addis Ababa and 60 km north of Debre Birhan. Debresina is located at 9085' N, 390076' E with altitude of 2660 meter above sea level. The altitude of this woreda ranges from 500 meters above sea level at the bottom of the canyon of the Nile ("Abay") River to 3200 meters above sea level in the northeast corner. Thymus serrulatus is a dominant plant on the hilly area growing together with the grass. The average temperature in Debre Sina is 15.1 °C. The average annual rainfall is 989 mm.

Trigonella foenum gracium "Abishe" was collected from the market of Bishoftu town (previously also called "Debre zeit"), which is located in the East Shewa Zone of the Oromia Region and has an elevation of 1,920 meters. Bishoftu is located at 90 N and 400 E. It is located 47 kilometers south of Addis Abeba. The plant *Trigonella foenum gracium* does not grow in the area but is commonly brought through trading routes from the southern region of the country.

2.2. Study design

This study was conducted at the Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University (AAU), using an experimental study design. The collected traditional medicinal plants, *Trigonella foenum gracium* (from Bishoftu market) and *Thymus serrulatus* (Debre

Sina), were brought to the ALIPB laboratory for extraction and an *in vitro* antimycobacterial effect study.

2.3. Plant materials used in the study

Two plants, namely *Thymus serrulatus* (leaves) and *Trigonella foenum gracium* (seeds), were used in this study. *Thymus serrulatus*, in Amharic “Tosigne," is a small plant, growing together with grass and a green leaf plant. The plant mostly grows in the northern part of Ethiopia, especially in the Debre Sina area of the Amhara region, and locally, people use it for different purposes, including as spices.

Trigonella foenum gracium, *known* in Amharic as "Abishe," is one of the plants commonly used for spice in the rural part of Ethiopia. The plant grows mostly in the summer. The leaf is small and green, and when the seed is ground, the flour is white.

2.4. Study Methodology

2.4.1. Plants material preparation and extraction

The collected *Thymus serrulatus leaves* were thoroughly washed with distilled water in order to remove any dirt and contaminants from the plant, shade-dried, not exposed to light to protect any loss in the activity of the plant, chopped into thin slices, and ground through a traditionally used grinder mortar to make fine powder. The powdered plant materials were stored until extraction. The extraction of plant material was done according to previously described protocols [8,9].

In the extraction, 165 grams of the leaf powder of *Thymus serrulatus* were added to 240 ml of each of the two solvents used, 99% methanol and 95% ethanol. Then the mixture was set into an electrical shaker for 24 hours to thoroughly mix the plant powder with the solvent. The supernatant of the solution was filtered through a 0.45 µm Whatman No.1 filter paper and stored at 4°C until further use. The sequential extraction was done by subjecting 200 ml of filtered leaf extract to a visible light source rotary evaporator (Laborata 4000, Germany) attached to a vacuum pump and set in a water bath at 40°C. The solvent and the crude extract were separated through a rotary evaporator, and the extracts were dried in an oven at 50°C for three weeks, after which the dried product was stored until further use.

Seeds of *Trigonella foenum graecum* were thoroughly washed with distilled water to remove dirt material, shade-dried, and ground to make a fine powder. The extraction of plant material was done according to previously described protocols [8,9]. Similarly, 165 grams of the seed powder of *Trigonella foenum graecum* was added to each 240 ml of solvents (99% methanol and 95% ethanol) in three conical flasks, for a total of 495 g to 700 ml of both methanol and ethanol solvents. The extracts were settled in an electrical shaker for 24 hours after being mixed with solvents. The well-mixed extracts were filtered through Whatman No.1 filter paper for each solvent to remove the fibrous portion of the plant. Then the plant material was extracted by a visible light source rotary evaporator (Laborata 4000, Germany) attached to a vacuum pump and set in a water bath at 40°C. The crude extract of the plant was allowed to dry in an oven at 50°C for three weeks, and the dried extract was stored in the refrigerator until used for the antimycobacterial assay (Table 1).

2.4.2 Growth media, test organisms and inoculums standardization

2.4.2.1 Growth Media

Commercial powder of both Lowenstein Jensen (L-J) medium and Middle brook 7H9 agar used for the antimicrobial assay were obtained from Aklilu lemma institute of pathobiology in tuberculosis laboratory. They were prepared according to manufacturer's instructions.

Plant species	Family	Vernacular name	Parts used	Voucher specimen	Place of collection
Thymus serrulatus	Lamiaceae lindl	Tosign (Amharic)	Leaves	FG-01	DebreSina
Trigonella foenum gracium	Fabaceae	Abish (Amharic)	Seeds	FG-02	Bishoftu

Table 1: Medicinal plants used for antimycobaterial activity against *Mycobacterium tuberculosis* and *Mycobacterium bovis* strains.

2.4.2.2 Test organisms

M. tuberculosis strains including (*H37Rv, SIT149*) and *M. bovis* strains (*BCG, SB1176*) were obtained from Tuberculosis laboratory of Aklilu lemma institute of patho biology and were used for evaluation of plants crude extracts.

2.4.2.3. Preparation of Inoculums

Mycobacterium tuberculosis (H37Rv, *SIT149*) and *Mycobacterium bovis* (BCG, SB1176) isolates obtained from TB laboratory of Aklilu Lemma Institute of Pathobiology were revived from deep freezer (-80°C) and subcultured in Lowenstein-Jensen media at 37°C for 14–21 days. Then the grown isolates were inoculated and then sub-cultured in Middle Brook 7H9 broth supplemented with OADC at 37°C for 14 – 21 days. The bacterial suspension was homogenized by vortex shakeup and the turbidity was adjusted in agreement with tube which is the scales of McFarland no.1 (3.2×10^6 cfu/mL). The inoculum was prepared diluting the bacterial suspension in the proportion of 1:20 in Middle Brook 7H9 broth medium. This diluted suspension (100 µL) was used to inoculate each well of the plate as described previously [9].

2.5. Microbiological Tests of Plant Extracts

Antimycobacterial activity of plants were tested by Microbroth dilution method (BMM) previously described by Mann et al. [10] with minor modification of some measurement were made by parts of method in Mohamad et al. [11]. *Mycobacterium tuberculosis* reference strain H37Rv was used to evaluate the preliminary screening of crude extracts of *Thymus serrulatus, Ttrigonella foenum gracium* at concentrations of 50 µg /ml. Prior to the bioassay, stock solutions of Rifampicin with the concentration of 10 µg/ml and isonized (10µg/ml) were prepared and stored to be used for the positive control. Prior to inoculation the crude extracts of the plant 30mg of the extract for each solvent were added to 1ml of DMSO and dissolved by using a vortex. Control wells without the tested extracts and a sterility control which contain only the media without extract and bacterial strain were assayed simultaneously. Rifampicin was prepared from the stock solution just prior to inoculation time to the concentration of 10µg/ml in the total volume of 200µl. The growth inhibition result was explained by Micro plate Alamar Blue Assay (MABA) using 1% Resazurine as previously described. The reagent allows the detection of microbial growth in micro titer plates without the use of spectrophotometer. The susceptibility test conducted by the Micro plate Alamar Blue Assay was using in 96 well microtitre plate to evaluate the susceptibility of *H37Rv, SIT149* for *Mycobacterium tuberculosis* and *SB1176, BCG* for *Mycobacterium bovis* isolates to the extract [12].

The inhibitory concentrations of both extracts were evaluated by the method with concentrations of 30mg/ml in the total volume of 200µl. Prior to inoculation the crude extracts of 30mg were measured to make the proposed concentration in 200µl. The measured extracts (30mg) were dissolved by in 1ml of DMSO by using a vortex. The working solution (1ml) with the bacterial suspension (100µl) and the diluents media (Middle broke

7H9) (100µl) were put on the 96 well and incubated for 5-7 days until the bacteria reach the lag phase and then 1% resazurin (25µl) was added in each well to observe any colour change after incubating for 24 hours. The alamar blue oxidation-reduction dye is a general indicator of cellular growth and/or viability; the blue, non-fluorescent, oxidized form becomes pink and fluorescent upon reduction. Growth can therefore be measured with a fluorometer or spectrophotometer or determined by a visual color change. The extracts were considered active (have inhibitory activity) for the well of the plate with unchanged color or the blue, nonfluorescent, oxidized form and if the color of the reagent or resazurine is changed to pink (fluorescent) the extract is considered to be inactive or the microorganism is considered resistant strain to the plant extract as described previously [13].

2.6. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration was determined by using the REMA (resazurin microplate alamar blue) in 96 well microtiter plates. 100 µL of Middle Brook7H9 broth with *M. tuberculosis* and *M. bovis* isolate inoculum were dispensed into all wells of a sterile 96-well microtitre plate. In the first column (no.1 well of all plate), 100 µL of extracts were added to each first wells using a unique pipette for each extract. The extracts were mixed thoroughly and 50 µL of extracts were transferred to well 3 to well 9 from which 50 µL were discarded. Well 10 up to well 11 were used as sterility control (with no inoculums and extract only media was added), and negative control wells. The working solution of extracts (100µg/ml) were diluted out across a 96- well in a two-fold serial dilution to give final testing concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.12mg/ml, 1.56mg/ml and 0.78µg/ml from well 3 to well 9 respectively. Rifampicine and isonizide (INH) (positive control) were added with concentration of 10µg/ml along the first and second coloumn of the 96 microplates well. The plates were then incubated for 5–7 days at 37°C. After the 7th day, 25 µl of resazurine was added to all wells and re-incubated overnight for development. The MIC was defined as the lowest concentration of the extracts/drugs that prevented a colour of resazurine to be changed from blue to pink (visual determination) [10,14].

2.7. Quality assurance

All aspects of procedures were performed in the safety cabinet. For laboratory activities like culturing, media preparation and sterilization (filtration) of extracts were carried out following recommended standard operating procedures of ALIPB laboratory and manufacturer's protocol. Measuring concentration and dilution were carried out at standard recommended level. Media purity was checked by 24 hrs incubation at 37°C before used in actual test. Proper biosaftey and aseptic procedures were followed to reduce risk of contamination of personnel and laboratory environment according to the laboratory safety protocols at ALIPB.

2.8. Data analysis

Data from experimental results on antimyocbacterial inhibitory effects of the extract with the minimum inhibitory concentrations were entered into MS excel 2007 spread sheets. Descriptive statistical analysis was used for data summarization and results were presented as tables and graphs.

3. Results

3.1. Antimycobacterial Activities

The antimycobacterial activities of crude methanol and ethanol extracts of seeds of *Trigonella foenum gracium* and leaves of *Thymus serrulatus* were investigated against *M. tuberculosis* (*H37Rv, SIT149*) *and M. bovis* (*BCG* and *SB1176*) isolates by using the REMA method, and the extracts showed promising antimycobacterial activity

with the mean minimum inhibitory concentration values ranging from 1.56 µg/ml to 50µg/ml of the extracts (Tables 2 and 3).

Ethanol extraction of *Trigonella foenum gracium* at a concentration of 50 µg/ml, and 25µg/ml and *Thymus serrulatus* at a concentration of 50µg/ml, 25µg/ml, 12.5 µg/ml, and 6.125µg/ml inhibit *Mycobacterium tuberculosis H37Rv.* While in methanol extract *Trigonella foenum gracium* at a concentration of 50µg/ml and 25µg/ml and *Thymus serrulatus* at a concentration of 50µg/ml, 25µg/ml and 12.5µg/ml inhibit the bacterial strain. Ethanol extraction of *Trigonella foenum gracium* at concentration of 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.125 µg/ml, 1.56µg/ml and 0.78µg/ml, and *Trigonella foenum gracium* at 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.125µg/ml, 1.56µg/ml inhibit *M. bovis* isolates (*SB1176* and *BCG*).

Mycobacterium species	**Strain name**	***Thymus serrulatus***		***Trigonella foenum gracium***	
		MEOH	**ethanol**	**MEOH**	**Ethanol**
M.TB	*H37rv*	+	+	+	+
M.TB	*SIT149*	+	+	+	+
M.B	*BCG*	+	+	+	+
M.B	*SB1176*	+	+	+	+
RIF (positive control)		+	+	+	+
INH (positive control)		+	+	+	+
EFI (negative control)		-	-	-	-
MO (sterility control)		NG	NG	NG	NG
Key: M. TB=*Mycobacterium tuberculosis*; M.B= *Mycobacterium bovis*; MeOH= methanol; + (visible inhibition observed); ─ (no visible inhibition observed at all); EFI (extract free isolate); Negative control (Media and bacteria without any extract); MO=sterile control (enriched media only); INH=isonized (positive control); RIF=rifampicin (positive control)					

Table 2: *Mycobacterium* species growth inhibition with respect to test extracts.

3.2. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of extracts with antimycobacterial activities against both *Mycobacterium* species fell in inclusive range of 50µg/ml-0.78 µg/ml (Table 3). The higher antimycobacterial activity with MIC was obtained in methanol extracts of *Thymus serrulatus* to SIT149 with 0.78 µg/ml. For *Trigonella foenum gracium* the MIC was 1.17µg/ml against *M.bovis* (sb1176) with methanol and ethanol extraction (Table 3, Figure 1).

Bacterial spp	**strain**	***Thymus Serrulatus***		***Trigonella Foenum***	
		methanol	**Ethanol**	**Methanol**	**Ethanol**
MTB	*HRv37*	4.31 **µg/m**	9.31 **µg/m**	18.75**µg/m**	9.31 **µg/m**
MTB	*SIT149*	0.78 **µg/m**	1.17**µg/m**	9.31**µg/m**	4.31**µg/m**
BTB	*BCG*	1.17 **µg/m**	1.17 **µg/m**	9.31 **µg/m**	1.17 **µg/m**
BTB	*SB1176*	1.17 **µg/m**	4.31 **µg/m**	1.17 **µg/m**	1.17 **µg/m**
Key: M. TB= *Mycobacterium tuberculosis,* M.B= *Mycobacterium bovis,*					

Table 3: MEAN MIC values of 99% methanol and 97% ethanol crude extracts of two medicinal plants against four *M. tuberculosis* strains and two M. *bovis* strains using visual REMA.

The higher activities or lowest MIC was obtained in methanol extracts of *thymus serrulatus* to all strains except ethanol extract against *M. tuberculosis H37rV*. For *Trigonella Foenum* the higher activity or lowest MIC was 1.17**µg/ml** against *M.TB (SIT149*) and 4.31**µg/ml** for *M. Bovis* with (*SB1176*) with methanol and ethanol extraction respectively. While this plant has the highest activity or lowest MIC (1.17**µg/ml)** with ethanol and methanol extraction against *SB1176* and ethanol extraction against *BCG*. The higher MIC or lowest activity of *Thymus Serrulatus* with methanol Extraction was 4.31**µg/ml against** *H37Rv*. In *Trigonella Foenum* the lowest activity obtained (18.75**µg/m)** in methanol extraction against *H37Rv*, as well as BCG. As shown in the chart above, *thymus serrulatus* had better activity against the mycobacterium species than *Trigonella Foenum*. *Thymus Serrulatus* with methanol extraction had better activity than ethanol one while *Trigonella Foenum Gracium* showed better activity in ethanol solvent than methanol (Figure 1).

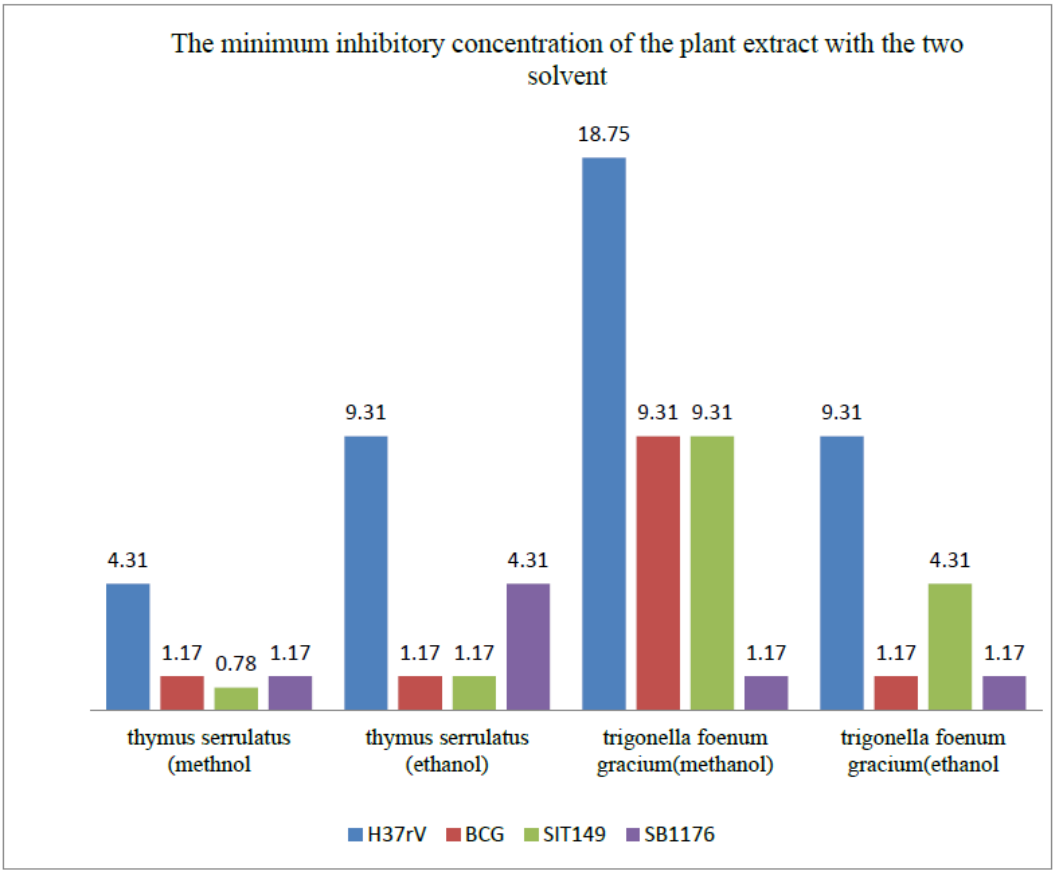

Figure 1: Graph showing the minimum inhibitory concentration of *Thymus Serrulatus* and *Trigonella Foenum Gracium*.

4. Discussion

Traditionally used plants were found to play great roles in the primary healthcare systems of the local people and animals in Ethiopia. This is because the resource-poor people of the country had little access and couldn't afford the cost of modern medications. The report shows that the local people have been seeking treatment in preference to modern medications and in connection with the

community's belief that they would not get better medications for some of the diseases in modern health services. Moreover, the previous report indicated that they served the community best as the distribution of health services was limited in the study area [15,16].

In the present study, the methanol and ethanol crude extract of *Thymus serrulatus* and *Trigonella foenum gracium* showed promising inhibitory effect on growth of *Mycobacterium tuberculosis* and *Mycobacterium bovis* isolates. Previous report confirms that the crude extract of the leave of *Trigonella foenum gracium* was active against malaria. In 2010 Navayath et al. reported that the aqueous extract of *Trigonella foenum gracium* (fenugreek) prevents cypermethrin-induced hepatotoxicity and nephrotoxicity. Another study also compared the effectiveness of *Trigonella foenum gracium* against two common pathogenic bacteria and it was found that *Trigonella foenum gracium* strongly inhibit the growth of *Staphylococcus aureaus* and *Pseudomonus aeruginosa in-vitro* [17]. Similarly, our study also confirmed that *Trigonella foenum gracium* had strong antibacterial activity against *Mycobacterium* species. On the other hand, the information on past biological activity of *Thymus serrulatus* was concentrated mostly on its activity on hypertension, anti-inflammatory, antiseptic and antispasmodic aspect [18].

No clear scientific report obtained from literature survey on antimycobacterial activity of *Thymus serrulatus*. Hence, the result of the present study which showed potential antimycobacterial effect of *Thymus serrulatus* could be the first report with this regard. Since, the plant contains valuable essential oils, phenol compounds, alkaloids, flavonoids, terpenoids, steroids, saponin and tannins [19] which have confirmed antimicrobial activity in other plants [20], this could support the result obtained in the present study.

In the present study both methanol and ethanol extraction of *Thymus serrulatus* and *Trigonella foenum gracium* had strong inhibitory effect against both *Mycobacterium* species at the concentration range from 0.78-50-µg/ml. This showed that *Thymus serrulatus* and *Trigonella foenum gracium* had got antimycobacterial active constituents that are readily soluble in both methanol and ethanol solvents.Even though, the inhibition concentration of crude extract of the plants had lower than that of rifampicin and isoniazid (positive controls during the experiment) the fact remains that the crude extract possess some antimycobacterial properties. Comparably, previously done research on the genus *Thymus* species, *Thymus sibthorpii* extract killed *M. tuberculosis* with the minimum bactericidal concentration value of 50µg/ml [21].

The higher antimycobacterial inhibition concentration of the crude extract comparing to the known commercially used antituberculosis drugs might be due to presence of impurities in the extract which may reduce the potency of the extract. The minimum inhibitory concentration of extracts having antimycobaterial activities against both test organisms fell in inclusive range of 50µg/ml-0.78 µg/ml (Table 3). This was comparable to MIC of 50 µg/ml- 0.78µg/ml with previously done research from crude extract of other plants against *M. tuberculosis* H37Rv strains [9].

Growing evidence suggests that minimum inhibitory concentration of the crude extracts may or may not be indicative for success full identification of active compound. This is because either an extract with a relatively low MIC (high activity) may contain large quantities of only very few moderately active major constituents or moderately active crude materials could lead to minor compounds with high activity [22].

Although the antimicrobial activity of *Thymus serrulatus* and *Trigonella foenum gracium* had been reported against other pathogenic bacteria, no report was found during a literature search against *Mycobacterium* strain apart from ethinobotanical report on these plants. Therefore, this investigation would be the first report on their antimycobacterial activities. The crude methanol extract of leaves of *Thymus serrulatus* exhibited the most antibacterial activity against both *Mycobacterium tuberculosis* and *Mycobacterium bovis* isolates. In general, this preliminary work on antimycobacterial activities of extracts of *Thymus serrulatus* and *Trigonella foenum gracium* demonstrates its potential use of these plants for further evaluation in the therapeutic value for the treatment of tuberculosis in affected host [23].

5. Conclusion and Recommendation

Thymus serrulatus and Trigonella foenum gracium have promising antimycobacterial activity. For the first time, methanol and ethanol crude extracts were found to be active within inclusive MIC range of 0.78 -50µg/m against all test organisms. This finding pointed out the scientific ground for the ethnomedicinal use of the plants against TB by the Ethiopian community. This finding can be used as baseline information for further antimycobacterial activity studies of these plants. Traditionally, *Thymus serrulatus* ("Tosigne") and *Trigonella foenum gracium ("Abishe")* have been used in various liquid and solid foods as flavors and for medicinal purposes. Our results indicated that it is a promising source of antimycobacterial agents. The broad antimicrobial activity of the plant extracts indicates the presence of bioactive antimycobacterial agents that can potentially be used to treat tuberculosis. Thus, these medicinal plants which have been used traditionally by Ethiopian communities might represent an inexpensive source of natural antibacterial substances for use in treating various diseases, as well as to prevent the growth of bacteria and extend the shelf life of the processed food.

Therefore, based on the above conclusions, the following recommendations were forwarded:

- Further investigation on the antimycobacterial activities of *Thymus serrulatus* and *Trigonella foenum gracium* in larger sample size and in well controlled repeated experiments should be carried out
- Further research should focus also on isolating the bioactive ingredient in both plants which are responsible for pharmacological activities
- Additional further research on in-vivo efficacy study based on animal model should be conducted to assess its *in vivo* efficacy and determine presence of any potential toxicity, dosage level and its pharmacodynamics in animal models.
- Even though this investigation supports the traditional medicinal uses of these plants in Ethiopia; further studies on the importance of the plants through active participation of the community should be carried out in order to assess how the community has been preparing and utilizing the plants and their associated effect on the antimycobacterial efficacy of the extracts.

Consent for publication

All authors had agreed for publication

Availability of data and materials

The data will be available if necessary

Competing interests

No conflict of interests between authors

Funding

No special fund received for this work

Authors' contributions

Fikir Gete collects plant material, laboratory work, data

entry and analysis while Temesgen Kassa participated in publication. review and editing, preparing the manuscript for

References

1. Heinrich M, Gibbons S. Ethnopharmacology in drug discovery: an analysis of its role and potential contribution. J Pharm Pharmacol 53 (2001): 425-32.
2. Tariku Y, Hymete A, Hailu A, et al. Essential-oil composition, antileishmanial, and toxicity study of Artemisia abyssinica and Satureja punctata ssp. punctata from Ethiopia. Chem Biodivers 7 (2010): 1009-18.
3. Gedif T, Hahn HJ. The use of medicinal plants in self-care in rural central Ethiopia. J Ethnopharmacol 87 (2003): 155-61.
4. Dye C, Scheele S, Dolin P, et al. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. JAMA 282 (1999): 677-86.
5. Walzl G, Ronacher K, Siawaya JFD, et al. Biomarkers for TB treatment response: challenges and future strategies. J Infect 57 (2008): 103-9.
6. WHO. Global tuberculosis report 2014. World Health Organization (2014).
7. WHO. Multidrug and extensively drug-resistant TB (M/XDR-TB). World Health Organization (2010).
8. Asres K, Bucar F, Edelsbrunner S, et al. Investigations on antimycobacterial activity of some Ethiopian medicinal plants. Phyther Res 15 (2001): 323-6.
9. Gemechu A, Giday M, Worku A, et al. In vitro anti-mycobacterial activity of selected medicinal plants against Mycobacterium tuberculosis and Mycobacterium bovis strains. BMC Complement Altern Med 13 (2013): 1-6.
10. Mann A, Amupitan JO, Oyewale AO, et al. Evaluation of in vitro antimycobacterial activity of Nigerian plants used for treatment of respiratory diseases. African J Biotechnol 7 (2008).
11. Mohamad S, Zin NM, Wahab HA, et al. Antituberculosis potential of some ethnobotanically selected Malaysian plants. J Ethnopharmacol 133 (2011): 1021-6.
12. Karuppusamy S, Rajasekaran KM. High throughput antibacterial screening of plant extracts by resazurin redox with special reference to medicinal plants of Western Ghats. Glob J Pharmacol 3 (2009): 63-8.
13. Collins LA, Franzblau SG. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium. Antimicrob Agents Chemother 41 (1997): 1004-9.
14. Yeung M, Lau CBS, Chan RCY, et al. Search for antimycobacterial constituents from a Tibetan medicinal plant, Gentianopsis paludosa. Phyther Res An Int J Devoted to Pharmacol Toxicol Eval Nat Prod Deriv 23 (2009): 123-5.
15. Kibebew F, Addis G. Utilization and conservation of medicinal plants in Ethiopia. In: Proceedings of the workshop on Development and Utilization of Herbal Remedies in Ethiopia: Addis Ababa (1996): 46-52.
16. Giday M, Teklehaymanot T, Animut A, et al. Medicinal plants of the Shinasha, Agew-awi and Amhara peoples in northwest Ethiopia. J Ethnopharmacol 110 (2007): 516-25.

17. Syeda BB, Muhammad IB, Shahabuddin M. Antioxidant activity from the extract of fenugreek seeds. Pak J Anal Environ Chem 9 (2008): 78-83.
18. Pinto E, Pina-Vaz C, Salgueiro L, et al. Antifungal activity of the essential oil of Thymus pulegioides on Candida, Aspergillus and dermatophyte species. J Med Microbiol 55 (2006): 1367-73.
19. Hudaib M, Speroni E, Di Pietra AM, et al. GC/MS evaluation of thyme (*Thymus vulgaris* L.) oil composition and variations during the vegetative cycle. J Pharm Biomed Anal 29 (2002): 691-700.
20. Wube AA, Bucar F, Gibbons S, et al. Sesquiterpenes from Warburgia ugandensis and their antimycobacterial activity. Phytochemistry 66 (2005): 2309-15.
21. Askun T, Tekwu EM, Satil F, et al. Preliminary antimycobacterial study on selected Turkish plants (Lamiaceae) against Mycobacterium tuberculosis and search for some phenolic constituents. BMC Complement Altern Med 13 (2013): 1-11.
22. Cantrell CL, Rajab MS, Franzblau SG, et al. Antimycobacterial ergosterol-5, 8-endoperoxide from Ajuga remota. Planta Med 65 (1999): 732-4.
23. Mehrafarin A, Rezazadeh SH, Naghdi Badi H, et al. A review on biology, cultivation and biotechnology of fenugreek (*Trigonella foenum-graecum* L.) as a valuable medicinal plant and multipurpose. Journal of Medicinal Plants 10 (2011): 6-24.