Evaluation of *In-vitro* Anti-Mycobacterial Activity of Selected Medicinal Plants in Mekelle, Ethiopia

Endale Balcha, Berhan Mengiste, Mebrahtom Gebrelibanos, Adane Worku and Gobena Ameni

Mekelle University College of Veterinary Medicine, P.O. Box: 2084, Mekelle, Ethiopia

Abstract: In the present study six medicinal plants: *Allium ursinum* (bulb), *Anethum graveolens* (areal part), *Buddleja polystachia* (leaf), *Croton macrostachys* (leaf), *Dodonaea angustifolia* (leaf) and *Pterolobium stellatum* (leaf), which are traditionally used to treat TB and related symptoms in Northern part of Ethiopia, were selected for the study. Crude extracts were prepared from the selected species by maceration using 80% ethanol. Various concentrations (250 mg/ml, 500 mg/ml and 1000 mg/ml) of the extracts were then screened for anti-mycobacterial activity against *Mycobacterium tuberculosis H37Rv* strain using Micro plate Alamar Blue Assay (MABA). Various concentrations (1, 3, 6, 12.5, 25, 50, 125, 250, 500, 1000 mg/ml) of the extracts from the plant species that showed anti-mycobacterial activity were used to determine their respective Minimum Inhibitory Concentrations (MICs). Only three plants (*A. ursinum*, *D. angustifolia* and *P. stellatum*) of the screened medicinal plants showed anti-mycobacterial activity. The MIC of *A. ursinum* and *P. Stellatum* extract was 250 mg/ml; while that of *D. angustifolia* was 12.5 mg/ml. It can be concluded that the present study provided a scientific support for the traditional use of *Allium ursinum*, *Dodonaea angustifolia* and *Pterolobium stellatum* for treatment of tuberculosis.

Key words: Anti-mycobacterial · Medicinal Plants · Minimum Inhibitory Concentration · Tuberculosis

INTRODUCTION

Tuberculosis (TB) is one of the oldest diseases known to humanity. It remains one of the major deadliest infectious diseases for humans [1]. The global TB epidemic situation has been further aggravated by the emergence of HIV infection and strains of drug-resistant TB. Multi drug-resistant TB (MDR-TB) has been reported in almost all parts of the world, primarily as a consequence of poor treatment services, which have not only increased the costs towards treatment, but also increased the risk of transmission of these resistant strains of the bacilli [2].

Of all the infectious diseases of man, none has been studied about more intensively than TB. The cornerstone of the modern treatment of TB is chemotherapy. Drugs used in the treatment of TB are isoniazid, rifampicin, ethambutol, streptomycin and pyrazinamidine etc. [3, 4]. These drugs have disadvantages of causing adverse side effects and organisms can gain easy resistance against these drugs [5].

Plants have long been a valuable source of novel drug compound. Plant derived chemicals have shown great promise in treatment of intractable infectious diseases including tuberculosis [6]. It has been discovered that higher plant extracts are promising source of anti-TB compounds. Herbal medicine is based on the fact that a single plant may contain thousands of constituent with possibility of discovering new drug [7].

In order to mitigate the shortcomings of allopathic medicine, a large percentage of patients in the African Region and East Africa in particular, seek remedy from traditional medical practitioners (TMPs) who use crude preparations of medicinal plants [8]. There have been claims of Traditional Medicine Practitioners being able to treat the symptoms of TB. Therefore there is need for scientific validation of the TMPs of the commonly used...
medicinal plants for treatment of TB and related symptoms in and around Mekelle. So far the anti-mycobacterial activity of herbal plants has not been assessed in and around Mekelle and the present research could serve as a spring board for future researches which attempt to find new drug for tuberculosis.

MATERIALS AND METHODS

Collection of Plants and Extract Preparation: Six plants commonly used medicinal plants for the treatment of tuberculosis and related illness were collected based on the information obtained from traditional healers (key informants). These plants are: Allium ursinum (bulb), Anethum graveolens (areal part), Buddleja polystachia (leaf), Croton macrostachys (leaf), Dodonaea angustifolia (leaf) and Pterolobium stellatum (leaf).

Methanolic extracts were prepared as per the method described by [9]. The plants were dried and powdered. The powdered materials were extracted by percolation for 72 hours at room temperature with methanol (400ml); mixture was stirred every 18 hour using a sterile glass rod. The extracts were then filtered with the help of Whatman filter paper No. 1. The extracts were concentrated under low pressure to dryness at 35-45°C using Rotary evaporator. The dried methanol extracts obtained from each plant was air-dried and then packed in glass bottles with proper labeling for future use.

Inoculum Preparation: The inoculums were prepared as per the method described by [10]. Mycobacterium tuberculosis reference strain H37Rv (the reference strain used in all DST for Mycobacterium tuberculosis ) was subcultured and incubated at 37°C using Middlebrook 7H9 broth which is supplemented with 0.2% glycerol and 10% OADC (to prepare 500ml 7H9 broth 50ml OADC was added ) enrichment for 21 days (until logarithmic phase). The standard inoculum was prepared in sterile 7H9-medium adjusted to a McFarland standard No.1 (equivalent to a standard suspension of 10^7 CFU/ml). This concentration was further diluted to 1: 25 and then used as an inoculum, to make that the bacteria were at the start of the log phase when the test commenced and 100µl was added to the inoculums to make the final volume of 200µl.

Anti-mycobacterial Activity: Mycobacterium tuberculosis reference strain H37Rv was used to evaluate the preliminary screening of crude extracts at concentrations of 250mg/mL, 500mg/mL and 1000mg/mL. The bacterial strains were obtained from Armauer Hansen Research Institute (AHRI), Ethiopia.

Microplate Alamar Blue Assay (MABA) method was used for the study as per the method described by [11]. Prior to the bioassay, stock solutions of Rifampicin with the concentration of 32 µg/mL was prepared and stored to be used for the positive control. Control wells without the tested extracts and sterility controls were assayed simultaneously. Rifampicin was prepared from the stock solution just prior to inoculation time to the concentration of 5 µg/mL in the total volume of 200µl. The growth inhibition result was explained by Microplate Alamar Blue Assay (MABA) using 1% resazurine. The reagent allows the detection of microbial growth in microtiter plates without the use of spectrophotometer.

The susceptibility test conducted by the Microplate Alamar Blue Assay was using 96 well microtitre plate to evaluate the susceptibility of H37Rv MTB reference strain to the extract. The inhibitory concentration of all extracts were evaluated with concentrations of 250 mg/ml, 500 mg/ml and 1000 mg/ml in the total volume of 200µl. Prior to inoculation the crude extracts of 50mg, 100mg and 200mg were measured to make the proposed concentration in 200µl. The measured extracts were mixed with the bacterial suspension and the diluent media (7H9) in the well.

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 was therefore 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, non-fluorescent, oxidized form and if the color of the reagent or resazurine is changed to pink (fluorescent) the extract is inactive or the micro organism is considered resistant strain to the plant extract [12].

Minimum inhibitory concentration (MIC) was determined for those extracts showing inhibitory effect. The MIC was conducted at various concentrations of 1, 3, 6, 12.5, 25, 50, 125, 250, 500, 1000 mg/ml of the extracts.

RESULTS AND DISCUSSION

Tuberculosis has been a major health problem in developing countries including Ethiopia. Due to increase in multi drug resistant (MDR) and extensive drug resistant (XDR) strains of M. tuberculosis, there is an urgent need of finding newer anti-mycobacterial agents to combat this problem [13].
Plants have provided many medicinal drugs in the past and remain a potential source of therapeutic agents to this day [14]. The use of plants with medicinal action has grown, despite all the advances made in medicine, even in developed countries, due to several factors such as the confidence of the populations that use them, their ease of acquisition and their low cost [15]. Public skepticism about the ability of allopathic medicines to be free from adverse effects, or to cure chronic conditions, have contributed to consumer demand for high quality herbal medicinal products [14]. Hence, indigenous plants are used worldwide as medicines, particularly in the developing countries.

In this study, six plant species used by traditional healers to treat TB related diseases were collected. The result of the anti-mycobacterium activity of the crude extracts at concentrations of 250mg/mL, 500mg/mL and 1000mg/mL revealed that only three plants namely Allium ursinum, Dodonaea angustifolia and Pterolobium stellatum inhibited the growth of the bacterium (Table 1). This is in agreement to previous reports, Pterolobium stellatum [16], Dodonaea angustifolia [17], Allium ursinum [18]. However, the other there species, Viz Anethum graveolens Buddleja polystachia and Croton macrostachys did not show activity against the organism. This might be due to the fact that different plant species are active against different species of Mycobacteria [19]. To better evaluate the plants growing naturally in North Ethiopia that are potentially useful resources, additional studies are necessary from the medicinal stand points. Plants produce a great diversity of substances that could be active in many fields of medicine [20].

The MIC of the extracts of the three plant species that showed inhibitory effect on M. tuberculosis H37Rv was determined at various concentrations of 1, 3, 6, 12.5, 25, 50, 125, 250, 500, 1000 mg/ml. Accordingly, the MIC of A. ursinum and P. Stellatum extract was 250 mg/ml; while that of D. angustifolia was 12.5 mg/ml (Figure 1). Hence, the findings indicate that A. ursinum, D. angustifolia and P. stellatum could be used as adjuvant therapy for TB.

Table 1: Anti-Mycobacterial activity of plant extracts

<table>
<thead>
<tr>
<th>Species of plants</th>
<th>Vernacular name (Amharic)</th>
<th>Activity against MTB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Croton macrostachys (leaf)</td>
<td>Bisana</td>
<td>Negative</td>
</tr>
<tr>
<td>Allium ursinum (bulb)</td>
<td>Yejib shinkurt</td>
<td>Positive</td>
</tr>
<tr>
<td>Buddleja polystachia (leaf)</td>
<td>Anfar</td>
<td>Negative</td>
</tr>
<tr>
<td>Dodonaea angustifolia (leaf)</td>
<td>Kendita</td>
<td>Positive</td>
</tr>
<tr>
<td>Anethum graveolens (arial part)</td>
<td>Ensial</td>
<td>Negative</td>
</tr>
<tr>
<td>Pterolobium stellatum (leaf)</td>
<td>Kentefa</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Conflict of interest: There is no conflict of interest among the authors.

ACKNOWLEDGEMENTS
The authors thank NORAD-III project for sponsoring the research work. We are also thankful to Mekelle University, College of Health Sciences, pharmacy department and Aklilu Lemma, Institute of Pathobiology, Addis Ababa University for providing the necessary facilities and assistance for the work.

REFERENCES