

SHORT COMMUNICATION

Antibacterial Activity of Medicinal Aqueous Plant Extracts against *Mycobacterium tuberculosis*

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ABSTRACT

Tuberculosis (TB) remains a serious health problem in many regions of the world, and the development of resistance to antibiotics by this microbe created the need for new drugs to replace those which have lost effectiveness. This study assesses the medicinal anti-*Mycobacterium tuberculosis* properties of natural products obtained from plants collected from Eastern Libya. In this study aqueous extracts of nine different plants were assayed for their *Mycobacterium tuberculosis* inhibitory activity using the BACTEC MGIT960 susceptibility test method. The aqueous extracts of *Ceratonia siliqua* L., *Helichrysum stoechas* (L.) Moench and *Thymus algeriensis* did not show any activity against *M. tuberculosis* in different concentrations. The aqueous extract of *Marrubium vulgare* L. from Syria showed high activity against *M. tuberculosis*. *Marrubium alysson* L., *Marrubium vulgare* L., *Pistacia lentiscus* L., *Quercus coccifera* L., *Thymus capitatus* (L.) Hoffm. & Link, showed varying degrees of activity against *M. tuberculosis*. The results of this study show that aqueous extracts from six different medicinal plants have different effects against *M. tuberculosis in vitro*.

Keywords: *Mycobacterium tuberculosis*, anti-mycobacterial activity, BACTEC MGIT 960 system, aqueous extracts of plants, Eastern Libya

INTRODUCTION

Tuberculosis remains an important public health problem worldwide, accounting for 8 million new cases per year. Its infectious agent, *Mycobacterium tuberculosis*, kills approximately three million people every year in the world (WHO, 2007). Despite the improvements in chemotherapy, the epidemiology of TB is severely affected by the emergence of multi-drug resistance in *M. tuberculosis* strains (Leite *et al.*, 2008). For a long time, medicinal plants and herbs were used intensively in folkloric medicine for treatment of various diseases. The scientific experiments which have been carried out on antimicrobial properties of plant components were first documented in the late 19th century (Zaika, 1975).

Recently, there has been widespread interest in drugs derived from plants. This interest primarily stems from the belief that medicinal plants are safe and dependable, as opposed to synthetic drugs that are costly and have adverse effects (Stuffness and Douros, 1982). In the past years several reports and review articles appeared in the literature about medicinal plants and natural products with anti-mycobacterium activity (Okunade *et al.*, 2004; Copp, 2003; Gautam *et al.*, 2007). Over 350 natural products, mainly from plant species, have been screened for their antimycobacterial activities. A number have demonstrated

significant *in vitro* anti-mycobacterial activity and active plant-derived compounds belonging to various chemical classes have been isolated (Newton *et al.*, 2000). In the present study, we used nine different plant extracts to test their effect on *M. tuberculosis* strains.

MATERIALS AND METHODS

Microbial strains

Eight *M. tuberculosis* samples were obtained from positive cultures of sputum in 2009 at the Tuberculosis laboratory of Benghazi. These *M. tuberculosis* strains were identified by BD Probe Tec system (Becton Dickinson-USA). Five of the eight strains were sensitive to first line drugs (Streptomycin, Isoniazid, Rifampin and Ethambutol) (SIRE) and three were multidrug resistant for first line drugs, determined by use of BD BACTEC MGIT 960 system.

Plant material

Ceratonia siliqua L., *Helichrysum stoechas* L., Moench, *Marrubium alysson* L., *Marrubium vulgare* L., *Pistacia lentiscus*, *Quercus coccifera* L., *Thymus capitatus* L., Hoffm & Link, *Thymus algeriensis* were collected from Eastern Libya in 2009 and *Marrubium vulgare* L. from

Syria in 2009 (commercial product). Different parts of the plant including leaves, seeds, fruits, young branches and stems were used, **Table 1**. The botanical nomenclature of the plants was identified by using standard flora of Libya (Jafri and El-Gadi, 1977, 1978, 1983 & 1985).

Table 1: Anti-*M. tuberculosis* activity of plant extract concentrations from Nine medicinal plants.

| Test Plants (Scientific name) | Plant Part used for extraction | Effect of different water plant extracts concentrations (%) | | | |
|--|--------------------------------|---|-------|-------|------|
| | | A | B | C | D |
| <i>Ceratonia siliqua</i> L. | Fruits | 0 | 0 | 0 | 0 |
| <i>Helichrysum stoechas</i> L. Moench | Flowers | 0 | 0 | 0 | 0 |
| <i>Marrubium alysson</i> L. | Leaves, branches and flowers | 0 | 0 | 0 | 12.5 |
| <i>Marrubium vulgare</i> L. | Leaves, branches and flowers | 0 | 0 | 12.5 | 75 |
| <i>Marrubium vulgare</i> L. from (Syria) | aerial part | 31.3 | 93.75 | 100 | 100 |
| <i>Quercus coccifera</i> L. | Fruits | 0 | 0 | 37.5 | 12.5 |
| <i>Pistacia lentiscus</i> L. | Leaves, branches, fruits, | 0 | 0 | 43.75 | 81.3 |
| <i>Thymus capitatus</i> L. Hoffm. & Link | Flowers | 0 | 0 | 56.3 | 75 |
| <i>Thymus algeriensis</i> | Leaves, branches and flowers | 0 | 0 | 0 | 0 |

Plant extraction

The plant parts were air-dried under shade at room temperature before extraction. The fruits of *Quercus coccifera* L and *Ceratonia siliqua* L plants were crushed to small pieces using a pestle and mortar. A total of 10 g of dry parts of each plant were infused in 150 mL deionized (DI) water, except for *Ceratonia siliqua* L. *Quercus coccifera* L, *Pistacia lentiscus* and *Marrubium vulgare* L. from Syria, which were infused in 100 mL DI water then heated for 30 min at 100 °C on a hot plate. The extract was filtered using sterile gauze and used directly after cooling.

Inoculum from growth on solid medium

Many colonies from fresh cultures were scraped from a Lowenstein Jensen solid medium with a sterile loop and then transferred into a sterile tube containing 4 mL of BBL Middlebrook 7H9 broth medium with 5-6 sterile glass beads (culture suspension tube). This tube was then vortexed for 2 min to break-up the clumps. The turbidity of the suspension was greater than the McFarland #1.0 standard. The tubes were allowed to rest for 20 min. The supernatant suspension was transferred with a pipette into another sterile tube, which stood for an additional 15 min

undisturbed. The supernatant was taken out with a pipette, without disturbing the sediment, and transferred into another sterile tube. The turbidity of this suspension was adjusted to 0.5 McFarland standard with sterile saline, and the adjusting was done by visual comparison. A 1:5 dilution of the above suspension in sterile saline was used as inoculum for plant extracts susceptibility testing.

Prepare MGIT 960 tubes for plant extracts

Four amounts of aqueous plant extracts (400 µL (A), 800 µL (B), 1600 µL (C) and 3200 µL (D)) from each plant were used 7 mL of BBL™ MGIT tube. Four BBL™ MGIT960 tubes were used for each plant, each tube containing a total volume of 7mL with different plant concentrations A, B, C and D. Also, one MGIT960 tube was used for each plant as growth control (GC).

Antimycobacterial susceptibility test using BACTEC MGIT 960 system

0.8 mL of BACTEC 960 growth supplement (Becton Dickinson) was added to each of the MGIT tubes. 0.5 mL of the culture suspension was added into each of the MGIT tubes containing the plant extracts. For the control, we first diluted the test culture suspension 1:100 by adding 0.1 mL of culture suspension to 10.0 mL of sterile saline. We then mixed well by inverting the tube 5-6 times. This diluted suspension was used to add 0.5 mL into the growth control tube. The cap was tightened and the inoculated broth was mixed well by gently inverting the tube several times. Labeled tubes were placed in the correct sequence in the set carrier (GC, A, B, C, D). The susceptibility set carrier entered into the BACTEC MGIT 960 instrument and the susceptibility test set entry feature was used. (Refer to the BACTEC MGIT 960 User's Manual, AST Instructions).

Instrument interprets results

The instrument monitors the entered susceptibility test set. Once the test is complete, the instrument will indicate that the results are ready. The instrument printout indicates susceptibility results for each plant extract. If the growth unit (GU) of the plant extract containing tube was more than 100 when the GU of the growth control was 400, the results were defined as resistant. If the GU values of the plant extract containing tubes were equal to or less than 100, the results were considered susceptible. For first line drugs, readings were automatically interpreted by the BACTEC MGIT960 instrument and reported as either susceptible or resistant. (For plant extract, readings were interpreted using the same algorithm as for first-line drugs).

RESULTS

In this study, aqueous extracts from nine different medicinal plants were tested against *M. tuberculosis* to determine their antimycobacterial activity. The

antimycobacterial activity results of all the aqueous extracts tested are presented in **Table 1**. Different aqueous extract concentrations (A, B, C, D) of *C. siliqua*, *H. stoechas* (L) Moench and *T. algeriensis* did not show any antimycobacterial activity against *M. tuberculosis* as represented by their complete degree of growth as the control. However, the aqueous extract of *M. vulgare* L. from Syria showed a significant, concentration dependent antimycobacterial activity which was represented by the decrease in the degree of bacterial growth. The antimycobacterial effect of the plant extract was increased by increased plant extract concentration (A= 31.3 %, B = 93.75 %, C =100 %, D =100 % respectively). *M. vulgar* L. from Libya exhibited similar antimycobacterial activity against *M. tuberculosis* at high concentrations C (12.5 %) and D (75 %), respectively. This effect was less potent than the effect obtained by the plant extract of *M. vulgare* L. from Syria at the same concentration.

The results also showed that there was no antimycobacterial activity in samples of *M. tuberculosis* treated with low concentration (A, B), except *M. vulgare* L. from Syria. At high concentrations these plants revealed an antimycobacterial effect against *M. tuberculosis* which was varied as function of plant species considered. *M. alysson* L showed no effect in A, B and C and only had very low effect against *M. tuberculosis* in D (12.5 %) concentration. The antimycobacterial activity of *Q. coccifera* L was very poor at concentration D (12.5 %) compared to the effect of *P. lentiscus* (81.3 %) and *T. capitatus* (75 %) aqueous extract at same concentration.

In general the anti-mycobacterial activity of tested plant extracts against *M. tuberculosis* was increased with increased dosage of plant extract except in *M. tuberculosis* strains treated with *Q. coccifera* L. The effect of C (37.5 %) concentration was more effective than D (12.5 %) concentration.

DISCUSSION

In this study an anti-*M. tuberculosis* activity was observed in Six medicinal plant extracts out of nine tested ones. This activity was ranged from very low (12.5 %) in *M. alysson* L. to highly significant effect in *M. vulgare* L. from Syria (100 %), depending on the applied concentration and type of tested plant extract. High concentration of most tested plants possessed greater antimycobacterial effects against TB than lower concentration in general. However, no antimycobacterial activity was observed at the same high concentration of *C. siliqua* L, *H. stoechas* L. Moench and *T. algeriensis* plant extracts. In fact, *H. stoechas* L. Moench had no effect on TB growth at all used plant extract concentrations. This is inconsistent with Meyer *et al* (2002) who found that *H. caespitium* has the ability to inhibit the growth of all the strains of *M. tuberculosis*. Also, Lall and Meyer (1999) showed that *H. melanacme* was active against TB. Bogdadi *et al.* (2007) found that *H. stoechas* exhibited the strongest activity against gram positive bacteria.

The difference in the results may be due to the treatment condition or due to the difference in concentration of active compound in tested plant material. The active compound concentration depends on time of collection and/or tested plant parts. The difference in the results may also be due to the difference in bacterial species. Many reports have recorded that *T. capitatus* L. Hoffm and Link possesses.

The most successful anti-*M. tuberculosis* effect was obtained from the aqueous extract of *M. vulgare* from Syria at all tested concentrations while *M. vulgare* from Libya exhibited similar, but less activity against *M. tuberculosis* at high concentration. *M. alysson* plant extract revealed a weak (12.5 %) anti-*M. tuberculosis* activity which was at only high concentration compared to other tested Marrubium plants. Similarly, *M. vulgare* whole plant extract antimicrobial activity had been observed against some gram positive (Masoodi *et al.*, 2008; Warda *et al.*, 2009) and gram negative bacteria (Masoodi *et al.*, 2008) but *M. tuberculosis* was not included. Also *Marrubium alysson* plant extract was found effective against different Enterobacteriaceae and different *Candida* species (Hayet *et al.*, 2007). Many other plants were found to be having antimicrobial activity against TB using MGIT960 (Mariita *et al.*, 2010) in the detection method while in this work antibiotics susceptibility test (AST) was used. In addition, they used different plant sources.

This study is the first report of anti-mycobacterial activity of aqueous extracts from medicinal plants collected from Eastern Libya against *M. tuberculosis* by using MGIT 960 system. The preliminary results presented in this study showed the potential of extracts from these medicinal plants to be used against *M. tuberculosis*. However, further studies are needed to identify the active compound(s) that cause(s) growth inhibition and to try to integrate the active compound(s) in the control programs used to reduce *M. tuberculosis*. Also needed are further investigations of the inhibitory activities of extracts from other medicinal plants on *M. tuberculosis*.

CONCLUSION

The results of this study show that aqueous extracts from six medicinal plants, *Marrubium alysson* L., *Marrubium vulgare* L., *Pistacia lentiscus*, *Quercus coccifera* L, *Thymus capitatus* L. Hoffm. & Link and *Marrubium vulgare* L. from Syria have an effect against *M. tuberculosis* *in vitro* using MGIT 960 system.

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