Original article

Antimycobacterial activity of partial purified extract of *Allium ascalonicum*

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Abstract

Introduction and objective: The World Health Organization estimates that about 8 to 10 million new Tuberculosis (TB) cases occur annually worldwide and its incidence is currently increasing. There are two million deaths from TB each year. The plants are an important source of new antimicrobial agents. In this study, antibacterial activity of *Allium ascalonicum* against *Mycobacterium tuberculosis* was evaluated.

Materials and methods: To extraction antibacterial agent from this plant, 100g of underground root of *A. ascalonicum* was mixed with 100ml ethyl acetate and shacked gently. Partially purified antibacterial compound was isolated by organic solvents. Antibacterial activity of this fraction against *M. tuberculosis* was performed using the E test.

Results: Shallot extract showed antimycobacterial activity with a minimum inhibitory concentration (MIC) value of 500µg/ml.

Conclusion: It is implied that *A. ascalonicum* extract could be used as an effective antibacterial agent against *M. tuberculosis,* which is a resistant infection in pulmonary tuberculosis.

Keywords: Antibacterial effect, *Allium ascalonicum*, *Mycobacterium tuberculosis*

Introduction

Scientific experiments since late 19th century have documented the antimicrobial properties of some spices, herbs, and their components. Studies in the past decade confirm that the growth of both Gram-positive and Gram-negative bacteria, yeasts and molds can be inhibited by garlic, onion, shallot, cinnamon, cloves, thyme, sage, and other spices. Effects of the presence of these spices/ herbs can be seen in food products such as pickles, bread, rice and meat products [1,2]. Tuberculosis (TB) is a disease known since antiquity and evidence of spinal TB in the form of fossil bones dates back to around 8000 BC. Today TB still remains the most prevalent cause of death in developing countries due to a single infectious agent [3]. TB is infecting about nine million people and kills approximately two million people annually [4]. Thus, there is an urgent need for the introduction of new effective TB control programs and for anti-TB agents with little toxicity to replace this warranty in use to

which mycobacterial resistance has occurred [3-5].

With respect to the past studies on antimicrobial activity of shallot (*Allium ascalonicum*) extract against wide range of Gram negative and positive bacteria and also yeast and filamentous fungi [6], in current study antibacterial activity of this plant was evaluated against *M. tuberculosis*. Shallot is a native of Palestine and is cultivated in USA and some European countries. It is commonly used as a folklore medicine, and used to cure earache, fever, antidote for snake venom and also as an aphrodisiac [7].

Materials and methods

Preparation of shallot extract

About 300g of white shallot bulbs (collected from Zagros Mountains, 50 Km of Dezful, a city south of Iran, in spring) were washed thoroughly in water and mashed properly in a kitchen mixer. The mashed shallot was mixed with 300ml of distilled water, and soaked with stirring by a magnetic stirrer for a period of five hours. The suspension was then filtered through Whatman No. 1 filter paper. This water extract was mixed with ethyl acetate in 50:50 proportions and kept for stirring on magnetic stirrer for a period of 10 minutes; the upper organic layer was separated in separating funnel and centrifuged at 5000 rpm for 10 minutes. The ethyl acetate layer was then removed and transferred to a clean flask. This process was repeated for three times and extracts pooled and dried in a rota evaporator (Heidolph-Germany) at 50ºC and the yield of the extract was measured. The dried extract was dissolved in methanol and subjected to antimicrobial activity.

Determination of minimal inhibitory concentration by E test

Ten clinically isolates of *M. tuberculosis* were collected from TB center in Ahvaz, Iran. A loopful of freshly grown culture was suspended in 5ml of Middlebrook 7H9 broth containing five glass beads, 1-2mm in diameter and vortex for 15S several times to break up cords. The turbidity was adjusted to that of a Mc Farland 0.5 standard to make a dilution of 1.5×10^8 [8]. The plate was inoculated by dipping a sterile cotton swab into the cell suspension and streaking it across the surface of the agar in three directions. The plates were dried at ambient temperature for 15 minutes before applying the discs. Five sterile discs (diameter 6 mm) were kept on the agar surface in a line. The shallot extract were serially diluted in methanol and 10µl of each dilution was separately used to impregnate the disc. The original dilution was 100mg/ml. The plates were incubated for 5-7 days at 37ºC. The minimum inhibitory concentration (MIC) values were read as the antibacterial concentration at the point where dense colonial growth intersected the disc [6,9]. The test was performed in quadruplicate for each culture.

Results and discussion

All tested isolates were sensitive to the shallot extracts with different MIC values. The mean of obtained MICs of shallot extract against *M. tuberculosis* strains was 500µg/ml (Fig 1). Hundreds of reports of resistance to anti-tuberculous drugs have been published during the last 50 years of TB chemotherapy. Until recently, a common feature of most of these reports has been that the efficacy of the drugs could not be compared in any meaningful way. There are several reasons for this, most importantly poor comparability of the groups of patients studied, undefined measures of treatment outcome and uncontrolled laboratory data [10].

Due to emergence of resistance to antibiotics amongst microorganisms especially *M. tuberculosis*, investigations for novel anti TB agents have always been one of the major preoccupations of medical

society. Additional chemotherapeutic agents were recognized in the early 20th century. The discovery of antibiotics and other antimicrobial chemicals, and studies on their mode of action, have allowed us to control a great variety of TB.

Fig. 1: Susceptibility of *M. tuberculosis* to shallot extract determined by E Test

There are many researches on antimycobacterial activities of plants but based on our investigation there was no any study of antimycobacterial evaluation on shallot compounds. Suksamrarn *et al*. [11] evaluated antimicrobial activity of Limnophila geoffrayi against *M. tuberculosis*. Both compounds isolated from this plant exhibited inhibition activity against *M. tuberculosis* with equal value of 200µg/ml. In next study several flavonoids isolated from Derris Indica exhibited antimycobacterial activity with MIC between 6.25 and 200 µg/ml [12].

To our knowledge there have been few reports detailing the investigation of antimicrobial compound in shallot bulbs. Wang and Ng [13] isolated an anti-fungal peptide from bulbs of shallot. This peptide inhibited mycelial growth in the fungus *Botrytis cinerea* but not in the fungi *Mycosphaerella arachidicola* and *Fusarium oxysporum*. Fattorusso *et al*. [14] also have done an extensive phytochemical analysis on the polar extracts from bulbs of shallot. These researchers isolated two new furostanol saponins but there is no antimicrobial investigation in their report. According to our past studies, shallot extract has high antimicrobial activity against wide range of pathogenic and non-pathogenic bacteria and fungi. The MIC values of this compound against tested microbes were between 1-10µg/ml [6].

Conclusion

The antimicrobial compound isolated from shallot can be combined with other anti TB drugs for treatment of TB patients. The shallot bulb is an edible part of shallot plant, according to that; we recommend consumption of this part of plant for TB patients.

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