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Smita D Rajani
Departments of Microbiology,
Shree Ram Krishna Institute of
Computer Education &
Applied Sciences, Surat.

Pratibha B Desai
Departments of Microbiology,
Shree Ram Krishna Institute of
Computer Education &
Applied Sciences, Surat.

Dhanji P Rajani
Microcare Laboratory, 105,
Manthan Point, Unapani
Road, Surat.

Correspondence
Smita D Rajani
Departments of Microbiology,
Shree Ram Krishna Institute of
Computer Education &
Applied Sciences, Surat.

Anti-mycobacterial activity of garlic (*Allium sativum*) against multi-drug resistant and reference strain of *Mycobacterium tuberculosis*

Smita D Rajani, Pratibha B Desai, Dhanji P Rajani

Abstract

Emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *Mycobacterium tuberculosis* has further complicated the problem of tuberculosis (TB) control. There is a basic need to look at alternative anti-TB agents. Medicinal plants present an anticipation for developing alternate medicines for the treatment of TB. Garlic (*Allium sativum*) is one of the natural plant which possesses variety of biological properties like anti-tumor, anti- hyperlipidemic and anti-microbial etc. The present study was completed to evaluate *in vitro* anti-tubercular activity of garlic against MDR, XDR and reference strain of *M. tuberculosis* H37Rv. Ethanolic extract of garlic was set by maceration method. Minimum inhibitory concentration (MIC) was performed by using 7H9 middle brook broth dilution technique on 48 MDR isolates out of total 230 clinical isolates of MTB and reference strain of *M. tuberculosis* H37Rv. MIC of garlic extract was ranged from 0.5 to 2 mg/ml, showing inhibitory effects of garlic against both MDR and XDR *M. tuberculosis* isolates. Alternate medicine practices with plant extracts including garlic should be considered to decrease the burden of drug resistance and cost in the management of diseases. The use of garlic against MDR-TB may be of great significance regarding to public health.

Keywords: TB, MDR, resistance, Garlic, Anti-tuberculosis activity.

Introduction

Tuberculosis is a highly infectious disease with about one third of the world's population including 40 per cent from India estimated to be infected it [1]. However, this problem has become serious as *Mycobacterium tuberculosis* developed resistance against both the first line as also the second line drugs. Due to this, there is emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *M. tuberculosis* all over the world including India [2].

Due to the increasing problem of antibiotic/drug resistance, WHO recommended exploring herbs or plants as alternative remedy for various bacterial infections? India is one of the few countries in the world which has unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for cure of various diseases [3, 4]. Garlic (*Allium sativum*) is natural plant being used as a food as well as folk medicine for centuries in all over the world [5]. It is a plant with various biological properties like antimicrobial, anti-cancer, and antioxidant, immuno-modulatory, anti-inflammatory, hypoglycemic, and anti-cardiovascular effects [6]. Allicin is thiosulfinate compound of garlic reported for its antibacterial activity. Allicin is proved to be anti-bacterial as it inhibits RNA synthesis [7]. According to Ayurvedic and Greek systems of medicine garlic is one of the established remedies for tuberculosis. In 1946 Rao *et al.* firstly described the *in-vitro* garlic activity against *Mycobacterium tuberculosis* [8]. A few studies have also been proving anti-mycobacterial activity of garlic against different species of *Mycobacteria* [9-15].

The aim of the present study was to evaluate anti-mycobacterial activity of Ethanolic garlic extract (EGE) against 48 clinical isolates of MDR and non-MDR *Mycobacterium tuberculosis* by using a recently discovered, most sensitive and rapid 7H9 middle brook broth dilution technique, which is a fluorescence based technique for detection of MTB.

2. Materials and Methods

2.1 Settings

Early morning sputum samples from 230 clinically suspected TB patients were collected from different hospitals of south Gujarat region, India, a period of two year from July 2009–June 2011. All Mycobacterial investigations were carried out at the Microcare Laboratory & Tuberculosis Research Laboratory, Surat. The laboratory is accredited for carrying out culture and Drug Susceptibility Testing (DST) by the Central TB Division, Ministry of Health and Family Welfare, Govt. of India.

2.2 Sample Collection and Processing:

230 patients suspected to be suffering from tuberculosis having clinical symptoms viz. Coughing, loss of weight, night sweat, fever, chest pain, dyspnea and anaemia were selected for study. Early morning sputum samples were collected in a sterile, leak-proof container. All the specimens were handled in class II bio safety cabinet in a bio-safety level (BSL)– 3 laboratory and were decontaminated by Modified Petroff's Method [16]. All the samples were subjected to smear examination for detection of acid fast bacilli (AFB) and culturing. Smears were made from the mucopurulent portion of sputum and stained by the conventional Ziehl Neelsen method. The smears were graded according to the number of bacilli seen on the slide, as per recommendations of the World Health Organization (WHO). For culturing of the specimen, two McCartney Bottles of Lowenstein – Jensen medium were inoculated with each sample and incubated at 37 °C until growth of mycobacterium was observed or were discarded as negative after 8 weeks. All the culture isolates were identified as *Mycobacterium tuberculosis* by their slow growth rate, colony morphology, inability to grow on L-J media containing p-nitro benzoic acid (500 mg/ml), niacin positive and catalase negative tests [17].

2.3 Antitubercular drugs

INH, STR, RIF, EMB, KA and of were obtained in powder form from Sigma (St Louis, Missouri, USA). Each drug was prepared at a concentration of 10 mg/mL in sterile distilled water, except RIF, which was dissolved in dimethyl formamide (DMF). Stock solutions were filter-sterilized and stored at -20 °C for not more than one month.

2.4 Drug Susceptibility test

The identified *Mycobacterium tuberculosis* were subjected to drug sensitivity using first line drugs viz. isoniazid (INH), rifampicin (RIF), ethambutol (EMB), streptomycin (SM) and screened for MDR strains, which were subjected to second line drugs, viz. kanamycin (KA) and Ofloxacin (OF) by proportion method as per standard procedure. The DST was carried out with the recommended critical concentrations of 40 µg/mL for RIF, 0.2 µg/mL for INH, 2 µg/mL for EMB, 4 µg/mL for STR 20 µg/mL for KA, and 2 µg/mL for of [18, 19]. The organisms showing growth while testing primary susceptibility against first line and second line drugs were interpreted as a MDR or XDR strains. Ethanolic extract of garlic was prepared by maceration method.

2.5 Garlic extract preparation

One kilogram (kg) of garlic (small cloves) was obtained and processed to get powder. The powder was soaked in 70% ethanol (Merck) for one week. After filtration by Whatman No.1 filter paper, filtrate was passed through 0.45 µm (Millipore) diameter pore size filter membrane to remove

any impurity. EGE was prepared by maceration method by processing filtrate for extraction by rotary evaporator to evaporate ethanol by a standard procedure [20]. EGE was semisolid, brown to black in colour with pungent smell. It was stored at -20 °C till use.

2.6. Determination of MIC for EGE

Stock solution and concentration of garlic extract

Garlic extract was dissolved in Di-methyl sulfoxide (DMSO) which has excellent solvating property [21]. Final concentration of stock solution of EGE was 10% (2g of EGE in 20 ml DMSO). The following concentrations 0.5, 1.0, 1.5 and 2.0mg/ml of the garlic extract were tested to determine its MIC against MDR and reference strain (H37Rv) of MTB.

2.7. Inoculation of middle brook 7H9 broth for MIC of EGE

On day 1 MGIT tubes were labeled with the respective concentration of EGE. To the GC tube 0.8 ml growth supplement was added, followed by 0.5 ml of 1:100 dilution of 0.5 McFarland adjusted suspension. No garlic was added to GC tube. The tubes for garlic concentrations were inoculated with 0.8 ml of Middle brook Oleic Albumin Dextrose Catalase growth supplement (OADC) and then with measured volumes of EGE. To each labeled tube of respective garlic concentrations 500 µl of 0.5 McFarland adjusted growth suspension was added. The tubes were incubated at 37 °C by putting the carrier racks; designed for multi tubes to be analyzed with same conditions inside the MGIT 960 analyzer.

2.8. Interpretation of results

The MGIT 960 instrument monitored the inoculated media and gave results within 4-13 days once the test was completed. The tubes were detected by MGIT UV detector and also stained by ZN technique to confirm whether organism present or inhibited.

3. Results

The results of inhibitory effect of EGE showed that different isolates of MDR was inhibited at different concentrations of garlic extract ranging from 1.0-2.0 mg/ml. Most of MDR isolates were inhibited at 2.0 mg/ml of garlic extract while minimum inhibition of H₃₇R_v was found at concentration of 0.25 mg/ml. According to table 1 most of MDR isolates resistant to any of the second line drugs were inhibited at 2.0 mg/ml of garlic extract. Table 2 shows that most of MDR isolates sensitive to second line drugs were also inhibited at 2.0 mg/ml of garlic extract.

Table 1: Antimycobacterial activity of Garlic extracts against MDR strains of *M. tuberculosis* resistant to any of the second line drugs (n=35).

Isolates of MTB (number)	Concentrations of garlic (mg/ml)				
	2.0	1.5	1.25	1.0	0.5
MDR (n=35)	14	8	7	6	Growth

Table 2: Antimycobacterial activity of Garlic extracts against MDR strains of *M. tuberculosis* which were sensitive to second line drugs (n=13).

Isolates of MTB (number)	Concentrations of garlic (mg/ml)				
	2.0	1.5	1.25	1.0	0.5
MDR (n=13)	8	4	1	Growth	Growth

4. Discussion

This study demonstrated that the garlic extract inhibited all MDR isolates of MTB resistant (n=35) and sensitive (n=13) to second line drugs at a concentrations ranging from 0.5 to 2.0 mg/ml. Garlic has showed the same effects against both MDR resistant and sensitive to second line drugs. It might be due to the difference in mechanisms of action of garlic; because the other drugs have single mode of action but garlic has been reported to have multi-factorial mechanisms due to various constituents that confer their effects at simultaneously [22].

Our results are in accordance with studies already done. In one of the previous studies, garlic extract inhibited six isolates of MTB at concentration of 1.34-2.68 mg/ml and Mycobacteria other than tuberculosis (MOTT) at 1.34-3.35 mg/ml [23]. Another study demonstrated the garlic activity against MOTT at MIC of 1.0-3.0 mg/ml [24]. However in contrast to this study, Rao *et al.* showed the inhibition of MTB at 2 mg/ml but he used only a single isolate [25]. Deshpand *et al.*, reported MIC of 1.0 mg/ml for aqueous garlic extract against MOTT [26].

The possible explanations for this difference in results among the studies might be due to various species of garlic which differ in concentration of active constituents, as the garlic cropped in China may have twice allicin as much as in Europe or United States [27]. A group of scientists also reported activity of purified allicin as anti-tuberculous agent against isolates of MTB with low MIC [28] but the present study was assumed that the crude extract retains its inhibitory activity due to various constituents against which resistance development might be difficult [29]. It is a need of hour to investigate extracts of allium species of different geographical locations for the most active ingredients responsible for their antibacterial activity.

5. Conclusion

This study demonstrated that the garlic extract has showed its effectiveness against clinical isolates of MDR *M. tuberculosis*. It is worthwhile to utilize garlic as natural supplement with other standard ATT. It is corresponding that substitute medicines practices with plant extracts including garlic as a means of decreasing the burden of drug resistance and reducing the cost of management of diseases would be of public health importance.

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