MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

# Effect of Sodium Bicarbonate on the Cellular Morphology of *Mycobacterium tuberculosis* by Using the Scanning Electron Microscope

***Aya G. Elgohary[1*], Mohammad K. ElBadrawy[2], Heba E. Eldegla[3], Gamal M. Abdel-Fattah[1]***

[1]*Microbiology and Botany Department, Faculty of science, Mansoura University, Mansoura, Egypt.*

[2]*Chest Department, Faculty of Medicine, Mansoura university, Mansoura, Egypt.*

[3]*Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura university, Mansoura, Egypt.*

[*]***Corresponding Author**: Prof. Dr. Gamal Abdel-Fattah E-mail: abdelfattaham@yahoo.com*

**Received:10/12/2022**
**Accepted:1/1/2023**

**Abstract:Introduction & aim:** There is a fundamental need to consider alternative anti-TB drugs because the emergence of multi-drug resistant (MDR) and extensively-drug hardy (XDR) strains of *Mycobacterium tuberculosis* has made the problem of tuberculosis control more challenging. This study was aimed to detect the effect of sodium bicarbonate 8.4% and some medicinal plants on the cellular morphology of *M. tuberculosis* by using the scanning electron microscope.

**Method:** This study was performed on 30 *M. tuberculosis* isolates from different samples, 20 isolates (17 sputum and 3 bronchoalveolar lavage) from patients who were admitted to the chest department in Mansoura University Hospitals and 10 *M. tuberculosis* cultures from Central Public Health Laboratories in Cairo. Isolation and identification of *M. tuberculosis*. Determination of minimum inhibitory concentration (MIC) of ethanolic plant extracts by using resazurin microtitre plate assay (REMA). Then the effect of sodium bicarbonate 8.4% and garlic at MIC=3.25 mg\ml on the cellular morphology of *M. tuberculosis* by using the scanning electron microscope.

**Results:** Sodium bicarbonate of 8.4% has a destructive effect on INH Isoniazid-resistant *M.tuberculosis* as indicated in the scanning electron micrographs. Garlic at MIC=3.25 mg\ml has an inhibitory effect on INH Isoniazid-resistant *M. tuberculosis*.

**Conclusions**: Sodium bicarbonate and garlic can be used as adjuvant drug to treat INH Isoniazid-resistant *M. tuberculosis* with the antituberculosis drug.

**keywords**: *Mycobacterium tuberculosis*, tuberculosis *(TB)*, sodium bicarbonate (SB), resazurin microtiter plate assay (REMA), *Allium sativum*

## 1.Introduction

*Mycobacterium tuberculosis* (*M. tuberculosis*) is a pathogenic bacterial species in the family Mycobacteriaceae and the causal manager of tuberculosis (TB). TB is a disease that mainly upsets the lungs, gathering respiratory disease the most community presentation **[1]**. But, TB is a multi-systemic disease with a protean appearance, the organs most normally artificial with TB are the respiratory system, gastrointestinal (GI) system, lymphoreticular arrangement, skin, central nervous system, musculoskeletal system, generative system, and liver **[2]**.

*M. tuberculosis* is traditional or marginally curved thin rod-shaped bacilli, non-sporing, non-motile, non-capsulated bacteria, obligate aerobic. acid-fast bacilli, which appears in single, pairs, or small clumps,─intracellular parasite, that reduces nitrate to nitrite and produces niacin **[3]**.

*M. tuberculosis* bacilli are endangered by the sharp media within the humanoid body as its standard position inside phagosomes of alveolar macrophages **[4]**.

Sodium bicarbonate (SB) is typically used as a pH buffering agent. It is useful in patients

with severe lactic acidosis, ketoacidosis, diarrhea, and renal tubular acidosis syndromes [5]. It has also destructive effects on tumor tissue. PH of Because lung cancer tissue is acidic, administering sodium bicarbonate (SB) through a bronchoscopy might alter the pH of the tissue, potentially causing tumor destruction [6]. SB with 8.4 % concentration is used as adjuvant therapy in the treatment of patients with reasonable clinical and radiological manifestations of COVID-19 pneumonia **[7]**.

Traditional medicines utilizing phytochemicals have been demonstrated to have enormous potential for treating TB, particularly in terms of eradicating M. tuberculosis, boosting natural resistance, and controlling the negative effects of anti-TB medications [8].

Scanning electron microscope (SEM) is a device at which obscure worlds of micro space and nano space can be seen. Details and density that are remote by light microscopy can be discovered by SEM **[9].**

## 2. Materials and methods

### 2.1. Collection of specimens:

This study was performed on 30 *M. Tuberculosis* isolates from different samples, 20 isolates (17 sputum and 3 bronchoalveolar lavage) from patients who were admitted to the chest Department in Mansoura University Hospitals and 10 *M. tuberculosis* cultures from the Central Public Health Laboratories in Cairo from May 2020 to September 2021. All the specimens were collected in sterile containers and transported to Microbiology Diagnostic and Infection Control Unit (MDICU) in the Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University.

### 2.2. Culture of *M.tuberculosis:*

BAL and sputum samples were processed using the NALC-NaOH (N-acetyl L-cysteine Sodium Hydroxide) technique. to decontaminate other bacteria and fungi in samples **[10]**. From the last suspension, 200 µl was inoculated into Lowenstein-Jensen Media (LJ). The LJ culture tubes were gestated at 37°C in 10 % $CO_2$ for an extreme of 8 weeks. Cultures presentating no growth after 8 weeks of gestation were stated as harmful **[11]**.

### 2.3. Identification of isolates

#### 2.3.1. Primary identification:

It was carried out by colony morphology **[12]** and Ziehl-Neelsen stain **[13]**.

#### 2.3.2 Biochemical identification (manual biochemical identificatification

It was carried out by Niacin accumulation test **[14]**, Nitrate reduction test **[15]**, catalase test **[16]**, Tween-80 hydrolysis test **[17]**.

### 2.4. Study the effect of sodium bicarbonate 8.4% on the cellular morphology of *M. tuberculosis* by using scanning electron microscope:

A volume of 2 mL McFarland no. 3 mycobacterial inoculum was transported into two screw-capped tubes. The first tube served as a control containing 6 mL of Middlebrook 7H9 broth. The second tube contained 6 mL of sodium bicarbonate 8.4%. The tubes were then incubated at 37 °C in 10 % CO2 for 12 days. The cell samples were harvested selectively on day 0, 7, and 12 for the minute remarks on the cellular morphology **[18**].

#### 2.4.1. Preparation of samples for examination under Scanning electron microscope:

The ball was made from placid bacterial cells from specific test dishes, as previously explained and was delayed in PBS (pH 7.2). The compartments were then centrifuged for five cycles at 5000 rpm. The ball was resuspended in McDowell-Trump setting solution prepared in 0.1 M PBS for at least two hours after the supernatant was wasted. The compartments were then washed twice with distilled water after being lapped with 0.1 M PBS, post-fixed with osmium tetraoxide equipped with PBS, and tracked [18].

#### 2.4.2. Scanning electron microscope (SEM) examination:

The morphology and surface assembly of M. tuberculosis were investigated using an examining electron microscopy approach. After that, a portion of the ball was dried using runs of 50%, 75%, 95%, and 100% ethanol, which were all monitored by hexamethyldisilazane (HMDS). After being emptied, the HMDS was placed in a desiccator at ambient temperature to air dry. With the use of double-sided sticky tape

and a gold coating, the dried cells were positioned over a SEM example tube [18]. After that, the cells were examined using a SEM JEOL-JEM 1200 EX II (Jeol, Ltd., Tokyo, Japan) with an 80 kV rushing voltage

### 2.5. Medicinal plants

#### 2.5.1. Preparation of plant extracts:

25 g of dried plants (garlic, ginger, green tea, and cinnamon) were saturated with 100 ml of ethanol for one week at chamber disease. After that, the follow-on extract was sifted over filter paper from Whitman (No.1). The same process was used twice more to mine the balance from the filters. The resulting filtrates were mixed, and using a rotary evaporator set at 40 °C, they were evaporated to dryness. By softening the simple extract in 5% dimethylsulfoxide (DMSO) stock solutions of the extract were obtained (DMSO). All extracts were sterilized by filtration through bacterial filter under positive pressure, then the filtrate was kept at -20$^{O}$C until used **[19]**.

#### 2.5.2. Stock solution and concentration of ethanolic plant extracts:

Ethanolic plant extracts were liquefied in Di-methyl sulfoixide (DMSO) which has admirable solvating stuff [20]. Final concentration of stock solution of ethanolic plant extract was 52 mg\ml (0.52g of EPE in 10 ml DMSO).

#### 2.5.3. Determination of minimum inhibitory concentration (MIC) of ethanolic plant extracts by using resazurin microtitre plate assay (REMA):

Minimum inhibition Whitman filtration paper (No.1). The filtering balance was mined twice more using the same method. Utilizing a rotary evaporator set at 40 °C, the acquired filtrates were mixed and evaporated to dryness. Simple extract stock solutions were obtained by softening in 5% Dimethylsulfoxide (DMSO). Columns (1,2,11 and 12) and rows (A, B, G and H) to maintain moisture during the incubation period. followed by100 µL Middlebrook 7H9 broth were added into shafts in pillars 4 until 10 in rows C to F. 100 µL of plant extracts were poured into the columns 3 and 4's wells, and from posts 4 to 5 until post 8, they were successively diluted. 100 µL of the remaining solution was discarded. Except for the test wells in the column, 100 µL of bacterial suspension was added to every other test well. 10 functioned as sterility control contained Middlebrook 7H9 broth only (without plant extracts and bacterial suspension). the wells in column 9 functioned as Growth controls which contained Middlebrook 7H9 broth and100 µL of bacterial suspension (without plant extracts). The plates were covered, sealed in the plastic bags, and incubated at 37°C in incubator with 10% $CO_2$ supply. After 7 days of incubation, 30 µl of 0.01% w/v sterile resazurin sodium solution was added to each well, and incubated overnight at 37°C. Plates were then observed for color change from blue to pink/colorless, which indicated the reduction of resazurin and therefore bacterial growth **[20, 21]**.

#### 2.5.4. Study the effect of *Allium sativum* (garlic) on the cellular morphology of *M. tuberculosis*

A bulk of 2 mL McFarland no. 3 mycobacterial inoculum was transmitted into two screw-capped tubes. The first tube which served as a control contained 6 mL of Middlebrook 7H9 broth. The second tube contained 6 mL of garlic with a MIC of 3.25 mg\ml the tubes were then incubated at 37 °C in 10% CO2 for 7 days. The cell examples were gathered selectively on days 0 and 7 **[18]**.

#### 2.5.5. Preparation of samples for examination under scanning electron microscope:

They were performed as previously described in Study the result of $NaCO_3$ at 8.4% on the cellular morphology of *M. tuberculosis* by using the scanning electron microscope **[18]**.

## 3. Results :

### 3.1. Isolation of *M. Tuberculosis*

This study was performed on 30 *M. Tuberculosis* isolates from different samples, 20 isolates (17 Sputum, 3 Bronchoalveolar Lavage) from patients who were admitted to chest department in Mansoura University Hospitals and 10 *M. Tuberculosis* cultures from Central Public Health Laboratories in Cairo. Each sample was cultured on Lowenstein-Jensen Media as shown in **Table (1)**.

### 3.2. Identification of *M.tuberculosis* of isolate

#### 3.2.1. Morphology of colony:

Culture is the gold standard for identification of *M.tuberculosis* .colonies are creamy white (buff colour), non-pigmented, dry, rough, and irregular with awrinkled surface, when cultured on Lowenstein Jensen (LJ) medium at 37°C in 10 %CO2 incubator as

**Table (1):** Distribution of *M. tuberculosis* in different clinical samples

| **sample** | **Number of *M.Tuberculosis* Total = 20** | **Percentage (%)** |
|---|---|---|
| Sputum | 17 | 85.0 |
| Bronchoalveolar Lavage (BAL) | 3 | 15.0 |
| Total | 20 | 100 |

The clinical *M. Tuberculosis* isolates includes 4 (20.0%) isolates from females and 16 (80.0%) isolates from males as shown in **Table (2).**

**Table (2):** Percentage of clinical samples collected from different genders.

| **Samples** | **Gender** | | | | **Total** | |
|---|---|---|---|---|---|---|
| | **Female** | | **Male** | | | |
| | **No.** | **%** | **No.** | **%** | **N.** | **%** |
| Sputum | 3 | 17.75 | 14 | 82.35 | 17 | 85.0 |
| BronchoalveolarLavage (BAL) | 1 | 33.33 | 2 | 66.67 | 3 | 15.0 |
| **Total** | 4 | 20.0 | 16 | 80.0 | 20.0 | 100 |

shown in photo (1).

**Photo (1):** (A) Lowenstein Jensen (LJ) medium before inoculation, (B) Growth of *M.tuberculosis* on Lowenstein Jensen (LJ) medium

#### 3.2.2. Ziehl-Neelsen stain

Microscopic examination of *M.tuberculosis* showed a typical character of acid-fast bacilli ( Red, straight or slightly curved rods with blue background) These findings were observed in all isolates as shown in **Photo (2).**

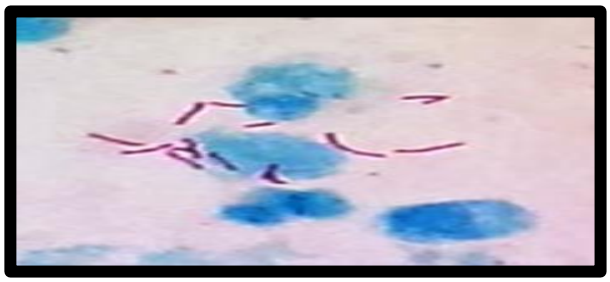

**Photo (2):** *M.tuberculosis* under light microscope using the Ziehl–Neelsen stain.

#### 3.2.3. Biochemical characterization of *M.tuberculosis:*

All isolates of *M.tuberculosis* were positive niacin and nitrate reductase test.

All isolates of *M.tuberculosis* were negative tween-80 hyrolysis test, but 24 isolates of *M.tuberculosis* were positive catalase test,and 6 isolates of *M.tuberculosis* were negative catalase test. that indicate the absence of catalase-peroxidase (katG), so 6 isolates were INH Isoniazid-resistant *M. tuberculosis.*

### 3.3. Scanning electron microscopic examination for study the effect of sodium bicarbonate 8.4% on INH Isoniazid-resistant *M. tuberculosis*:

Sodium bicarbonate 8.4% has destructive effect on INH Isoniazid-resistant *M. tuberculosis that indicate* in scanning electron micrographs **photo (3).**

Result in **photo (3A**) before being treated with sodium bicarbonate 8.4% (control), INH Isoniazid-resistant *M. tuberculosis* cells showed typical long rod-shaped structures with a smooth surface and an intact cell wall.

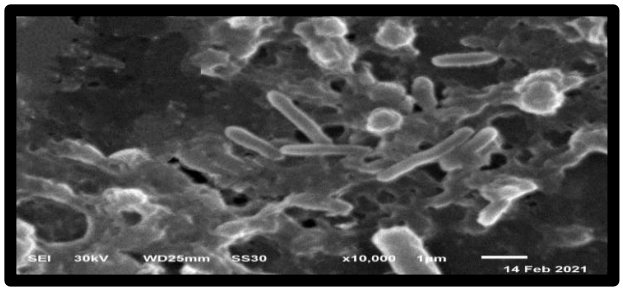


**Photo 3 (A):** INH Isoniazid-resistant *M. tuberculosis* before treated with sodium bicarbonate 8.4% (control).

**After being treated with sodium bicarbonate 8.4% for 7 days:**

INH Isoniazid-resistant *M. tuberculosis* cells increased in size and appeared many of pores on cell surfaces. Cells shape changed from bacilli to coccobacilli. the damaged cell morphology of INH Isoniazid-resistant *M. tuberculosis* showed large surface collapse, abnormal cell breakage, and wrinkled cell walls **(Photo 3 B).**

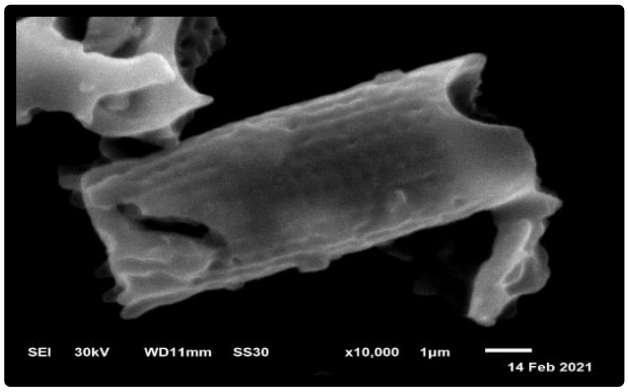


**Photo 3 (B):** INH Isoniazid-resistant *M. tuberculosis* After treated with sodium bicarbonate 8.4% for 7 days.

**After being treated with sodium bicarbonate 8.4% for 12 days:**

There are many changes in cells with increasing exposure time for 12 days. Pores on cell surfaces increased in volume, rupture of cells and complete lysis of INH Isoniazid-resistant *M. tuberculosis* cells **(Photo 3 C)**.

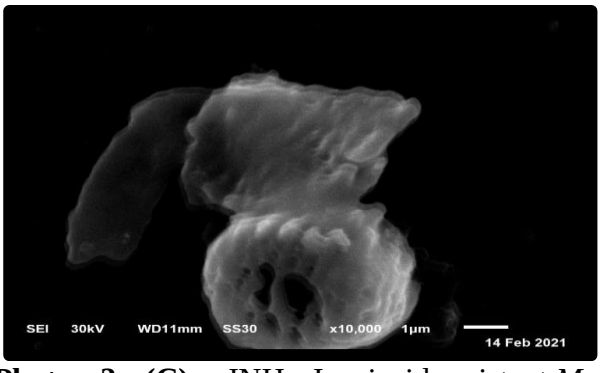


**Photo 3 (C):** INH Isoniazid-resistant *M. tuberculosis* After treated with sodium bicarbonate 8.4% for 12 days.

**3.4. Determination of minimum inhibitory concentration (MIC) of some medicinal plants (garlic, ginger, green tea and cinnamon) by using resazurin microtiter plate assay (REMA).**

All medicinal plants used have effect against clinical isolates of INH Isoniazid-resistant *M. tuberculosis*, but ethanolic extract of garlic was the most active one with MIC=3.25 mg\ml followed by cinnamon, green tea and ginger respectively as shown in **Table (3**) and **photo (4).**

**Table 3.** Effect of some medicinal plants on INH Isoniazid-resistant *M. tuberculosis.*

| Medicinal plants used | MIC (mg/ml) |
|---|---|
| Garlic | 3.25 |
| Ginger | 26 |
| green tea | 13 |
| Cinnamon | 6.5 |

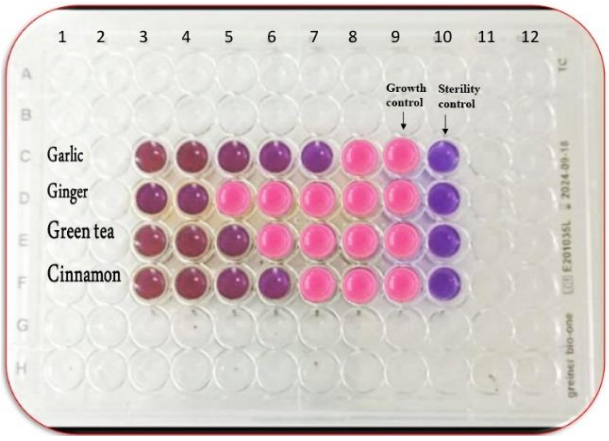


**Photo (4):** Determination of minimum inhibitory concentration (MIC) of some medicinal plants by using (REMA).

**3.4.2. Scanning electron microscopic examination for study the effect of Garlic extract on INH Isoniazid-resistant *M. tuberculosis.***

The cells have a smooth surface and a clearly defined stiff rod structure before treatment with garlic extract, as shown in the photo (5A). As illustrated in the photograph, the cells became crumpled, scruffy, clustered together, and reduced in volume, all of which are telltale signs of damage to the cell wall photo (5 B).

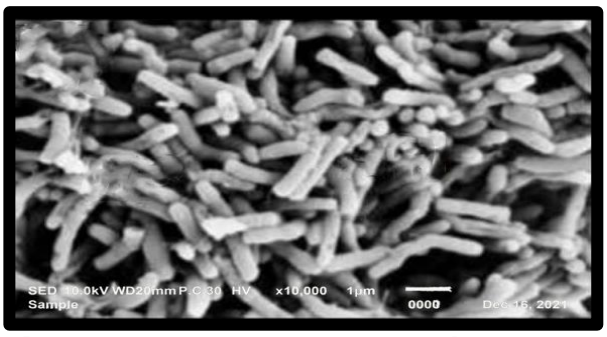


**Photo 5 (A):** INH Isoniazid-resistant *M. tuberculosis* before treated with garlic extract (control).

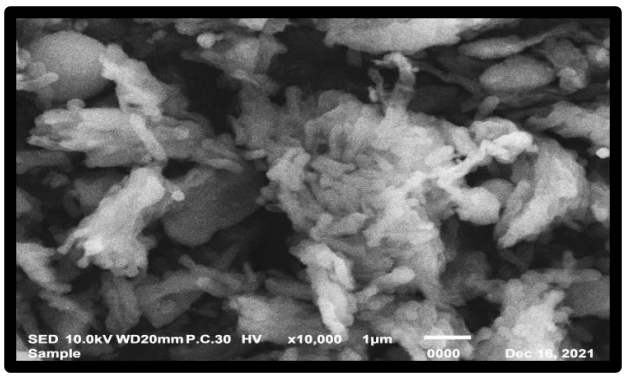


**Photo 5 (B):** INH Isoniazid-resistant *M. tuberculosis* After treated with garlic extract MIC=3.25 mg\ml for 7 days.

**4. Discussion:**

Inside macrophages, *M. tuberculosis* can thrive in an acidic medium. Depending on the macrophage's degree of activation, the estimated pH of the compartment where M. tuberculosis is thought to live ranges from pH 4.5 to 6.2. *M. tuberculosis* grew best in an enriched liquid medium when the pH was somewhat acidic, between 5.8 and 6.7 **[22]**.

According to **Metchnikoff (1905)**, the waxy cell wall of *M. tuberculosis* acts as a crucial barrier against the acid stress experienced by phagocytes. A layer of peptidoglycan-arabinogalactan covalently linked to mycolic acids in the lipid-rich cell wall of *M. tuberculosis* follows a conventional bilayered plasma membrane. Previous studies showed that *M. tuberculosis* was surrounded by an extra outer lipid bilayer in 2008 **[24]**.This intricate cell wall serves as a significant permeability barrier for protons and other antibacterial effectors.

Because SB 8.4% is safe for humans and has a high alkaline pH, it was chosen for this investigation. Based on the previously cited results, we hypothesized that altering the acidic environment in which *M. tuberculosis* lives may affect the TB bacilli's wall, leading to its destruction. It frequently functions as a pH buffering agent. Patients with renal tubular acidosis syndromes or diarrhea can safely use chronic bicarbonate replacement. in patients with ketoacidosis and severe lactic acidosis **[5]**.

In this study, sodium bicarbonate 8.4% had a destructive effect on INH Isoniazid-resistant *M. tuberculosis.* These findings were in agreement with earlier research **[24]** which showed that sodium bicarbonate 8.4% inhibits bacterial, fungal, and mycobacterial growth in the specific cultures and interferes with ZN staining of mycobacteria, and **[25]** which showed that inhaling SB 8.4% as an adjunctive therapy to standard anti-TB drugs reduces the time needed for smear and TB culture conversion and achieves rapid clinical and radiologic improvement in patients with drug-sensitive pulmonary TB.

Medicinal plants play an important role in the treatment of human infections due to their high antibacterial activity. Medicinal plants are used specifically in developing countries due to their local availability, high efficiency and their low price Plants are rich with phytochemicals including tannins, alkaloids, terpenoids, and flavonoids which have antimicrobial properties **[26].**

Using the traditional resazurin microtitre plate assay (REMA), the anti-mycobacterial activity of plants was quantified in terms of minimum inhibitory concentration (MIC) **[20]**. The MIC was established as the lowest amount of plant extracts required to prevent blue to pink color change **[21]**.

In the current study four ethanolic extracts derived from different parts of four medicinal plants traditionally used in Egyptian folk medicine and belonging to different four families were screened for their anti-mycobacterial activity alongside clinical isolates of INH Isoniazid-resistant *M. tuberculosis* by REMA.

All the extracts of medicinal plants have an effect against clinical isolates of INH Isoniazid-resistant *M. tuberculosis*, but the ethanolic extract of garlic was the most active one with a MIC=3.25 mg\ml followed by cinnamon, green tea and ginger respectively.

These results were in agreement with the previous studies (Delaha and Garagusi, 1985) who reported that garlic extract inhibited *M. tuberculosis* at a MIC 1.34-3.35mg/ml. Another study demonstrated garlic activity against MDR isolates of M. tuberculosis resistant at a MIC 1.0-3.0 mg/ml **[28].** Whereas, (Rao *et al*., 1946 ) in contrast to our result showed the inhibition of M. tuberculosis at 2 mg/ml but he used only a single isolate, and (Rajani et al., 2015), who reported that ethanol extract of garlic against MDR isolates

of M. tuberculosis resistant showed MIC values in the range of 0.5-2.0 mg/mL .

The most effective medicinal plant extract against INH Isoniazid-resistant *M. tuberculosis* was garlic. This isolate was examined under an electron microscope before and after treatment with a plant extract.

**5. Conclusions:**

According to the results of our investigation, it was determined that sodium bicarbonate (8.4%) may be applied to the respiratory tract via inhalation or instillation via bronchoscope or endotracheal tube and utilized as an adjuvant to antitubercular medications. It makes sense that alternative medicine techniques using plant extracts, such as garlic, would be important for public health in order to lessen the cost of treating illnesses and ease the burden of drug resistance.

**6. References:**


**1** Terracciano E, Amadori F, Zaratti L, Franco E (2020). [Tuberculosis: an ever present disease but difficult to prevent]. Ig Sanita Pubbl. Jan-Feb;**76**(1):59-66.

**2** Mbuh TP, Ane-Anyangwe I, Adeline W, Thumamo Pokam BD, Meriki HD, Mbacham WF. .( 2019) Bacteriologically confirmed extra pulmonary tuberculosis and treatment outcome of patients consulted and treated under program conditions in the littoral region of Cameroon. BMC Pulm MedJan 17;**19(1):**17.

**3**. Dunn.JJ, Starke.JR,and Revell.PA(2016). Laboratory Diagnosis of *Mycobacterium tuberculosis* Infection and Disease in Children. *J Clin Microbiol*.( 2016) Jun; **54(6):** 1434–1441.

**4** Saviola B. (2012) Response of Mycobacterial species to an acidic environment. In: Cardona PJ, editor. Understanding tuberculosis − deciphering the secret life of the bacilli. InTech;. pp. 89–102

**5**. Sabatini S, Kurtzman NA. (2009) Bicarbonate therapy in severe metabolic acidosis. *J Am Soc Nephrol* **20**:692–695.

**6**. M, Arram E, Elhadidy T, Hassan M, Habashy H and El Badrawy M, (2022) Electron Microscopic and Pathological Changes in Lung Cancer after Intratumoral Injection of Sodium Bicarbonate, Current Cancer Therapy Reviews**. p .**123 - 130.

**8**. Fatima S, Kumari A, Dwivedi VP. (2021);Advances in adjunct therapy against tuberculosis: Deciphering the emerging role of phytochemicals. MedComm. **2(4):**494-513.

**9.** Pearce R, Goldstein J, Newbury D, Joy D, lyman, c., echlin, p., lifshin, E., SAWYER, L. & MICHAEL, J. .(2003), Scanning Electron Microscopy and X-Ray Microanalysis, Geological Magazine**140(6)**, 728-729.

**10**. Sharma M, Misra RN, Gandham NR, Jadhav SV, Angadi K, Wilson V. (.2012)Comparison of modified Petroff's and *N*-acetyl-l-cysteine-sodium hydroxide methods for sputum decontamination in tertiary care hospital in India. *Med. J. DY Patil Univ*. **5**:97–100.

**11**. Kassaza K, Orikiriza P, Llosa A, Bazira J, Nyehangane D, Page AL, Boum Y (2014) 2nd. Lowenstein-Jensen Selective Medium for Reducing Contamination in Mycobacterium tuberculosis Culture.*J Clin Microbiol.* Jul; **52(7):** 2671–2673.

**12** Al-mohanna M. (2016). Identification of mycobacterium.P 205.

**13**. Van Deun A, Hossain MA, Gumusboga M, Rieder HL. ( 2008) Ziehl-Neelsen staining: theory and practice. *Int J Tuberc Lung Dis*Jan;**12(1):**108-10.

**14** Kent, P. T., and G. P. Kubica**.** (1985). Public health mycobacteriology: a guide for the level III laboratory, U.S. Department of Health and Human Services, Atlanta, Ga. p. 91-95

**15**. Virtanen S. (1960); A study of nitrate reduction by mycobacteria. The use of the nitrate reduction test in the identification of mycobacteria. Acta Tuberculosea Scandinavica. Supplementum. **48**:1-119.

**16** Reiner K. (2010.) Catalase Test Protocol. ASM Conference for Undergraduate Educators B, VIII

**17**. Cox. Et al. (1978) Rapid Tween-80 Hydrolysis Test for *Mycobacteria.Journal of Clinical Microbiology*; Vol.: **7**, No.: 1; Jan, p. 104 -105.

**18** Parumasivam T,Naveen Kumar HS, Mohamad S, Ibrahim P, SADIKUN A (2013) .Effects of a lipophilic isoniazid derivative on the growth and cellular morphogenesis of Mycobacterium tuberculosis H37Rv. *Int J Pharm Pharm Sci*, Vol **5**, Supp l4, 43-50.

**19**. Thakare M. (2004) Pharmacological screening of some medicinal plants as antimicrobial and feed additives (Thesis). Virginia Polytechnic Institute and State University, Blacksburg, Virginia USA,. P. 15.

**20** Nair SS, Gaikwad SS, Kulkarni SP, Mukne AP. (2017) Allium sativum constituents exhibit anti-tubercular activity *In vitro* and in RAW 264.7 mouse macrophage cells infected with Mycobacterium tuberculosis H37Rv. Phcog Mag;**13**, Suppl S2:209-15.

**21**. Ang CF, Mendoza MT, Bulatao WC: (2010) Evaluation of the Resazurin Microtiter Assay for Drug Susceptibility Testing of Clinical Isolates of *Mycobacterium tuberculosis*. *Philipp J Microbiol and Infect Dis.*, **39:** 59-65.

**22**. Vandal OH, Nathan CF, Ehrt S. (2009) Acid resistance in Mycobacterium tuberculosis. *J Bacteriol*; 191:4714–4721.

**23**. Metchnikoff E. (1905) Immunity to infective disease. Cambridge, UK: Cambridge University Press. p. 182.

**24** El Badrawy MK, Elela MA, Yousef AM, et al. (2018); Effect of sodium bicarbonate 8.4% on respiratory tract pathogens. Chest Lung Res **1(1):**3-7.

**25** El-Badrawy M.K, Arram E.O., Abdalla D.A. *et al* (2019). Effect of adding inhalation of sodium bicarbonate 8.4% to the usual treatment on smear-positive pulmonary tuberculosis: a prospective controlled study. *Egypt J Bronchol*. **13**, 531–538.

26 . Alviano DS, Alviano CS. (2009) Plant extracts: search for new alternatives to treat microbial diseases. Curr Pharm Biotechnol Jan;**10(1)**:106-21.

**27** .Delaha EC, Garagusi VF. (1985) Inhibition of mycobacteria by garlic extract (Allium sativum). Antimicrob Agents Chemother. Apr;**27(4):**485-6.

**28** Abbruzzese MR, Delaha EC, Garagusi VF. (1987); Absence of anti-mycobacterial synergism between garlic extract and anti-tuberculosis drugs. Diagn Microbiol Infect Dis **8**:79-85.

**29** .Rao RR, Rao SS, Natarajan S, Venkataraman PR. (1946) Inhibition of Mycobacterium tuberculosis by garlic extract. Nature,; 157:441.

**30** .Rajani SD, Desai PB, Rajani DP. (2015) Anti-mycobacterial activity of garlic (Allium sativum) against multi-drug resistant and reference strain of Mycobacterium tuberculosis. IJAR; **1(13):** 767-770.