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Antimycobacterial and antibiofilm activity of garlic essential oil using vapor phase techniques

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ABSTRACT

Garlic is a well-known species of the genus *Allium* that acts as a natural chemotherapeutic agent and used for cure and prevention against enteric diseases. In addition, its essential oil has provided a promising way to treat different human associated diseases. Consequently, *Mycobacterium tuberculosis* causes tuberculosis among immunosuppressed individuals and develops resistance rapidly due to inappropriate therapy. Indeed, an alternative therapy is need of the hour to control resistance of mycobacterial strains. Concerned with the development of drug-resistant strains, garlic essential oil (GEO) in liquid and volatile phase was investigated against *Mycobacterium smegmatis* using *in-vitro* techniques. Furthermore, validation of anti-mycobacterial effect using GEO was also determined on *M. tuberculosis* with its drug resistant variants. Different *in-vitro* techniques, that is, extraction and identification of five major volatile constituents from GEO using gas chromatography-mass spectrometry, were initially analyzed and screened out for exploration of anti-mycobacterial susceptibility tests. In addition, inverted disc method and anti-biofilm assay by GEO vapor were determined for evaluating its volatile efficacy. The efficiency of GEO in liquid phase showed growth inhibiting value at 0.03 mg/mL and 0.5 mg/mL as bactericidal concentration against *M. smegmatis*. Whereas, *M. tuberculosis* (H37Rv), isoniazid, and rifampicin resistant strains were found to be inhibitory concentration at 0.003, 0.06, and 0.03 mg/mL, respectively. Furthermore, 1 mg for inverted disc-vapor assay and 0.125 mg for anti-biofilm assay in air-liquid interface were found to prevent *M. smegmatis* growth efficiently. Thus, vapor contact of GEO serves as a novel strategy for anti-mycobacterial activity for TB-disease. In addition, it might be introducing a novel volatile therapy technique against different pulmonary infection.

1. INTRODUCTION

Garlic (*Allium sativum* [L.]) is a widely used foodstuff that has been acclaimed over a period of 1000 years for its beneficial contribution to human health. According to worldwide trends, it has been used for therapeutic purposes and has potential for further expansion of its beneficial properties. On the flipside, its strong odor and fragrance have inspired a myriad of food delights as well as natural remedies for a wide range of health concern [1,2]. Its insightful qualities are rooted in amazing sulfur chemical properties and provide a raising diversity of sulfur effective compounds through different extraction procedures [3]. Although, with the rapid advancement of scientific research, different activities of garlic clove products have been established, many activities are not yet to be established for treating human acute and chronic diseases [4]. Consistent with earlier research, there is reason to believe that garlic components improve different human associated diseases. In addition, preclinical evidence suggests that garlic constituents may help to improve cardiovascular and cancer diseases [5]. On the other hand, essential oils also play a key role in promising pharmaceutical alternative and conventional therapy for related purposes [6-8]. In contrary to antimicrobial drugs, inhalation immunotherapy of volatile oil employed in health-care system to alleviate acute and chronic bronchitis-related disorders [9]. According to epidemiological research and randomized trials, garlic essential oil (GEO) has shown diverse pharmacological effects that are closely connected to its distinct active components [10]. Indeed, the efficacy of GEO has also been identified against some enteric bacterial stains, utilizing liquid, and disc volatilization approaches [11]. Altogether, GEO holds a promising future toward antibiotic remedies and conventional medicine for comparable objectives [9].

Notably, *Mycobacterium tuberculosis,* the causative agent of tuberculosis (TB), develops mainly pulmonary infections, although extrapulmonary infections are also found. The constantly growing antibiotic resistance strains of *M. tuberculosis* outlined as serious threat to community well-being as well as impacts on developed and developing countries [12]. According to the contemporary World Health Organization database 2021, there is a global incidence of 10 million affected individuals with tuberculosis infection that lead

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to 1.5 million deaths worldwide in 2020 [13]. The lipid rich surface layer makes this bacterium restricted from drug incorporation. Even if, recent findings reveal that *M. tuberculosis* develops biofilm in lesions of the human lungs [14]. Different non-tubercular *Mycobacterium* also displayed similar characteristics with *M. tuberculosis*. Therefore, non-tubercular *Mycobacterium* consider as a working model for drug discoveries. Furthermore, different experiments also revealed that organosulphur molecule in garlic clove, such as allicin or ajoene have imperative antimycobacterial effects and can even suppresses the growth of drug resistant mycobacterium [15]. However, these molecules are quite unstable and change into different compounds with time. Garlic oil contains different volatile substances. Therefore, volatile oil might overcome the problem as a useful antimycobacterial agent. There are almost 150 species of *Mycobacterium* that favors to surface adhesion and growth over aqueous suspension [16]. Its polymeric substances including lipid and polysaccharide that help to adhere surface wall with bacteria are called biofilm. Apparently, *Mycobacterium* develops strong biofilms on air-liquid interfaces [17,18]. Most importantly in biofilm, there is a heterogeneous population of bacteria which includes drug susceptible replicating bacteria, slow growing or non-replicating persistent bacteria that have different levels of phenotypic drug resistance [19]. Most importantly, recent studies have revealed that *M. tuberculosis* and *M. smegmatis* develop similar kinds of pelicular biofilm. Drug-resistant *tuberculosis* remains a challenging task to combat in the battle against tuberculosis. Therefore, new therapeutic strategies or drugs are need of the hour to combat battle against *M. tuberculosis*. According to garlic potential some of research studies revealed that volatile effect of garlic essential oil has effective antimicrobial activities and could be used as volatile antibiotic in combination with normal antibiotics [20]. Therefore, volatile effect of natural garlic essential oil might be helpful as an effective antimycobacterial agent. As per our comprehensive knowledge, no such apparent findings of anti-mycobacterial activity of GEO by vapor phase evaluation assay have been recorded. Thus, the goal of this study was to evaluate the efficacy of volatile and liquid usage of essential oil against *M. smegmatis* as a model organism. In addition, different *M. tuberculosis* strains including H37Rv, isoniazid (INH) resistant, and rifampicin (RIF) resistant clinical isolates were assessed with GEO for their susceptibility.

2. MATERIALS AND METHODS

2.1. Bacterial Strain and Culture Conditions

The non-tubercular bacterium used in this study, *M. smegmatis* mc^2 155 (ATCC14468), was initially procured from MTCC Chandigarh. *M. tuberculosis* H37Rv, one clinical isolates of isoniazid resistant and one rifampicin resistant isolate, was obtained from ICMR-Regional Medical Research Centre (RMRC), Bhubaneswar. All cultures were maintained in Middlebrook 7H9 broth medium with supplements to 10% oleic acid/bovine albumin, dextrose, catalase (OADC), 0.05% Tween 80, and 0.2% glycerol at 37°C for 48 h shaking incubation period for *M. smegmatis* and 15 days for *M. tuberculosis*. The bacteria cells containing 1×10^5 CFU/mL were maintained throughout this study for assessment of antimicrobial activities in liquid phase assay. Furthermore, other chemical reagents used in this study were obtained from HiMedia Pvt. Ltd. (Mumbai, India) and used as respective evaluation *in vitro* volatilization effects.

2.2. Herbal Collection and Extraction of Volatile Essential Oil

Commercially available garlic bulbs (*Allium sativum* (L.)) were procured from the local region of Chandrasekharpur market, Bhubaneswar, India at a longitudinal position of 20.3338° North, and 85.8241° East. Afterward, 300 g of fresh garlic bulbs were peeled out and crushed in a homogenizer mixture with double distilled water for further extraction of essential oil processes. Subsequently, homogenized garlic bulbs mixture was transferred to a round bottom flask and hydrodistillation procedures were initiated for 5 h at ambient temperature for the extraction of volatile essential oil from shredded garlic bulbs [21]. After that, the purified volatile oil was collected and distributed into separate vials for further investigation and analysis of anti-mycobacterial and anti-biofilm activities.

2.3. Assessment of Essential Oil Analysis Using Gas Chromatography-Mass Spectrometry (GC-MS) Equipment

Chemical analysis of volatile essential oil from garlic bulbs was performed using Clarus 580 GC coupled with SQ8S MS detector equipped with Elite-5 capillary column (30 m length × 0.25 mm I.D., film thickness 0.25 μm). Helium was used as a carrier gas and kept at a constant flow rate of 1 mL/min. The GC-MS was used under the following conditions: The oven temperature was first set at 60°C and subsequently increased to 220°C at a rate of 3°C/min; the electron ionization voltage was set at 70 eV, and the ion source temperature was set at 250°C. Following that, a series of carbon-n-alkanes (C8-C20) chains was used for calculating individual peak areas and side by side experimental retention index values reported in Satyal *et al.* (2017) literature and the NIST library were assessed for identification of volatile components from GC-MS analysis [22,23].

2.4. Assessment of Anti-Mycobacterial Effect of Garlic Essential Oil

Bacilli were grown to log phase, that is, 0.6–0.8 optical density at 600 nm and seeded 10^5 bacteria/well into a 96-well plate having serial dilution of garlic oil. Incubation was carried out at 37°C for 48 h for *M.smegmatis* and 10 days for *M. tuberculosis*. For minimum inhibitory concentration (MIC), 1X alamar blue (Invitrogen) per well was added and a change in color from blue to pink was recorded after 1 h of incubation for *M. smegmatis* and overnight for *M. tuberculosis*. Wells containing only media and bacteria served as positive control and wells having only media served as negative control. For minimum bactericidal concentration (MBC) of *M. smegmatis,* an appropriate dilution of each well was plated on 7H11 agar plates, incubated for 72 h at 37°C. Meanwhile, MIC and MBC tests were performed in triplicates and displayed as same interpreted result vales.

2.5. Activity of Garlic Essential Oil Vapor

To determine the fume effect of essential oil using inverted disc technique, *M. smegmatis* was initially cultured (7H9 medium with supplemented to OADC (10% W/V)) and allowed to grow to optical density of 0.8 at 600 nm (OD600) at 37°C. 10^5 bacterial cells were spread over 7H11 agar plate and a sterilized empty filter paper (6 mm) was infused with different concentrations of garlic oil ranging from 5–0.01 mg on the lid of the petri dish. The solidified agar plate containing bacteria was kept in inverted form and the bacterial growth inhibition was determined based on the fume effectives of GEO in between 48 and 72 h of incubation period at 37°C. The vapor experiment was executed in three independent triplicates and recorded to be same result.

2.6. Establishment of *M. smegmatis* Biofilm and Its Biomass Estimation

To evaluate the fume effect of GEO on biofilm and pellicle formation, mycobacteria *(M. smegmatis)* were grown in a specific 7H9 medium supplemented with OADC (10% W/V) for 36 h to reach an optical density (OD) of 0.7–1 at 600 nm. The mycobacterial culture was transferred to Sauton's media at a ratio of 1:100–10 mL glass vials and added different concentrations (2–0.007 mg) of essential oil on the inner side of the cap of each vial for anti-biofilm outcomes. After that, the experimental vials were incubated for 5 days at 37°C and examined for each type of sub-population biomass using crystal violet (1%W/V) solution. Subsequently, the absorbance was taken at 592 nm using UV-visible spectrophotometer and the results were determined. Afterward, the same technique was repeated for 3 times and recorded as performed results in an excel sheet for further interpretation of analysis.

2.7. Statistical Interpretation

The MIC, MBC, and inverted disc fume tests were carried out in three replications and the result was found to be consistent. The percentage of biofilm inhibition at different volatile concentrations of GEO was examined using one-way ANOVA followed by Tukey's *post hoc* multiple comparison test with IBM SPSS software version 26 and expressed as mean ± standard deviation at $P < 0.05$ significant level.

3. RESULTS

3.1. Phytochemical Composition of Essential Oil

The yield percentage of GEO obtained from hydrodistillation (Clevenger type apparatus) was noted to be 0.17% (v/w), on a dry weight basis. Following that, an extracted garlic clove essential oil was characterized using gas chromatography-mass spectrometry and identified volatile constituents in clove essential oil. GC-MS analyses revealed the major five volatile compounds accounting for 85.51% of total oil, including diallyl trisulfide (DTS) (37%), followed by diallyl disulfide (DDS) (25.9%), allyl methyl trisulfide (AMT) (13.16), allyl methyl disulfide (5.2%), and allyl (E)-1-propenyl disulfide (4.25%) as specified in [Table 1].

Perhaps, it was fascinating to be noted that DTT, DDS, and AMT are abundantly identified in this essential oil and denoted as higher percentages of volatile sulfur compounds among other ingredients. As a result, DTT and DDS represents 62.9% of total essential oil and denoted as key highlight for abundant sulfur component present in essential oil.

3.2. Susceptibility Activity of Garlic Essential Oil

Initially, essential oil was synthesized through hydrodistillation technique, appeared to be thick yellow in color with a density of 1 g/mL (W/V). The extracted essential oil was further exploited to evaluate antimycobacterial activity against *M. smegmatis* using broth microdilution technique. Representative outcomes from the abovementioned strategies are illustrated in Figure 1. It shows that the MIC value of GEO at 0.03 mg/mL found to be effective with this efficient concentration. Consequently, it was also utilized for analyzing its bactericidal activity, which displayed that at a 0.5 mg/mL concentration level was sufficient to breakdown *M. smegmatis* growth development. Overall, garlic oil has a notable growth inhibitory and cidal effect against *M. smegmatis*.

3.3. Effect of GEO Using Inverted Disc Technique

Considering the positive results obtained from antimicrobial activity of garlic essential oil, it was interesting to correlate these results in gaseous contact against *M. smegmatis* using an inverted disc volatilization assay. As disc volatilization technique is one of the

Table 1: Volatile constituents of essential oil from *Allium sativum* L. identified by GC-MS.

S. No.	RT (min)	Compound	RI[a]	RI[b]	Obtained Area % from GC-MS
1	5.172	Allyl methyl disulphide	912	916	5.2±0.01
2	10.412	Diallyl disulphide	1074	1080	25.9±0.02
3	11.082	Allyl (E)-1-propenyl disulphide	1095	1100	4.25±0.03
4	12.668	Allyl methyl trisulfide	1131	1138	13.16±0.01
5	19.643	Diallyl trisulfide	1295	1301	37.00±0.01
		Total			85.51±0.08%

[a]RI: Retention index calculated from homologous series n-alkane (C8–C20) using Elite-5 column. [b]RI: Retention index from literature Satyal *et al.* (2017). GC-MS: Gas chromatography-mass spectrometry

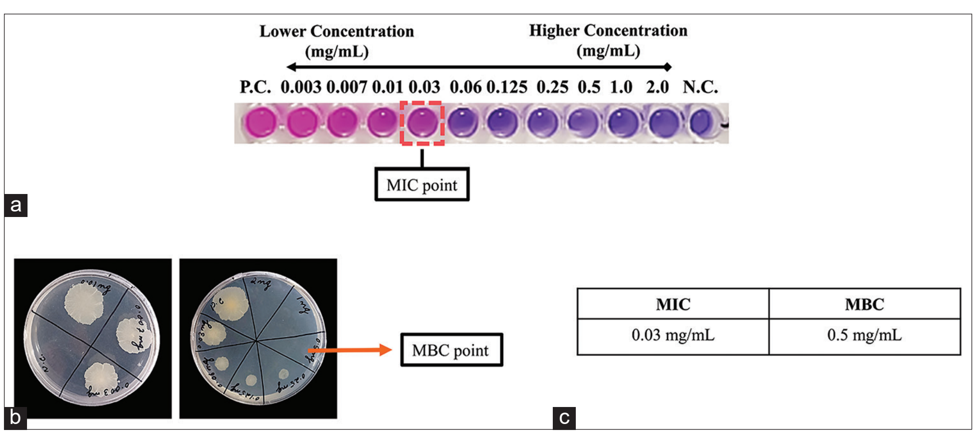

Figure 1: In vitro growth effect of Mycobacterium smegmatis using *garlic essential oil* (a) *minimum* inhibitory *concentration (MIC)* in liquid phase, (b) *minimum* bactericidal concentration *(MBC)* in 7H11 agar medium, and (c) MIC and MBC representative values of garlic essential oil.

fundamental experiment for evaluating the antimicrobial properties of essential oil in vapor-phase, as well as also used as a preliminary screening method to select the most promising oil [24]. Considering the above remark, our results reflect that there was dose dependent inhibitory activity of volatile GEO. The minimum inhibitory dose (MID) of essential oil in vapor concentration is 1 mg (1 μL), which was able to restrict the growth of *M. smegmatis*. However, below 1 mg (1 μL) of GEO showed growth of bacteria in plate but in a dose dependent manner [Figure 2].

3.4. Assessment of Anti-biofilm Effect Using Fume of GEO Against *M. smegmatis*

Due to their strong affinity toward abiotic materials such as, polyvinyl chloride or polycarbonate, mycobacterium thrive in environments with stress factors and form floating biofilms at air-liquid interface [25,26]. The carbon-nitrogen ratio in the nutritional medium can be a major consideration in mycobacterial coaggregation and biofilm development [26]. As efficient result was obtained from above inverted disc susceptibility examination, it was thought to be interesting to explore whether EO treatment through vapor contact had an effect on biofilm viability or not. As a result, to find out the mystery behind unknown facts of GEO efficacy, it was treated with *M. smegmatis* biofilm. Phenotypic observations showed that 0.06 mg (0.06 μL) GEO was enough to inhibit biofilm production by vapor phase contact. Crystal Violet estimation showed that 50% inhibition of GEO was observed at a concentration of 0.03 mg (0.03 μL) [Figure 3]. The activity of EO was found to reduce with decreasing concentrations (<0.06 mg), which promotes biofilm formation and biomass production on the surface of liquid media under ideal conditions (37°C). As a whole, *M. smegmatis* biofilm treated with GEO through vapor contact presented itself as a potential inhibitor of pellicle biofilm formation effectively.

3.5. Validation of the Effect of GEO on *M. tuberculosis* (H37Rv), INH and RIF Resistant Clinical Isolates

To confirm our observation found in *M. smegmatis,* we conducted a susceptibility test using garlic essential oil on H37Rv, the virulent strain of laboratory, as well as one isoniazid and one rifampicin resistant clinical isolate [Table 2]. The results indicate that MIC value of GEO at 0.003 mg/mL was determined to be lowest inhibitory dose against H37Rv virulent strain, followed by 0.06 mg/mL against INH resistance, and 0.03 mg/mL against RIF resistance strain. Looking with above considerable remark, it highlights that GEO is highly sensitive to H37Rv virulent strain as well as exhibits notable inhibitory activity against INH resistance and RIF resistance strains. Thus, the efficiency of essential oil was found to be quite effective against drug sensitive as well as drug resistant tuberculosis developing *M. tuberculosis*.

4. DISCUSSION

In the past few years, inhaling essential oils (EOs) vapor has gained rapidly with visions for a wide range of purposes, including adequate solutions for folk remedies and aromatherapy. Different studies also highlighted potential antimicrobial effects of essential oils in the literature, but very few number of studies have indicated an existing concern about antimicrobial activity of essential oil in vapor phase [11,24]. Indeed, the use of garlic is known for centuries to treat different infectious diseases. Different extraction procedures of garlic have been utilized for the evaluation of antibacterial activities against commensal pathogens and respiratory associated bacteria in infectious diseases. In addition, its essential oil demonstrated remarkable antibacterial activity against different enteric pathogens and commensal bacteria [3]. As a result, the above positive reports show that GEO is a promising antimicrobial agent along with antimicrobial drugs with a low risk of emergence of resistance in microbes.

The constituents of EO as well as their respective volatilities influence the properties of their vapors, which, in turn, promotes their antibacterial potential [25,26]. Different studied strains such as *Escherichia coli,*

Table 2: Efficacy of garlic essential oil against different *Mycobacterium tuberculosis* strains.

Tested microorganisms	MIC value (mg/mL)
Mycobacterium tuberculosis (H_{37}Rv)	0.003
INH resistance	0.06
RIF resistance	0.03

MIC: Minimum inhibitory concentration, INH: Isoniazid, RIF: Rifampicin

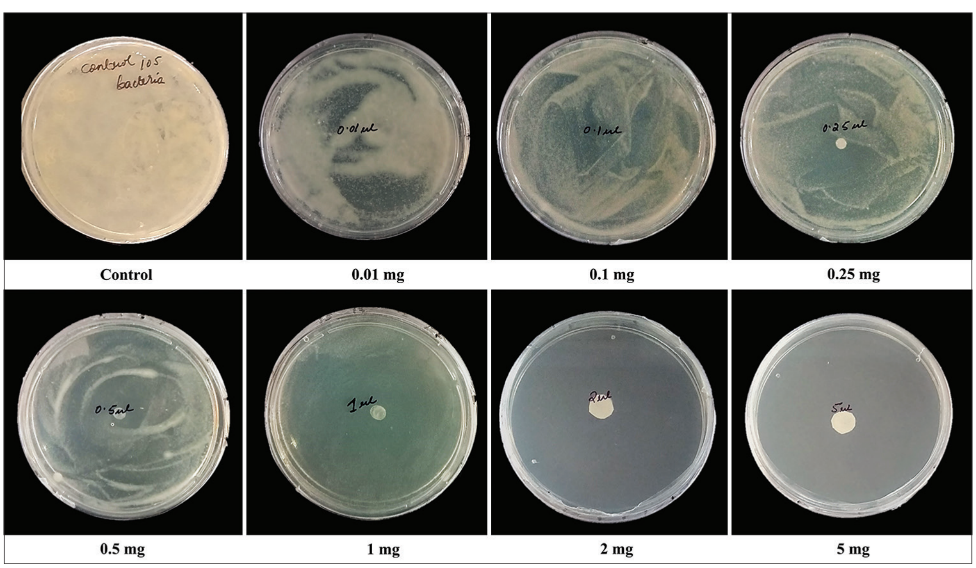

Figure 2: Volatile effect of garlic essential oil using inverted disc assay against *Mycobacterium smegmatis* strain.

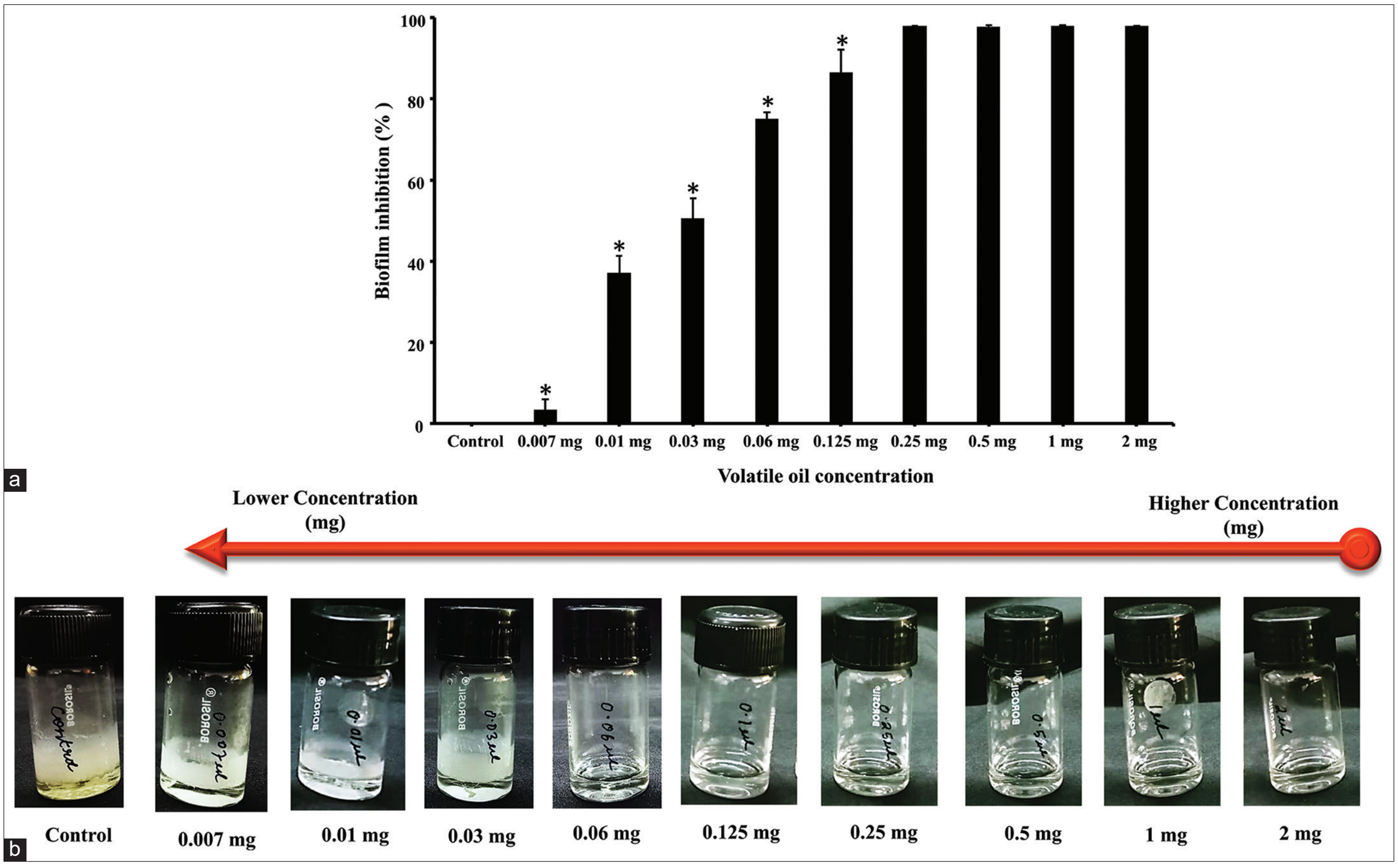

Figure 3: Effect of air-liquid interface in eradication of NTB (*Mycobacterium smegmatis*) biofilm using volatilization assay. (a) Percentage of biofilm inhibition at different volatile concentration of garlic essential oil (GEO); data are presented as means (n = 3), error bars represent SD. *Indicates statically significant at $P < 0.05$. (b) Phenotypic observations of anti-biofilm activity using fume GEO.

Penicillium corylophilum, Bacillus cereus, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella enteritidis, Staphylococcus aureus, Alternaria alternate, Ascophaera apis, Aspergillus niger, and *Penicillium digitatum* are quite susceptible to GEO (MIC ranges from 10–250 μL/L) using vapor of GEO with airtight disc volatilization techniques [10,11]. However, effect of garlic oil on any *Mycobacterium* spp. is not revealed. *M. tuberculosis* is one of the important pathogens and causes the second most highest death rate among different infectious diseases [15]. Recently, one of the reports from Reiter *et al.* states that the volatile substance of garlic extract, that is, allicin has antimicrobial activity against lung infection caused by *Pseudomonas*, *Streptococcus*, and *Staphylococcus* strains [20]. Regarding the use of garlic oil vapor, one hypothesis suggested that the vapor phase is more efficient because the lipophilic molecules present in the aqueous phase form micelles and so prevent the EOs from attaching to the organism, but the vapor phase allows for unfettered attachment [27]. Altogether, evidence of GEO in liquid phase has been extensively investigated, but its vapor potential remains relatively unexplored, which is now gaining interest in the medical field. A few studies have revealed that the vapor phase of garlic has anti-microbial effect [11] and concepts for the use of volatile antibiotic are slowly emerging. Furthermore, no preceding evidence for GEO anti-mycobacterial activity in liquid and vapor phase has been discovered. Therefore, we decided to explore the effectiveness of GEO against *M. smegmatis* using MIC, MBC, and inverted disc volatilization and anti-biofilm assay by vapor interaction approaches. Furthermore, we confirmed that GEO restricted the growth of *M. tuberculosis*.

In the present study, we have chosen fast growing *M. smegmatis* as a model organism in our experiment. It shows for the first time the anti-mycobacterial potential of GEO against *M. smegmatis* and represents that essential oil has direct effect on tested strain. While Figure 1 illustrates MIC value of essential oil (GEO), at 0.03 mg/mL with MBC value, at 0.5 mg/mL are found to be effective as both growth inhibitors as well as bactericidal. Furthermore, for the proof of the concept, we have performed the antimicrobial susceptibility test of *M. tuberculosis* virulent strain H37Rv and one each of INH and RIF resistant clinical isolates and found that GEO was able to restrict the growth of all the virulent strains [Table 2]. In contrast to the above findings in liquid phase, GEO by gaseous contact is evident with an effective antimicrobial action against *M. smegmatis* with good solubility and diffusibility rate of active compounds contained in garlic essential oil, as depicted in Figure 2. The volatile substance of 1 mg garlic oil restricted the bacterial growth and it works in dose dependent manner. As the, antimicrobial drug resistance results in more pathogenicity and virulence factors in mycobacterial infections, an effective approach is required to combat the rapid progress of the disease. Consequently, biofilms contain a heterogeneous population of replicating and non-replicating persistent bacteria [14]. Moreover, biofilm model was found to be an ideal way to execute and evaluate the effector doses of anti-tubercular drugs against mycolic acid produced by the-pathogens [28,29]. Considering the above proclamation, we found that GEO in the vapor phase exhibited bactericidal potential on *M. smegmatis* biofilm at a dose of 0.25 mg of GEO. While at the lowermost concentration, 0.03 mg MID dose of essential oil performs

50% of inhibition against biofilm formation in *M. smegmatis* strain. Altogether, vapor phase of GEO delivers a potential mark for inhibitory agent against *M. smegmatis* biofilm.

In brief, our findings support the anti-microbial action of GEO by vapor contact and it was found to be more efficient when it was exposed to high vapor concentration for a short period of time. In addition, the results also suggested that at a maximal vapor level of 0.1 mg GEO is quite enough to prevent normal inhibition of biofilm forming *M. smegmatis* strain as well as 1 mg (inverted disc vaporization concentration) GEO efficiently prevented the replicating *M. smegmatis* [Figure 3].

From the previous literature surveys, it was perceived that in GEO the percentage of DTS and DDS were found to be high, which can be considered as a probable cause of essential oil's antibacterial property. In addition, its sulfur containing compounds such as DDS and DATS make GEO highly fragrant and make essential volatile components in garlic oil a novel source of antimicrobial therapy [15,30]. Notably, our GC/MS analysis also showed similar types of active volatile constituents [Table 1] and resembles novel sources of remarkable therapy for all of the above tested activities. Thus, GEO vapor therapy can be useful in anti-mycobacterial activity. Although we have done these experiments in *M. smegmatis* as a model organism, these data suggest that use of vapor phase garlic oil could restrict *M. tuberculosis* (H37Rv) and its drug resistant form.

5. CONCLUSION

As garlic bulbs have been well known to possess antibacterial properties from ancient civilization times, it makes considerable interest among researchers and found to be one of the most effective approaches to combating antibiotic resistance strains of pathogenic bacteria. Its organosulfur compounds make more appealing in the direction of bactericidal effects through multiple mechanisms and signifies difficult for bacteria to develop resistance. Furthermore, GEO is found to be effective against some other clinical pathogens using vapor phase method, but it has not been examined against any Mycobacterial spp. In view of the rapid rise of antibiotic resistance in TB disease, it is necessary to evaluate the efficacy of essential oil, to know its utilization in vapor phase with desire effect. On contrary, the findings of these results suggest that GEO exhibits remarkable anti-mycobacterial activity in vapor phase, which attributes the presence of organosulfur mixtures causes a potent antimicrobial effect on *M. smegmatis* and *M. tuberculosis* strains. Furthermore, the potential effectiveness of vapor phase through inverted disc and anti-biofilm effect by volatilization approaches shows that it could be a potential source of growth inhibitor in mycobacterial biofilm. Therefore, GEO vapor could be used as a vapor antibiotic and might be used with normal antibiotics as combination therapy mainly for different pulmonary diseases. Although tuberculosis is one of the deadliest pulmonary diseases, this strategy could be a new stratagem to eliminate the tubercular pathogen faster from human civilization.

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7. AUTHORS' CONTRIBUTIONS

Ashirbad Sarangi and Bhabani Shankar Das helped in conceptualization, data curation, formal analysis, validation, writing, and editing of the manuscript. Ambika Kumar Sahoo, Sunil Swick Rout, Sidhartha Giri helped in investigation, validation, visualization, and writing and editing of the manuscript. Debapriya Bhattacharya generate the idea designed, wrote and edited the manuscript.

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9. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interests exist regarding the publication of this paper.

10. ETHICAL APPROVAL

Institutional ethical approval was taken from the ethics committee.

11. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

12. PUBLISHER'S NOTE

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