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Effectiveness of Black Cumin (Nigella Sativa) Seed Extract as an Antibacterial Agent Against Mycobacterium Tuberculosis Using The Resazurin Microtiter Assay (REMA) Method

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Abstract

Tuberculosis remains a major global health problem caused by Mycobacterium tuberculosis. The rising prevalence of multidrug-resistant tuberculosis and the adverse effects associated with prolonged first-line therapy like rifampicin, highlight the need for alternative or adjunctive treatments. Nigella sativa, a medicinal plant rich in bioactive compounds, has attracted considerable attention because of its antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties, which are primarily attributed to its major active compound, thymoquinone. This study aimed to determine the antimicrobial activity and minimum inhibitory concentration of Nigella sativa seed extract against Mycobacterium tuberculosis using the resazurin microtiter assay method. A true experimental study was conducted using the H37Rv strain of Mycobacterium tuberculosis. A methanolic extract of Nigella sativa seeds was prepared using the maceration method. Antibacterial activity was assessed using the resazurin microtiter assay (REMA) after 10 days of incubation. Bacterial growth inhibition was determined through visual observation of resazurin color change and confirmed using a microplate reader at 630 nm. Wells remaining blue indicated inhibition, whereas pink indicated growth. Rifampicin and sterile water were included as positive and negative controls respectively to validate experimental conditions and assay performance consistency throughout the study. Nigella sativa seed extract demonstrated antimicrobial activity with a minimum inhibitory concentration of approximately 0.125 mg/mL (31-62 µg/mL), indicated by the absence of color change and supported by lower absorbance values at 630 nm. Rifampicin showed inhibitory activity at significantly lower concentrations 0.125µg/mL, confirming its higher antimicrobial potency. Nigella sativa seed extract exhibits measurable antibacterial activity against Mycobacterium tuberculosis.

INTRODUCTION

Tuberculosis (TB) remains one of the most serious infectious diseases worldwide and continues to be a major public health concern, particularly in developing countries. According to the Global TB Report 2023, tuberculosis ranked as the second leading cause of death in the world after COVID-19 in 2022, with approximately 4,400 deaths and nearly 30,000 new infections occurring each day despite the disease being preventable and treatable. Indonesia is

among the countries with the highest TB burden worldwide, ranking second after India, with an estimated 1,060,000 new cases and approximately 134,000 deaths annually (World Health Organization, 2023). The increasing number of cases highlights the persistent challenge in TB control, particularly in densely populated urban areas such as Surabaya, where overcrowding, poor ventilation, delayed diagnosis, and limited access to healthcare contribute to rapid disease transmission. Data from the Surabaya Health Office reported approximately 5,800 TB cases as of June 2024 (Patoppoi, 2024). Tuberculosis is caused by Mycobacterium tuberculosis, an acid-fast bacillus transmitted through airborne droplets released when an infected individual coughs, sneezes, or speaks (World Health Organization, 2022). Although the disease primarily affects the lungs, it may also spread to other organs and lead to chronic infection and severe complications.

One of the major challenges in tuberculosis management is the growing emergence of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. MDR-TB is resistant to at least rifampicin and isoniazid, the two most potent first-line anti-TB drugs, while XDR-TB demonstrates additional resistance to second-line agents (Omollo et al., 2021). Approximately 450,000 new MDR-TB cases are reported each year globally. Drug-resistant TB significantly complicates treatment because patients require longer treatment regimens involving more toxic, expensive, and less effective drugs (World Health Organization, 2022).

In recent years, medicinal plants have attracted considerable scientific interest as potential sources of novel antimicrobial compounds. Traditional herbal medicine has been used for centuries in various cultures to prevent and treat human diseases. According to Sultani et al. (2021), approximately 75-80% of the global population still relies on plant-based medicines as part of primary healthcare. Herbal Medicines are particularly important in developing countries because they are relatively affordable, accessible, and culturally accepted.

Among medicinal plants, Nigella sativa, commonly known as black cumin, has received substantial attention due to its broad pharmacological activities. The seed contains numerous biologically active compounds, including thymoquinone, flavonoids, alkaloids, and the therapeutic effects of Nigella sativa, including antibacterial, antioxidant, antitumor, anti-inflammatory, analgesic, antipyretic, bronchodilator, hepatoprotective, Reno protective, antidiabetic, antidiarrheal, spasmolytic, and gastroprotective effects and immunomodulatory activities (Nurchollifah et al., 2021). Several studies have shown that thymoquinone exerts antibacterial activity through multiple mechanisms. One important mechanism involves disruption of bacterial cell membrane integrity, leading to leakage of intracellular components and bacterial cell death. Thymoquinone may also induce oxidative stress by generating reactive oxygen species (ROS), which damage bacterial proteins, lipids, and DNA. In addition, several studies suggest that thymoquinone may inhibit biofilm formation, which plays an important role in bacterial resistance and persistence (Mahmud et al., 2017; Goel and Mishra, 2018).

Further investigation is required to evaluate the effectiveness of Nigella sativa against Mycobacterium tuberculosis using a reliable antimicrobial susceptibility testing methods. One method increasingly used in antimycobacterial research is the Resazurin Microtiter Assay (REMA). Resazurin Microtiter Assay is a simple, rapid, sensitive, cost-effective, and non-radioactive method that is tolerant to plant pigments, oils, and resins, supports bioactivity-guided fractionation, provides both qualitative and quantitative data, enables accurate minimum inhibitory concentration (MIC) detection, and is suitable for testing crude and semi-

purified Nigella sativa extracts (Sarker et al., 2007). The assay utilizes resazurin dye, which changes color based on bacterial metabolic activity. Viable bacteria reduce blue resazurin into pink resorufin, whereas inhibition of bacterial growth prevents the color change (Martin et al., 2003).

Given the increasing global burden of tuberculosis, there is an urgent need to identify alternative therapeutic agents with potential antimycobacterial activity. Although Nigella sativa has demonstrated promising antibacterial, antioxidant, anti-inflammatory, and immunomodulatory properties (Nurchollifah et al., 2021), evidence regarding its effectiveness against Mycobacterium tuberculosis remains limited and requires further investigation. Therefore, the present study aims to evaluate the effectiveness of Nigella sativa seed extract as an antibacterial agent against Mycobacterium tuberculosis using the Resazurin Microtiter Assay (REMA) method and determine its minimum inhibitory concentration. By addressing the limited data regarding plant-based anti-tuberculosis therapies, this study is expected to contribute to the development of alternative or complementary therapeutic strategies for tuberculosis management and support future antimicrobial research involving medicinal plants.

Specifically, this study aims to determine the antimicrobial activity and minimum inhibitory concentration (MIC) of Nigella sativa seed extract against Mycobacterium tuberculosis using the Resazurin Microtiter Assay (REMA) method, as well as compare its antibacterial potential with rifampicin as a positive control. The benefit of this study is that it provides scientific evidence on the potential of Nigella sativa as an antibacterial agent against Mycobacterium tuberculosis, which may support the development of adjuvant therapy for tuberculosis. This research is also expected to serve as a basis for further research on the characterization of active compounds, in vivo assays, and clinical investigations to ensure the efficacy, safety, and therapeutic application of Nigella sativa in the management of tuberculosis.

METHOD

Study design

This study employed a true experimental laboratory design to evaluate the effectiveness of *Nigella sativa* seed extract as an antibacterial agent against *Mycobacterium tuberculosis* using the Resazurin Microtiter Assay (REMA) method. This design is commonly used in antimicrobial susceptibility testing to determine the effectiveness and minimum inhibitory concentration (MIC) of natural compounds against pathogenic microorganisms.

Sterilization of Tools and Materials

Sterilization procedures were performed prior to all experimental setups to prevent contamination. Equipment, including micropipettes, micropipette tips, test tubes, and stirring rods were wrapped in aluminum foil and sterilized using an autoclave at 121°C and 15 psi for 15-20 minutes. Similarly, the Middlebrook 7H9 broth medium was sterilized via autoclaving under these identical conditions before bacterial culture preparation (Rutala and Weber, 2010).

Preparation of Nigella sativa seed extract

Plant extracts were prepared using the maceration process. *Nigella sativa* seeds were thoroughly rinsed with distilled water to remove adhering impurities and shade-dried for 3-5 days at ambient temperature. The cleaned seeds were then coarsely ground into powder form using a mortar and pestle or grinder. The powdered seed material was subsequently soaked in

an extraction solvent consisting of 80% methanol and 20% distilled water at a ratio of 1:10 (v/v %). The extraction process was carried out at room temperature for 24 hours with continuous agitation at 150 rpm. Maceration was performed for 1-3 days and could be extended up to 7 days to maximize extraction efficiency. Following extraction, the solution was filtered using Whatman No. 42 filter paper, and the obtained extract was stored at 4°C until further use (Daoudi *et al.,* 2022).

Preparation of Bacterial Inoculum

The bacterial inoculum was prepared using *Mycobacterium tuberculosis* H37Rv ATCC strain previously cultured on Löwenstein-Jensen medium for 14 to 21 days at 37°C. The cultured bacteria were transferred into fresh Middlebrook 7H9 broth supplemented with 10% ADC (Albumin Dextrose Catalase) or OADC, 0.2% glycerol. The bacterial suspension was vortexed vigorously with sterile glass beads for 3 minutes and incubated at room temperature for 15 to 30 minutes. Following sedimentation, the homogenous upper supernatant was aseptically transferred into a new sterile tube, and the turbidity of the single-cell suspension was adjusted using fresh supplemented Middlebrook 7H9 broth to match a 0.5 McFarland standard, equivalent to approximately 1.5×10^8 CFU/mL (Sawicki *et al.*, 2022)

Preparation of working solution

The solutions used in this study consisted of rifampicin solution as the positive control and *Nigella sativa* seed extract solution as the test sample. Rifampicin concentrations prepared were 16; 8; 4; 2; 1; 0.5; 0.25; 0.125; 0.0625; 0.0312; 0.0156; 0.0078; 0.0039 µg/mL, while *Nigella sativa* seed extract prepared were 10; 5; 1; 0.5; 0.25; 0.125; 0.06; 0.03; 0.01 mg/mL, and 500; 125; 62; 31; 16; 8; 4; 2; 1 µg/mL. The extract solutions were mixed with 1% dimethyl sulfoxide (DMSO) to improve solubility.

Antibacterial activity test using the Resazurin Microtiter Assay method

The antibacterial activity of *Nigella sativa* seed extract against *Mycobacterium tuberculosis* was evaluated using the Resazurin Microtiter Assay method. Initially, 100 µL of supplemented Middlebrook 7H9 broth was dispensed into each well of the microplate, followed by direct two-fold serial dilutions of the *Nigella sativa* seed extract to achieve the desired concentration range. Each well was then inoculated with an equal volume (100 µL) of the standardized mycobacterial suspension. To maintain experimental accuracy and reproducibility, extract concentrations were evaluated in triplicate. To minimize evaporation, the microplate was covered, sealed inside a plastic bag, and incubated at 37°C for 7 days. After this initial incubation phase, 30 µL of freshly prepared resazurin indicator was added to every well, and the plate was incubated overnight at 37°C. Antibacterial activity was evaluated based on color changes observed in the wells. visible color change of the dye from blue (growth inhibition) to pink (viable bacterial growth), and the minimal inhibitory concentration (MIC) was defined as the lowest concentration of the drug that prevented this change in color (Khalifa *et al*., 2013).

Data Analysis

The minimum inhibitory concentration (MIC) values and bacterial growth inhibition were evaluated across the different *Nigella sativa* seed extract concentrations and corresponding control groups. The antibacterial efficacy of the extract was determined based on the lowest concentration capable of completely suppressing mycobacterial growth, as indicated by the retention of the blue dye and the total absence of a color change from blue to

pink. This growth inhibition was initially assessed via visual observation of the resazurin colorimetric transition and subsequently confirmed quantitatively by measuring the absorbance of each well at 630 nm using an automated microplate reader.

Ethical Considerations

Ethical approval for this study was granted by the Health Research Ethics Committee of the Faculty of Medicine, Airlangga University, Surabaya, Indonesia (Approval No. 289/EC/KEPK/FKUA/2025). All laboratory procedures involving *Mycobacterium tuberculosis* were conducted under sterile conditions and strict biosafety regulations at the Institute of Tropical Disease (ITD), Universitas Airlangga, to minimize contamination, ensure experimental reliability, and comply with institutional safety protocols.

RESULTS AND DISCUSSION

After the incubation, the antibacterial activity of Nigella sativa seed extract was analyzed using the REMA method, 18 concentrations ranging from 10 mg/mL down to 0.01 mg/mL and 500 µg/mL down to 1 µg/mL were evaluated in triplicate. The visual assessment of the resazurin color change is documented in Figure 1, whereas the visual interpretation and quantitative validation obtained via microplate reader absorbance measurements are presented in Table 1.

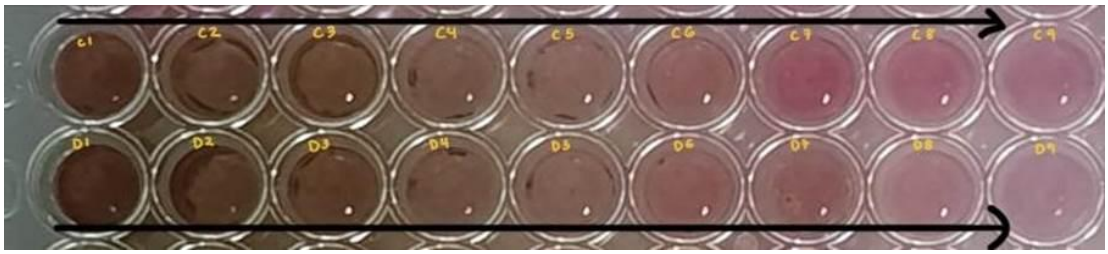

Figure 1. REMA results of Nigella sativa

Table 1. REMA + Microplate reader result interpretation of the Nigella sativa seed extract solution

Well Code	Concentration	Optical Density	Results
C1	10mg/mL	0.218	Inhibited
C2	5mg/mL	0.297	Inhibited
C3	1mg/mL	0.239	Inhibited
C4	0.5mg/mL	0.395	Inhibited
C5	0.25mg/mL	0.437	Inhibited
C6	0.125mg/mL	0.486	Inhibited (MIC)
C7	0.06mg/mL	1.159	Growth
C8	0.03mg/mL	1.481	Growth
C9	0.01mg/mL	1.819	Growth
D1	500µg/mL	0.272	Inhibited
D2	125µg/mL	0.303	Inhibited
D3	62µg/mL	0.532	Inhibited (MIC)
D4	31µg/mL	0.651	Transition (MIC)
D5	16µg/mL	1.353	Growth
D6	8µg/mL	1.643	Growth
D7	4µg/mL	1.86	Growth
D8	2µg/mL	2.193	Growth
D9	1µg/mL	2.862	Growth

The minimum inhibitory concentration (MIC) was determined to be approximately 0.125 mg/mL, which corresponds to a range of 31-62 µg/mL. This concentration was identified as the lowest at which no visible color change from blue to pink occurred, indicating effective inhibition of microbial growth.

The rifampicin, which served as the positive control, was analyzed using the REMA method, the antibiotic was evaluated across a concentration range extending from 16 µg/mL down to 0.0039 µg/mL. The visual assessment of the resazurin color change is documented in Figure 2, whereas the interpretation and quantitative validation obtained via microplate reader absorbance measurements at 630 nm are presented in Table 2.

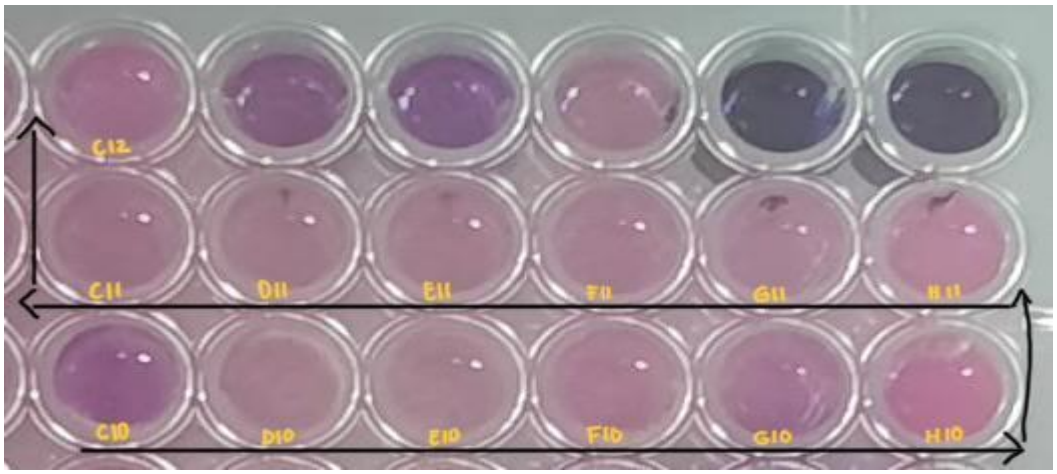

Figure 2. REMA results of rifampicin

Table 2. REMA + Microplate reader result interpretation of Rifampicin

Well Code	Concentration	Optical Density	Results
C10	16µg/mL	0.219	Inhibited
D10	8µg/mL	0.242	Inhibited
E10	4µg/mL	0.276	Inhibited
F10	2µg/mL	0.313	Inhibited
G10	1µg/mL	0.365	Inhibited
H10	0.5µg/mL	0.394	Inhibited
H11	0.25µg/mL	0.375	Inhibited
G11	0.125µg/mL	0.459	Inhibited (MIC)
F11	0.0625µg/mL	0.896	Growth
E11	0.0312 g/mL	1.216	Growth
D11	0.0156µg/mL	1.648	Growth
C11	0.0078µg/mL	2.114	Growth
C12	0.0039µg/mL	2.227	Growth

The minimum inhibitory concentration (MIC) was determined to be approximately 0.125 µg/mL, as evidenced by the retention of the original coloration without transition to pink, indicating effective inhibition of microbial growth. The rifampicin possessed greater antibacterial potency than the Nigella sativa seed extract. Nevertheless, the plant extract exhibited measurable inhibitory activity against Mycobacterium tuberculosis, indicating the presence of bioactive compounds with potential antibacterial properties.

Antibacterial Activity of Nigella sativa Against Mycobacterium tuberculosis

The present study demonstrated that Nigella sativa seed extract exhibited inhibitory activity against Mycobacterium tuberculosis, as evidenced by the results of the Resazurin Microtiter Assay (REMA). The minimum inhibitory concentration (MIC) of Nigella sativa seed extract was determined to be 0.125 mg/mL, approximately (31-62ug/mL), indicated by the persistence of blue coloration in the assay wells and supported by a microplate reader at 630 nm. These findings confirm that Nigella sativa possesses measurable antibacterial activity and support its potential role as a source of natural compounds with therapeutic relevance in tuberculosis management.

The antibacterial effect observed in this study is likely associated with thymoquinone, the principal bioactive constituent of Nigella sativa. Thymoquinone has been reported to exhibit antimicrobial activity through several mechanisms, including disruption of bacterial cell membrane integrity, induction of oxidative stress, inhibition of biofilm formation, and interference with essential metabolic pathways (Hannan et al., 2021; Abbas et al., 2024). Since the cell wall of Mycobacterium tuberculosis is rich in mycolic acids and contributes significantly to bacterial survival and drug resistance, disruption of membrane permeability by thymoquinone may impair bacterial viability and inhibit growth. Mahmud et al. (2017) further reported that thymoquinone increases membrane permeability, resulting in leakage of intracellular contents and subsequent bacterial death.

An additional factor that may explain the antibacterial activity observed in this study is the phytochemical composition of the Nigella sativa seeds used. Previous studies have demonstrated considerable variation in thymoquinone concentration among Nigella sativa samples originating from different geographical regions. Comparative analyses reported that seeds cultivated in China contain approximately 21.01% thymoquinone (Albakry et al., 2022), while samples from Bangladesh contain concentrations ranging from 29.77% to 39.52%, and samples from India contain lower concentrations ranging from 10.27% to 27.51% (Kabir et al., 2020). Similarly, Nigella sativa from Habasyah (Ethiopia) was reported to contain approximately 39.52% thymoquinone (Mahfur, 2018). In contrast, Nigella sativa from Indonesia has been reported to contain thymoquinone levels ranging from 27.8% to 57%, representing one of the highest concentrations among the countries evaluated (Webadmin, 2021). Higher thymoquinone levels may contribute to stronger bacterial growth inhibition and lower minimum inhibitory concentration (MIC) values against Mycobacterium tuberculosis. Since thymoquinone is recognized as the principal bioactive compound responsible for the antibacterial, antioxidant, and anti-inflammatory properties of Nigella sativa seed, variations in its concentration may directly influence the antibacterial potency of the extract. Therefore, the relatively high thymoquinone content reported in Nigella sativa from Indonesia may partly explain the inhibitory activity observed in the present study.

Comparison with Previous Studies and Potential Mechanisms

The findings of this study are consistent with previous investigations reporting the antibacterial activity of Nigella sativa and its active constituents. Randhawa (2011) suggested that black cumin possesses significant therapeutic potential in tuberculosis due to its antimicrobial and immunomodulatory properties. Likewise, Abbas et al. (2024) demonstrated that thymoquinone administration reduced bacterial burden and improved histopathological outcomes in experimental tuberculosis models. Similar inhibitory effects against both drug-

sensitive and multidrug-resistant Mycobacterium tuberculosis strains have also been reported in previous in vitro studies.

The consistency between the present findings and previous reports strengthens the evidence supporting the antibacterial potential of Nigella sativa seed extract. Although MIC values have been reported to vary across studies, these differences may be attributed to variations in extraction methods, solvent polarity, phytochemical composition, bacterial strains, and experimental conditions. Nevertheless, the collective evidence indicates that thymoquinone-containing extracts possess biologically relevant inhibitory effects against Mycobacterium tuberculosis.

The REMA method employed in this study proved to be a reliable and effective approach for evaluating the antibacterial activity of Nigella sativa seed extract. As a colorimetric assay based on bacterial metabolic activity, REMA is highly sensitive and can detect even minimal growth inhibition. The method offers several advantages, including rapid turnaround time, low cost, simplicity, and suitability for screening natural products. In comparison with conventional culture techniques for M. tuberculosis, which may require several weeks to produce results, REMA can provide susceptibility data within a much shorter period while simultaneously allowing accurate determination of MIC values. The incorporation of spectrophotometric measurements at 630 nm in the present study further improved the objectivity and reproducibility of the results by providing quantitative confirmation of visual observations. The consistency between visual color changes and absorbance readings supports the reliability of the assay and strengthens confidence in the MIC values obtained.

Clinical Implications and Comparison with Rifampicin

Although Nigella sativa seed extract demonstrated measurable antibacterial activity, its potency remained substantially lower than that of rifampicin. In the present study, rifampicin exhibited an MIC of 0.125 µg/mL, whereas Nigella sativa seed extract required 0.125 mg/mL approximately (31-62µg/mL) to achieve bacterial inhibition. This difference is expected because rifampicin acts through a highly specific mechanism involving inhibition of DNA-dependent RNA polymerase, thereby directly suppressing bacterial RNA synthesis (Anderson et al., 2012). Previous studies have reported rifampicin MIC values ranging from 0.002 to 0.006 µg/mL against susceptible Mycobacterium tuberculosis strains (Dookie et al., 2018; Kapp et al., 2021), highlighting its superior efficacy as a first-line anti-tuberculosis drug.

Despite its lower antimicrobial potency, the significance of Nigella sativa seed extract may extend beyond direct bacterial inhibition. Previous studies have demonstrated that Nigella sativa seed extract possesses antioxidant, anti-inflammatory, immunomodulatory, and hepatoprotective properties (Nurchollifah et al., 2021). These characteristics are particularly relevant in tuberculosis treatment because prolonged anti-tuberculosis therapy is frequently associated with adverse drug reactions, especially hepatotoxicity.

In addition, thymoquinone has been shown to stimulate macrophage activity, enhance natural killer cell function, and increase the production of cytokines involved in host defense against Mycobacterium tuberculosis, including interferon-γ and interleukin-12 (Salem, 2005; Majdalawieh & Fayyad, 2015).

Nigella sativa are generally considered to possess low toxicity and are relatively safe for human consumption at traditional dietary doses. Common oral doses of whole Nigella sativa seeds range from approximately 1-3 g/day, while seed oil is commonly administered at

doses of 1-5 mL/day in adults. Animal studies and limited human data have demonstrated a wide safety margin with no consistent evidence of significant organ damage at typical intake levels. Compared with concentrated extracts or isolated compounds, whole seeds are regarded as the safest commonly used form due to their lower overall toxicity risk. Adverse effects are uncommon but may occur at high or concentrated doses, including mild gastrointestinal irritation such as nausea, vomiting, diarrhea, abdominal discomfort, and heartburn. In addition, rare hypersensitivity reactions, including dermatitis and severe allergic skin reactions, have been documented. Animal toxicity studies demonstrated that thymoquinone doses above 50 mg/kg body weight may increase the risk of toxicity, whereas lower doses were generally well tolerated. Despite its favorable safety profile, caution is still advised (Jantz et al., 2025; Ramadani, 2016). Nevertheless, herbal medicines should generally be consumed to maintain health, support recovery from illness, or reduce the risk of disease rather than being considered to cure illness. Therefore, herbal medicines, including Nigella sativa seed extract, are more appropriately regarded as complementary rather than replacement therapies for conventional chemical drugs. Given its immunomodulatory and antimicrobial activities, Nigella sativa seed extract may provide meaningful therapeutic benefits as an adjunctive treatment by enhancing bacterial clearance, reducing excessive inflammatory responses, and potentially improving overall clinical outcomes when used alongside standard anti-tuberculosis therapy.

Overall, the findings of this study demonstrate that Nigella sativa seed extract possesses measurable antibacterial activity against Mycobacterium tuberculosis. With an MIC of 0.125 mg/mL approximately (31-62µg/mL), its antibacterial potency remains lower than that of rifampicin. The combination of antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, and hepatoprotective properties suggests that Nigella sativa seed extract may have potential as an adjunctive therapeutic agent in tuberculosis management. These findings contribute to the growing body of evidence supporting medicinal plants as complementary approaches in combating tuberculosis and provide a foundation for future investigations into plant-derived anti-tuberculosis therapies.

CONCLUSION

The findings of this study indicate that Nigella sativa seed extract exhibits measurable antibacterial activity against Mycobacterium tuberculosis using the REMA method at a minimum inhibitory concentration (MIC) of 0.125 mg/mL approximately (31-62µg/mL). The observed antibacterial activity is likely associated with thymoquinone, the principal active constituent of Nigella sativa. Previous studies have reported that Nigella sativa from Indonesia contains relatively high thymoquinone concentrations (27.8-57%) compared with samples from several other geographical regions. Given that thymoquinone is the major compound responsible for the antibacterial activity of Nigella sativa, the Nigella sativa from Indonesia may have a greater potential as a source of anti-tuberculosis agents. Although the Nigella sativa seed extract demonstrated lower antibacterial potency than rifampicin, its combined antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, and hepatoprotective properties suggest considerable promise as an adjunctive therapy in tuberculosis treatment. Further studies involving active compound characterization, in vivo evaluation, and clinical investigation are warranted to confirm its efficacy, safety, and therapeutic applicability in

tuberculosis management not contain vertical lines, while horizontal lines are allowed but only for essential lines.

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