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Exploring *Aloe vera* (L.) Burm.f. as a Source of Antimycobacterial Agents: Phytochemical and *In-vitro* Assessment

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ABSTRACT

Tuberculosis (TB) and related mycobacterial infections remain major global health concerns, necessitating the search for novel antimycobacterial agents. *Aloe vera*, a widely used medicinal plant, contains a variety of bioactive phytochemicals with potential therapeutic properties. This study aimed to evaluate the phytochemical composition and antimycobacterial activity of the ethanolic crude extract of *Aloe vera* against *Mycobacterium tuberculosis*, *Mycobacterium bovis*, and *Mycobacterium smegmatis*. Ethanolic extract of *Aloe vera* leaves were prepared and subjected to qualitative phytochemical screening. The antimycobacterial efficacy of the extracts was assessed using standard broth microdilution techniques to determine the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against the selected mycobacterial strains. Phytochemical analysis revealed the presence of flavonoids, alkaloids, saponins, tannins, phenols, and steroids. The extract exhibited antimycobacterial activity with MIC values of 1560 µg/mL ($p<0.05$) for *M. bovis* and *M. smegmatis*, and 6250 µg/mL for *M. tuberculosis*. MBC values were 3125 µg/mL ($p<0.05$) for *M. bovis* and *M. smegmatis*, and 12500 µg/mL for *M. tuberculosis*. The results support the traditional use of *Aloe vera* in treating infections and highlight the potential of its bioactive constituents as leads for future antitubercular drug development. Further studies involving compound isolation and mechanism-based assays are recommended.

Keywords: *Aloe vera*, antituberculosis, *M. tuberculosis*, phytochemicals, MIC, MBC

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INTRODUCTION

Tuberculosis (TB) remains one of the most significant global public health threats, caused primarily by *M. tuberculosis*. According to the World Health Organization [1], TB was responsible for 1.3 million deaths among HIV-negative individuals and 167,000 deaths among people living with HIV in 2022. The emergence and spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tuberculosis* have further complicated TB control efforts, underscoring the urgent need for novel, affordable, and effective antimycobacterial agents [2]. In light of challenges like drug resistance and the adverse effects of current TB treatments, there is increasing interest in natural products especially medicinal plants as promising sources for new anti-TB agents [3].

Humans have relied on herbs and plant-based remedies since ancient times to enhance immunity and treat ailments such as colds, joint pain, and fever [4]. Medicinal plants have long served as a valuable source of therapeutic compounds, particularly in the treatment of infectious diseases. Phytochemicals such as alkaloids, flavonoids, tannins, saponins, and phenolics play essential roles in the antimicrobial efficacy of plant-derived treatments [5]. These bioactive compounds have demonstrated diverse mechanisms of action against pathogens, including disruption of microbial cell walls, inhibition of nucleic acid synthesis, and modulation of host immune responses [6, 7].

Aloe vera (*Aloe barbadensis Miller*) is a widely used medicinal plant with established antimicrobial, anti-inflammatory, and immunomodulatory properties. Several studies have confirmed the presence of pharmacologically active compounds in *Aloe vera*, including anthraquinones (e.g., aloe-emodin), flavonoids, and polysaccharides, which contribute to its therapeutic potential [8, 9]. Previous research has shown that *Aloe vera* extracts possess antibacterial and antifungal activity, including efficacy against mycobacterial species [10, 11].

In the search for alternative therapies against TB and related mycobacterial infections, *M. smegmatis* is often employed as a non-pathogenic model due to its genetic and structural similarities to *M. tuberculosis*, as well as its rapid growth rate [12]. Additionally, *M. bovis*, the causative agent of bovine TB, shares over 99% genomic similarity with *M. tuberculosis* and poses zoonotic risks [13]. This study aims to analyze the phytochemical constituents of the ethanolic crude extract of *Aloe vera* and to determine its *in-vitro* antimycobacterial activity against *M. tuberculosis*, *M. bovis*, and *M. smegmatis*. The findings will contribute to the growing body of evidence supporting the use of plant-based therapies in managing drug-resistant TB infections and serve a s a lead for research and development of new drug for tuberculosis related diseases.

MATERIALS AND METHODS

Plant Collection and Preparation

Fresh *Aloe vera* plant leaves were collected in February, 2023 from a garden in Abuja Municipal Area Council, Abuja, Federal Capital Territory. *Aloe vera* leaves identification and authentication was done at the Herbarium unit of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The leaves were washed with distilled water, sliced and dried in a shaded area to prevent direct sunlight from deteriorating some of the plant's components. The dried leaves were pulverized to a powder form using a mortar and pestle. The powdered sample was weighed using an analytical scale and kept at room temperature until needed.

Crude Extract Preparation

A total of 500 g of the powdered sample was transferred to a 2000 mL flask and macerated in ethanol until the sample was completely submerged. Maceration was performed for 72 hours with periodic shaking to ensure exhaustive extraction of the crude extract. The crude extract was filtered using Whatman No. 1 filter paper, and the resulting solution was concentrated using a rotary evaporator at 40 °C. The crude extract was stored in an airtight container at 4 °C until needed.

Qualitative Phytochemical analysis

Qualitative screening of alkaloids, flavonoids, tannins, saponins, phenols and steroids in the *Aloe vera* crude extract was performed according to previous studies following standard procedures [14, 15, 2].

Test Organisms

Clinical *M. tuberculosis (*H37Rv*)* isolate was obtained from the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. *M*ycobacterium *bovis* (BCG) (ATCC 27290) and *M*ycobacterium *smegmatis* (ATCC 607) supplied by Microbiologics, (200 Cooper Avenue North, St Cloud, MN 56303, United States) were also used in this study.

Inoculum Preparation

An inoculum of the test organisms (*M. tuberculosis, M. bovis*, and *M. smegmatis*) was prepared by inoculating each into Middlebrook 7H9 broth supplemented with Albumin Dextrose Catalase (ADC). The cultures were incubated aerobically at 37 °C in a shaking incubator for 7–14 days, with daily observation during the incubation period. Growth was monitored until an optical density (OD) of 0.2–0.3 at 600 nm was achieved using a UV spectrophotometer, corresponding to approximately 10^7 CFU/mL. The cultures were then diluted 1:1000 by adding 50 µL of the culture to 50 mL of fresh 7H9/ADC broth.

Determination of Antituberculosis Activity

The MIC values of the crude extract was determined using the microbroth dilution method, as described by [2]. The susceptibility test was performed in 96 wells microtiter plate using tetrazolium dye as an indicator of cell viability or growth inhibition. Approximately 200 mg of the extract was dissolved in 0.5 mL Dimethyl sulfoxide (DMSO) and 0.5 mL sterile Middlebrook 7H9/ADC broth to give a stock concentration of 200 mg/mL. To prepare the microtiter plate, 50 µL of Middlebrook 7H9/ADC broth was added to wells 2–12. In well 1, 100 µL of the extract stock solution was added, followed by a two-fold serial dilution across wells 2 to 11. Each well (1–12) was then inoculated with 50 µL of the standardized bacterial suspension. The final row (well 12), which did not contain extract, served as the organism viability control (containing only Middlebrook 7H9 broth and the microorganism). The positive control, rifampicin (25 µg/mL; Sigma-Aldrich Inc.), was prepared by dissolving 250 mg of the drug in 10 mL of dimethyl sulfoxide (DMSO), followed by dilution of 25 µL of this stock solution in 25 mL of Middlebrook 7H9 broth. Negative controls included wells containing Middlebrook 7H9 broth alone and those with the organism viability control. Each microtiter plate was incubated aerobically at 37 °C for 5–7 days. After incubation, 25 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well, and the plates were re-incubated at 37 °C for 24 hours. MIC was determined as the lowest concentration of the extract that inhibited color change, where a change in color indicated observable bacterial growth. The test was performed in triplicate.

Minimum Bactericidal Concentration Determination

The MBC of the extract was determined based on the MIC results, following the method described by [2]. The neutralization technique was used by transferring 50 µL from the well preceding the MIC (i.e., the well with the lowest concentration showing visible growth) into 0.5 mL of polysorbate 20 to neutralize residual antimicrobial activity. The mixture was then inoculated into freshly prepared Middlebrook 7H9 broth supplemented with ADC and incubated aerobically at 37 °C for 5–7 days. The absence of turbidity in the broth after incubation indicated a bactericidal effect.

Data Analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation. Data analysis was conducted using GraphPad Prism version 8.0. Differences between means were assessed using two-way ANOVA, followed by Bonferroni post hoc test for multiple comparisons.

RESULTS

The qualitative phytochemical compositions of the ethanolic crude extract of *Aloe vera* leaves are

presented in Table 1. Bioactive constituents such as flavonoids, tannins, alkaloids, saponins, phenols, and steroids were all present in the ethanolic crude extract of the plant (Table 1).

Table1: Qualitative Phytochemical Analysis of *Aloe vera* Crude extract

Phytochemicals	**Ethanol Extract**
Flavonoids	+
Tannins	+
Alkaloids	+
Saponins	+
Phenols	+
Steroids	+

Key: (+) Present

Table 2 shows the MIC values of the crude extracts against *M. tuberculosis*, *M. bovis*, and *M. smegmatis*. The ethanolic crude extract exhibited varying degrees of inhibitory activities ranging between 1560 – 6250 µg/mL. The crude extract had inhibitory effect against *M. bovis* and *M. smegmatis* at concentration of 1560 µg/mL. The extract was also active against *M. tuberculosis* at concentration of 6250 µg/mL**.** Rifampicin (control drug) inhibited the growth of *M. tuberculosis*, *M. bovis*, and *M. smegmatis* at concentrations of 0.02, 0.02, and 0.05 µg/mL, respectively**.**

Table 2: MIC of *Aloe vera* Crude Extract against the Different Strains of Mycobacterial.

Strains	**MIC ± SD (µg/mL)**	**Rifampicin (µg/mL)**
M. tuberculosis H37Rv	6250 ± 0.00	0.02 ± 0.00
M. bovis	1560 ± 0.00	0.02 ± 0.00
M. smegmatis	1560 ± 0.00	0.05 ± 0.00

Data are represented as Mean ± SD (n = 3); SD = ±0.00 for all values; $p<0.05$ represent significant difference across groups (microorganisms).

Table 3 revealed the MBC values of the crude extracts against *M. tuberculosis*, *M. bovis*, and *M. smegmatis*. The crude extract was found to possess MBC against the test organisms with activities ranging between 3125 - 12500 µg/ml. The extract exhibited MBC against *M. bovis* and *M. smegmatis* at 3125 µg/mL concentration. Additionally, the extract showed bactericidal concentration against *M. tuberculosis* at 12500 µg/mL. Rifampicin was effective against *M. tuberculosis*, *M. bovis*, and *M. smegmatis* with MBCs of 0.01, 0.01, and 0.02 µg/mL, respectively**.**

Table 3: MBC of *Aloe vera* Crude Extract against the Different Strains of Mycobacterial.

Strains	**MBC ± SD (µg/mL)**	**Rifampicin (µg/mL)**
M. tuberculosis H37Rv	12500 ± 0.00	0.01 ± 0.00
M. bovis	3125 ± 0.00	0.01 ± 0.00
M. smegmatis	3125 ± 0.00	0.02 ± 0.00

Data are represented as Mean ± SD (n = 3); SD = ±0.00 for all values; $p<0.05$ represent significant difference across groups (microorganisms).

DISCUSSION

Phytochemicals play crucial roles in the bioactivity of medicinal plants. The therapeutic properties of such plants are largely attributed to the presence of various bioactive compounds [5]. The qualitative phytochemical analysis in this study revealed the presence of flavonoids, tannins, alkaloids, saponins, phenols, and steroids in the ethanolic crude extract of *Aloe vera*. This finding is consistent with the reports of [9] and [5], who also documented similar phytoconstituents in *Aloe vera* extracts.

Flavonoids, a diverse class of polyphenolic compounds, have been widely studied for their pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, and antituberculosis activities. They have been reported to inhibit enzymes critical for mycobacterial cell wall biosynthesis [16], and to enhance host immunity by stimulating macrophage activity and cytokine production [17]. Alkaloids, nitrogen-containing compounds found abundantly in medicinal plants, also exhibit potent antimycobacterial activity. Berberine, for instance, disrupts mycolic acid synthesis in *M. tuberculosis* [18], while other alkaloids induce oxidative stress through reactive oxygen species (ROS), damaging bacterial membranes [19].

Phenolic compounds (phenols) destabilize the lipid-rich cell walls of mycobacteria, increasing membrane permeability and leading to cell death [20]. Saponins, natural glycosides, exhibit immunomodulatory and antimicrobial activities. Their ability to stimulate macrophage activation and cytokine release may aid in mycobacterial clearance [21]. Tannins, high-molecular-weight polyphenols, can alter membrane permeability by binding to bacterial wall proteins and lipids, causing leakage of intracellular contents [22].

This study showed that the ethanolic crude extract of *Aloe vera* exhibited antimycobacterial activity against *M. tuberculosis*, *M. bovis*, and *M. smegmatis*, though with varying degrees of efficacy. The MIC values revealed that *M. bovis* and *M. smegmatis* were inhibited at a lower concentration of 1560 µg/mL ($p<0.05$), while *M. tuberculosis* required a higher concentration (6250 µg/mL). Similarly, the extract exhibited bactericidal effect against *M. bovis* and *M. smegmatis* at 3125 µg/mL ($p<0.05$), and *M. tuberculosis* at 12500 µg/mL. These differences may be attributed to the more complex lipid-rich cell wall of *M. tuberculosis*, which provides intrinsic resistance to many antimicrobial agents [23]. The observed MIC value for *M. tuberculosis* (6250 µg/mL) aligns with findings by [11], who reported MIC values of 5000–7000 µg/mL for *Aloe vera* extract against multidrug-resistant *M. tuberculosis* strains. [24], also documented MIC values of 5, 10, and 2.5 mg/mL for *Aloe vera* against *M. tuberculosis* H37Rv, *M. smegmatis*, and *M. tuberculosis* H37Ra, respectively.

Mycobacterium smegmatis, though non-pathogenic, is widely used as a surrogate for *M. tuberculosis* in early antimycobacterial screening due to its comparable cell wall architecture and rapid growth [12]. The sensitivity of *M. smegmatis* observed in this study aligns with earlier findings by [25], who reported activity of *Aloe vera* juice, and [26], who demonstrated the effectiveness of the methanolic extract against this organism.

Furthermore, [8] reported that aloe-emodin, a major anthraquinone in *Aloe vera*, exhibited synergistic antimycobacterial activity against *M. bovis* when combined with vancomycin, achieving MICs of 12.5-25 µg/mL. This suggests that isolated compounds from *Aloe vera* may offer stronger antimycobacterial potential than crude extracts, highlighting the need for further fractionation and compound-specific investigations. In contrast, the standard drug rifampicin showed significantly higher potency, with MICs of 0.02 µg/mL for *M. tuberculosis* and *M. bovis*, and 0.05 µg/mL for *M. smegmatis*. This dramatic difference in activity emphasizes the need for bioassay-guided fractionation to concentrate and identify the active constituents within the extract.

CONCLUSION

The ethanolic crude extract of *Aloe vera* demonstrated moderate but significant antimycobacterial activity against both pathogenic and non-pathogenic mycobacterial strains. The presence of key phytochemicals such as flavonoids, alkaloids, saponins, phenols, steroids, and tannins likely contributes to this activity. While the crude extract showed promising effects, particularly against *M. bovis* and *M. smegmatis*, the higher MIC required for *M. tuberculosis* underscores the pathogen's intrinsic resistance mechanisms. These findings support the traditional use of *Aloe vera* and warrant further studies to isolate and characterize its active constituents for potential development into effective anti-TB agents.

CONFLICTS OF INTEREST: The article has no conflict of interest.

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