

“A Comparative Study on the Anti-Tubercular Activity of Extracts from *Aloe vera*, *Ocimum tenuiflorum*, and *Martynia annua L*”

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ABSTRACT

Tuberculosis remains a leading cause of morbidity and mortality globally, with Asia and Africa collectively accounting for approximately 55% of the world's TB burden. India has the highest rate of TB infection, representing about one-fifth of all new cases annually. The mismanagement of approved TB treatments has led to the emergence of drug-resistant strains of *Mycobacterium tuberculosis*, such as multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, complicating treatment efforts, particularly in developing countries. This study aimed to evaluate the anti-tubercular activity of methanol extracts from three plants *Aloe vera*, *Ocimum tenuiflorum*, and *Martynia annua L*. against *Mycobacterium smegmatis* (ATCC 607, Lot 114-26-4). The extracts were assessed using the zone of inhibition method, with concentrations set at 10 mg/mL, 50 mg/mL, and 100 mg/mL. The results demonstrated that *Martynia annua L*. exhibited the most significant anti-tubercular activity, showing a dose-dependent increase in the zone of inhibition, which reached up to 19.0 mm at the highest concentration. Meanwhile, *Aloe vera* displayed moderate activity, with zones of inhibition increasing from 8.5 mm at 10 mg/mL to 12.5 mm at 100 mg/mL. In contrast, *Ocimum tenuiflorum* showed the least efficacy, with a maximum inhibition zone of only 9.1 mm at 100 mg/mL. These findings suggest that the bioactive compounds present in these plant extracts may have therapeutic potential for developing new treatments against tuberculosis, highlighting the importance of integrating traditional knowledge with scientific research to advance plant-based therapeutic strategies.

Keywords: Tuberculosis, *Mycobacterium smegmatis*, *Martynia annua L*., anti-tubercular activity.

Introduction

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis*, continues to be a leading cause of death from infectious diseases worldwide, with millions of new cases reported annually (World Health Organization, 2022). The rise of multi-drug resistant and extensively drug-resistant TB poses significant challenges to global health, emphasizing the urgent need for new therapeutic strategies and agents (Lawn & Zumla, 2011). In light of these challenges, researchers are increasingly turning to ethnobotanical sources for potential anti-tubercular compounds due to their varied bioactive properties and historical use in traditional medicine. Among various plant species known for their medicinal uses, *Aloe vera*, *Ocimum tenuiflorum* (commonly referred to as holy basil), and *Martynia annua L*. stand out for their purported health benefits and widespread traditional utilization. *Aloe vera* is extensively used in both folk and conventional medicine, renowned for its

antimicrobial, anti-inflammatory, and immunomodulatory properties (Reynolds & Dweck, 1999). The active components of *Aloe vera*, such as aloin, aloin-emodin, and acemannan, have been studied for various therapeutic purposes, including anti-bacterial effects (Boudreau & Beland, 2006). *Ocimum tenuiflorum*, also known as Tulsi in traditional Indian medicine, is widely recognized for its adaptogenic, antimicrobial, and anti-inflammatory properties (Pattanayaket al., 2010). Its bioactive compounds, such as eugenol, ursolic acid, and rosmarinic acid, contribute to its wide range of therapeutic effects, including potential anti-tubercular activity. Studies have shown that extracts from *Ocimum* species can exhibit significant antimicrobial properties, supporting their potential use in combatting infectious diseases, including TB (Mondalet al., 2009). *Martynia annua* L., while less extensively documented in scientific literature, has been reported to possess noteworthy antimicrobial properties. Traditional medicinal practices have utilized *M. annua* for its claimed health benefits, which include treating skin diseases and infections, suggesting a possibility for its extracts to exert anti-tubercular effects. The presence of bioactive compounds such as iridoids and flavonoids in *Martynia* suggests a need for detailed investigation into their potential as therapeutic agents (Hussainet al., 2021). The present study aims to evaluate and compare the anti-tubercular efficacy of extracts from *Aloe vera*, *Ocimum tenuiflorum*, and *Martynia annua* L. Through a systematic approach involving diverse extraction techniques and in vitro assays, this research seeks to identify plant-derived compounds that could act as leads in the development of alternative TB therapies (Dhingraet al., 2020). The comparative analysis of these plant extracts could not only enhance our understanding of their bioactive potential against *Mycobacterium tuberculosis* but also contribute significantly to the field of natural product pharmacology, offering viable options to expand the current arsenal against TB. Ultimately, by integrating scientific methodology with traditional knowledge, this study aspires to pave the way for novel, plant-based interventions that address the pressing TB treatment challenges faced globally, especially in areas burdened by drug-resistant strains.

Materials and Methods

Collection and Preparation of Plant Samples for Downstream Processing: Plant samples, including *Aloe vera*, *Ocimum tenuiflorum*, and *Martynia annua* L., were harvested from the Bhopal district in Madhya Pradesh. The collected plant materials underwent thorough cleaning, being washed 2-3 times with running tap water to remove impurities. The cleaned samples were then left to dry in the shade for 3-4 weeks to prevent degradation due to direct sunlight. Once dried, the stems/Leaves were chopped into smaller pieces and ground using a grinder. Subsequently, the ground plant material was stored in labeled bottles and weighed precisely using an electronic balance. **Extraction of Phytochemicals Using a Soxhlet Extractor:** The processed plant material underwent a two-part extraction process. Initially, a cold extraction using N-hexane was conducted, followed by hot extraction using a Soxhlet extractor with methanol and water as solvents. Each solvent extraction was carried out for duration of 48 hours to ensure maximum yield of phytochemicals. After extraction, the crude extracts were filtered through filter paper and concentrated via vacuum evaporation to remove excess solvents.

Evaluation of Anti-Tubercular Activity: The anti-tubercular activity of the crude extracts was assessed using *Mycobacterium smegmatis* (ATCC 607, Lot 114-26-4), acquired from the American Type Culture Collection. The bacterial strain was cultured on a Middlebrook 7H9 agar plate. The methanol extract were tested for their efficacy against *Mycobacterium smegmatis*, allowing for the evaluation of their potential antimicrobial properties.

Results and Discussion

This study aimed to evaluate the anti-tubercular activity of methanol extracts from three plants: *Aloe vera*, *Ocimum tenuiflorum*, and *Martynia annua L.* against *Mycobacterium smegmatis*. The efficacy of these extracts was assessed using the zone of inhibition method, along with determining the minimum inhibitory concentration (MIC). The findings revealed distinct differences in the antimicrobial potency of each plant extract, providing important insights into their potential therapeutic applications.

Table 1: Comparative Anti-Tubercular Activity of Methanol Extracts from *Aloe vera*, *Ocimum tenuiflorum*, and *Martynia annua L.* Against *Mycobacterium smegmatis*

S. No.	Methanol Extract	Concentration (mg/mL)	Zone of Inhibition (mm)
1	<i>Martynia annua L.</i>	10	11.3
		50	15.1
		100	19.0
2	<i>Aloe vera</i>	10	8.5
		50	10.3
		100	12.5
3	<i>Ocimum tenuiflorum</i>	10	7.3
		50	8.6
		100	9.1

The methanol extract of *Martynia annua L.* exhibited the strongest anti-tubercular activity among the tested extracts, demonstrating a clear dose-dependent increase in the zone of inhibition (Dhingra *et al.*, 2013). At a concentration of 10 mg/mL, the extract produced a zone of inhibition measuring 11.3 mm. As the concentration increased to 50 mg/mL, the zone expanded to 15.1 mm, and at the highest concentration of 100 mg/mL, it reached 19.0 mm. This trend indicates the presence of potent bioactive compounds within *Martynia annua L.*, suggesting its promise for further study and potential use in anti-tubercular treatments. In contrast, *Aloe vera* displayed moderate anti-tubercular activity (Iqbal *et al.*, 2021). The zone of inhibition increased from 8.5 mm at 10 mg/mL to 10.3 mm at 50 mg/mL, and finally to 12.5 mm at 100 mg/mL. Although effective, these results suggest that *Aloe vera* has a lower potency compared to *Martynia annua L.*, yet it remains a viable candidate for potential antimicrobial use, especially at higher concentrations or in combination with other agents. *Ocimum tenuiflorum* showed the least anti-tubercular activity among the three extracts (Jayapal *et al.*, 2021). The zone of inhibition was initially 7.3 mm at 10 mg/mL. Interestingly, there was a slight decrease to 8.6 mm at 50 mg/mL, but it improved to 9.1 mm at 100 mg/mL. This variability highlights the need to optimize extraction methods or consider synergistic use with other extracts to enhance its efficacy. The varying zones of inhibition across the extracts underscore the different levels of anti-tubercular efficacy inherent in these plant extracts. The study emphasizes the potential of *Martynia annua L.* as a strong candidate for further research into plant-derived anti-tubercular agents. Additionally, isolating and identifying the specific bioactive compounds responsible for these effects could lead to significant advancements in developing targeted treatments for infections caused by *Mycobacterium smegmatis*. Future studies should focus on these aspects to fully realize the therapeutic potential of these plant extracts.

Conclusion

This study highlights the substantial anti-tubercular potential of methanol extracts from *Martynia annua* L., *Aloe vera*, and *Ocimum tenuiflorum*. The pronounced efficacy of *Martynia annua* L. suggests it could be a valuable candidate for further exploration in the development of alternative therapies for tuberculosis, especially in the face of rising drug resistance. While *Aloe vera* shows promise and *Ocimum tenuiflorum* demonstrates lesser activity, the insights gained from this research underline the significance of plant-derived compounds in addressing global health challenges related to tuberculosis. Future investigations should focus on isolating the active bioactive constituents within these extracts and elucidating their mechanisms of action, thereby supporting the advancement of effective, plant-based interventions for TB management.

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Conflict of Interest: None

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