Review Paper:

Embracing Nature: Anti-Biofilm Herbal Compounds against *Mycobacterium tuberculosis* (Mtb) used in Tuberculosis Treatment

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Abstract

The rise of multidrug-resistant Mycobacterium tuberculosis (Mtb) strains has increased the global burden of tuberculosis (TB), making the investigation of alternate therapeutic options necessary. Using herbal substances with anti-biofilm characteristics is one possible approach. Antibiotic resistance and the duration of chronic infections are greatly influenced by biofilms. This review looks at the potential of different herbal ingredients to fight Mtb biofilms. Curcumin, hypericin, quercetin, ursolic acid, thymoquinone and berberine are important herbal components that are emphasized for their noteworthy anti-biofilm qualities. Through a variety of methods, such as quorum sensing modulation, biofilm matrix synthesis inhibition and immune response amplification, these drugs diminish bacterial viability, limit biofilm development and break existing biofilms.

To sum up, these herbal remedies provide a comprehensive strategy for treating tuberculosis that targets the bacterial cells as well as the biofilm matrix. Including phyto therapeutic substances in current treatment plans may improve effectiveness, lower drug resistance and lessen adverse effects linked to long-term antibiotic use. To maximize the usage of these drugs, to investigate synergistic effects and to assess their clinical usefulness, more research is required. In the fight against tuberculosis, this review highlights the potential of herbal anti-biofilm compounds as a promising non-antibiotic approach.

Keywords: Herbal compounds, Biofilm disruption, Mycobacteria and Anti- Biofilm agents.

Introduction

Overview of tuberculosis and the challenge of drug resistance: Tuberculosis (TB) continues to pose a major global health challenge due to its contagious characteristics. Despite nearly a century of vaccination efforts and successful control measures targeting the causative agent, *Mycobacterium tuberculosis* (*Mtb*), through chemotherapy, directly observed treatment (DOTS) and various antibiotics such as isoniazid, rifampicin and ethionamide, this disease remains the leading cause of mortality worldwide[4,6,23]. The major challenge involved in TB treatment is drug- resistance as it involves a lengthy regimen of multiple antibiotics[15]. The rise of extensively drug-resistant (XDR) and multidrug-resistant (MDR) strains of *Mtb* has made treating the illness more challenging as well as less effective and has created significant public health problems[13].

There are many factors which contribute to the problem of drug-resistance and thereby create a lasting impact on drug-resistant TB[11]. For example, incomplete treatment in which the bacteria may persist and become resistant if patients do not finish their entire term of TB treatment[35]. Secondly, inadequate treatment plans, which is often linked to poor-quality medications, incorrect dosages and incorrect prescriptions can lead to the emergence of drug resistance [35]. Lastly, transmission in which resistance strains of TB can proliferate as a result of drug-resistant TB being passed from person to person[22]. All the above-mentioned factors certainly impact drug-resistant TB. The increase in number of severe side effects, high mortality rates and lengthy treatment durations are frequently linked to conventional antibiotic therapies. In addition, the treatment also becomes expensive[15].

Importance of biofilms in the persistence and resistance of *Mtb*.: One of the basic reasons for antibiotic resistance and chronic TB infections is that they are greatly exacerbated by biofilms[1,24,32]. They play a crucial role in the persistence and drug resistance of Mtb, making them a key target for novel therapeutic strategies. This review investigates the possibility of anti-biofilm herbal substances as a new therapeutic strategy for tuberculosis.

Biofilm Formation and its Role in TB: Biofilms may be defined as intricate bacterial assemblages that shield the bacteria from external stressors like antibiotic drugs and the host immune system[50]. They play an impacting role in the chronicity as well as recurrence of the disease by providing a physical barrier. The extracellular matrix actually reduces the effectiveness of antibiotics by limiting their penetration[47]. Secondly, they induce metabolic dormancy[8]. This means that the antibiotics that target actively dividing cells, are less effective against bacteria in biofilms because they frequently go into a dormant condition. And lastly, the biofilm matrix may prevent immune cells from identifying and phagocytosing bacterial cells which further result in evasion of immune response[2].

Herbal anti-biofilm agents: Herbal anti-biofilm agents are organic substances made from plants that can either break up

or stop biofilms from forming[28]. Structured populations of bacteria known as biofilms are shielded from environmental stressors like antibiotics and the human immune system by an extracellular matrix that they manufacture on their own[20]. Thus, biofilms play a significant role in the survival of chronic diseases like tuberculosis (TB) and drug resistance.

Biofilms are essential to the survival and resilience of *Mycobacterium tuberculosis* (*Mtb*), the bacterium that causes tuberculosis (TB)[20]. The limitations and difficulties related to drug resistance and prolonged antibiotic use are addressed by the use of herbal anti-biofilm medicines, which present a promising substitute or supplement to traditional antibiotic treatments[41].

There are several advantages of herbal anti-biofilm compounds[42]. First, they are safe and are easily accessible[42]. In general, herbal substances are thought to be less harmful and have fewer adverse effects than synthetic medications[42]. They are frequently easier to obtain, particularly in environments with limited resources where access to traditional antibiotics may be limited[42]. Secondly, these plant-based compounds can be easily used with multi-targeted approach[42]. A variety of active components found in herbal substances usually target different facets of bacterial physiology and biofilm development[42]. This multifaceted strategy can lower the likelihood of drug resistance[37]. Thirdly, these can have synergistic effects[7]. That means when combined with traditional antibiotics, a number of herbal substances can increase their effectiveness and produce a synergistic effect that enhances treatment results[7]. And lastly, they can modulate immune system[45]. This means that by strengthening the host's immune system, certain herbal substances can help to eradicate germs linked to biofilms and to increase the effectiveness of treatment as a whole[46].

Key Herbal Compounds with anti-biofilm properties: Using herbal compounds to fight biofilms, particularly those made by *Mycobacterium tuberculosis* (*Mtb*), is a promising way to combat drug-resistant TB. Table 1 indicates certain herbal compounds that are being researched for TB treatment.

Curcumin: Curcumin is an herbal polyphenolic compound which is readily extracted from turmeric (*Curcuma longa*). It already demonstrated significant potential as an anti-biofilm agent for treating tuberculosis (TB)[9,51]. This compound shows inhibitory as well as antibacterial type of mechanism of action towards *mycobacterium spp*. By preventing the synthesis of biofilm matrix and altering quorum sensing, curcumin breaks down the structure of biofilms. And then the bacteria become more vulnerable to antibiotics and the host immune system as a result of this disruption[25].

There have been many researches that indicate that curcumin can improve the penetration of traditional antibiotics into biofilms, thereby increasing their effectiveness. Because biofilms contribute to persistence and resistance, this is especially crucial when treating drug-resistant TB strains.

Table 1
Herbal Compounds with Anti-Biofilm Activity

Herbal Compound	Source	Mechanism of Action	Efficacy Against *Mtb* Biofilms
Curcumin[21]	Turmeric	Disrupts biofilm structure, reduces viability	High
Hypericin[30]	St. John's Wort	Modulates quorum sensing, enhances immune response	Moderate
Quercetin[39]	Fruits and Vegetables	Inhibits biofilm formation, anti-inflammatory	Moderate
Ursolic Acid[31]	Apples	Disrupts biofilm integrity	High
Thymoquinone[12]	Black Seed	Exhibits strong anti-mycobacterial activity	High
Berberine[52]	Goldenseal and Barberry	Reduces bacterial resistance, inhibits biofilm matrix	Moderate

Table 2
Curcumin with its Anti-Biofilm Properties.

Source	*Curcuma longa* (Turmeric)
Mechanism	Alters quorum sensing, interferes with the formation of biofilm matrix and breaks down biofilm structure[14].
Methodology	In vitro biofilm disruption assay[14].
Key Findings	Significant reduction in biofilm mass[14].
Therapeutic Potential	Studies have shown that curcumin exhibit significant anti- biofilm activity, lowers bacterial viability and thereby improve immune response[14].

Marini et al[25] called curcumin as an antibiotic resistance breaker against one clinical isolate of the mycobacterium species (*Mycobacterium abscessus*). Their research made four major key findings. First, curcumin totally inhibited 4- as well as 8-day mature biofilms at four times the minimum inhibitory concentration (MIC)[25]. Secondly, this compound also shows synergistic effects with antibiotics like linezolid, ciprofloxacin, amikacin and clarithromycin[25], thus, increasing their efficacy against resistant strains of *Mycobacterium abscessus*.

Additionally, it dramatically decreased the motility of bacteria. Lastly, whether curcumin is used alone or along with antibiotics, their results support its potential as an antibiotic resistance breaker. Thus, attributes presented by Marini et al[25] make curcumin a promising candidate for addressing biofilm-associated infections and combating antibiotic resistance.

However, curcumin's poor (low) bioavailability is a problem. But with the help of nano technological developments, curcumin nanoparticles have been created, which enhance its solubility and tissue delivery[44]. Its therapeutic potential for the treatment of tuberculosis is thus increased. In addition, curcumin's therapeutic potential is further enhanced by its anti-inflammatory and antioxidant characteristics[3]. This compound can enhance treatment results and promote the general health of TB patients by lowering oxidative stress and inflammation[3].

Curcumin is a promising option for treating tuberculosis because of its capacity to break down biofilms and improve the effectiveness of antibiotics. To fully grasp its potential and maximize its use in conjunction with current TB therapies, more investigation and clinical trials are required.
Hypericin: Hypericin is an herbal compound which is readily extracted from *Hypericum perforatum* (St. John's Wort). It already demonstrated significant potential as an anti-biofilm agent against mycobacteria[48]. This compound also shows inhibitory type of mechanism of action towards mycobacterium spp. By disrupting the biofilm matrix and altering quorum sensing, it inhibits the formation of biofilms which, in turn, makes the bacteria more vulnerable to antibiotics and the host immune system[30].

In 2012, Mortensen et al[30] conducted research on the effectiveness of St. John's Wort (*Hypericum perforatum*) as an antimicrobial agent against mycobacteria showing promising results. They performed the SEM analysis of the herbal extract which contains compounds such as hyperforin, hypericin and pseudohypericin and found that they contribute to its antimicrobial properties which, in turn, are actually effective at inhibiting five non-pathogenic *Mycobacterium* isolates and the minimal bactericidal concentrations (MBC) ranged from 0.33 to 2.66 mg extract/mL.

Thus, hypericin is a promising treatment option for mycobacterial infections, such as tuberculosis (TB), due to its capacity to break down biofilms and improve the effectiveness of antibiotics. To completely comprehend its mechanisms and maximize its application in clinical settings, more research is required.

Quercetin: Numerous fruits and vegetables like onions, kale, cherry tomatoes, broccoli, blueberries, apples, buckwheat and grapes contain quercetin which is a flavonoid that has shown promising result as an anti-biofilm agent against mycobacteria[29]. This particular flavonoid shows inhibitory type of mechanism of action towards mycobacterium spp. by changing the permeability of cell membranes, suppressing enzyme activity and interfering with bacterial communication systems (quorum sensing). Quercetin prevents the formation of biofilms[27,43].

Table 3
Hypericin with its Anti-Biofilm Properties.

Source	*Hypericum perforatum* (St. John's Wort)[19,48].
Mechanism	Alters quorum sensing and improves immune response[30].
Methodology	Biofilm inhibition assay[30].
Key Findings	Reduced biofilm formation, enhanced immune response[30].
Therapeutic Potential	Studies have shown that hypericin is a useful adjunct in the treatment of tuberculosis because it prevents the formation and persistence of biofilms[48].

Table 4
Quercetin with its Anti-Biofilm Properties.

Source	Onions, kale, cherry tomatoes, broccoli, blueberries, apples, buckwheat, grapes[29].
Mechanism	It breaks the biofilm and alters communication of bacteria (quorum sensing) and in turn, improves immune response[27].
Methodology	Biofilm inhibition assay[27].
Key Findings	Decreased biofilm formation, anti-inflammatory effects[27].
Therapeutic Potential	Studies have shown that quercetin is a useful flavonoid in TB treatment because it prevents the formation and persistence of biofilms. In addition, it exhibits both anti-inflammatory as well as biofilm-inhibitory effects, thus enhancing the efficacy of conventional antibiotics[38].

In 2018, Sasikumar et al[39] showed promising findings on quercetin as anti-mycobacterial agent. Their team studied the antimycobacterial properties of quercetin against *Mycobacterium tuberculosis H37Rv* and showed 56.21% inhibition in the broth microdilution (BMD) assay at 50 µg/mL and 99.30% inhibition in the Lowenstein-Jensen proportion technique (LRP) assay at 200 µg/mL. 6.25 µg/mL was determined to be the minimal inhibitory concentration (MIC). Thus, according to the study, quercetin has superior inhibitory action against *Mtb H37Rv*. Although many other compounds shows promising results as anti-mycobacterial drugs, yet quercetin seems to work better[39].

In conclusion, quercetin is a promising option for treating tuberculosis because of its capacity to break down biofilms and improve the effectiveness of antibiotics. In addition, it also shows synergistic effects with antibiotics. Further research and clinical studies are necessary to fully understand its potential and optimize its use in conjunction with existing TB medications.

Ursolic Acid: Jyoti et al[18] studied *in vitro* effect of ursolic acid on *Mycobacterium tuberculosis* as well as its cell wall mycolic acid. The researchers successfully demonstrated that by using a resazurin assay, ursolic acid showed an inhibitory activity against *Mycobacterium tuberculosis H37Ra*, with an inhibitory concentration ranging between 10 and 20 µg/mL. Also, quantitative LC-MS/MS data confirmed that ursolic acid decreased mycolic acid biosynthesis in a dose-dependent manner, which is similar to the effects of isoniazid (INH)[18].

However, electron microscopy images verified that ursolic acid treatment affected both the cell and intracellular content of Mycobacteria. This research suggests that ursolic acid has encouraging antimycobacterial activity by preventing the manufacture of mycolic acid, which is essential for maintaining the integrity of *Mycobacterium tuberculosis*' cell wall.

Thymoquinone: A naturally occurring substance in *Nigella sativa* (black seed), thymoquinone, has demonstrated encouraging promise as an anti-biofilm agent against mycobacteria[10]. Thymoquinone has an antibacterial mechanism of action by changing the permeability of cell membranes, producing reactive oxygen species (ROS) and interfering with bacterial communication systems (quorum sensing), thus preventing the formation of biofilms[10,16]. This particular compound has already been researched with number of bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* in order to gain knowledge on prevention of biofilm production.

Actually, the quorum sensing genes including lasI, lasR, rhlI and rhlR, which are essential for biofilm formation, are downregulated by thymoquinone[16]. Additionally, it also increases the efficacy of antibiotics against bacterial strains that are resistant to them when taken in conjunction with them[16]. Very recently in 2024, Jankowski et al[16] studied about a molecular mechanism of thymoquinone that works against *Mycobacterium tuberculosis* and discovered that it quickly triggers the synthesis of sigma factor genes that respond to stress, especially sigE and sigF. Furthermore, it disrupts energy generation by depleting ATP and NAD pools. It also compromises cell integrity by downregulating plasma membrane lipids[16]. Thus, thymoquinone is a strong antimycobacterial agent because of its capacity to cause stress reactions, exhaust energy stores and to compromise membrane integrity[16]. One can say that this compound has the potential to cure Mycobacterium tuberculosis strains that are highly and multi drug resistant[16].

Table 5
Ursolic Acid with its Anti-Biofilm Properties

Source	Apples and medicinal plants[40].
Mechanism	It breaks the biofilm and alters communication of bacteria (quorum sensing) and in turn, improves immune response. Thus, making it easier for the immune system to eradicate bacteria[49].
Methodology	*In vivo* TB model[18].
Key Findings	Reduced bacterial load and biofilm presence.
Therapeutic Potential	Studies have shown that Ursolic acid is a useful compound in TB treatment as it prevents the formation of biofilms. In addition, it exhibits biofilm-inhibitory effects, thus enhancing the host immune response[36].

Table 6
Thymoquinone with its Anti-Biofilm Properties

Source	*Nigella sativa* (Black seed)[10].
Mechanism	It reduces the mass of biofilm as well as bacterial viability and in turn, improves immune response[16].
Therapeutic Potential	Studies have shown that Thymoquinone demonstrates strong anti-mycobacterial activity and could be a potent anti-biofilm agent in TB treatment, thus enhancing the host immune response[12].

Table 7
Berberine with its Anti-Biofilm Properties

Source	*Berberis* spp. (Barberry)[33].
Mechanism	It interferes with the production of biofilm matrix as well as the bacterial resistance mechanisms and in turn, improves immune response[33].
Therapeutic Potential	Studies have shown that berberine enhances the effectiveness of antibiotics. Thus, shows synergistic effect and disrupts biofilm-associated resistance and in turn enhances the host immune response[33].

Berberine: Plants known as Berberis vulgaris (barberry) contain the isoquinoline alkaloid berberine which has demonstrated promise as an anti-biofilm agent against mycobacteria[33]. It has an anti-bacterial mechanism of action. Berberine prevents the formation of biofilms by affecting bacterial communication mechanisms (quorum sensing), changing the permeability of cell membranes and blocking enzyme functions[33]. It has already been demonstrated that berberine readily inhibits biofilm formation in various bacterial pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In fact, it also shows an excellent synergistic effect[33]. That means when used with other antibiotic drugs, this compound enhances their efficacy against resistant strains of bacteria. In addition, it shows good broad-spectrum antimicrobial activity, making it effective against multidrug-resistant pathogens.

Ozturk et al[33] evaluated berberine for treatment of tuberculosis. They evaluated the potential of berberine using a C57BL/6 mouse model for pulmonary tuberculosis. While it did not influence the growth of *Mycobacterium tuberculosis* (*Mtb*) in axenic cultures, it demonstrated enhanced bacterial eradication in primary murine bone marrow-derived macrophages as well as in human monocyte-derived macrophages. Secondly, the administration of berberine alongside rifampicin and isoniazid proved advantageous for the host, leading to a reduction in lung pathology. Lastly, according to this study, berberine also showed impacting immunomodulatory effects as its administration resulted in a reduction of neutrophils, CD11b+ dendritic cells and the quantity of recruited interstitial macrophages within the pulmonary tissue [33].

Thus, one can say that berberine may serve as an immunomodulatory agent to improve the effectiveness of conventional tuberculosis treatments, contingent upon the stage of the disease and the inflammatory condition of the host. Actually, berberine's capacity to break the biofilms and improve the effectiveness of antibiotics makes it as a promising option for the treatment of bacterial infections, particularly those induced by mycobacteria. Additional research is essential to comprehensively elucidate its mechanisms and to refine its application in clinical environments.

Discussion

In the past, there have been a considerable scientific investigation regarding the efficacy of anti-biofilm herbal compounds in combating *Mycobacterium tuberculosis* (*Mtb*) for the treatment of tuberculosis (TB). Matlala et al[26] studied the antioxidant, anti-mycobacterial and anti-biofilm properties of the acetone extract and its subfraction from *Artemisia afra*. The subfraction exhibited a lower minimum inhibitory concentration (MIC) and demonstrated a concentration-dependent inhibitory effect on the adherence of mycobacterial cells and the formation of early biofilms[26].

Pakadang et al[34] evaluated the MIC and MKC (Minimum Killing Concentration) values of various herbal remedies against *Mycobacterium tuberculosis*. Their study included 25 plants such as Meniran leaves, banana peels, basil leaves, turmeric rhizomes and others showing promise as potential anti-tuberculosis agents. But their study does not specifically focus on biofilms. However, Bhunu et al[5] investigated on the leaf extracts of *Parinari curatellifolia* and revealed their ability to inhibit biofilm formation in *Mycobacterium smegmatis*, which serves as a model organism in the discovery of anti-mycobacterial drugs. The combination of the ethanol extract with kanamycin significantly enhanced the extract's effectiveness in preventing biofilm formation.

Future Perspectives

In this study, a novel approach involves the utilization of herbal compounds sourced from nature as anti-biofilm agents. These compounds are becoming more accessible due to advancements in analytical technology, particularly when compared to traditional chemical alternatives, which tend to be more expensive. It is reasonable to anticipate that these herbal substances may function as effective anti- biofilm agents and exhibit comparable efficacy to their chemical counterparts. As the identification of herbal products continues to grow, it is likely that numerous new herbal PSs will be uncovered in the near future. Phyto-therapy is gaining attention as a therapeutic method that not only ensures effective treatment but also aids in diagnosis.

Nonetheless, herbal compounds may encounter challenges in penetrating bodily tissues. To address this, combining other treatments, such as enhancing the circulation of nanoparticles, may prove beneficial. Creating nano-formulations of these herbal substances improve their delivery mechanisms and effectiveness. One may use combination therapies like exploring the synergistic interactions between herbal compounds and current antibiotics to address multidrug-resistant strains. Lastly, the clinical trials can help implementing preclinical and clinical trials to confirm the safety and effectiveness of these compounds in real-world applications.

To conclude, the incorporation of herbal compounds into tuberculosis treatment protocols presents considerable promise for combating drug resistance and enhancing treatment efficacy. Continued research and development are crucial to fully exploit the therapeutic advantages offered by these natural substances.

Conclusion

Herbal compounds exhibiting anti-biofilm characteristics represent a promising non-antibiotic approach in combating tuberculosis. By addressing both the bacterial cells and the biofilm matrix, these compounds provide a comprehensive strategy for TB management. Incorporating phyto therapeutic agents into current treatment protocols may improve effectiveness, may decrease the likelihood of drug resistance and may lessen the adverse effects linked to extended antibiotic therapy. Additional research is essential to refine the application of these compounds, to investigate potential synergistic effects and to assess their clinical relevance.

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