

# The Effect of Polyherbal Medicines Used for the Treatment of Tuberculosis on Other Opportunistic Organisms of Humans Infected with Tuberculosis

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## ABSTRACT

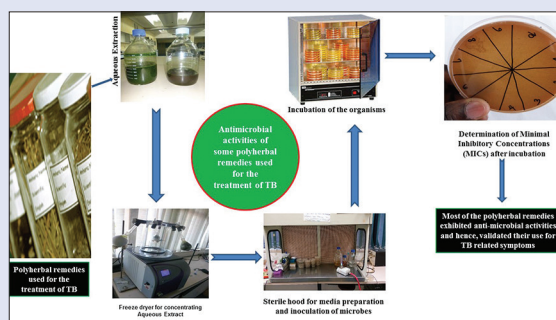
**Background:** In many immunocompromised patients, opportunistic bacterial and fungal infections are common. Polyherbal medicines examined in this study are used by the indigenous people of South Africa for the treatment of tuberculosis (TB) and other opportunistic infections associated with TB. **Objective:** To evaluate the antibacterial and antifungal activity of nine polyherbal remedies against four Gram-positive and Gram-negative bacteria respectively and three fungi. **Materials and Methods:** Agar dilution method was used to determine the minimum inhibitory concentration (MIC) of the remedies against the organisms. **Results:** The inhibitory activity of the polyherbal medicines based on the overall MIC revealed that HBfs and FB remedies were the most active remedies against the bacterial isolates at the concentration of 2.5 mg/mL, followed by HBts remedy at 5.0 mg/mL. However, the MIC values of KWTa, KWTb, KWTc, HBss, EL and AL remedies were higher than 5.0 mg/mL which was the highest concentration used. Only KWTa remedy showed activity against *Aspergillus niger* and *Aspergillus fumigatus* with the MIC value of 2.5 mg/mL. While KWTc and HBts had the highest activity at 1.25 mg/mL against *Candida albicans*, the remaining remedies were active at 2.5 mg/mL. **Conclusion:** This study revealed that some of these polyherbal formulations have activities against some of the opportunistic bacterial and fungal isolates associated with TB patients. The capability of these remedies to inhibit the organisms is an indication that they are a potential broad-spectrum antimicrobial agent. However, the remedies that are inactive might contain stimulant effects on the immune system.

**Key words:** Antibacterial, antifungal, polyherbal medicines, tuberculosis

## SUMMARY

- In the Eastern Cape Province of South Africa, no study has been reported

on the effect of polyherbal remedies used for the treatment of TB on the opportunistic pathogen. This study therefore revealed that some of the polyherbal medicines possess activity against bacterial and fungal pathogens.



**Abbreviations used:** TB: Tuberculosis; MIC: Minimum Inhibitory Concentration; CFU/ML: Colony Forming Unit Per Mill.

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## INTRODUCTION

The use of herbal medicines for the prevention and cure of various diseases has increased tremendously all over the world in the recent years. They are believed to be safe, effective, accessible, and free from serious adverse reactions.<sup>[1,2]</sup> These medicines are often “polyherbal” preparations made from the mixtures of various medicinal plants. Hence, they contain multiple bioactive constituents that interact with each other in the formulation and achieve extra therapeutic effectiveness.<sup>[3]</sup> Due to the wide therapeutic range and high efficiency of polyherbal formulations, they are used for the treatment of a vast number of diseases such as diabetics, arthritis, liver and kidney disorders, cough, asthma, fever, respiratory disorders, and tuberculosis (TB).<sup>[4-9]</sup>

TB is a chronic infectious disease and has remained one of the major public health problems in South Africa. The country ranks the third highest in the world with a high incidence of TB with about 80% of the population infected with latent TB.<sup>[10]</sup> The chronic nature of tubercular infection coupled with long-term administration of

antibiotics not only leave the patients with impaired immunity but also predispose them to opportunistic pathogens.<sup>[11,12]</sup> The suppression of human defense mechanism during the course of active TB makes the patients vulnerable to pathogenic and opportunistic pathogens, and, as such, acquires fungal infection in addition to bacterial, viral and parasitic infections.<sup>[13,14]</sup> These organisms invade any part of the body and cause secondary infections.<sup>[15]</sup> In addition, with the increasing incidence of resistant strains of bacteria and fungi with commonly used anti-infective agents and the persistence of these

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organisms in immunocompromised patients, it is of great importance to find effective antimicrobial agents against the infections caused by these organisms.

The most common opportunistic bacterial infections in immunocompromised TB patients are members of the family Enterobacteriaceae, *Pseudomonas* spp. and *Staphylococcus* spp.<sup>[14]</sup> These bacteria have been extensively studied and found to cause infections in patients undergoing prolonged use of antibiotics and immunosuppressed individual.<sup>[14,16,17]</sup> The synergistic growth promoting association of fungi and *Mycobacterium* has been reported in pulmonary TB patients.<sup>[18]</sup> This is due to the prolonged use of chemotherapy with or without corticosteroids which promotes the growth and reproduction of opportunistic fungi and, in turn, aggravates the course of the underlying process in the lung tissues.<sup>[19]</sup> The major opportunistic fungal pathogens that have been reported to cause infections in TB patients include *Aspergillus* spp. causing aspergillosis, *Candida* spp. causing candidiasis, *Cryptococcus neoformans* causing cryptococcosis, and *Mucor* spp. causing mucormycosis.<sup>[12,18,20]</sup> Several researchers have investigated the antibacterial and antifungal activity of medicinal plants used for the treatment of TB in the province.<sup>[21,22]</sup> However, no study has been reported on the antimicrobial effect of polyherbal remedies used for the treatment of TB. Since opportunistic bacterial and fungal pathogens tend to cause diseases in TB patients, the study was, therefore, aimed at evaluating the antimicrobial effect of polyherbal medicines used for the treatment of TB in the Eastern Cape Province of South Africa, in order to determine whether they could also serve as antibacterial and antifungal agents.

## MATERIALS AND METHODS

### Sample collection

Polyherbal medicines evaluated in this study were purchased from herbal healers in five communities, namely, Alice (AL), East London (EL), Fort Beaufort (FB), Hogsback, and King Williams Town. These are some of the towns within the Amathole District Municipality of Eastern Cape Province, South Africa. Each of the aqueous formulation was already prepared and packaged into a clean 2-L container by the herbal healers. The remedies were called TB healing remedy. Thus, they were code-named according to their respective place of collection as follows; King Williams Town site A (KWTa), King Williams Town site B (KWTb), King Williams Town site C (KWTc), Hogsback first site (HBfs), Hogsback second site (HBss), Hogsback third site (HBts), EL, AL, and FB. The samples were transported to the laboratory for analysis.

### Sample preparation

All the polyherbal preparations were filtered using Buchner funnel and Whatman No. 1 filter paper. The filtrate obtained was frozen at  $-40^{\circ}\text{C}$  and freeze-dried for 48 h using a freeze-dryer (VirTis BenchTop K, VirTis Co., Gardiner, NY, USA). Each of the samples was re-suspended in distilled water to yield 100 mg/mL stock solution.<sup>[23]</sup>

### Microorganisms and media

The bacteria and fungi used in this study were chosen primarily on the basis of their importance as opportunistic pathogens of humans infected with TB.<sup>[12,22,24]</sup> Strains from the American Type Culture Collection (ATCC) were used for both assays. The bacteria used include four Gram-positive and Gram-negative bacteria, namely, *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 10702, *Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 100031, *Escherichia coli* ATCC 8739, and *Salmonella typhimurium* ATCC 13311.

For the antifungal analysis, the fungal strains used include *Candida albicans* ATCC 10231, *Aspergillus fumigatus* ATCC 204305, and *Aspergillus niger* ATCC 16888. Mueller-Hinton Agar (MHA), Mueller Hinton Broth (MHB), Sabouraud Dextrose Broth (SDB), and Sabouraud Dextrose Agar (SDA) used were obtained from BioLab and were prepared according to the manufacturer's instructions.

### Preparation of inocula

For the bacterial inoculum preparation, all the test bacteria strains that were maintained on nutrient agar slants were recovered in sterile MHB and incubated overnight at  $37^{\circ}\text{C}$ . In order to obtain distinct colonies, the 24 h old cultures were diluted 1:100 v/v in fresh sterile MHB and cultured on MHA overnight at  $37^{\circ}\text{C}$ . The colony suspension method of EUCAST<sup>[25]</sup> was used for the preparation of the inoculum. Identical colonies from the culture were suspended in 0.85% sterile saline, adjusted with saline and compared with 0.5 McFarland standards to obtain a suspension density equivalent to  $10^6$  CFU/mL. The suspensions were confirmed by spectrophotometric reading at 600 nm. The cell suspensions were finally diluted 1:100 by transferring 0.1 mL of the bacterial suspension into 9.9 mL of sterile broth to give an approximate inoculum of  $10^4$  CFU/spot.<sup>[26]</sup> The inocula suspensions were used for inoculation within 15 min.

For the fungal inoculum preparation, modified method of Therese *et al.*<sup>[27]</sup> was used for the analysis. The fungi strains were freshly sub-cultured on SDA and incubated at  $25^{\circ}\text{C}$  for 72 h. About 1 cm<sup>2</sup> of 3-day-old spore producing cultures were dropped in sterile distilled water and vortexed for 30 s to release the fungal spores. The spore density of each fungus was adjusted with a spectrophotometer at 580 nm to obtain a final concentration of approximately  $10^5$  spores/mL. Cell suspensions were finally diluted to  $10^4$  CFU/spot.<sup>[28]</sup> For the *Candida* spp., the inocula were prepared by adding 1.0 mL of overnight *Candida* cultures to 9.0 mL of SDB to yield  $10^4$  CFU/spot of the inoculum.<sup>[29]</sup>

### Antibacterial and antifungal minimum inhibitory concentration assays

#### Determination of the minimum inhibitory concentration of the herbal remedies against bacterial isolates

The antibacterial activity of the polyherbal remedies was carried out using the agar dilution method of Afolayan and Meyer<sup>[30]</sup> with slight modification. MHA was prepared according to the manufacturer's instructions and placed in a water bath at  $50^{\circ}\text{C}$  to prevent solidification. From the stock solution of each remedy (100 mg/mL), different concentrations of 5.0, 2.5, 1.25, 0.625, 0.3125, and 0.15625 mg/mL were prepared and incorporated in the molten agar (three replicates). The agar containing the remedy was poured into sterile Petri dishes, swirled carefully and allowed to cool. The controls used were blank plates containing only MHA, another plates containing MHA and 1.0 mL of distilled water which is the solvent of extraction (negative control), and plates containing 0.0125–0.000391 mg/mL of ciprofloxacin serves as the positive controls. Ten microliters of the standardized bacterial cultures were streaked in a radial pattern on the solidified agar remedy plates. The plates were incubated at  $37^{\circ}\text{C}$  for 24–72 h. The concentration at which there was no visible growth of the organism on the agar plates was considered the minimum inhibitory concentration (MIC) of the remedy.<sup>[25]</sup>

#### Determination of the minimum inhibitory concentration of the herbal remedies against fungal isolates

The antifungal activity of the remedies was evaluated using the agar dilution method of Therese *et al.*<sup>[27]</sup> with slight modification. SDA was prepared according to the manufacturer's instructions and placed

in a water bath at 50°C to prevent solidification. Before congealing, different volumes of the herbal remedies were added to the SDA to have concentrations ranging between 0.15625 and 5.0 mg/mL. The controls used were blank plates containing only SDA, another containing SDA and 1.0 mL of distilled water (negative control), and plates containing 12.5–0.391 µg/mL of amphotericin-B (positive controls). Ten microliters of the final suspensions were placed on the solidified agar remedy plates. The plates were incubated at 28°C for 48–96 h. The concentration at which there was no visible growth of the organism on the agar plates was considered the MIC of the remedy.<sup>[27]</sup>

## RESULTS

### Effect of the herbal remedies on bacterial isolates

Evaluation of the antibacterial activity of the nine polyherbal remedies against eight bacterial isolates revealed that three of the herbal formulations, namely, HBfs, FB, and HBts exhibited antibacterial properties [Table 1]. The growth of *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *E. faecalis*, *B. cereus*, and *S. aureus* was inhibited by HBfs remedy at a concentration of 2.5 mg/mL while *Salmonella typhimurium* and *Streptococcus pyogenes* were inhibited at the MIC of 5.0 mg/mL. Furthermore, FB preparation was active against six bacteria, namely, *P. aeruginosa*, *S. typhimurium*, *E. faecalis*, *S. pyogenes*, *B. cereus*, and *S. aureus* at the MIC of 2.5 mg/mL, while *K. pneumoniae* and *E. coli* were inhibited at 5.0 mg/mL. HBts remedy showed activity at 5.0 mg/mL against eight isolates except on *B. cereus* which was resistant to the remedy. The MIC of KWTa, KWTb, KWTc, HBss, EL, and AL remedies against the bacterial isolates was higher than 5 mg/mL which was the highest concentration used. However, the standard antibiotic (ciprofloxacin) used as a positive control inhibited the isolates at the concentration of 0.0015625 mg/mL except on *S. typhimurium* were the MIC at 0.003125 mg/mL [Table 1]. The inhibitory activity of the herbal formulations based on the overall MIC revealed that HBfs and FB remedies were the most active against the bacterial isolates followed by HBts, while the remaining formulations showed no activity at the concentration used [Table 1].

### Effect of the herbal remedies on fungal isolates

The effects of the remedies against the fungal isolates are shown in Table 2. Among the nine herbal formulations tested, only KWTa remedy was active against *A. niger* and *A. fumigatus* with the MIC value of 2.5 mg/mL [Table 2]. Other remedies; KWTb, KWTc, HBfs, HBss, HBts, AL, EL, and FB were inactive against *Aspergillus* spp. at the concentration used in this study. However, all the remedies showed activity against *C. albicans*. The highest activity was recorded in KWTc and HBts remedies with the MIC value of 1.25 mg/mL while the remaining remedies had the MIC of 2.5 mg/mL. The activity of these remedies against the yeast (*C. albicans*) alone suggest that the remedies might not have high efficacy with a broad spectrum of antifungal activity compared to the drug (amphotericin B) used as a positive control which inhibited the isolates at the concentration of 0.391 µg/mL [Table 2].

## DISCUSSION

Polyherbal formulations are well known used Ayurvedic medicines for their effectiveness against a wide range of diseases.<sup>[31]</sup> They contain multiple bioactive constituents that act synergistically and achieve extra therapeutic effectiveness. Polyherbal formulations have been reported to possess activity against opportunistic antibacterial and antifungal isolates.<sup>[32–36]</sup> However, herbal formulations examined in this study are used by TB-patients for the treatment and management of TB, especially, in the Eastern Cape Province, South Africa.

**Table 1:** Minimum inhibitory concentration of polyherbal remedies used for the treatment of tuberculosis on bacterial isolates associated with tuberculosis infection

Bacteria species	Gram-positive/negative	KWTa (mg/mL)	KWTb (mg/mL)	KWTc (mg/mL)	HBfs (mg/mL)	HBss (mg/mL)	HBts (mg/mL)	AL (mg/mL)	EL (mg/mL)	FB (mg/mL)	Ciprofloxacin (mg/mL)
<i>Klebsiella pneumoniae</i> ATCC 10031	Negative	>5	>5	>5	2.5 <sup>a</sup>	>5 <sup>c</sup>	5	>5	>5	5	0.0015625
<i>Pseudomonas aeruginosa</i> ATCC 27853	Negative	>5	>5	>5	2.5	>5	5	>5	>5	2.5	0.0015625
<i>Salmonella typhimurium</i> ATCC 1331	Negative	>5	>5	>5	5 <sup>b</sup>	>5	5	>5	>5	2.5	0.0031250
<i>Escherichia coli</i> ATCC 8739	Negative	>5	>5	>5	2.5	>5	5	>5	>5	5	0.0015625
<i>Enterococcus faecalis</i> ATCC 29212	Positive	>5	>5	>5	2.5	>5	5	>5	>5	2.5	0.0015625
<i>Streptococcus pyogenes</i>	Positive	>5	>5	>5	5	>5	5	>5	>5	2.5	0.0015625
<i>Bacillus cereus</i> ATCC 10702	Positive	>5	>5	>5	2.5	>5	5	>5	>5	2.5	0.0015625
<i>Staphylococcus aureus</i> ATCC 29213	Positive	>5	>5	>5	2.5	>5	>5	>5	>5	2.5	0.0015625

<sup>a</sup>MIC; <sup>b</sup>Maximum concentration of the polyherbal medicines tested; <sup>c</sup>Not active. KWTa: King Williams Town site A; KWTb: King Williams Town site B; KWTc: King Williams Town site C; HBfs: Hogsback first site; HBss: Hogsback second site; HBts: Hogsback third site; AL: Alice; EL: East London; FB: Fort Beaufort; ATCC: America Type Culture Collection; MIC: Minimum inhibitory concentration



**Table 2:** Minimum inhibitory concentration of polyherbal remedies used for the treatment of tuberculosis on fungal isolates associated with tuberculosis infection

Fungi species	KWTa (mg/mL)	KWTb (mg/mL)	KWTC (mg/mL)	HBfs (mg/mL)	HBss (mg/mL)	HBts (mg/mL)	AL (mg/mL)	EL (mg/mL)	FB (mg/mL)	Amphotericin-B (µg/mL)
<i>Aspergillus niger</i> ATCC 16888	2.5	>5	>5	>5	>5 <sup>c</sup>	>5	>5	>5	>5	0.78
<i>Aspergillus fumigatus</i> ATCC 204305	2.5	>5	>5	>5	>5	>5	>5	>5	>5	0.78
<i>Candida albicans</i> ATCC 10231	2.5	2.5	1.25 <sup>a</sup>	2.5 <sup>b</sup>	2.5	1.25	2.5	2.5	2.5	0.39

<sup>a</sup>MIC; <sup>b</sup>Maximum inhibitory concentration; <sup>c</sup>Not active. KWTa: King Williams Town site A; KWTb: King Williams Town site B; KWTC: King Williams Town site C; HBfs: Hogsback first site; HBss: Hogsback second site; HBts: Hogsback third site; AL: Alice; EL: East London; FB: Fort Beaufort; ATCC: American Type Culture Collection; MIC: Minimum inhibitory concentration

In this study, three of the herbal formulations exhibited antibacterial properties against both Gram-negative (*P. aeruginosa*, *K. pneumoniae*, *E. coli*, and *S. typhimurium*) and Gram-positive bacteria (*E. faecalis*, *S. pyogenes*, *B. cereus*, and *S. aureus*). This is an indication that the herbal preparations possess good antibacterial activity against the opportunistic bacteria capable of causing infection in TB-patients when compared with other remedies. From the results, the response of the bacteria to the remedies varied among the isolates and the concentrations. The variation in the MIC of the polyherbal remedies could be due to the mode of action, the differences in cell wall composition and/or genetic content of the organisms<sup>[37]</sup> as well as the synergistic effects of different phytochemicals present in the herbal formulations. The antibacterial activity of HBfs, FB, and HBts remedies at relatively minimal concentrations could be attributed to the bioactive constituents present in the remedies at different concentrations, its ability to damage the cell walls to allow the active compounds to absorb, diffuse, penetrate and interact with the target sites and potent enough to inhibit, or kill microbial agents.<sup>[38]</sup>

Furthermore, the results showed that all the remedies were active against the growth of only the yeast (*C. albicans*). The resistance of *Aspergillus* spp. suggests that the remedies might not have high efficacy with a broad spectrum of antifungal activity when compared with amphotericin-B which showed activity against the three fungi. However, the activity of KWTa remedy could be attributed to the presence of bioactive antifungal agents which are not in the other remedies. In general, the poor growth inhibitory activity against some of the organisms demonstrated by some of these remedies could be attributed to the solvent (water) used by the herbal healers for their preparation. Aqueous extracts have been reported to be generally less active as described in many previous studies.<sup>[37,39,40]</sup> The activity of these remedies on the opportunistic pathogens does not mean that they are inactive. They may act by stimulating the immune system of the patient, or by creating internal conditions that are unfavorable for the multiplication of the microorganism.<sup>[21]</sup>

## CONCLUSION

This study revealed that some of these polyherbal formulations have activities against some of the opportunistic bacterial and fungal isolates associated with TB patients. The capability of these remedies to inhibit the organisms is an indication that they are a potential broad-spectrum antimicrobial agent. However, the remedies that are inactive might contain stimulant effects on the immune system.

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## Conflicts of interest

There are no conflicts of interest.

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