The In-Vitro Activity of Manuka Honey and Indian Costus (Saussurea Costus) on the Growth of Bacteria that Cause Pulmonary Tuberculosis

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Abstract

The Mycobacterium Tuberculosis complex is a group of species of Mycobacteria involved in a serious bacterial disease which infects the respiratory system and organs. Multi-drug resistant (MDR) strains have been produced as a result of the fast emergence of resistance against first-line anti-tuberculosis medicines in recent years worldwide. The objective of this study is to determine alternatives to anti-mycobacterial antibiotics, such as Manuka honey and Indian costus. MTB isolates (n = 15) were evaluated for their susceptibility to Manuka honey graded as +15 and Indian costus by using different concentrations. Among the TB isolates (n = 15) tested, four (27%) isolates were resistant at 15% v/v honey, while, at 18% v/v of Manuka honey, two (13%) isolates were found to be resistant. Resistance was not seen at honey concentrations of 20% v/v. On the other hand, Indian costus had a slight effectiveness at concentrations of 20% and 25%, affecting only two and five isolates of Mycobacteria, respectively; however, all isolates were inhibited at a concentration of 33% v/v of Indian costus. The present study clearly demonstrates that supposed alternatives (Manuka honey graded as +15 and Indian costus) possess significant anti-mycobacterial activity in vitro; however, further clinical trials should be performed to corroborate our initial findings.

Keywords: Mycobacterium Tuberculosis; Mycobacterium Tuberculosis Complex, Activity of Manuka honey; Antimicrobial resistance; Indian costus.

1. Introduction

As a persistent bacterial infectious illness, tuberculosis has emerged as a major global public health concern. For many years, a mixture of medications has been used to treat tuberculosis (Daniel 2006). Single-drug regimens cause resistance to develop quickly, which leads to treatment failure (Wang et al. 2006). According to Lawn and Wilkinson (2006), "the new threat in the management of tuberculosis is the emergence of resistance to standard anti-TB drugs that leads to multidrug resistance". Multi-drug resistance (MDR) has emerged as a significant issue for tuberculosis control, especially in poor nations (Cohn et al.1997). Higher death rates from MDR-TB than from TB treated with sensitive medications make it a developing global issue (Diraa et al. 2003). An estimated 450,000 new cases of MDR-TB are reported each year (Thaver and Ogunbanj, 2006). Antibiotic therapy for bacterial infections is typically successful when prescribed with conventional medications. Nonetheless, the issue of antibiotic resistance is worsening, and we still require new remedies. Worldwide, folk medicine uses plants, herbs, and other natural materials to cure bacterial illnesses. Honey is a naturally occurring substance that is widely available, reasonably priced, and liked by all. Honey is often used in medicine and it has been approved as a therapeutic agent for burns, diabetic foot ulcers, surgical wounds, leg ulcers, and skin graft donor sites. Researchers should further investigate this natural remedy's medicinal potential for a variety of uses, as it is currently incredibly underutilized (Zumla and Lulat 1989). The antibacterial characteristics of honey were first described by Van Ketel in 1892 (Dustmann 1979). Dold conducted a thorough investigation into the antibacterial properties of honey in 1937 before coining the term "inhibine." Inhibine was later discovered to be hydrogen peroxide produced by a bee gland enzyme (Aliyazicioglu and Boukraa 2015). Since then, a number of other research studies on the topic have been published, all of which have proven honey's antibacterial properties (Efem et al. 1992). According to Molan (2019), honey's acidity, high osmolarity, hydrogen peroxide produced by glucose oxidase (a bee enzyme), and non-peroxide components (of plant origin) are the primary causes of its antibacterial effects. The bee's hypo-pharyngeal gland secretes the heat- and light-sensitive enzyme glucose oxidase, which is shown to be dormant in unadulterated honey (Dustmann 1979). When honey is diluted, this enzyme changes water and sugar into hydrogen peroxide (Franchini et al. 2007). Because exudates from wound surfaces dilute honey and, hence, increase its antibacterial activity, undiluted honey is therefore a good therapeutic agent. It is astonishing that no two honeys are exactly the same, with over a hundred botanical species known to yield European unifloral honeys (Oddo and Bogdanov 2004). Amazingly, honey both promotes the growth of the normal flora in the gastrointestinal tract and inhibits over sixty different species of harmful bacteria (Olofsson and Vasquez 2008). Given the various tactics honey uses to both directly and indirectly combat harmful organisms, honey consumed orally could be a great option for illness prevention and treatment in the future. Honeys with dark hues, like avocado, chestnut, manuka, and jelly bush from Australia and Canada, are well-known for having strong antibacterial properties (Gheldof et al. 2002). Lastly, honey has a well-established role as a topical therapeutic agent for skin and other pathogenic agents (Okeniyi et al. 2005). The other medication of plant origin recommended for TB infection is Indian costus (the scientific name of which is Saussurea costus), which has been approved for its efficacy against fungi and yeasts, which have similar

cell wall constructions (Soliman et al. 2022). In this research, both aforementioned materials, Manuka honey and Indian costus, will be tested against some isolates of Mycobacterium.

2. Material and Methods:

Study design: This study is an empirical study, which was performed in the regional laboratory of Makkah, Saudi Arabia. Ethical approval was obtained from the Ethics Committee of the Ministry of Health, KSA (ethical approval reference no. (H-02-K076-0624-1143). The study was designed to assess the antibacterial activity of Manuka honey against clinical isolates of Mycobacterium tuberculosis. The duration of the study spanned from May 2024 to May 2025.

Bacterial Isolates. The samples of Mycobacteria were collected from patients from hospitals in Makkah al-Mukarramah during the 3-month period of the study.

Isolation of MTB. The isolation procedure of MTB isolates was applied as recommended by the BD® company. In brief, NaOH–NALC–sodium citrate solution was added directly to the specimen in a screw-cap container. The mixture was vortexed for 15 minutes (up to 25 minutes' maximum) to make sure that the specimen was completely liquefied. Phosphate buffer (pH 6.8) was added to the mixture to reach the volume of 50 ml. Then, it was mixed well by being lightly vortexed or inverted several times. Using refrigerated centrifugation at a higher speed, the tubes were centrifuged at a speed of 3000 g or more for 15–20 minutes. Tubes were left after centrifugation for 5 minutes to allow the aerosols to settle down. The supernatant was decanted carefully into a suitable container containing a myco-bactericidal disinfectant. A small quantity (1–2 ml) phosphate buffer (pH 6.8) was added and the sediment was resuspended using a pipette or vortex mixer. Of the resuspended mixture, 0.5 ml was added to a MGIT vial, and 0.8 mL of PANTA (comprising Polymyxin B (6000 units), which works against Gram-negative bacteria; Amphotericin B (600 µg), which acts against yeast and fungi; Nalidixic acid (2400 µg), which works against Gram-negative bacteria; and Trimethoprim (600 µg) and Azlocillin (600 µg), which work against both Gram-negative and Gram-positive bacteria), which is a lyophilized five-antibiotic cocktail powder covering a broad spectrum of organisms that commonly contaminate cultures, was added as well. Negative and positive growth control vials were prepared with each batch.

Identification of MTB: Positive vials are tested using Geneexpert® to identify all complexes of tuberculosis, through the PCR technique. In addition, negative samples to PCR were secluded. The PCR technique detects DNA sequences specific to Mycobacterium tuberculosis and rifampicin resistance by polymerase chain reaction. It is based on the Cepheid GeneXpert system, a platform for rapid and simple-to-use nucleic acid amplification tests (NAATs). The system detects the rpoB gene, which contains 81 base pair core regions common to all types of Mycobacterium complex.

Drug Susceptibility Testing (DST) of Mycobacterial isolates: All the isolates were subjected to standard concentrations of streptomycin (332 μg), isoniazid (33.2 μg), ethambutol (1660 μg), rifampin (332 μg), and PZA (100 mg/liter) and tested using MGIT 960, according to protocols in MGIT procedure manual.

Activity of Manuka honey graded as +15 and Indian costus test: 15 samples of mycobacteria were tested for their susceptibility to Manuka honey graded as +15 and Indian costus. Stock solutions of Manuka honey and Indian costus were prepared and added directly to the tubes, which already contained mycobacteria, to obtain final dilutions of Manuka honey (13%, 15%, 18%, and 20%) and Indian costus (10%, 20%, 25%, and 33%). All tubes were incubated for 7 days at 37^0C. After that, all tubes were treated as original samples and all steps of TB isolation were followed. The tubes were monitored for 42 days, according to the standard protocol, before declaring them negative. The collected data were plotted on Excel sheets to display the results and graphs. A descriptive statistical analysis was performed, also using Excel software. The results are presented as mean values and standard deviations.

3. Results:

A total of fifteen isolates of Mycobacteria were isolated from hospitalized patients in hospitals of Makkah city. Testing revealed that the tested isolates (n=15) have variable resistant profiles, as shown in Table 1.

Specimen No	Specimen Type	PCR Result	Sensitivity Test Result				
			S	I	R	E	PZA
1	Sputum	RS	S	S	S	S	S
2	Sputum	RS	S	S	S	S	S
3	Sputum	RS	S	S	S	S	S
4	Pleural Fluid	RS	S	S	S	S	S
5	Sputum	RS	R	R	S	S	R
6	Sputum	RS	S	S	S	S	S
7	Sputum	RS	S	S	S	S	R
8	Sputum	RS	S	S	S	S	S
9	Sputum	RS	S	S	S	S	R
10	Sputum	RS	S	S	S	S	S
11	Sputum	RS	S	S	S	S	S
12	Sputum	RS	S	S	S	S	S
13	Sputum	RS	S	S	S	S	S
14	Sputum	RS	S	S	S	S	S
15	Sputum	RS	S	S	S	S	R

Table (1): Results of the samples included in the research (RS = M. tuberculosis detected rifampicin resistance not detected, S = Streptomycin, I = Isoniazid, R = Rifampin, E = Ethambutol, PZA = Pyrazinamide, S = Sensitive, R = Resistance).

The efficacy of Manuka honey graded as +15 was tested against all isolates of Mycobacteria. A honey concentration of 20% suppressed all isolates. However, only four and two isolates could grow in concentrations of 18% and 15%, respectively. A Manuka honey concentration of 13% has no efficacy against Mycobacteria at all (Figure 1).

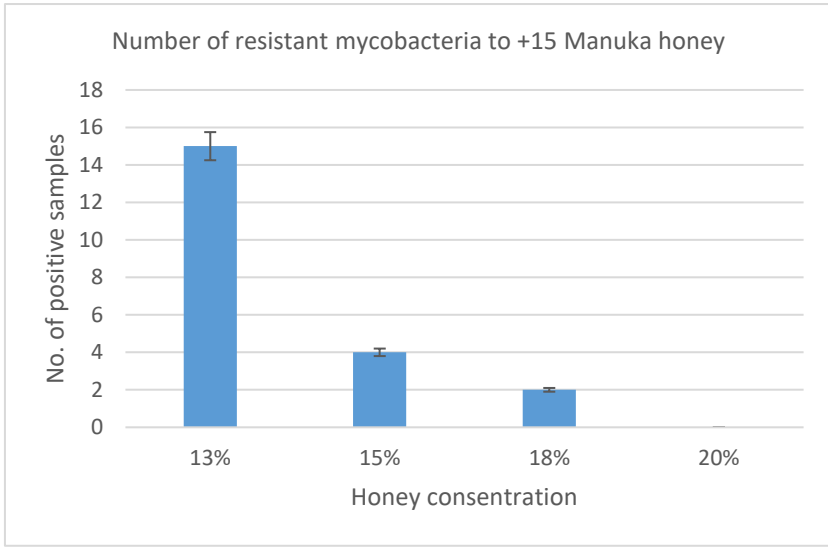

Figure (1): Susceptibility of Mycobacterium isolates to different concentrations of +15 UMF Manuka honey.

At a 33% concentration, all isolates were found to be affected by Indian costus. At a 25% concentration, 13 isolates out of 15 were found sensitive, and 10 out of 15 were found sensitive at a 20% concentration. However, a 10% concentration had no efficacy on isolates of Mycobacteria (Figure 2).

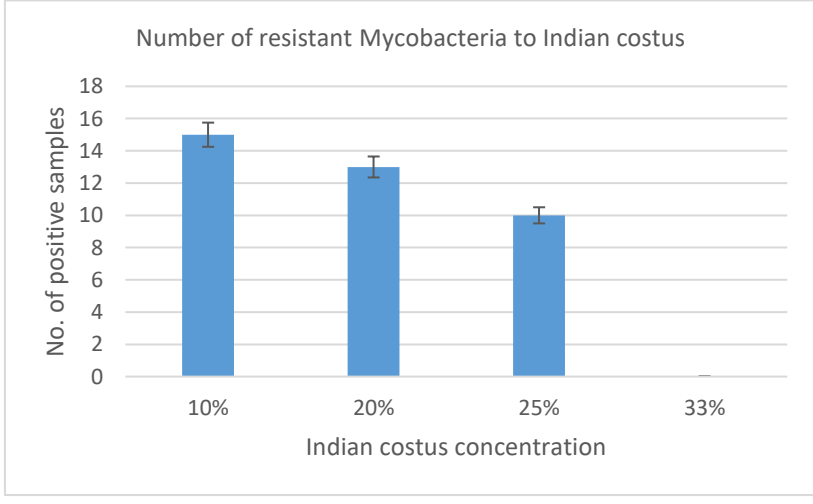

Figure (2): Susceptibility of Mycobacterium isolates to different concentrations of Indian costus (Saussurea costus).

4. Discussion:

Honey has long been used as a traditional treatment for microbiological diseases (Molan 1992). Studies on Manuka honey have revealed its efficacy against a number of human infections, such as Salmonella typhimurium, Enterobacter aerogenes, Escherichia coli, and Staphylococcus aureus. This honey has been shown to be effective against vancomycin-resistant enterococci (VRE), hemolytic streptococci, and methicillin-resistant S. aureus (MRSA) in laboratory trials carried out by Rani et al. in 2017. Manuka honey and Indian costus are natural products which have no side effects on the human body. Manuka honey graded as +15 was chosen in this research because it had the lowest MIC among the grades tested in previous experiments (Girma et al. 2019). Our results concluded that a 20% concentration of Manuka honey graded as +15 inhibited all isolates of Mycobacteria, and the number of resistant isolates increased with a decreasing honey concentration. The same outcome was obtained with Indian costus; all isolates were inhibited at a 33% concentration of Indian costus, but a 10% concentration had no activity at all on any isolates of Mycobacteria. The same result was obtained by a researcher with a different type of honey, namely Avicenna honey, as they found that, at concentrations of 10% to 20%, Avicenna honey suppresses MTB strains (Hannan et al. 2014). Nolan et al. 2022 approved the remarkable efficacy of Manuka honey against Mycobacterium abscessus when it was used in vitro in combination with Amikacin and in combination with Azithromycin. Like Manuka honey, Indian costus has been proven to be effective against highly resistant bacteria such as E. coli, Pseudomonas, and Acientobacter (Abdullah et al. 2021). Suriati et al. 2011 tested the anti-tuberculosis activity of Indian costus, wherein the MIC of Indian costus was recorded as 800 μg/ml. The chemical constituents of Indian costus are flavonoids; however, the extract which might have an effect on mycobacteria has yet to be identified (Dukre et al. 2021). It is proposed that this work is extended to assess a greater number of strains in a suitable animal model. Eventually, one can anticipate that the results will be advantageous for clinical trials.

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