

Analysis of the Antimicrobial Properties of Thanaka, a Burmese Powder Used to Treat Acne

Elizabeth V. Seiverling, Jordan P. Trubiano, Jacqueline C. Williams, Hadjh T. Ahrns, David W. Craft, Matthew R. England

Departments of Dermatology, Family and Community Medicine, and Pathology, Pennsylvania State University - Milton S. Hershey Medical Center, Hershey, PA, USA
Email: eseiverling@pennstatehealth.psu.edu

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Abstract

Thanaka, powder from *Hesperethusa crenulata* tree bark, has been used in Burmese culture for acne treatment and prevention for over 2000 years. The purpose of this study was to evaluate the antimicrobial properties of thanaka against *Staphylococcus aureus*, *Escherichia coli*, and *Propionibacterium acnes*. Kirby-Bauer disk diffusion revealed no zones of inhibition for thanaka against the tested microorganisms. Disk diffusion may not be the best modality for definitive analysis of the antimicrobial activity of thanaka. Furthermore, the utility of thanaka in acne treatment may be related to anti-inflammatory, rather than antimicrobial properties.

Keywords

Thanaka, Myanmar, Disk Diffusion, *Propionibacterium acnes*

1. Introduction

Thanaka (also spelled thanakha) is a powder produced from the bark of *Hesperethusa crenulata* or *Naringi crenulata* trees (**Figure 1(a)**). Thanaka is used for a variety of cosmetic and dermatologic purposes including photo-protection, acne treatment and prevention, skin cooling, skin lightening, pruritus relief, scar reduction, mosquito repellent, and odor prevention (**Figure 1(b)**) [1] [2] [3] [4]. Acne treatment and prevention were the most commonly reported reason for applying thanaka to the skin in persons 12 - 16 years old [4]. *P. acnes* is involved in the release of proinflammatory mediators in the pilosebaceous unit and leads to inflammatory papules, pustules, and nodules on the skin as seen in acne vulgaris [5]. Thus, inhibition of *P. acnes* with topical antibacterial agents such as

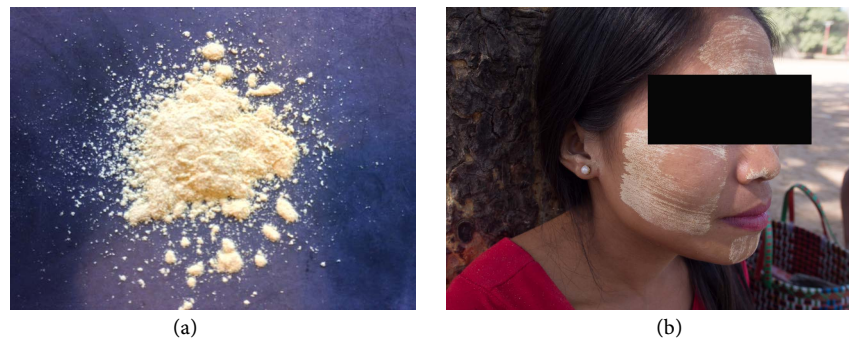


Figure 1. Thanaka powder and application: (a) An example of thanaka powder produced from the bark of *Hesperethusa crenulata* or *Naringi crenulata* trees and (b) An example of direct topical application of thanaka on the face of a Burmese woman.

benzoyl peroxide or blockade of its downstream inflammatory effects is key in treatment for acne [6]. One previous study reported that thanaka possesses some anti-microbial properties against *E. coli* and *S. aureus* using a minimum inhibitory concentration (MIC) assay [7]. The purpose of our study was to evaluate the antimicrobial properties of thanaka against *P. acnes*, *S. aureus*, and *E. coli* using Kirby-Bauer disk diffusion and Clinical and Laboratory Standards Institute (CLSI) breakpoints [8].

2. Methods

2.1. Preparation of Antimicrobial Disks

Kirby-Bauer disks impregnated with thanaka are not commercially available. Thus, thanaka-laced antimicrobial disks were first created mimicking how thanaka is typically used in Myanmar: ground into powder, mixed with water, and applied directly to the face as a paste (Figure 1(b)). However, the thanaka was not completely soluble in water. In an attempt to improve solubility, thanaka was dissolved into 95% ethanol: water: tween 20 (5:93.5:1.5), to create 1, 5, and 10 mg/ml solutions, as reported in Wangthong *et al.* [7]. Sterile 6 mm blank paper disks (Becton Dickinson, Cat #231039) were impregnated by soaking in the thanaka solutions for 30 min.

2.2. Clinical and Laboratory Standards Institute (CLSI) Kirby-Bauer Disk Diffusion

Isolates of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *P. acnes* (clinical isolates identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) were isolated on 5% sheep-blood agar (Becton Dickinson, Cat# 221261). Mueller-Hinton agar plates were inoculated with 1:10 dilutions of 0.5 McFarland bacterial solutions [8]. The following antimicrobial disks were applied to each plate: 1 each of clindamycin (positive control, Beckton Dickinson, Cat #231213), ethanol: water: tween 20 (5:93.5:1.5) (negative control) and 2 each of the 1, 5, and 10 mg/mL thanaka-impregnated disks (Figure 2). Plates were incubated at 24 h at 35°C for *E. coli* and *S. aureus* and 48 h under anaerobic conditions at 35°C for *P. acnes* following CLSI protocol.

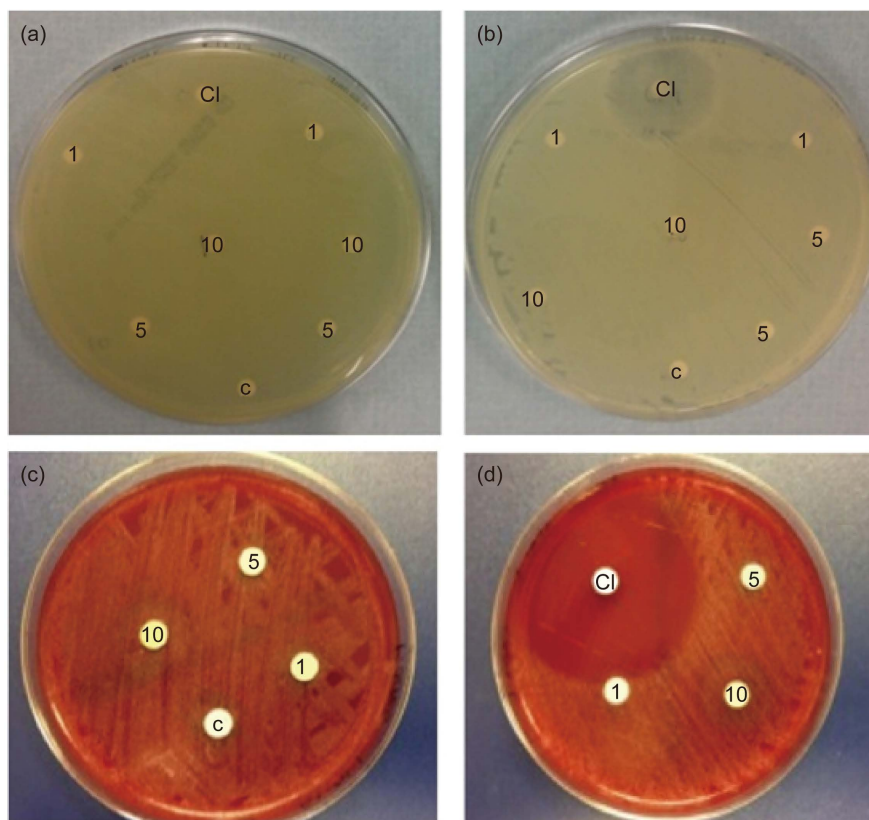


Figure 2. Kirby-Bauer disk diffusion results: (a) *E. coli* and (b) *S. aureus* on MHA with clindamycin and buffer controls and varying concentrations of thanaka disks (values listed in mg/ml) (c) and (d) *P. acnes* on Brucella blood agar with the clindamycin and buffer controls and varying concentrations of thanaka disks (values listed in mg/ml, c = control, Cl = clindamycin).

3. Results

The measured zone of inhibition for clindamycin was 33 mm against *S. aureus*, consistent with CLSI guidelines (Figure 2) [8]. The measured zones of inhibition for clindamycin against *E. coli* was 0 mm and against *P. acnes* was 48 mm; however there are no disk diffusion guidelines published for clindamycin against *E. coli* or *P. acnes* (Figure 2) [8]. There were no zones of inhibition for the disks of 1, 5, and 10 mg/ml thanaka or the negative control disks against *S. aureus*, *E. coli*, or *P. acnes* (Figure 2).

4. Discussion

In an attempt to study the antimicrobial properties of thanaka, the Kirby-Bauer disk diffusion protocol was utilized [8] [9] [10] [11]. The lack of a zone of inhibition for the thanaka disks of all three tested concentrations could be attributed to a number of possibilities. First, thanaka is not completely soluble in the sterile, deionized water (Figure 3(a)). In order to create thanaka-impregnated disks, thanaka was first dissolved in sterile, de-ionized water. Mixing thanaka with water mimics how the powder is typically used in Myanmar: the thanaka bark is ground into powder, mixed with water, and applied directly to the face as a paste

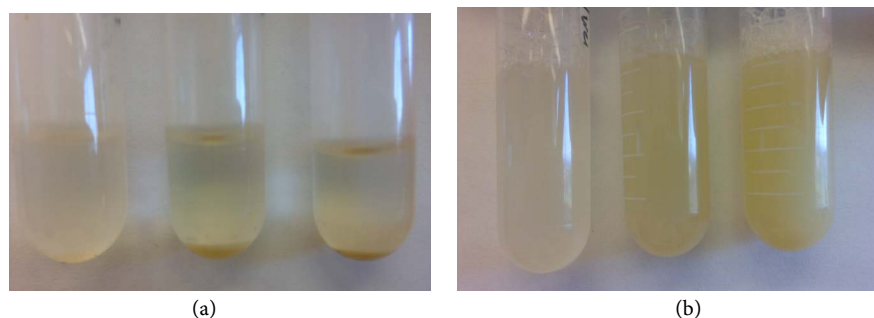


Figure 3. Solubility testing: Solutions of thanaka dissolved in (a) sterile, de-ionized water or (b) 95% Ethanol: Water: Tween 20 (5:93.5:1.5) at 1, 5, and 10 mg/ml.

(**Figure 2**). In an attempt to improve solubility, the protocol was modified by dissolving the thanaka into 95% ethanol: water: tween 20 (5:93.5:1.5), similar to that used by Wangthong *et al.* [7]. Of note, Wangthong *et al.* utilized extracted compounds from thanaka rather than testing the powder itself. Addition of alcohol and detergent led to improved solubility (**Figure 3(b)**), but may have impacted the ability to measure antimicrobial activity. Second, the concentrations previously demonstrated by MIC interpretations may not exhibit an equivalent Kirby Bauer disk diffusion *in vitro* activity. This is not uncommon in the CLSI tables [8]. Third, the thanaka product may not have direct antimicrobial properties against *E. coli*, *S. aureus*, or *P. acnes*. One of the chemical constituents of thanaka is coumarin, which has known anti-oxidant and anti-inflammatory activity [12] [13]. Consequently, the role thanaka plays in acne treatment may be related to anti-inflammatory properties, rather than antimicrobial activity. Fourth, it is possible that the thanaka, or one of its active ingredients, has poor agar diffusion similar to other large, bulky antibiotics, like colistin or other polymyxins [14]. Finally, it is important to note that commercially available thanaka powder is sometimes mixed with other ingredients such as sandalwood or apple wood. Although we attempted to test ‘pure’ unmixed thanaka, we did not have a method to validate its purity. Future research into thanaka would benefit from a standardized product.

5. Conclusion

Thanaka powder is used for acne treatment and prevention, amongst other cosmetic and dermatologic uses. In this study, the antimicrobial properties of thanaka were evaluated according to published CLSI breakpoint interpretation for Kirby-Bauer disk diffusion. No zones of inhibition were observed when testing an alcoholic solution of thanaka against *E. coli*, *S. aureus* and *P. acnes*. Thanaka may have poor agar diffusion or may simply not have antimicrobial activity. Aqueous and alcoholic extracts of thanaka have, however, been shown to inhibit inflammation by blocking free radical release which may be beneficial in acne [7]. Standardizing a system for obtaining extracts of thanaka would benefit future investigations of its anti-acne properties. However, even with a standardized product, disk diffusion may not be the best modality for testing the efficacy of

thanaka against *P. acnes* or skin flora. Ultimately, studies exploring the anti-inflammatory properties of thanaka may provide greater insight into its therapeutic potential.

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