



Antimicrobial Activity of Steamed Lime and Honey Remedy against *Streptococcus pneumoniae*

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ABSTRACT

This study was conducted to determine the antimicrobial activity of steamed lime and honey against *Streptococcus pneumoniae*. The inhibition zone of pure steamed honey concoction against *Streptococcus pneumoniae* ATCC 49619 was 17.75 mm and the pure steamed lime was 26.5 mm. The combination of both pure steamed honey and lime was 25.25 mm. The concoction have shown that the antimicrobial activity of pure steamed lime and honey remedy highly significant and compare favorably with the effect of steamed pure lime alone and pure steamed honey against the *Streptococcus pneumoniae*. The dilution exhibit mild outcome against *Streptococcus pneumoniae* ATCC 49619.

KEYWORDS: *Streptococcus pneumoniae*, Lime, Honey, Zone of inhibition

Introduction

Globally, the bacteria known as *Streptococcus pneumoniae* (pneumococcus) is a significant reason of morbidity and mortality especially in infants and also elderly people (Ghasemi *et al.*, 2009). It is the most vital of human pathogens and is accountable for bacterial meningitis, sepsis, pneumonia, and also acute otitis media (Brugger *et al.*, 2009 ; Daka *et al.*, 2011). According to the immunochemistry, this bacteria can be divided into 90 serotypes and more (Ghasemi *et al.*, 2009).

Penicillin is the antibiotic in which *S. pneumoniae* is susceptible. Not only to penicillin but also to other antimicrobial agents during the first half of 20th century. *S. pneumoniae* highly resistant to normal agents including penicillin, erythromycin, chloramphenicol, and extended spectrum cephalosporins in the recent years. Antibiotics have been employed widely first since the World War II era which has saved a lot of lives and reduced lots of serious complications of many scared infectious ailments, and since it's a highly employed multi drug resistant strains become highly prevalent, the treatment options becomes narrow (Daka *et al.*, 2011).

Lime (*Citrus aurantifolia*), a fruit which can be either with the taste of sweet or sour. Citric acid plus with sugar content highly present in sour lime than lemons and feature a tart and acidic taste. Amino acids, fatty acids, sodium, insoluble and soluble fiber, sugar, minerals, carbohydrates, vitamins and more antioxidant and anti-cancer properties are included in the nutritional summary. The main organic acid in the juices are identified as Citric Acid which was found to be accountable for inhibiting the growth (Bina *et al.*, 2010).

Honey has therapeutic potential. Antimicrobial activity of honey is due to the hydrogen peroxide. The honey can inhibit the growth of a wide range of viruses, protozoa, fungi and bacteria. Honey can clear infection in various ways, moreover its antimicrobial possessions which includes enhancing the immune system, having anti-inflammatory, antioxidant activities and via stimulation of cell growth (Tan *et al.*, 2009).

Honey is slightly acidic, pH ranging between 3.2 and 4.5 (Raied, 2009). In current times, honey has been recounted to have an inhibitory outcome to about 60 species of bacteria comprising anaerobes and aerobes, gram-negatives and gram-positives. It destroys and/or prevents the growth of some pathogenic vegetative micro-organisms (Peter *et al.*, 2007).

Material and methods

Collection and preparation of test material

The lime (*Citrus aurantifolia*) fruits (unripe) were purchased from

a fruit shop at Petaling Street in Kuala Lumpur. The fruits were sent for identification at Herbarium Institute of Bioscience, University Putra Malaysia and was evaluated by Mr. Shamsul Khamis and the voucher number (SK 2164/13) was given. Whereas the lime (*Citrus aurantifolia*) fruits that was free of decay and mold were obtained. The lime were washed with distilled water several times to remove soil and other extraneous matter so that any dirt or bacteria residing on the surface will not be transferred to the fruit's interior (Bina *et al.*, 2010). The honey was purchased from a shop at Petaling Street in Kuala Lumpur. The honey used was Tualang honey.

Test organism

Streptococcus pneumoniae ATCC 49619 was obtained from ASIA Metropolitan University's laboratory.

Steaming method

The tip of the lime was cut and then steamed. Honey also steamed. Whereas for the combination of lime and honey were done by cutting of the tip of the lime and placing the 15ml of honey into it. The combination were steamed together. After that the juice was squeezed and a serial dilution was performed on the concoction.

Preparation of test materials was done by diluting the steam lime juice, steam honey and combination of steam honey with steam lime juice at different dilutions, 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} . All dilutions were carried out with distilled water. Usually the dilution factor at each step was constant (Tumin *et al.*, 2005).

After obtaining the culture sample of *streptococcus pneumoniae*, the bacteria was then subcultured on the blood agar plate. Then a few single *streptococcus pneumoniae* colonies from the culture on the blood agar were inoculated into peptone water to attain McFarland standard suspension turbidity of 0.5 of the microorganism to get hold of the pure culture of *streptococcus pneumoniae* (Tan *et al.*, 2009).

Well diffusion method

Firstly the blood agar was prepared using the commercially manufactured blood agar powder. The *Streptococcus pneumoniae* was then uniformly streaked on the blood agar plate surface using sterile swab which has been dipped in the organism suspension. In this approach, small circular holes were prepared using sterilized cork borer on agar plates seeded with test organisms. These holes were filled with different concentrations of the lime and honey remedy using micropipette (Tumin *et al.*, 2005). The antimicrobials present in the lime and honey is permitted to diffuse out into the medium and intermingle with the agar plate freshly seeded with the test organisms *Streptococcus pneumoniae*.

Result and discussion

Table 1: Zone of inhibition of Honey, Lime, Honey + Lime in mm ± SD

	HONEY	LIME	HONEY + LIME
PURE	17.75 ± 0.50	26.50 ± 0.58	25.25 ± 0.50
10 ⁻¹	15.75 ± 0.50	15.00 ± 0.00	16.50 ± 0.58
10 ⁻²	14.00 ± 0.00	14.50 ± 0.58	14.50 ± 0.58
10 ⁻³	12.00 ± 0.00	12.00 ± 0.00	14.25 ± 0.50
10 ⁻⁴	11.50 ± 0.58	11.75 ± 0.50	14.00 ± 0.00
10 ⁻⁵	11.25 ± 0.50	11.50 ± 0.58	12.00 ± 0.00

From the table 1, it was clearly stated that pure honey, lime and combination of honey and lime produced better inhibition zone compared to other dilutions because the honey and lime purely introduced and it does not contain any foreign substances like in dilutions, where in dilution, the concentration of pure substances were reduced gradually using serial dilution method.

Combination of honey and lime however does not produced any synergistic or additive effects. When narrowly looked at the decreasing dilution concentration from 10⁻¹ to 10⁻⁵, the zone of inhibition also were decreasing from as high as 15.00- 16.50mm in 10⁻¹ to 11.25- 12.00 mm in 10⁻⁵.

Streptococcus pneumonia is also known as pneumococcus which is a round-oval to lanceolate cocci, gram-positive with a size of 0.5 to 1.25 millimeter (mm). The cells are surrounded by a polysaccharide capsule. The carrier percentage in healthy adults flanked by 40% and 70% (<http://streptococcus-pneumoniae.org/>, 2010).

Lime can achieve its bacterial inhibition because the lime juice contains a group of flavonoid phytochemicals with anti-bacterial which is flavonol glycosides (<http://www.livestrong.com>, 2012). Investigation of the antimicrobial activity of lime juice in combination with other herbs and alone has been studied (Onyeagba *et al.*, 2004) and lime juice has high antimicrobial activity which have been identified (Aibinu *et al.*, 2007). In current study, it was further proven lime has antibacterial activity.

Honey has therapeutic potential. Antimicrobial activity of honey is due to the hydrogen peroxide. The honey can inhibit the progress of a wide range of viruses, protozoa, bacteria and fungi (Tan *et al.*, 2009). Apitherapy or therapy using bee products is an age-old therapeutic practice as recognized by some ancient civilizations. Indeed, in the world's oldest medical literatures, honey's medicinal importance has been recorded. Honey has further been found to own good antimycobacterial activity (Tumin *et al.*, 2005). Honey is a natural antiseptic and also holds antimicrobial agent (Needham, 2010). In this study honey inhibit the growth of the bacteria.

In the dilution from 10⁻¹ to 10⁻⁵, the zone of inhibition were gradually reducing because the phytochemicals of the lime and honey were reduced everytime while doing the serial dilution from 10⁻¹ to 10⁻⁵.

Conclusion

This study provides evidence that pure steamed of lime (*Citrus aurantifolia*) and Tualang honey concoction exhibits an antimicrobial activity against *Streptococcus pneumonia* and the combination of steamed lime and honey possess effective inhibition. Whereas the dilution of steamed lime and honey shows less effective compared to the combination of steamed lime with honey which is much better in inhibiting the *Streptococcus pneumonia*.

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