Lime juice and soap inhibit growth of dermatophytes *in-vitro*

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Abstract

Trichophytons are common causes of tinea pedis. There is a belief among rural communities in Sri Lanka that cleaning the toe webs with a piece of fresh lime fruit (*Citrus acida*) is helpful in preventing infections on the feet. *Cassia alata* ('Eth thora'- Sinhala) leaf has also been claimed to have antifungal properties. We planned to evaluate the efficacy of fresh lime juice, *Cassia alata* (CA) leaf, lime juice with CA leaf and various soaps, in inhibiting dermatophyte growth *in vitro*. CA leaf pulp, pieces of soap (Lux, Pears Baby Soap, Lifebuoy, Sunlight etc.), CA leaf pulp with lime juice and lime alone were placed in small wells cut out on Sabouraud's agar plates with a topical spread of dermatophyte spores. Miconazole and econazole discs were used as posive controls. Fungal cultures were observed on day 3,5 and 7. There were definite zones of inhibition of fungal growth around lime juice, 'lime juice with CA leaf pulp', all the soaps tested and the positive controls. There was no inhibition around CA leaf pulp. pH of lime juice was 2.32 and that of Lux^{R} soap saturated solution was 10.28. We conclude that lime juice and soaps have antifungal properties against dermatophytes in vitro. It may be due to the fact that dermatophytes favour a pH of around 5.5 (normal body pH) for growth. This finding indicates that use of a piece of lime probably reduces the chances of colonization of dermatophytes on the toe webs, which has important public health implications. *Cassia alata* did not show any antifungal effect in this study.

Introduction

Trichophyton, epidermophyton and microsporum are the genera that cause dermatophytosis. Trichophytons are the commonest cause of tinea pedis worldwide[1]. It occurs commonly on toe webs. There is a belief in some rural areas in Sri Lanka, that when a person walks in muddy water in a rainy day, it is a good habit to clean the toe webs with a piece of lime fruit. Lime is well known to have antibacterial properties.

In Ayurvedic medicine, which has been the major form of medical system practiced in the country before the Western allopathic system became widespread, *Cassia alata* or winged senna ('Eth Thora' in Sinhalese) has been used as an antifungal preparation for superficial fungal infections, sometimes with lime juice[2,3].

The normal skin pH is around 5.5. Dermatophytes also grow well in culture around this pH (pH of mycobiotic agar (Difco) is 6.5 +/- 0.2 and pH of Sabouraud agar (Difco) is 5.6 +/- 0.2). There is no data available on whether dermatophytes will grow in high acid or high base pH environments, although it is unlikely.

The authors went on to assess whether there is a scientific basis for these practices.

Aims

To assess in-vitro, whether *Cassia alata* leaf pulp, fresh lime juice or their combination or soaps have antifungal properties against dermatophyte fungi.

Materials and Methods

Naturally occurring mature *Cassia alata* ('Eth Thora') plant leaves (fresh) were obtained from a growth on a bank of a waterway at Nawala, (bordering Colombo city). The characteristics given in Medicinal Plants Used in Ceylon (Jayaweera 1981) were used to confirm the identity of the plant[2]. The leaves were washed in plain tap water and crushed and homogenized in a blender at the Natural Products Division of the Medical Research Institute. A voucher specimen of *Cassia alata* was kept for identification. Ehtanol extracts were prepared with some *Cassia alata* leaves. Sabouraud agar was prepared in petri dishes and after the agar settled down, a suspension containing *Trichophyton rubrum, Trichophyton mentagrophytes and Microsporum canis* spores (equivalent of 0.5% McFarland turbidity) was spread evenly (in separate petridishes) on the surface. Several small 'wells' were cut when the medium became settled in the petri-dishes. Into these wells, small flakes/pieces (to fit the well in the agar plate) of

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Lux[R] soap (Unilever, Colombo), Pears baby soap (Unilever), Eth thora soap (Harischandra Industries, Matara - this soap has 1% extract of eththora aqueous extract according to manufacturer's claims), Lifebuoy carbolic soap and Sunlight soap (Unilever, Colombo) were placed. Commercially available laboratory standard miconazole (Janssen) and econazole discs were placed in two other wells in the petri-dish. Similarly, inside other dermatophyte spore impregnated agar containing petridishes, crushed *C. alata* leaf pulp, *C alata* leaf pulp with lime juice (*Citrus acida*), fresh lime juice, methanolic extract of *C. alata* leaf and miconazole and econazole discs were placed in the 'wells' cut out. The growth of the fungus was observed after 3 days, 5 days and after 1 week.

These tests were done with different species of dermatophytes separately (e.g. *T. mentagrophytes, M. canis*)

In a subsequent study, pH of lime (*Citrus acida*) was tested with a pH meter (CyberScan pH1000 Bench pH Meter, Eutech Instruments, Singapore) at room temperature, and pH of saturated solution of Lux soap prepared at room temperature was also tested. pH of serial dilutions of 50% and 25% lime juice was also tested with the same pH meter.

Results

There was a significant zone of inhibition surrounding miconazole, econazole, lime with *C. alata* and lime alone. Pure *C. alata* leaf pulp or the ethanolic extract of *C. alata* did not show any significant zone of inhibition. The zone of inhibition of fungal growth was greater for miconazole and econazole discs, compared to lime juice and *C. alata* and lime juice containing wells on the petri-dish. Figure 1 shows zones of inhibition of *Trichophyton mentagrophytes* growth due to *C. alata* leaf pulp with lime and miconazole and econazole discs.

A compounding factor in the case of '*C. alata* leaf pulp and lime' and '*C. alata* leaf pulp alone' were the unexpected growth of Penicillium like unrelated fungus within the leaf pulp. When the experiment was repeated, this contaminant fungus grew again after several days.

There was a zone of inhibition of fungal growth around the wells of all the soaps tested. Figure 2 shows clear zones of inhibition of *T. mentagrophytes* growth around the miconazole disc, and also around a soap, whilst there is no significant inhibition around *C. alata* leaf pulp with distilled water. The area of inhibition of fungal growth was similar with all soaps. There was no additional inhibitory effect of 'eth thora soap' over other soaps.

pH of lime at room temperature was 2.32. The saturated Lux soap solution had a pH of 10.28 at room temperature. Dilutions of 50% and 25% lime juice had a pH of 2.41 and 2.46 respectively, indicating that even 25% dilution of lime juice maintains a strong acid pH.

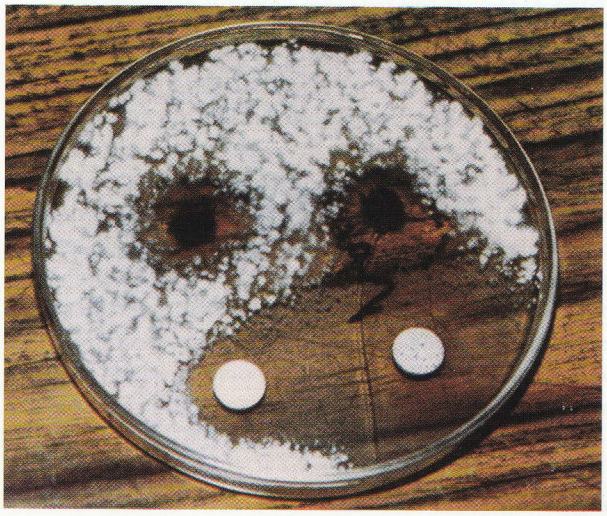

Figure 1. *Miconazole and econazole discs as well as C. alata leaf pulp with lime juice show zones of inhibition of growth of Trichophyton mentagrophytes.*

Figure 2. *Miconazole disc (above) and soap (below) show clear zones of inhibition of fungal growth (T. mentagrophytes) around them, but C. alata leaf pulp (2 dark spots on the photograph) does not show any zones of inhibition.*

Discussion

Some previous authors have reported that *C. alata* leaf has antifungal properties[4-7]. The efficacy against dermatophye fungi have been variable according to published data. The first author became interested in this plant after hearing from patients presenting with tinea corporis or cruris that they used the 'Eth thora' leaf extract for their 'ring worm' as a home Ayurvedic remedy, although with partial success. However according to this study we are unable to confirm any definite antifungal activity against dermatophytes. Our negative conclusion in this regard may be premature, as there was penicillum like fungal contamination of the eth thora sample. This is probably due to the contaminants present on the leaf itself before making the pulp. In the clinical setting when the patient uses freshly ground leaf with lime, the effect could be different. Lime juice has also been reported to have immunomodulatory properties which may be useful in fighting against fungi and other microbes[8].

It was interesting to note that lime alone as well as lime with eth thora leaf had a zone of inhibition to fungal growth indicating that at least lime has an antifungal property against common dermatophytes. This effect may be due to an inherent property of lime juice or due to the acidic pH of the lime. All the soaps tested also had a zone of inhibition. Here again it is not clear whether it is a special quality of soaps or the high (basic) pH of soaps.

The authors feel that it is more likely that it is the high or low pH which inhibits fungal growth. In the clinical situation however, if the fungus has penetrated into the epidermal cell layers it is unlikely that these topical preparations will be of much use due to the dilution factor and the barrier effect of the epidermis. However, before the fungus gets a hold on to the epidermal cells it is likely that both soap and lime have beneficial effects. In the subsequent observation with serial dilutions of lime juice, we found that 50% lime had a pH of 2.41 and 25% lime juice had a pH of 2.46, still within high acidic range. Thus the practice of using a piece of lime on toe webs after walking in muddy waters may have a prophylactic effect on tinea pedis rather than as a form of treatment.

Lime has antimicrobial activities. Considering the antibacterial properties of lime, the practice of cleaning toe webs and soles with a piece of lime may also reduce the chances of Corynebacterium sp. causing pitted keratolysis of the feet as well. This needs further evaluation. The use of lime juice as a food garnisher has proven to be antibacterial to *Vibrio cholera*, and it has been proven to reduce probability of spread of cholera in the community[8-10]. Similarly, finding a scientific value in indigenous practices is important from the community point of view.

Lime juice may also cause irritation of sensitive skin as well as cause a phyto-photodermatitis due to presence of psoralens[11]. However both these issues are not clinically very important, unlike in sun exposed areas, if it is used momentarily on the toe webs before washing off.

Interestingly these findings give some credence to the age-old practice of using a piece of lime to clean the toe webs in the rural areas of the country. Likewise there may be some other pharmacologically or prophylactically valuable 'natural secrets' hidden in some of the practices in indigenous medicine. As increasing microbial resistance to standard drugs is a growing concern, finding newer substances with anti-microbial properties is of great importance. However these claims have to be scientifically proven before blindly believing in indigenous practices. More scientific research is necessary for antifungal properties of lime.

Conclusions

Lime juice and soaps of various types have antifungal properties against dermatophytes. This effect may be due to the fact that dermatophyte fungi do not grow at extremes of the pH scale. These findings suggest that there is a scientific basis for the traditional practice of 'use of a piece of lime on toe webs as a healthy cleanser' to keep away the microbes. The herbal plant *Cassia alata* did not demonstrate antifungal effects against dermatophyte fungi.

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