

1. INTRODUCTION

In 1989 a Nepalese publication documented a tome of South Asian traditional medicine, pertaining to natural methods for rendering groundwater both potable and palatable (Bhattarai, 1989). The main disinfectant isolated was *Luffa cylindrica*, a tropical and subtropical gourd. This investigation aimed to quantitatively verify any antimicrobial properties of the plant, for potential usage in developing countries as an alternative water disinfectant. To date, no tests have been conducted on *L. cylindrica*'s potential in water disinfection. This investigation sought to observe any disinfectant effects of *L. cylindrica* extracts on water samples. River water from both Nepal and London were thus tested with *L. cylindrica* extracts, using the Membrane Filtration Procedure to ascertain bactericidal activity. *E. coli* samples were also tested against the *L. cylindrica* extracts.

2. OBJECTIVES

- To make a simple aqueous extract of *L. cylindrica*, using the plant's seeds, leaves and fruit.
- To test the antibacterial effect on total and faecal coliforms ("TC" and "FC") upon addition of these extracts using the Membrane Filtration Procedure.
- To optimise the antibacterial effect, if found, by varying extract concentration, volume-added, and contact time.
- To assess whether the extracts tested could be viably used in a developing country context, by working at both Imperial College London and Nepal Engineering College.

3. METHODOLOGY

Extracts were made with dried and powdered samples of *L. cylindrica* seeds, fruits, and leaves. Seeds and leaves were mainly tested in Nepal, while the fruits were mostly tested in London. In London, fruit samples were boiled for 1 minute, and then centrifuged for 5 minutes, and the supernatant forming the final extract. In Nepal 30 minute's boiling time on seed and leaf extracts due to a lack of antibacterial activity after 1 minute's boiling time.

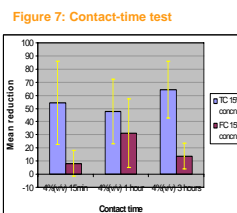
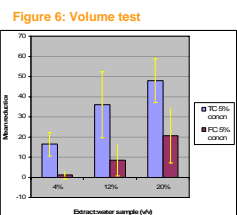
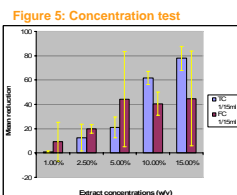
Extracts were then added in different volumes, concentrations, and for different contact times with river water samples. Volumes were shown as extract volume (ml) / water sample volume (ml), or volume/volume (v/v). Concentrations were shown as dried sample weight (g) / 100ml distilled water, or weight/volume (w/v). The contact time consisted of the minutes a water sample was left in contact with *L. cylindrica* extract before filtering out.

The Membrane Filtration Procedure consisted of filtering the water sample (with or without extract) through a special membrane capable of capturing the bacteria in the sample. This membrane was then plated on selective medium and incubated. Resulting bacteria were counted by enumerating the colonies formed. Two mediums were used, mFC and mENDO, respectively selecting for faecal (FC) and total coliforms (TC).

4. RESULTS

4.1 London vs Nepal

A significant gap between utilities and facilities was seen between the laboratories in London and Nepal. This resulted in most of the results in Nepal showing heavy contamination, despite measures to keep the environment aseptic.



4.2 Tests on *L. cylindrica* fruit

Conducted in London, these tests revealed the least error. A clear relationship was seen between extract addition and antibacterial reduction of total (TC) and faecal (FC) coliforms.

Extract concentration and volume-added were seen to have strong relationships with bacterial reduction. The effect of contact-time, however, was less clear. All values in figures 6,7 and 8 were mean values, with 3 replicates each. The yellow error bars represent the standard deviations.

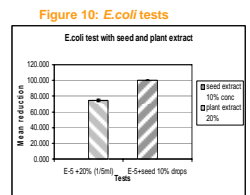
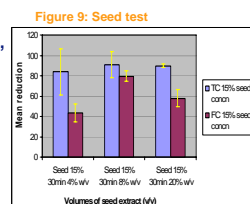
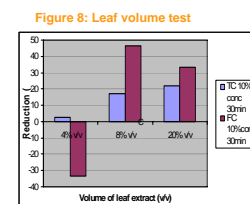
Figure 5 illustrates the effect of extract concentration on TC and FC reduction. TC reduction was increasingly greater as higher concentrations were added, nearing 80% coliform reduction. FC reductions seemed to peak around 5% w/v concentrations, and then fluctuated around the 40% reduction mark from then on.

Figure 6 shows a clear increase in reduction for both TC and FC as the volume of extract-added was increased. TC reductions were always higher than FC by a minimum factor of 2.

Figure 7, showing the relationship between coliform reduction and contact time reveals a less clear trend. The FC bars suggest a peak in reduction at 1 hour contact time, whereas the TC bars show a peak at 3 hours. However, further tests were conducted with more time increments (though no replicates), suggesting a peak between 1 to 2 hours.

4.3 Tests on *L. cylindrica* seeds and leaves

Most of these tests were conducted in Nepal, with a few tests replicated in London to see whether location could account for a valid difference. The replicates showed that while the rougher conditions in Nepal accounted for significant error, there were clear trends apparent in both sets of data.



Leaves were seen to have little effect, with only high volumes having an effect (figure 8). Seeds were seen to have strong reduction potential, reaching the highest average reductions seen (up to 90%) in both total and faecal coliforms.

Tests against *E. coli* showed a good reduction with seed extracts (over 75%). Plant extract drops added to a bacterial lawn (though not directly representative of tests in solution) showed 100% bacterial reduction.

4.4 Problem: bacterial growth

Importantly, it was noted that when river water was left overnight, coliform counts reduced. When added to extract however, exponential growth was seen. This was also seen in many of the seed and leaf trials.

5. CONCLUSIONS AND RECOMMENDATIONS

The antibacterial effect of *L. cylindrica* was clearly established. However, its potential for water disinfection is debatable. Fruit extracts, particularly in high concentrations and volumes showed the least variable reduction. Seed extracts on the other hand showed the highest reductions, but with many trials having negative reduction and high reduction variation. Leaf tests showed the least activity and highest error bars. Though the addition of plant, seed, and leaf extract all exhibited some level of antibacterial activity, the growth of bacteria in many cases (particularly with time) suggested another element at play. It was thus hypothesised that the bacterial reduction – due to certain active chemicals in the plant – was in competition with nutrients in the extract which stimulated bacterial growth. The extract used was effectively an aqueous solution of water and plant nutrients, which is an environment in which bacteria can thrive. It was thus concluded that the present extract was not suitable for disinfection. Tests should be conducted to elucidate the active chemicals bearing antibacterial activity, in order to devise a simple extraction method which would also avoid the issue of bacterial growth.