Abstract

Northern Peruvian curanderos have traditionally used mixtures of local plants to treat infectious diseases. We hypothesized that 2-plant mixtures would exhibit lower toxicity than the individual plants, and tested this using brine shrimp toxicity assays in three 2-plant mixtures (Pimpinella anisum/Mentha spicata, Tagetes filifolia/Mentha spicata and Apium graveolens/Petroselium crispum). Serial dilutions of plant extracts were added to brine shrimp, and the dead shrimp were counted one day later. The LC₅₀, the concentration necessary to kill 50% of the shrimp, was calculated from percent survival curves. LC₅₀ values ranged from 0.49 to 1.35 mg/mL in the tested plant extracts. The extract of the 2-plant mixture (T. filifolia/M. spicata) exhibited lower toxicity than the extracts of either component plant (higher LC₅₀). The 2-plant mixture that exhibited lower toxicity when compared to its individual components merits further study and demonstrates the use of this toxicity assay in the search for new antibiotics.

Methods

Extraction Preparation: Plants were purchased in local markets, dried and ground. Voucher specimens were prepared for storage at the herbariums at the National University of Trujillo and the Missouri Botanical Garden. The plant material was incubated in ethanol for 24-72 hours at room temperature in the dark, before being filtered through filter paper and concentrated in a rotary evaporator and then dried in an oven at 50°C. The dried extract is then reconstituted in boiling distilled water and centrifuged, although it may be cotton filtered or re-heated prior to centrifugation. The extract is then filter-sterilized through a syringe filter and placed in a sterile test tube. A dry weight is taken to determine extract concentration. Plant mixture extracts were prepared with 50% of each component plant.

Results

Brine Shrimp Toxicity Assay: Brine shrimp eggs (Fig. 3) are hatched overnight under light in the commercial instant ocean solution. Fig. 4 shows a hatched shrimp. The following day, 32 test tubes are prepared, in 4 sets of matching replicates. These include 6 serial dilutions of the plant extract being tested. The plant extracts are diluted with instant ocean solution. Each replicate additionally contains one tube with only instant ocean solution as a negative control (all shrimp should survive) and one tube with KClO₃ as a positive control (all shrimp should be killed). A pipette was used to add about 20 brine shrimp to each tube, and then they are left under light for 24 hours. The following day, the number of dead shrimp extracted from each tube is counted in wells. Ethanol is then added to each well, which kills the remaining shrimp so that the total number of shrimp can be counted, allowing for the determination of percent living and dead shrimp in each tube to be made. The LC₅₀ is determined by graphing percent survival vs. extract concentration and fitting a linear equation to the area of the curve with the most change. This allowed the concentration at 50% shrimp death (LC₅₀) to be calculated. In this case, it was presented as the mean ± standard deviation for 4 replicates in each experiment or for more replicates if experiments were combined.

Brine Shrimp Toxicity Assay (continued):

The other two plant mixtures tested exhibited toxicity values intermediate between those of their components. Neither of the three mixtures tested exhibited toxicity values greater than both of their two components, as can be seen in Table 1 showing the mean and standard deviation of LC₅₀ values. All experiments were performed at least twice; so there are at least 8 replicates. The data were used to calculate the average LC₅₀ for all plants and plant mixtures, with the exception of the P. anisum/M. spicata mixture. The trends observed in these experiments need to be verified by statistical analysis.

Conclusions

• One of the three plant mixtures (T. filifolia/M. spicata) exhibited a trend of lower toxicity than its component plants, and shows promise as a potential antibiotic if antibiotic properties can be verified.

• The other two plant mixtures exhibited a trend of toxicity values in between those of their two components.

• The reproducible results shown in these experiments verify the use of the brine shrimp assay in measuring plant extract toxicity.

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References


Table 1: Summary of toxicity data for plant mixtures and components

<table>
<thead>
<tr>
<th>Plant Mix</th>
<th>LC₅₀ (mg/mL)</th>
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</thead>
<tbody>
<tr>
<td>P. anisum/M. spicata</td>
<td>1.34 ± 0.54 (n=20)</td>
</tr>
<tr>
<td>T. filifolia/M. spicata</td>
<td>0.97 ± 0.23 (n=8)</td>
</tr>
<tr>
<td>T. filifolia</td>
<td>1.25 ± 0.15 (n=7)</td>
</tr>
<tr>
<td>A. graveolens</td>
<td>1.05 ± 0.20 (n=8)</td>
</tr>
<tr>
<td>A. graveolens/P. crispum</td>
<td>1.45 ± 0.62 (n=19)</td>
</tr>
<tr>
<td>A. graveolens/P. anisum</td>
<td>1.08 ± 0.19 (n=8)</td>
</tr>
</tbody>
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Figure 6: Percent survival curve for T. filifolia

Figure 7: Raw data percent survival curve for M. spicata

Figure 8: Percent survival curve for T. filifolia

Figure 9: Percent survival curve for Tagetes filifolia

Figure 10: Percent survival curve for Apium graveolens/Petroselium crispum.