Comparison of the Toxicity of Individual Plants and Plant Mixtures Used to Treat Infectious Disease by the *Curanderos* of Northern Peru

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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 graveiolen/Petroseliumum crispum*.) Serial dilutions of plant extracts were added to brine shrimp, and the dead shrimp were counted one day later. The LC_{50}, the concentration necessary to kill 50% of the shrimp, was calculated from percent survival curves. LC_{50} values ranged from 0.49 to 1.35 mg/mL in the tested plant extracts. The extract of one 2-plant mixture (*T.filifolia/M.spicata*) exhibited lower toxicity than the extracts of either component plant (higher LC_{50}). 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.

Introduction

The *curanderos* of Northern Peru (Fig. 2) are practitioners of a tradition of medicine that has been ongoing for thousands of years [2]. The location provides close access to many different plants from several different types of environments (Fig. 1). Northern Peru is in fact considered to be the epicenter of traditional healing knowledge held by the old Central Andean culture [3]. *Curanderos* treat a wide variety of illnesses, including some that are believed by allopathic medicine to be caused by infectious agents [1]. These treatments often involve the use of medicinal plant extracts, suggesting that toxicology and microbiology studies would help characterize the antibiotic potential of these extracts.

The toxicity team hypothesized that plant mixtures would exhibit lower levels of toxicity than would the individual plants making up the mixture and that the simple brine shrimp assay could be used in the existing Peruvian laboratory conditions. Toxicity research is important because it helps to ensure that the extracts will not also be as damaging to multicellular organisms as they are to bacteria, which helps to ensure that plant extracts demonstrating antibacterial properties could potentially be used as safe antibiotics.

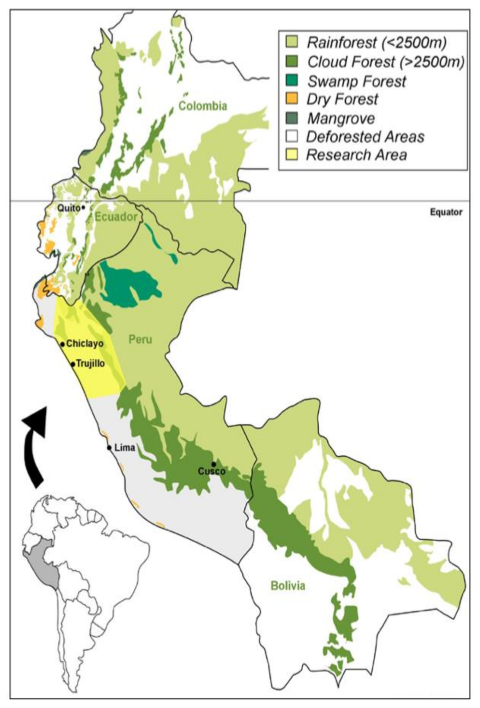

Figure 1- Range of ecosystems in Northern Peru

Figure 2- *Curandera* Julia Calderón performs a traditional healing ceremony. (Photo credit Katelyn Henson)

Methods

Extract 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 resuspended 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.

Figure 3- Brine shrimp eggs being rehydrated prior to hatching

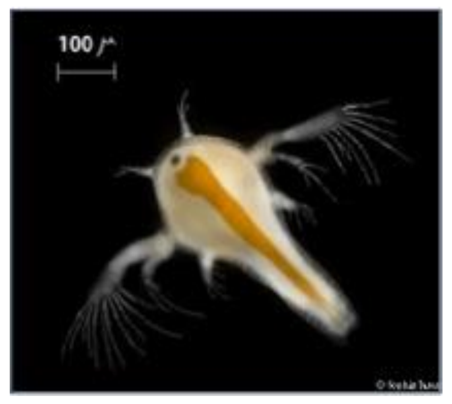

Figure 4- A close-up of a brine shrimp (*Artemia franciscana)*

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 for a negative control (all shrimp should survive) and one tube with $K_2Cr_2O_7$ 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 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 LC_{50} 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 (LC_{50}) to be calculated, and this value was presented as the mean ± standard deviation for the 4 replicates in each experiment or for more replicates if experiments were combined.

Figure 5- Giselle Rodriguez and Emily Bakaj work on a toxicity assay setup. (Photo credit Katelyn Henson).

Results

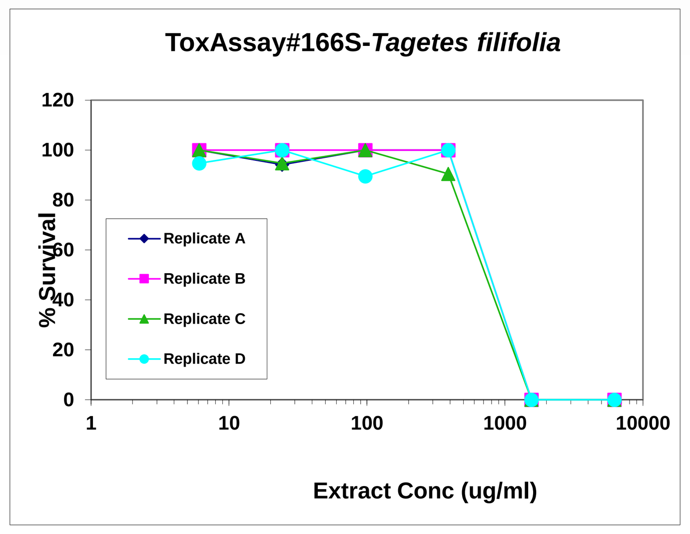

Figure 6- Percent survival curve for *T. filifolia*

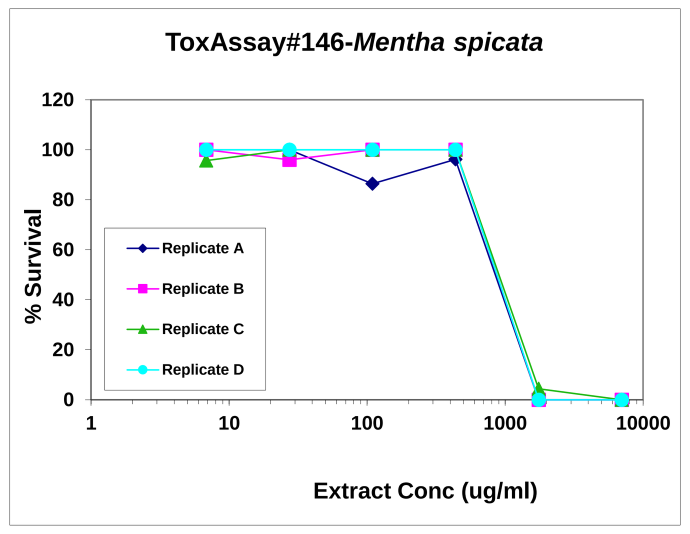

Figure 7- Raw data percent survival curve for *M. spicata*

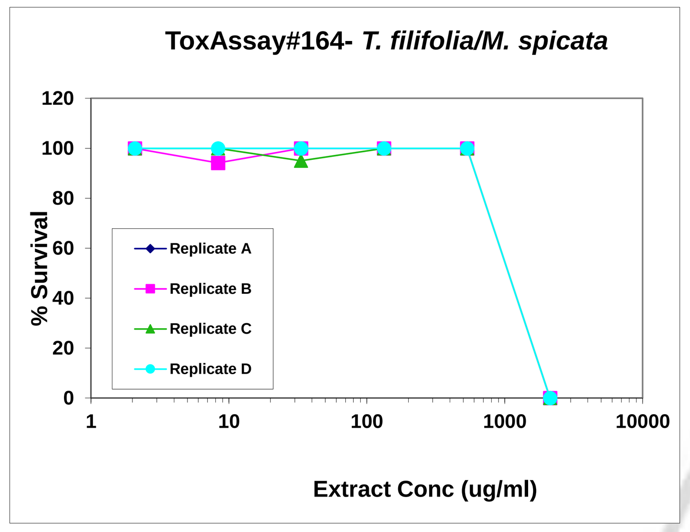

Figure 8- Raw data percent survival curve for *T. filifolia/M. spicata*

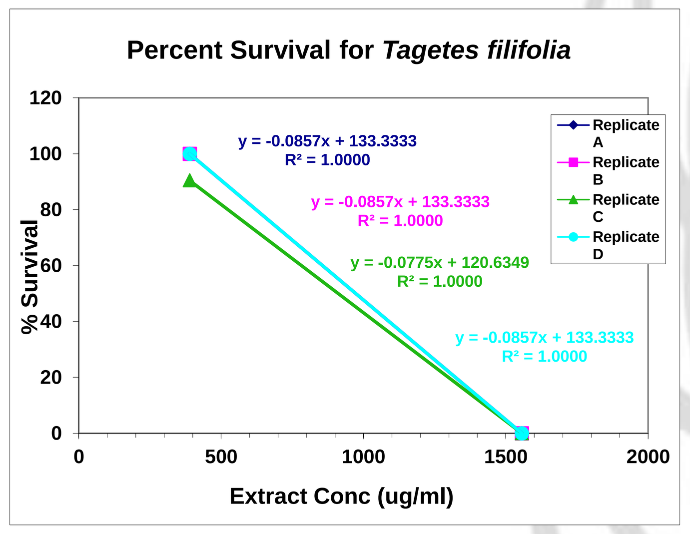

Figure 9- Percent survival for *Tagetes filifolia*

The percent survival curves shown (Fig. 6, 7, and 8) are examples of the raw data used to create the curves from which LC_{50} values are calculated. The part of the curve with the largest amount of change was graphed separately in order to determine the LC_{50}. This was calculated to be the x-variable on the line at which the shrimp survival (y-variable) was 50%. Fig. 9 shows the LC_{50} calculation from one full experiment of 4 replicates. In the examples shown at left, the individual plants *T. filifolia* and *M. spicata* were shown exhibited lower LC_{50} values than did the extract of the mixture of the two plants in this experiment. A lower concentration of plant was required to reach 50% shrimp death which means it was more toxic. Both of the component plants in this mixture are traditionally used to treat diseases that may be infectious in nature [4].

The other two plant mixtures tested exhibited toxicity values intermediate between those of their components. None 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 for LC_{50} values. All experiments were performed at least twice, so there are at least 8 replicates being used to calculate the average LC_{50} 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.

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

Plant	LC50 (mg/mL)
P. anisum	1.34 ± 0.54 (n=20)
M. spicata	0.97 ± 0.23 (n=8)
P. anisum/M. spicata	1.25 ± 0.15 (n=7)
T. filifolia	0.95 ± 0.022(n=8)
T. filifolia/M. spicata	1.34 ± 0.063 (n=28)
A. graveiolen	0.69 ± 0.061 (n=8)
P. crispum	1.45 ± 0.62 (n=19)
A. graveiolen/P. crispum	1.08 ± 0.19 (n=8)

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 antibacterial 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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