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# Ethnobotanical Promotion of Fibroblast Growth Using Yerba Santa Extract

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# Ethnobotanical Promotion of Fibroblast Growth Using Yerba Santa Extract

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## Abstract:

Ethnobotany is a promising method for discovering new drugs, drawing on the knowledge of generations of traditional healers. For hundreds of years, the Chumash people have lived in the coastal regions of California, becoming experts on the many uses of its natural resources. One such resource is the Yerba Santa plant (*Eriodictyon crassifolium)*, which was used by the Chumash to treat a myriad of conditions including coughs, chest pain, and fever. It was also used as a poultice on wounds and cuts, suggesting that the plant has a stimulating effect on the growth of skin cells. Because of these qualities, this experiment quantitatively tested the potential of Yerba Santa to encourage fibroblast growth using a goldfish scale keratocyte assay. The extract tested was made by grinding Yerba Santa leaves and storing them overnight in methanol to allow the release of potentially bioactive molecules from the cells. After methanol extraction, the remaining material was then resuspended in a modified solution of PBS (phosphate buffered saline with MgCl, CaCl, and 10% mass by volume dextrose). Individual goldfish (*Carassius auratus*) scales were then treated with either the modified PBS with extract or the modified PBS alone as a control. After 48 hours, ImageJ software was used to compare the areas of new cell growth. The group treated with extract were found to have enhanced growth relative to the control. The mean growth for control scales was 0.246 $mm^2$ compared to 1.014$mm^2$ for scales treated with the Yerba Santa extract. Mean values were significantly different by a two tailed Student's t-test, P = 0.0063. These results are consistent with the Chumash's use of Yerba Santa to treat wounds and skin abrasions indicating that it may be a viable option as a topical treatment of skin disorders.

## Introduction:

This study quantified the effects of Yerba Santa extract *(Eriodictyon crassifoliu)* on fibroblast growth in fish scales. This plant was chosen for the ethnobotanical properties observed in the past by the Chumash Indians. The Chumash used Yerba Santa in a tea to treat numerous internal ailments. They also used the leaves externally to treat wounds or soreness. *E. crassifolium* is currently used as an herbal drug in the form of tea or extract for similar purposes to that of the Chumash (Timbrook 2007). Due to the rise in popularity of herbal medicines as an alternative to Western medicine, quantitative analysis of the potential effects of Yerba Santa extracts might prove beneficial.

In order to determine the effect of Yerba Santa on fibroblast sheet migration, fibroblast outgrowth from *Carassius auratus* scales were used as an assay (Thompson). These scales were selected because their fibroblasts are known to proliferate rapidly making them easily observable in the time frame of this study (Graham et al. 2014). Scales treated with the Yerba Santa extract were expected to exhibit different amounts of fibroblast growth than scales treated with only a PBS control.

## Acknowledgements and References:

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Graham, David M. et al. "Epidermal Keratinocyte Polarity and Motility Require Ca2+ Influx through TRPV1." *Journal of Cell Science* 126.20 (2013): 4602–4613. *HighWire*. Web. 16 Apr. 2014.

Phan, T T, M A Hughes, and G W Cherry. "Enhanced Proliferation of Fibroblasts and Endothelial Cells Treated with an Extract of the Leaves of Chromolaena Odorata (Eupolin), an Herbal Remedy for Treating Wounds." *Plastic and reconstructive surgery* 101.3 (1998): 756–765. Print.

Thompson, Christine. Biotechnology Fish Keratocyte Cell Culture. Public Schools of Northborough and Southborough MA. http://webcache.googleusercontent.com/search?q=cache:m038nbpIXvAJ:web.nsboro.k12.ma.us/algonquin/faculty/scienceteachers/thompson/documents/FishKeratocyteCellCulture.doc+&cd=5&hl=en&ct=clnk&gl=uk

Timbrook, Jan. *Chumash Ethnobotany*. Berkeley, CA: Heyday Books, 2007.

**Figure 1.** *Eriodictyon crassifolium,* also known as Yerba Santa, has been recognized for its healing qualities by Chumash Indians.

**Figure 2.** Example of slides containing scales treated with modified PBS with or without Yerba Santa extract.

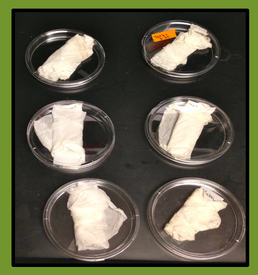

**Figure 3.** Humidity chambers were constructed by using dampened Kim wipes and covering petri dishes in aluminum foil.

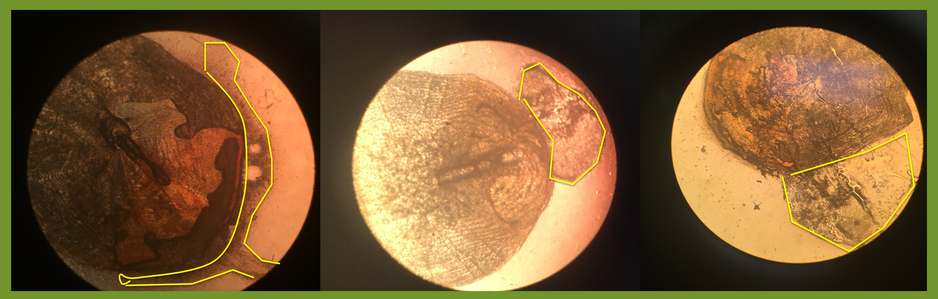

**Figure 4.** Three photographs display representative samples of photographs taken of scales treated with Yerba Santa extract. Migration of keratocyte sheet is outlined in yellow and measured with Image J software.

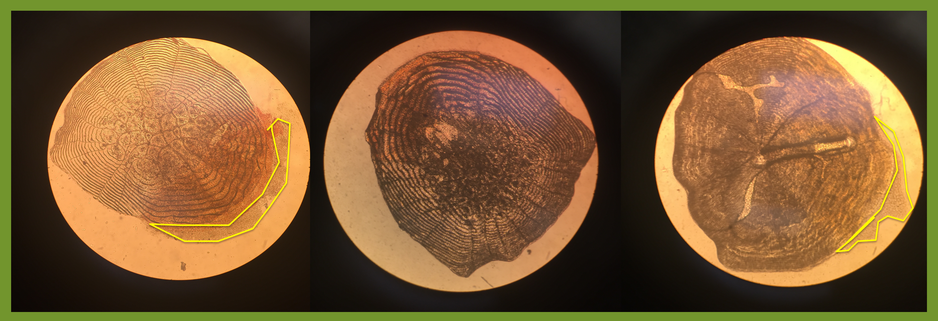

**Figure 5.** Three photographs display representative samples of photographs taken of scales treated only with PBS control. Migration of keratocyte sheet is outlined in yellow and measured with Image J software.

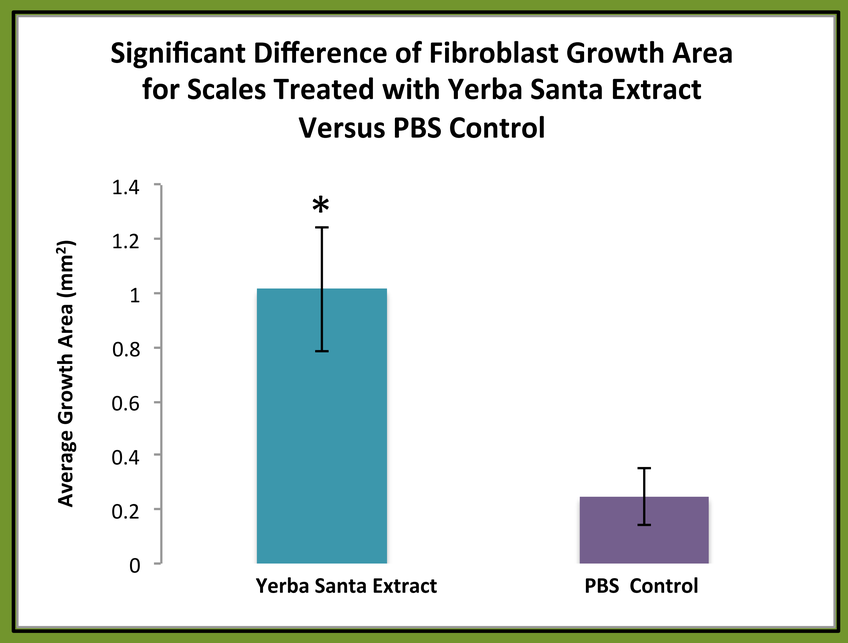


**Figure 6.** Comparison of mean fibroblast growth of 12 different scales incubated with and without Yerba Santa extract. Error bars indicate a standard error of 0.250 $mm^2$ for extract and 0.117 $mm^2$ for the control. Yerba Santa positively affects the growth of fibroblasts on goldfish scales. Statistical difference (indicated by asterisk) was determined by the Student's t-test which produced a p value of P= 0.0063, n=12.

## Methods and Materials:

An extract from *Eriodictyon crassifolium* (Figure 1) was made by first combining approximately 2 g of ground leaf tissue with 10 mL of methanol and vortexing it before storing it overnight. The extract was then placed in the speed vac to evaporate the methanol. Next, the extract was resuspended with 1 mL of modified PBS (phosphate buffered saline). Modified PBS consisted of 75 mL PBS, 0.03 g MgCl, 0.017 g CaCl, and 10% dextrose.

This extract was tested using a fish keratocyte assay. The same sized scales from various *Carassius auratus* fish were used for the assay. After placing a fish on ice for approximately 90 seconds, sterile forceps were used to remove a single scale from the fish before being placed in a 15 $\mu L$ drop of media on a slide. There were two scales placed on each slide as seen in Figure 2. Six slides contained drops of PBS with extract while a different 6 slides contained the PBS media alone. This made a total of 12 scales per group, and cover slips were placed over each scale. After making the slides, they were placed in humidity chambers (Figure 3). Humidity chambers consisted of dampened Kim wipes wrapped around each slide that were placed in petri dishes, stacked, covered with aluminum foil, and incubated at room temperature. Following a 24 hour incubation period, Image J software was used to measure the surface area of the migration of the keratocyte sheet off the fish scale. The software determined surface area by calculating the area outlined in yellow as seen in Figure 4 and 5. With these measurements, difference in surface area was analyzed using a Student's t-test and considered statistically different at $P<0.05$.

## Discussion:

The results showed the area of fibroblast migration to be significantly greater in scales treated with Yerba Santa extract compared to PBS alone. The control average area of growth was 0.246$mm^2$ compared to 1.014$mm^2$ per scale treated with the extract. Using a a two-tailed Student's t-test, the resulting P value was 0.0063, which indicated significant difference between the two groups. The greater surface area of the keratocyte epithelial sheet suggests that there may be a compound present in the extract that causes the fibroblasts to proliferate and/or migrate at a greater rate than in modified PBS alone. The standard deviation for the Yerba Santa treatment group was 0.791 $mm^2$, which showed that there was a fairly high amount of variation about the mean. However, the control group had a standard deviation of 0.340 $mm^2$.

These results are similar to those found in a study of *Chromolaena odorata* which had traditionally been used in Vietnam as a topical treatment for wounds and burns. *Chromolaena odorata* was found to enhance fibroblast growth compared to fibroblasts grown in medium supplemented only with fetal calf serum (Phan, Hughes, and Cherry 1998). In both cases, fibroblast growth was enhanced by application of an aqueous extract of the leaves of plants used in traditional medicine. With the results from this study, further research is needed to understand both the active component of the extract as well as the molecular mechanisms behind the stimulation of cell proliferation and migration that are associated with wound healing.

## Conclusions:

- Average growth area for the PBS control was 0.246$mm^2$ compared to 1.014$mm^2$ for the Yerba Santa extract; more growth occurred in the treatment group
- The growth of fibroblast migration sheet from scales treated with the Yerba Santa extract was significantly different than growth of those in the control group (p=0.0063)
- These findings suggest that there are compounds in Yerba Santa that may stimulate migration or proliferation with migration in fibroblast cells
- Yerba Santa may be a viable option as a topical treatment for wounds