



# GUAYAQUIL UNIVERSITY

## DEPARTMENT OF CHEMICAL SCIENCES

Ciudad Universitaria "Dr. Salvador Allende"

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Guayaquil, Ecuador

### FINAL REPORT

**CODE: CA 88/07**

**TITLE:**

Establishment of the potential anti-inflammatory effect of the product known as **PARSLEY**, originating from NutraMedix Laboratories, LLC. Florida

**OBJECTIVES:**

To study the possible anti-inflammatory effect of PARSLEY, measured by auricular<sup>1</sup> edemas<sup>2</sup> in laboratory mice.

**BACKGROUND**

The auricular edema is achieved by applying 12-0- Tetradecanoil Forbol-13 Acetate (TPA), one of the components responsible for the irritating action of croton oil, into the auditory pavilion of the mouse. The inflammatory reaction consists of erythema<sup>3</sup>, edema and infiltration by polymorphonuclear leukocytes<sup>4</sup>. As such, eicosanoid<sup>5</sup>-type mediators are freed, inducing degranulation of the mast cell<sup>6</sup>. This technique thus allows the evaluation of the inhibiting substances of the biosynthesis of prostaglandins<sup>7</sup> and leukotrienes<sup>8</sup>.

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<sup>1</sup> Of or pertaining to the outer ear.

<sup>2</sup> The swelling of soft tissues as a result of excess water accumulation

<sup>3</sup> Redness of the skin.

<sup>4</sup> A type of white blood cell with a nucleus that is so deeply lobated or divided that the cell looks to have multiple nuclei. Informally called a poly.

<sup>5</sup> A lipid mediator of inflammation derived from the 20-carbon atom arachidonic acid or a similar fatty acid. The eicosanoids include the prostaglandins, prostacyclin, thromboxane, and leukotrienes.

<sup>6</sup> A connective tissue cell whose normal function is unknown but which is frequently injured in allergic reactions, releasing chemicals including histamine that are very irritating and cause itching, swelling, and fluid leakage from cells.

<sup>7</sup> One of a number of hormone-like substances that participate in a wide range of body functions such as the contraction and relaxation of smooth muscle, the dilation and constriction of blood vessels, control of blood pressure, and modulation of inflammation. Prostaglandins are derived from a chemical called arachidonic acid.

<sup>8</sup> One of a group of hormones that cause the symptoms of hayfever and [asthma](#). Derived from arachidonic acid, the leukotrienes act by mediating immediate hypersensitivity. Leukotriene modifiers that prevent the production or action of leukotrienes are used to treat hayfever and asthma.



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As discussed in numerous international works, the pharmacological study of the above-mentioned effect is indispensable, and guarantees (within the margin of error associated with the technique) that the potential for producing anti-inflammatory effects in humans will be learned.

The basis of this work is the pharmacological effect as an anti-inflammatory, as described in international literature (1, 2).

#### **TECHNICAL, SCIENTIFIC AND SOCIOECONOMIC BENEFITS**

The demonstration of this product as an anti-inflammatory is important due to its potential as a new, plant-based medication, with its associated low toxicity. This was demonstrated by us in a previous work, allowing us to enter the product as a new medication in the appropriate Register.

#### **VARIABLES TO MEASURE:**

- 1. Weight of the treated and untreated ears**
- 2. % of Inflammation**
- 3. % of inhibition**

#### **PROCEDURES TO FOLLOW:**

**TEST MATERIALS: PARSLEY** The procedure followed was that described by CYTED (1996) and the Gerhard Voegel (1997).

#### **CHANGES IN THE CURRICULUM:**

Changes did not take place in protocol proposed to the Unity of Quality Guarantee, whose number is referred to on Page 1.



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#### DATA FROM THE SAMPLE:

**Organization soliciting services:** NutraMedix Laboratories, LLC.

**Person in charge of the Organization's application:** Jose Icaza

**Date of application:** 4/20/05

**Person in charge in the Executor Organization:** MSc. Gastón Garci'a Simón.

**Storage:** The product was stored at room temperature with controlled access.

**Organization that carried out the work:** University of Guayaquil, Department of Chemical Sciences.

**Address:** Ciudadela Universitaria "Dr. Salvador Allende"

**Form of presentation of the product:** amber glass drop bottle containing 30 milliliters

**Storage:** The product was maintained at room temperature before and during the experiment, and as indicated was protected from light and kept in a locked cabinet.

#### INFORMATION WITH RESPECT TO THE HANDLING

No special handling instructions were needed.

#### COMPOSITION OF THE PRODUCT:

PARSLEY leaf extract

Mineral water

Ethanol



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#### EXPERIMENTAL PROCEDURE:

##### INTRODUCTION

This experiment was carried out with the intention of determining the possible anti-inflammatory effect of PARSLEY, utilizing croton oil as the inflammatory agent.

##### DOSAGE USED IN THE TEST

0.20ml of PARSLEY per 20 g of body weight was utilized via oral in this study.

##### PRINCIPAL TEST

##### METHODS AND TECHNIQUES

###### Study Material: PARSLEY

**Animal Model:** A single rodent species (mouse) was utilized, with a minimum of 5 animals of a single sex in each group. In this case, male mice with an average weight within  $28g \pm 20\%$  (3), belonging to the Swiss line and coming from the Chemistry Department of the University of Guayaquil were appropriate and were utilized in the experiment.

The animals were maintained in quarantine conditions and were acclimated according to established procedures (4,5), said period having a duration of five days minimum.

Access to the water and the food was "ad libitum."(6,7)



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The animals were randomly distributed from within the different groups.(8)

Food was denied 4 hours before exposure to the test material.

The experiment lasted 6 days (5 of acclimation and 1 of test)

#### DEVELOPMENT OF THE METHOD

The following three groups were constructed for the test:

Test groups	
1	Oil of croton 40 $\mu$ L
2	Oil of croton 40 $\mu$ L + Feldene <sup>9</sup> that covered the two sides of the auditory pavilion.
3	Oil of croton 20 $\mu$ L + 0.20ml of PARSLEY

The mice were denied food for four hours then weighed, after which began the experiment.

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<sup>9</sup> Feldene (generic name Piroxicam) is a nonsteroidal anti-inflammatory drug (NSAID) effective in treating fever, pain, and inflammation in the body.



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The irritant solution of 5% croton oil in acetone was applied topically to the group 1 in the right ear, at the indicated volume, using an automatic pipette.

The composite solutions were administered topically to the group 2 in the right ear immediately after the irritant, in the indicated volume.

The irritant solution of 5% croton oil in acetone was applied topically to the group 3 in the right ear, at the indicated volume, and PARSLEY was immediately administered via oral.

3 hours after the application of the irritant, the animals are euthanized in a saturated ether atmosphere, and their ears are cut along the edge. 6 mm discs were cut with a punch then weighed.

**RESULTS CALCULATIONS:**

Outcomes are rated by calculating the weight of each mouse's ears, both the treated and untreated.

The **percentage of inflammation** of the treated as opposed to the untreated ear is calculated using the following formula:

$$\% \text{ Inflammation} = \frac{T \times 100}{ST} - 100$$

Where T is the average of the weights of the treated ears (right) and ST is the average of the weights of the untreated ears (left).



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$$\% \text{ Inhibition of inflammation} = \frac{C - T}{C} \times 100$$

Where C is the average value of % of inflammation of the animals of the control group and T is the average value of % of inflammation of the animals of the control problem group or control.

**DESCRIPTION OF THE DOSAGE, METHOD OF ADMINISTRATION AND DURATION OF THE TEST.**

The test was achieved by following the method established by CYTED and using the indicated dose for each mouse.

PARSLEY was administered as indicated on the development of the method information.

The composite solutions were applied in the right auditory pavilion of the study animals, the left auditory pavilion being the control.

**ANALITICAL RESULTS**

The results of the average value of the weights of the right and left ears are found in Table #1.



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**Table # 1**  
**Study of the possible Anti-inflammatory effect of PARSLEY**  
**Weight of ears in mg**

<b>GROUP</b>	<b>RIGHT EAR Average <math>\pm</math> SD</b>	<b>LEFT EAR Average <math>\pm</math> SD</b>	<b>WEIGHT DIFFERENCE Average <math>\pm</math> SD</b>	<b>STATISTICAL SIGNIFICANCE Average <math>\pm</math> SD</b>
<b>CROTON OIL ONLY</b>	<b>22.0 <math>\pm</math> 0.9</b>	<b>11.2 <math>\pm</math> 1.2</b>	<b>10.8 <math>\pm</math> 0.3</b>	<b>a</b>
<b>CROTON + FELDENE</b>	<b>12.3 <math>\pm</math> 1.5</b>	<b>10.3 <math>\pm</math> 1.3</b>	<b>2.0 <math>\pm</math> 0.2</b>	<b>b</b>
<b>CROTON + PARSLEY</b>	<b>13.6 <math>\pm</math> 1.8</b>	<b>10.6 <math>\pm</math> 0.9</b>	<b>3.0 <math>\pm</math> 0.3</b>	<b>c</b>

Statistical significance: a, b:  $p < 0.05$

As can be seen from table 1 regarding the difference between the treated ears and not treated ears, animals treated with Feldene and croton oil (group 2), and those treated with PARSLEY and croton oil (group 3) show good anti-inflammatory results than the animal treated with croton oil only (group 1).

The percentages of inflammation and of inhibition of inflammation, which appear in Table 2, were calculated using the values on table 1, as follows:





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**Table # 2**

**Study of the possible anti-inflammatory effect of PARSLEY, % of Inhibition of Inflammation and of Inflammation**

<b>Group</b>	<b>% Inflammation</b>	<b>% Inhibition</b>
<b>Croton</b>	<b>96.4</b>	<b>-</b>
<b>Croton + Feldene</b>	<b>19.5</b>	<b>79.7</b>
<b>Croton + PARSLEY</b>	<b>28.1</b>	<b>70.8</b>

As can be seen from the table, Feldene 0.5% (Piroxicam) demonstrated an anti-inflammatory effect of 79.7% as same as PARSLEY demonstrated an anti-inflammatory effect of 70.8%.

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**CONCLUSIONS**

1. **PARSLEY** was demonstrated to have an anti-inflammatory effect in the animal subjects as it was able to diminish swelling from croton oil as it was supposed in the objectives of this study.
2. Feldene was also shown to have the effect for which it is sold.

**GENERAL CONCLUSIONS**

**PARSLEY** was demonstrated to have an anti-inflammatory effect, less than Feldene, but able to diminish inflammation from irritant agents like croton oil.

**PERSONNEL RESPONSIBLE FOR THE STUDY:**

**STUDY DIRECTOR:**  
Dr. Walter Herrera

**Signature:**

**PROFESSIONAL IN CHARGE:**  
Dra. Ma. Fernanda Mora F.

**Signature:**

**Date: 16/02/07**



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**BIBLIOGRAPHY**

CYTED course for Researchers in the Discovery of new medicines, Lima  
November 1996, Edema Earpiece pp 83.

Drugs Discovery, Gerhard Voegel (1997).

Procedure. Bodily weight of mice

Procedure. Guide for the care of laboratory animals

Procedure. Quarantine

Procedure. Sub-minister of Water, Usage Manual.

Procedure. Random Allocation of rodent species

Procedure. Euthanesia

Pink W. "Statistics for toxicology," in *Principles and methods of Toxicology*, W.  
Hayes, ed. Raven Press, N:Y: 1994