



## Derivated products from *Achyrocline satureioides* and *Arnica montana*, with immunomodulating effects

Flavia S. Alaniz<sup>1</sup>, Luis A. Mazzarini<sup>2</sup>, Mirta S. Demo<sup>3</sup>, Liliana I. Sabini<sup>4</sup> and Ana M. Maldonado<sup>5</sup>

<sup>1-2-3-4-5</sup> Department of Microbiology & Immunology. Facultad de Ciencias Exactas, Físicas, Químicas y Naturales

### ABSTRACT

In previous studies we have demonstrated that essential oil and infusions of leaves and flowers of *Achyrocline satureioides* had antimicrobial and antiallergic properties. The yellow flowers shining of *Arnica montana* own anti-inflammatory properties. Vegetal derivatives of *Achyrocline satureioides* and *Arnica montana* are medicinal grass of the Compositae family, subfamily Asteraceae, which could regulate the altered immune system.

Mononuclear cells of 11 allergic patients to anemophilous fungi and 8 healthy controls from 5 to 45 years old were isolated. The test by colorimetric assay with equipment for MTT Cell Proliferation Assay Kit was used. Cell cultures without or with stimulus: PHA 10 µg/ml, anemophilous fungi extract: 10 PNU/ml, 3mg/ml *Achyrocline satureioides* flowers infusion and 100 µg/ml *Arnica montana* were tried.

The cells of the allergic ones proliferated by anemophilous fungi stimulated. The cells of controls ones stimulated by allergens, did not proliferate. The PI was majors ( $p < 0.01$ ) that those of the cells without stimulus and minors ( $p < 0.01$ ) that those of the cells PHA or vegetal derivate stimulated. The cells stimulated with *Achyrocline satureioides* or *Arnica Montana* with allergen added, reduced PI ( $p < 0.037$ ), reaching the values of the cultures without stimulus. These findings demonstrated that the investigated vegetal derivatives had immunomodulating effects.

**Keywords:** *Achyrocline satureioides*, *Arnica montana*, Immunomodulating, Anemophilous fungi, Cell proliferation with allergens

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**Corresponding author:** Ana Maria Maldonado. Email address: amaldonado@exa.unrc.edu.ar

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## Introduction

Some vegetal derivatives have shown anti-inflammatory effects (Felix Berumen *et al.*, 2004). Others have shown properties on the immune system (Cariddi *et al.*, 2006, Cariddi *et al.*, 2009). We demonstrated that *Achyrocline satureioides*: essential oil and leaves and flowers decoction had antimicrobial and antiallergic properties by *in Vitro* assays. (González Pereyra *et al.*, 2006, Calvo *et al.*, 2006, Cariddi *et al.*, 2007). The yellow flowers shining of *Arnica montana* own anti-inflammatory properties. In dose of  $\mu\text{g/ml}$  it presents/displays stimulating effects of the phagocytosis increasing the reactive intermediaries of oxygen and nitrogen (Kasuhisa *et al.*, 2007). Vegetal derivatives of *Achyrocline satureioides* and *Arnica montana*, are medicinal herbs that belong to the family of the Compositae, subfamily Asteraceae. *Achyrocline satureioides* and *Arnica Montana* vegetal derivatives, could to regulate the modified immune system in allergic patients.

**Experimental:** Mononuclear cells of 11 allergic patients to anemophilous fungi (*Af*) and 8 healthy controls of 5 to 45 years old were isolated. In 2007, *Achyrocline satureioides* samples from Alpa Corral were collected. Voucher specimens have been deposited under number # 6362 at the Herbarium of the Universidad de San Luis, Argentina and identified by Dr. Luis Del Vitto. The allergens of Anemophilous fungi were from commercial origin (*Diater Laboratorios, IPI SA y Allergo-Pharma SRL*). Allergens watery extract of *Alternaria t.*, *Aspergillus f.*, *Rizophus a.*, *Rizophus o.*, *Hormodendrum h.*, *Penicillium n.* and *Cladosporium s.*, were applied. The test by colorimetric assay was realized, in cultures without stimulus or stimulated with: PHA10  $\mu\text{g/ml}$ , main allergen fungi: 10PNU/ml, flowers infusion of *Achyrocline satureioides* 3mg/ml. Arnica S (Heel, Deal D12), 100 $\mu\text{g/ml}$  was applied. The equipment Vybrant<sup>®</sup> MTT Cell Proliferation Assay Kit was used (Molecular Probes Invitrogen Detection Technologies, Eugene, Oregon, the USA), (Tuchscherer *et al.*, 2002).

**Results and Discussion:** The proliferation indices (IP) by PHA stimulated cells were:  $>1.2$  from allergic patients and  $>1.5$  from controls. Similar proliferation indices PI of cells from the allergic patients as from controls, stimulated with PHA or vegetal derivatives they were observed. Only the cells of the allergic ones proliferated by Anemophilous fungi stimulated. The PI was major that those of the cells without stimulus ( $p<0.01$ ), and minors that those of the cells PHA or vegetal derivate stimulated ( $p<0.01$ ).

The cells stimulated with *Achyrocline satureioides* or *Arnica montana* and the allergen, reduced PI ( $p<0.037$ ), reaching the values of the cultures without stimulus (**Figures 1 and 2**).

The single vegetal derivatives, stimulated the lymphocyte proliferation, but against the allergen, they inhibited the cellular stimulation. In the regulation of the allergic processes they participate: regulating dendritic cell. IL-10, TGF- $\beta$  1 and soluble CD23 level began to decrease during the improvement of allergic disease (Ye *et al.*, 2006). Recently others authors demonstrated an essential role of IL-18 in CD8 T cell-mediated suppression of IgE responses (Salagianni *et al.*, 2007). Following investigations shown regulators cells abolish Th2-mediated IgE production and allergic inflammation inhibiting the IgE response through the activation of diverse types of Th cell responses. Treg cells CD4+CD25+FoxP3+  $\gamma$  CD8 T cell-mediated suppression of IgE responses (Fujita *et al.*, 2008). Different findings observe other authors: In PBMCs from healthy donors, *Achyrocline satureioides* infusion, reduced PHA-induced proliferation and production of IFN- $\gamma$  and IL-4. Lower concentrations of the infusion were ineffective on cell proliferation. Nevertheless these concentrations significantly increased the production of both IFN- $\gamma$  and IL-4 (Cosentino M *et al.*, 2008). Our findings suggest a T cells subpopulation deviation, secretors of suppressor cytokines: IL-10, TGF- $\beta$ , and IFN- $\gamma$  and involved in IL-4 and IgE production inhibition.

## Conclusions

The vegetal derivatives showed stimulating properties of the lymphocyte proliferation similar to the one of classic mitogens. Nevertheless both products modulated the proliferative answer induced by the specific allergen indicating effects modulators, possibly by the synthesis of suppressor cytokines. These findings demonstrated that the investigated vegetal derivatives: *Achyrocline satureioides* and *Arnica montana* had immunomodulating effects on allergen challenged cells from allergic patients, inhibiting the specific cellular answer.

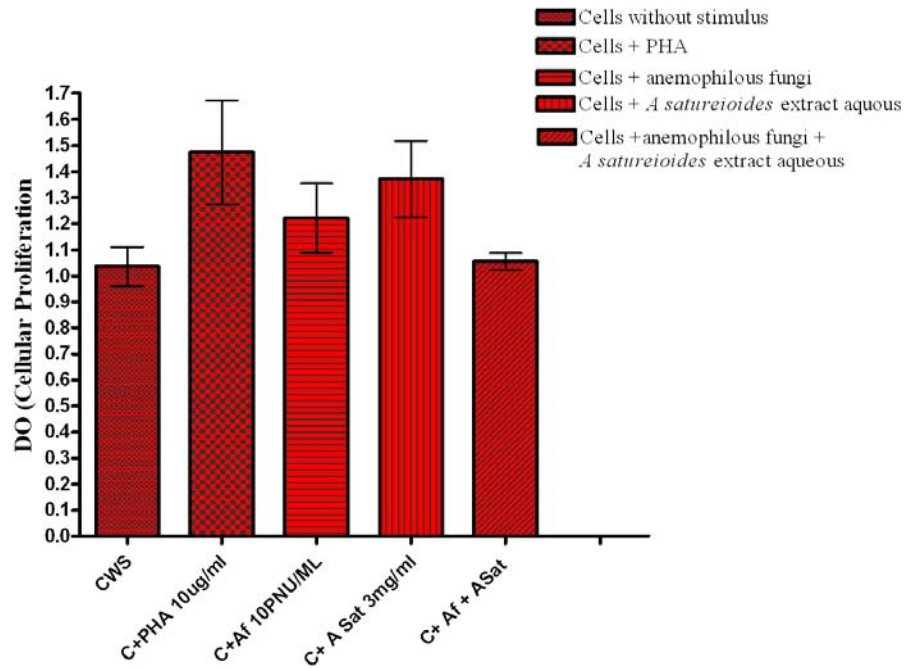
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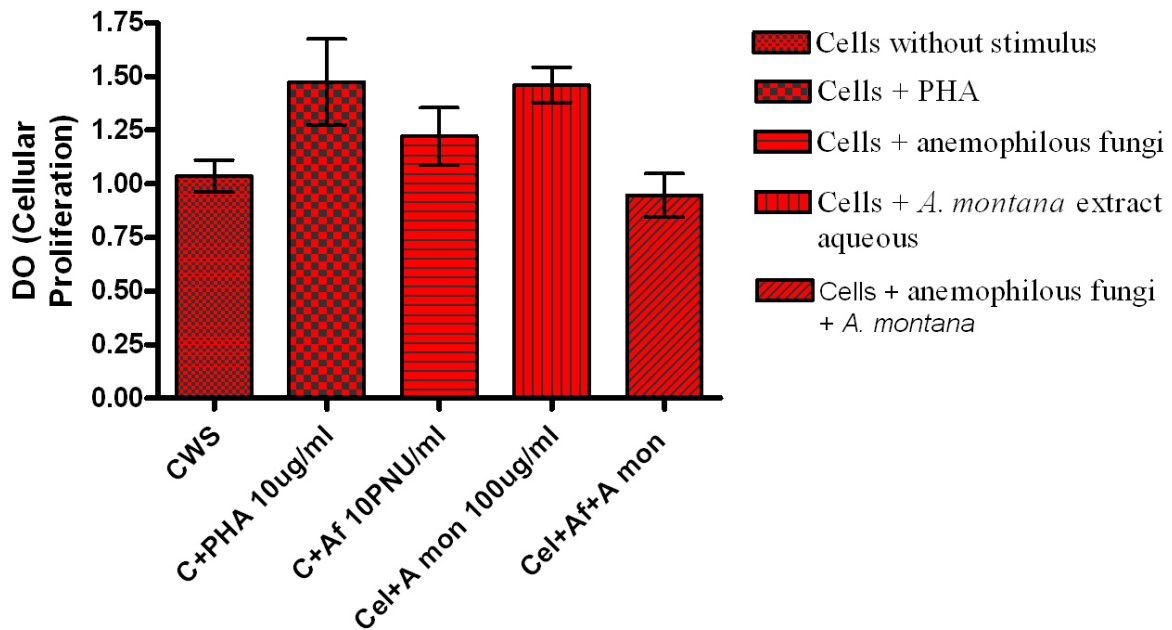


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**Figure 1:** Effects of *Achyrocline saturoioides* decoction on PBMC culture allergen stimulated. PBMC were cultured without stimulus, with PHA, and separated or together: anemophilous fungi and *Achyrocline saturoioides*.



**Figure 2:** Effects of *Arnica montana* extract on PBMC culture allergen stimulated. PBMC were cultured without stimulus, with PHA, and separated or together: anemophilous fungi and *Arnica Montana*.