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In vitro Anti-Arthritic Activity of Extracts in Water, Ethanol and Diethyl Ether of the Rhizomes from *Anredera Vesicaria*

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Abstract: *This work was directed to evaluate the in vitro anti-arthritic activity of extracts in water, ethanol and diethylether of the rhizomes from Anredera vesicaria. Solutions at 125, 250 and 500 µg/mL from the dry extract of the rhizomes were prepared, these solution were subjected to evaluation of the anti-arthritic activity in vitro by the method of inhibition of protein denaturation. According with the obtained results all the products are able to inhibit the denaturation of proteins, therefore, they present anti-arthritic activity, there being significant differences among the tested products. All the extracts evaluated showed anti-arthritic activity. The greatest activities were presented by the ethanol extracts.*

Keywords: *Anti-arthritic activity, inhibition of protein denaturation, Anredera vesicaria.*

1. INTRODUCTION

Rheumatoid arthritis is a systemic autoimmune disease with chronic inflammation characterized by hyperplasia of the synovial cells and angiogenesis in the affected joints, which leads to the destruction of cartilage and bone[1]. This disease can cause a severe disability, limiting the performance of a person in their daily routine, so it affects the quality of life. The main methods applied to its treatment include analgesic and anti-inflammatory drugs, most of which are steroids that can suppress the symptoms of the disease, although antirheumatics are able to modify the disease; treatments associated with side effects are also employed, such as anti-tumor necrosis factor (α-TNF) and anti-CD20 therapy [2].

Plants are a great source for obtaining new medicines and herbal products are promising for the development of effective and safe drugs against arthritis[3-4].Cuba, with a biodiversity of more than 7500 plant species and more than 50 % of endemism, has great resources that could be used in obtaining products with antiarthritic activity.

Anredera vesicaria (Figure 1), a medicinal plant traditionally used in eastern of Cuba as a remedy for inflammatory diseases, can serve as a raw material to obtain new anti-arthritic drugs.

This work is aimed to evaluate the in vitro anti-arthritic activity of extracts in water, ethanol and diethylether from the rhizomes of this plant.

Fig1. *Rhizomes of A. vesicaria.*

2. Materials and Methods

2.1. Plant Material

Rhizomes of *A. vesicaria*, collected in the north slope of the perimeter fence of the Beisbol Stadium "Wilfredo Pagés", in Manzanillo city, Granma province, Cuba, were authenticated in the Botanical Garden of Guisa, Granma. The biomass was dried at room temperature andthen in astove with recirculated air at 38 °C. A fine powder was obtained using a mill on a laboratory scale.

2.2. Obtaining Extracts

The aqueous, ethanolic and ethereal extracts of the powdered rhizomes were prepared using an ultrasound device, at 40 °C for 2 hours. The obtained extracts were concentrated in a rotary evaporator and dissolved in dimethylsulfoxide (DMSO) with a vortex shaker, by this way three solution with doses of 125, 250 and 500 µg/mL were obtained.

2.3. *In Vitro* Anti-Arthritic Activity

The anti-arthritic activity of the extracts was determined by the method of inhibition of protein denaturation reported by Williams *et al*[5].

The test solution (0.5 mL) was constituted by 0.45 mL of bovine serum albumin (5 % aqueous solution w/v) and 0.05 mL of the extracts in water, ethanol and diethyl ether of the rhizomes of *A. vesicaria* in 3 doses (125, 250 and 500 µg/mL).

The control solution (0.5 mL) was constituted by 0.45 mL of bovine serum albumin (aqueous solution at 5 % w/v) and 0.05 mL of distilled water.

The positive control (0.5 mL) was constituted by 0.45 mL of bovine serum albumin (aqueous solution at 5 % w/v) and 0.05 mL of sodium diclofenac at various doses (125, 250, 500 µg/mL).

All solutions were adjusted to pH 6.3 using HCl 1N, incubated at 37 °C for 20 minutes and heated at 57 °C for 3 minutes. After cooling, 2.5 mL of phosphate buffer (pH 6.3) were added.

Absorbance was measured at 416 nm, using a UV-visible spectrophotometer. The percent inhibition of protein denaturation was calculated according to the equation:

$$\% \text{ Protein inhibition } = \frac{\text{Abs (Control) - Abs (Sample)}}{\text{Abs (Control)}} \times 100$$

2.4. Statistical Processing

The results were processed with the statistical package Statgraphics Centurion XV. The experiment was organized according to a factorial design, in which the effects of the i^{th} levels of several quantitative factors and their interactions on the response of interest were studied. The variables that represented the factors to be studied were: extracts (τ), doses (β) and products (γ); Meanwhile, the other parameter of the model characterizes the effect of the interactions between both factors.

$$y_{ijkl} = \mu + \tau_i + \beta_i + \gamma_i + (\tau\beta\gamma)_{ij} + \epsilon_{ijkl} \begin{cases} i = 1,2,3,4 \\ j = 1,2,3 \end{cases}$$

The levels of the factors were defined as follows: 3 types of extracts (water, ethanol and diethyl ether), 3 dose levels (125, 250 and 500 µg/mL) and 2 products (extracts of rhizomes and diclofenac sodium). For the statistical analysis we used the analysis of variance (ANOVA), where the value of F-test was calculated for a probability value ($p<0.05$), in order to determine the significant differences between the means of the main effects and interactions.

3. Results and Discussion

In the process of denaturation, proteins lose their secondary and tertiary structures through the application of external actions such as strong acidic and basic environment, concentrated inorganic salts, organic solvents and heat. Protein denaturation is a well-documented cause of inflammation in rheumatoid arthritis conditions, and protection against this process is the main mechanism that accompanies the action of non-steroidal anti-rheumatic anti-inflammatory drugs (NSAIDs)[6].

The results of the inhibition assays of protein denaturation are reported in Figure2. All the products tested inhibited protein denaturation (Figure2a), according to a dose-response dependence (Figure2b).

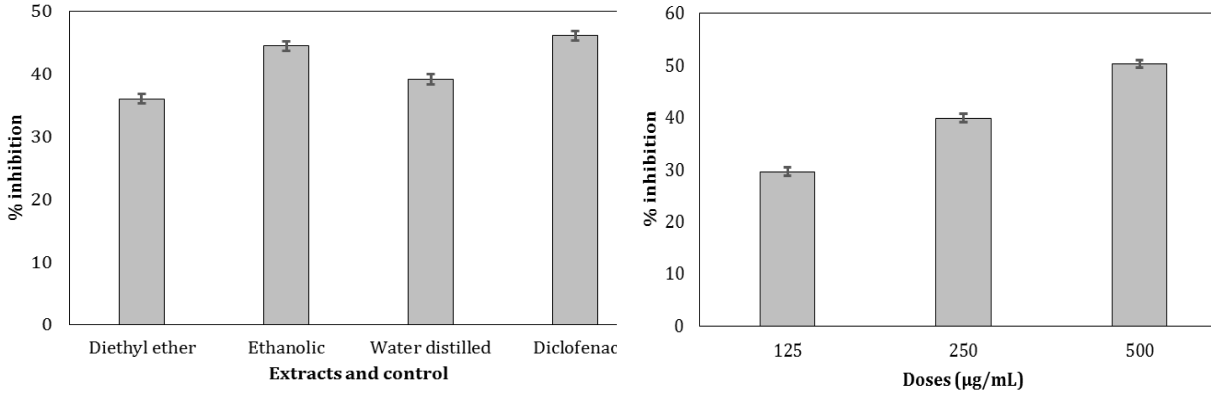

Fig2. *Percentage of inhibition of protein denaturation: a) Activity of the products; b) Dose-response dependence.*

Figure 3 shows significant interactions between solvents, doses and products. The solvent used in the preparation of the extract does not limit the increasing behavior of the percentage of inhibition of protein denaturation, achieving the highest biological activity at a dose of 500 µg/mL (Figure 3a).

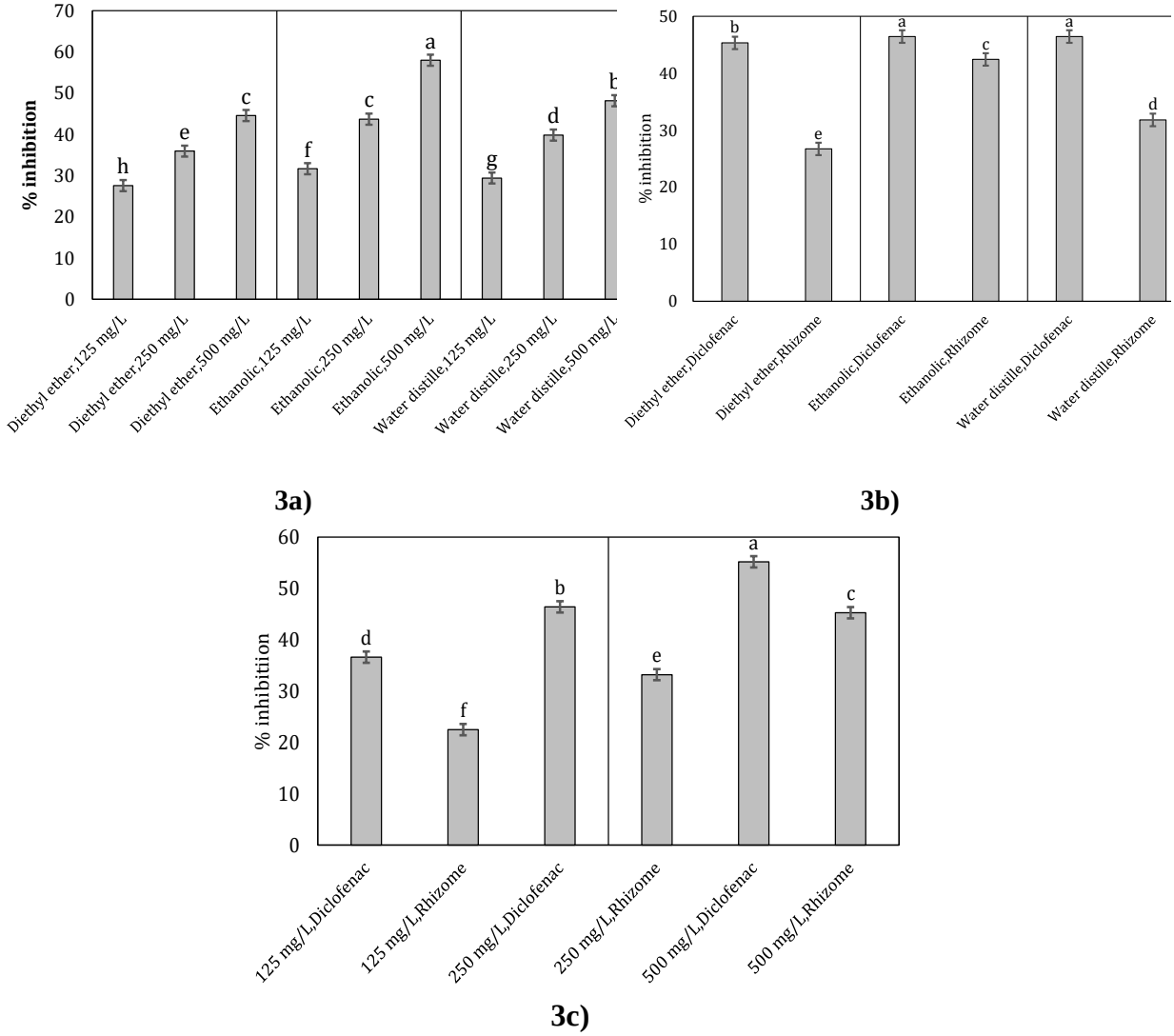

Fig3. *Significant interactions between products, doses and extracts in the percentage of inhibition of protein denaturation: 3a) extracts against doses; 3b) extracts against products; and 3c) dose against products.*

Even with the existence of significant differences, the closeness of the values of the percentage of inhibition of protein denaturation at the dose of 500 µg/mL, between diclofenac and the extract in ethanol (3b, 3c) is relevant. This result is remarkable because it is not frequent in products obtained from plants [6].

The anti-arthritic activity of the rhizomes could be related to their anti-inflammatory property and both can be attributed to the presence of oleanolic acid [6-7].In previous studies, the relationship between this triterpenoid and the anti-inflammatory activity of *Anredera* species was reported [8-10].

The analysis of variance (ANOVA) was carried out to study the significance of the 3 factors (extracts, doses and products) on the variable responses for the experimental data obtained. Table 1 summarizes the experimental results obtained in various combinations of ANOVA treatment.

Table1. *Analysis of variance (ANOVA)*

Source	Sum of Squares	Gl	Middle Square	F-Reason	Value-P
MAIN EFFECTS					
A:Extracts	651.239	2	325.619	29.94	0.0000
B:Doses	3846.75	2	1923.37	176.85	0.0000
C:Products	2080.78	1	2080.78	191.32	0.0000
INTERACTIONS					
AB	157.146	4	39.2866	3.61	0.0132
AC	512.636	2	256.318	23.57	0.0000
BC	43.8574	2	21.9287	2.02	0.1465
WASTE	435.026	40	10.8757		
TOTAL (CORRECTED)	7727.43	53			

All F-ratios are based on the mean square of the residual error

The effect of the factors was significant in the response evaluated for a 95 % probability level; significant differences were also detected between all the factors and among the evaluated levels. These results show that there is an influence of the 3 factors on protein denaturation.

Table 2 shows a summary of the multiple regression procedure applied to construct a statistical model that describes the relationship between the variables extracts, products and doses with the percentage of inhibition. In the procedure, the stepwise regression methodology was applied in order to include variables that were statistically significant in the final model. In this procedure the non-significant variables were eliminated at a given step, if their F-test values were lower than the F-test value defined for $p<0.05$. All the variables were significant for $p<0.05$; therefore, a multiple regression model that shows the relationship among them was determined.

Table2. *Analysis of variance for the multiple regression model*

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	6578.76	5	1315.75	54.98	0.0000*
Extracts	651.239	2	325.619	13.61	0.0000*
Products	2080.78	1	2080.78	86.95	0.0000*
Doses	3846.75	2	1923.37	80.37	0.0000*
Residual	1148.67	48	23.9305		
Total (corrected)	7727.43	53			
* p-values <0.05 were considered to be significant					
R2	85.14 %				
R2 adjusted	83.59				
Standard error of estimation	4.89				
Mean absolute error	3.48				

*% inhibition = 39.8702 - 3.83545*Diethyl ether + 4.57422*Ethanolic extract + 6.20749*Diclofenac - 10.3119*Dose 125 µg/mL - 0.0501563*Dose 250 µg/mL*

As indicated, the model obtained had a low p-value, which indicates a significance of 95 %, which explains 85.14 % of the variability of the data; while, the adjusted R2 also showed a high value of 83.59 % and low values of standard errors and average absolute of 4.89 and 3.48, respectively. These results indicate an adequate adjustment of the statistical model and a good correlation between the

values observed and predicted by the multiple regression model. In addition, the cluster of points around the diagonal line indicates a good fit of the regression model (Figure 4).

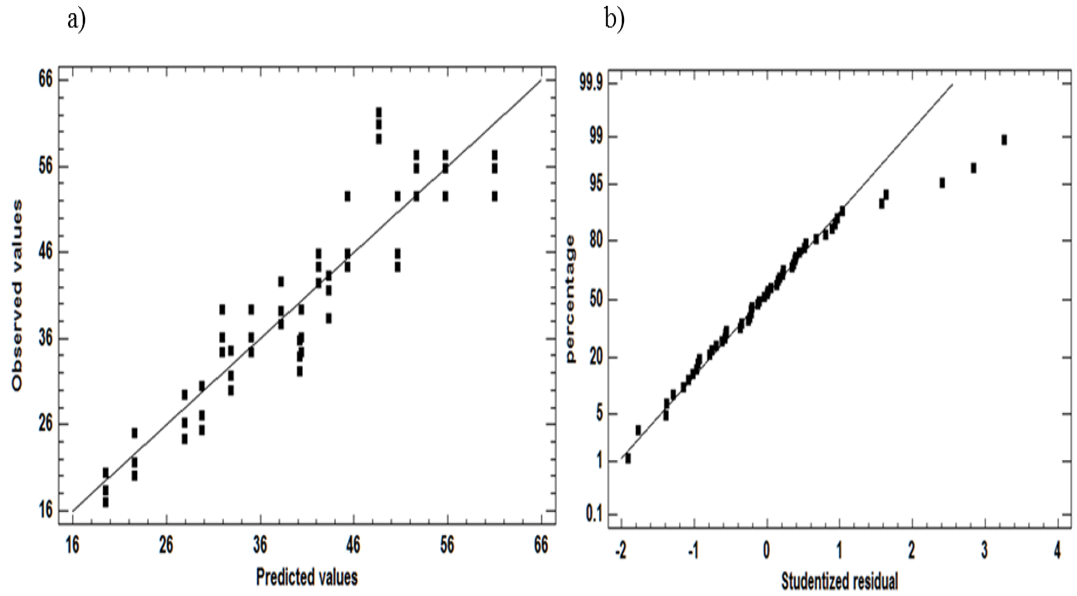

Fig4. *Checking the suitability of the model: a) Parity plot showing the correlation between the experimental and predicted values; b) normal probability of the residues studied.*

4. CONCLUSION

The rhizomes of *A. vesicaria* showed anti-arthritic activity in all evaluated extracts. The greatest anti-arthritic activities were corresponded to the ethanol extracts. The biological activity may be related to the presence of oleanolic acid in this part of the plant.

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