

## Diuretic Activity of *Artemisia annua* L. Extracts

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**SUMMARY.** Different *Artemisia annua* L. extracts were evaluated and tested in rats as diuretics and saluretics. The methanolic extract caused an increase in the urine volume and a selective saluretic effect. Basic and neutral fractions produced a significant increase of the urine volume and of its electrolytes content, while the total methanol extract show diuretic and saluretic activity, acting as potassium economizer.

**RESUMEN.** "Actividad Diuretica de Extractos de *Artemisia annua* L.". Diferentes extractos metanólicos obtenidos de *Artemisia annua* L., fraccionados según su comportamiento ácido, básico o neutro, fueron evaluados y testeados en ratas como diuréticos y saluréticos. Las fracciones básica y neutra producen un significativo aumento de la diuresis y en el contenido de electrolitos urinarios, mientras que el extracto metanólico total presenta actividad diurética y salurética, actuando como economizador de potasio respecto a las otras dos fracciones activas.

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### INTRODUCTION

Synthetic drugs, used at present as diuretic agents, frequently produce several collateral effects such as, for instance, hyperglycemia<sup>1</sup>, ototoxicity<sup>2</sup>, and hypokalemia<sup>3</sup>. These limitations, not yet resolved, open a perspective to the introduction of new chemical structures that may surpass, therapeutically, those existing now. On the basis of these considerations, we have decided to study some species belonging to the flora of South America in order to find new diuretic agents. We have chosen *Artemisia annua* L. as the first specie to be studied.

The genus *Artemisia* includes a great number of species<sup>4</sup>, some of them used in traditional medicine. Sesquiterpene lactones<sup>5,6</sup> and other compounds as flavonoids<sup>7</sup>, alkaloids, tannins and volatile oils<sup>8</sup>, are found among their constituents.

*Artemisia annua* L. is a herb that belongs to the Compositae family which, originally belonging to Asia (known as Quinghao), is nowadays spread to Europe and America<sup>9</sup>. It has been used for centuries in the traditional Chinese medicine

**KEY WORDS:** *Artemisia annua*, Diuretic, Potassium economizer, Saluretic,.

**PALABRAS CLAVE:** *Artemisia annua*, Diurético, Economizador de potasio, Salurético.

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for the treatment of both fever and malaria<sup>10-12</sup>. Its active antimalaric principle has been isolated and identified. It has received different names, depending on the researchers involved: arteanuina<sup>13</sup>, qinghaosu<sup>14</sup>, or artemisinin<sup>15-17</sup>.

Our aim was to demonstrate that *A. annua* extracts have diuretic activity, as well as characterize the ionic mobility in rats.

Plant material was extracted with methanol and its fractions separated according to the characteristic of its constituents (basic, acid, or neutral). The different fractions were pharmacologically tested. The isolation of the compounds responsible for the activity of each fraction is currently carrying out in our laboratories.

## **MATERIAL AND METHODS**

### ***Plant material***

The plant was collected in May 1985-93, in Puerto Ibicuy (Entre Ríos Province, Argentina). It was identified as *Artemisia annua* L. (Compositae -Anthemidae), (Leg. Spegazzini E.D. N351-352. LPE).

The material consists in the root and the aerial parts, air dried at room temperature in a fresh place, with low humidity, and then dried until constant weight under vacuum at 40 °C.

### ***Extraction***

The material was ground and soxhlet extracted with hexane (hexanic fraction, HF), and the residue was re-extracted with methanol (TMF). The methanolic extract was concentrated *in vacuo* until it reaches 1/5 of the initial volume. Then was cooled (0 °C), an equal volume of benzene-chloroform (3:2) was added, and it was acidified with 1N hydrochloric acid (Congo Red). The acid aqueous phase (AAP) was separated. The organic phase was washed with water and the aqueous extractive added to the AAP. The addition of 1N sodium hydroxide to the organic phase formed the alkaline aqueous phase (ALAP). The organic phase was dried with sodium sulfate to give the neutral fraction (NF).

AAP was ice cooled, an equal volume of benzene-chloroform (3:2) was added and brought to pH 10-11 with 1 N sodium hydroxide. After filtering the organic phase, it was dried with sodium sulfate, to give the basic fraction (BF). The aqueous phase was discarded.

1 N Hydrochloric acid was added to ALAP (Congo Red). It was then extracted with ethyl ether and dried with sodium sulfate to give the acid fraction (AF).

### ***Diuretic activity***

Five groups of female albino Sprague-Dawley rats, weighing 200-250 g with 24h fast but water ad libitum were kept in pairs in metabolic cages.

The different extract solutions (TMF, AF, BF, and NF) were prepared at the moment of the test, eliminating the organic solvent in a rotary evaporator at room temperature until constant weight. The final product was solubilized with ethanolic solution (10% ethanol 96% v/v, with 0.85% NaCl) in order to obtain a concentration of 0.5 mg/ml.

The treated rats received 10 mg/kg of the extract by oral route using a gastric

catheter (2.6 mm external diameter). The control rats received 20 ml/kg of physiological solution (0.85% NaCl with 10% ethanol of 96%).

The experiments were carried out in a quiet area of the laboratory, and at a controlled temperature (17.5-18.5 °C) during a four hours period.

Diuresis, sodium, potassium and chloride ions as well as urine pH were determined in all cases.

Sodium and potassium ions were quantified by means of a flame photometer (Wayers 2.000). A mercurimetric method was used to determine the chloride ions<sup>18</sup>.

All reagents and solvents were of analytical grade.

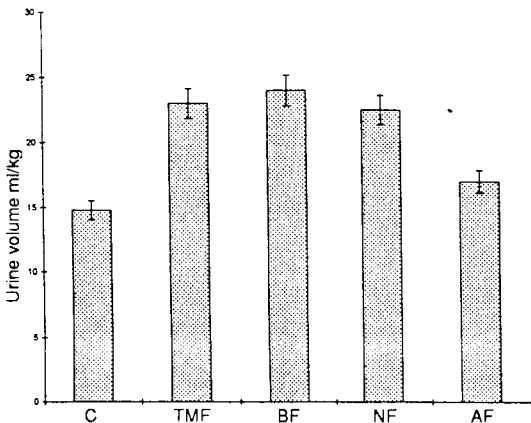
**Statistical analysis**

Variance analysis was used to calculate the statistical significance of the results.

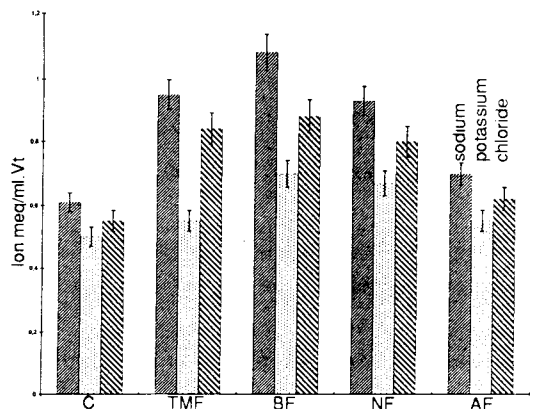
**RESULTS AND DISCUSSION**

The results show that *Artemisia annua* L. has a significant diuretic activity. The activity of TMF and of the fractions AF, BF and NF were compared in order to find the most active extract. From the analysis of the results (Figs. 1 and 2), no significant differences in the diuretic, natriuretic and chloruretic activities of BF, NF and TMF were found. All of them were significantly higher than the control. AF, on the other hand, caused no change in the urine volume or its electrolyte content.

In all cases pH values did not differ significantly from the control group, being  $6.7 \pm 0.5$ . It is interesting to remark the kaliuresis produced by the different treatments. We have found that the kaliuretic activity of BF and NF was significantly higher than that of the TMF and the control group. TMF showed a selective saluretic effect increasing the excretion of sodium but not potassium ion, keeping the level at its physiological value. This is a desirable effect in a diuretic agent because it avoids the hipokalemia associated to diuretics of moderate and high efficiency, as well as hyperkalemia produced by potassium economizer agents.



**Figure 1.** Effects of various treatments on urine volume. C = control. Other abbreviations: see the text. Values are mean ± S.E. \*\*p < 0.01.



**Figure 2.** Effects of various treatments on ions urine excretion. C: control; other abbreviations: see the text. Vt: urine volume (4 h). Values are mean ± S.E. \*\*p < 0.01.

This paper gives conclusive evidence that *Artemisia annua* L. has diuretic activity. Present research in our Laboratory is focused on the isolation of the active principle(s) responsible for that activity.

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