

## QUALITATIVE AND QUANTITATIVE STUDY OF TANNINS FROM THE HYDRO-ALCOHOLIC EXTRACTS OF AGRIMONIA EUPATORIA, VISCUM ALBUM AND VERONICA OFFICINALIS

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### Abstract

*Tannins from the hidroalcoholic extracts of medicinal plants (Agrimonia eupatoria, Viscum album and Veronica officinalis) were determined qualitatively by color and precipitation reactions and quantitatively by cold titration with  $KMnO_4$  0.01N, in the presence of carmine indigo, used as a redox indicator. These extracts have antioxidant and antimicrobial properties.*

**Keywords:** gallic acid, catechin, polyphenolic compounds.

### Introduction

Agrimonia eupatoria, Viscum album and Veronica officinalis are well known medicinal plants for their therapeutic properties in different affections. [1].

Tannins are organic compounds, with a heterogenic structure of polyphenolic type. [7].

They are useful in the skin dressing industry, having properties to transform the crude skin into tanned skin.

At the origin of tannins are the gallic acid and catechin respectively, with a polyphenolic structure, showed in figure 1. [7].

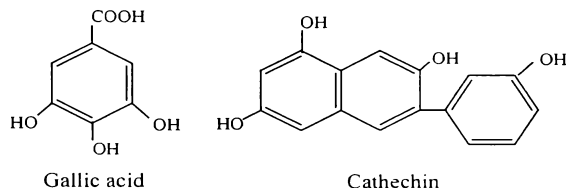


Fig. 1: The structure of the main compounds present in diverse types of tannins

## **Experimental**

Portions of 5 g of vegetal dry product were submitted to extraction (maceration), with 50 ml solution of ethylic alcohol (96%), 10 days, at room temperature (22-24°C), making 3-4 stirrings a day, in the dark. [4]. The dry vegetal products were gathered in 2006.

After 10 days, the solutions were filtered, the filtrate being used for the qualitative and quantitative study.

Reagents: gelatin solution 1%;  $(\text{CH}_3\text{-COO})_2\text{Pb}$  solution 10%,  $\text{FeCl}_3$  solution 1%, hydrochloric vanillin solution 1%, bromine water solution.

For the identification reactions, portions of 1 ml of vegetal extract solution were mixed with: 2-3 drops of gelatin solution 1%, 0.5 ml  $(\text{CH}_3\text{-COO})_2\text{Pb}$  solution 10%, 0.5 ml  $\text{FeCl}_3$  solution 1%, 0.5 ml hydrochloric vanillin solution 1% and 2 ml bromine water solution respectively.

The quantitative determination method consisted in the cold titration with  $\text{KMnO}_4$  of the tanning substances. [2, 3, 5].

The titration was made parallel with a control sample, in the presence of carmine indigo used as a redox indicator. As reagents  $\text{KMnO}_4$  solution 0.01N freshly prepared with a known factor; alcoholic tartaric solution: 5g/l tartaric acid, neutralized with KOH solution 1N in 10% (volumes) of ethylic alcohol and carmine indigo solution 0.15g/l freshly prepared were used. This last solution was prepared from a filtered carmine indigo solution 3g/l in the following way: in a calibrated flask of 1000 ml 50 ml carmine indigo solution 3g/l, 50 ml  $\text{H}_2\text{SO}_4$  (1:2  $\text{H}_2\text{SO}_4\text{:H}_2\text{O}$ ) were measured and it was completed with distilled water to the mark.

The fresh carmine indigo solution must have a blue color. Solutions with a blue-yellow color or dark blue were not utilized.

In a 100 ml flask 10 ml carmine indigo solution 0.15% and 0.4 ml vegetal extract solution are dropped.

The content is titrated with a  $\text{KMnO}_4$  solution 0.01N until the blue color disappears and appears the yellow color.

The number of ml of  $\text{KMnO}_4$  solution 0.01N used for titration is noted with  $V_1$ . The titrated sample is kept to compare the obtained coloration with the coloration of the next sample.

In another 100 ml flask, 10 ml of carmine indigo 0.15% and 0.4 ml tartaric alcoholic solution are added and are titrated with a  $\text{KMnO}_4$  solution 0.01N until identical coloration is obtained comparative with the previous sample.

The number of ml of  $\text{KMnO}_4$  solution 0.01N used for this titration is noted with  $V_2$ .

The result expressing is made by the number of ml  $\text{KMnO}_4$  solution 0.01N consumed at a litter of extractive solution.

The  $\text{KMnO}_4$  coefficient is calculated with the formula:

$$I_{\text{KMnO}_4} = 5(V_1 - V_2) \text{ ml KMnO}_4 \text{ 0.01N}$$

The  $\text{KMnO}_4$  coefficient can be expressed under tannins form, knowing that at 1 g of tannin corresponds a value of the coefficient equal with 25 ml solution of  $\text{KMnO}_4$  0.01N. [2, 6, 8].

### Results and Discussion

In table 1 are presented the results of the identification reactions for the vegetal material collected in 2006: *Agrimonia eupatoria*, *Viscum album* and *Veronica officinalis*.

**Table 1:** Identification reactions

Reagent used	<i>Agrimonia eupatoria</i>	<i>Viscum album</i>	<i>Veronica officinalis</i>
Gelatin solution 1%	White precipitate	White precipitate	White precipitate
$(\text{CH}_3\text{-COO})_2\text{Pb}$ solution 10%	Yellow-green precipitate	Yellow-brown precipitate	Yellow-brown precipitate
Hydrochloric vanillin solution 1%	Red-yellow color	Red color	Res-orange color
$\text{FeCl}_3$ solution 1%	White-violet precipitate	White-violet precipitate	Dark green precipitate
Bromine water	Yellow precipitate	Yellow precipitate	Yellow precipitate

In table 2 is presented the content in tannins of the analyzed vegetal extracts.

**Table 2:** The content in tannins of the vegetal extracts

Medicinal plant	Content in tannins g/100 g vegetal product
<i>Agrimonia eupatoria</i>	1.30
<i>Viscum album</i>	0.52
<i>Veronica officinalis</i>	0.04

By making a comparison between the three extracts, it can be noticed that the first one contains the highest quantity of tanants and polyphenols. By calculating the ratio between the dried substance and total polyphenols it has been noticed that the second extract has the lowest value, which means that it

contains a higher quantity of active polyphenolic substances with low polymerization degree.

### Conclusions

Through the color reactions were shown the following aspects: all three analyzed vegetal materials contain tannins; Agrimonia eupatoria contains the highest amount of tannins which was shown by the abundance of the formed precipitates and by the intensity of their color comparative with the other extracts.

The hydro-alcoholic extracts of Agrimonia eupatoria and Viscum album have the higher values in tannins, values confirmed also by the identification reactions.

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