COLORIMETRIC METHOD FOR THE QUANTITATIVE DETERMINATION OF HYDROLYZABLE TANNIN SUBSTANCES

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A colorimetric method of determining tannins is proposed for the analysis of Geraniaceae raw material and its extracts, and also of other tanning plants the tannides of which belong to the hydrolyzable series.

One of the important problems of the leather industry is the development of a rapid and convenient method of determining the quality of tanning plants and tanning agents.

Many comparatively rapid methods of determining tanning substances exist: titrimetric [1-5], colorimetric [6-10], gravimetric [11-13], spectrophotometric [14], nephelometric [15, 16], oxidimetric [17], and others. However, they are not universal and cannot be used in all cases of determining phenolic compounds.

The All-Union Unit Method (VEM) of determining tannides [12], which is based on their capacity for being bound by rawhide powder, is very lengthy and laborious.

In the present paper we give the results of the use of an iron-tartrate reagent (a mixture of a 0.1% solution of $FeSO_4$ and a 0.5% solution of $KNaC_4H_4O_6$) to determine tanning substances of the hydrolyzable series on the basis of the determination of the tannides in the epigeal part of *Geranium rectum* Trautv.

It is known that polyphenols from stable red complexes with the iron-tartrate reagent [18]. This reagent is widely used for determining the amounts of ortho and ordinary hydroxyls in various phenolic compounds.

For polyphenols containing ordinary hydroxy groups, the maximum optical density is observed at pH 6.24, and for polyphenols with ortho-hydroxyls at pH 8.06.

The results of our investigations have shown that the tannides of *Geranium* belong to the hydrolyzable tanning substances and give the coloration at pH 6.25.

The essence of the proposed method is as follows. The total amount of polyphenols, including both the tanning substances and the polyphenols not possessing tanning properties, in the extract are determined. An aliquot of the extract is treated with rawhide powder. The tannides bind to it and the phenolic substances possessing no tanning properties remain in solution. The amount of the latter is determined colorimetrically by means of the color reaction with the iron-tartrate reagent. The difference between the total polyphenols and the amount of polyphenol not binding with rawhide powder will be the true amount of tanning substances.

Determination Procedure. A 20-g sample of raw material weighed with an accuracy of 0.01 g is extracted with 150 ml of water at 50°C for 2 h three times. The extract is filtered into a 500-ml measuring flask, the plant material is washed with hot water (50-60 ml), and the wash-waters are combined with the extract and the volume is made up to the mark.

The contents of the flask are carefully mixed and filtered through a folded paper filter. The first portion of filtrate (50-60 ml) is discarded. To determine the total amount of polypenols 2-ml portions are taken from the filtered extract and are diluted with water in a

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TABLE 1. Results of the Analysis of Samples of Geranium Obtained by the Standard and the Proposed Methods

Sample	Tannides content, %		Sample	Tannides content, %	
	standard	colorimetric		standard	colorimetric
1	12,70	12,90	9	9,80	10,40
2	8,90	9,70	10	14,30	15,00
3	13,50	14,20	11	11,70	12,00
4	11,90	12,70	12	10,80	11,00
5	10,65	11,10	13	11,30	12,00
6	12,80	13,70	14	8,60	8,95
7	10,50	10,90	15	13,70	14,10
8	10,30	10,60	16	12,35	13,00

measuring flask to 100 ml. Then, in each case, 1 ml of the diluted extract is transferred to a 50-ml measuring flask, and 5 ml of phosphate buffer with pH 6.25 and 2 ml of the iron-tartrate reagent are added and the total is made up to the mark with water.

The contents of the flask are carefully mixed and the optical density of the solution is measured on an FÉK-56 M photoelectric colorimeter in cells with a layer thickness of 2 cm with a No. 7 filter. The amount of polyphenols is determined from a calibration graph drawn up from results obtained on a preparation of the total polyphenols isolated from an ethyl extract of an aqueous extract of the geranium.

The initial extract (100 ml) is treated with rawhide powder (according to VEM) and is filtered, and the amount of nonadsorbed polyphenols in the filtrate is determined colorimetrically. For this purpose, two 1-ml samples of the filtrate are transferred to 50-ml measuring flasks, and in each case 5 ml of phosphate buffer and 2 ml of iron-tartrate are added and the total volume is made up to the mark with water. The optical density of the solution is measured as described above. The amount of nonadsorbed polyphenols is also determined from the calibration graph.

The total amount of polyphenols (x) is found from the formula

$$x = \frac{a \cdot V_2 \cdot V \cdot 100}{V_1 \cdot b \cdot V_3} \%,$$

where a is the amount of polyphenols found from the calibration graph; V is the initial volume of the extract, ml; V_1 is the volume of extract taken for dilution, ml; V_2 is the volume of diluted extract, ml; V_3 is the volume of diluted extract taken for the determination, ml; and b is the weight of the raw material, g.

The amount of polyphenols not adsorbed by the rawhide powder (y) is found from the formula

$$y = \frac{a \cdot V \cdot 1{,}3 \cdot 100}{b} \%,$$

where a is the amount of polyphenols found from the calibration graph, g; V is the initial volume of extract, ml; 1.3 is the dilution factor; and b is the weight of raw material.

The tannide content of the extracts (TC) is determined from the formula

$$TC = x - y$$

Table 1 gives the comparative results of an analysis of various samples of geranium obtained by the standard and the proposed methods. As we see, the discrepancy in the amounts of tannides by the standard and the proposed methods does not exceed that permitted by OST-17-535-75 for parallel determinations.

Consequently, the colorimetric method of determining tannides can be used as a rapid method for the analysis of geranium raw material and its extracts, and also for other tanning plants the tannides of which belong to the hydrolyzable series.

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