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Tannin quantification in industrial waste of tobacco production and selection of optimal extraction parameters

Abstract: Optimum technological parameters of extraction for the sum of tannins from tobacco dust (*Nicotiana tabacum* L) waste of tobacco production were established. The basic process parameters to obtain the highest yield of tannins from raw materials such as selection of optimum extractant ratio of raw material to solvent, extraction time, temperature and mode, selection of the optimal precipitator for tannins and the optimal ratio of extract to precipitator. A process technological scheme of tannins extraction from tobacco dust waste was suggested.

Key words: tobacco dust (*Nicotiana tabacum* L.), tobacco extraction, tannins, technological block diagram of tannins extraction

Introduction

Although tannin is frequently cited as an example of a plant defensive chemical [1, 2] experimental documentation of a defensive role for tannin is limited. Clear demonstration of a defensive function for tannin depends on accurate quantitation of tannin. When adequate analytic methods are available, it will become possible to determine whether there is any correlation between tannin content and patterns of herbivory. The accuracy with which a chemical component in a biological matrix, such as tannin in tobacco dust, can be determined depending on several factors. The tobacco dust of interest must be collected and preserved so that the component is not altered or destroyed. The component must be extracted from tobacco in high yield. The method of assaying the component must be free of interferences from other materials present in the extract. However, there is no consensus on the best method for preserving tobacco or the most efficient solvents for extracting tannins [1, 2]. The purpose of this study was to compare and to quantitate the extractability of tannin with various solvents as well as developing technological scheme for extraction of tannins.

Experimental

Selection of the optimal technological scheme of producing substance from medicinal plants is based on complete exhaustion. This object is achieved by selecting a suitable extracting agent, optimum ratio between extracting agent and plant raw materials

as well as the extraction duration, temperature and mode.

Selection of the optimal extractant. Selecting the optimal solvent had the following objective: to choose a solvent that extracts the greatest quantity of tannins from the tobacco waste dust. The following solvents were used: Ethanol 80% water, 2-Butanol, 50% dioxane, dioxane, ethyl acetate, ethanol 50%, ethanol 30%, ethanol 70%, dimethyl. The results are shown in Table 1.

Determination of the optimum ratio between raw material and the solvent (80% ethanol). Obviously, keeping a constant amount of the plant material and increasing the amount of the extractant in the extraction process will lead to higher extraction yield. However, increasing the amount of extractant will also reduce the concentration of biologically active substances in the extract, thus addition of the extractant cannot be infinite.

Therefore, in determining the optimal amount of extractant selected, the next ratio of raw material to extractant were used 1:5, 1:7, 1:9 and 1:10. Controlled variables of the extraction process are: the mass of raw material (1 g) and extraction time (3 hours). Results are presented as a histogram (Figure 1).

Methodology of tannins yield determination. The exact portion of the dry extract (1-2g) or liquid substance(3-5g) is placed into 100 ml flask, then 30 ml of purified water and 30 ml of a 10% solution of copper acetate (II) added, heated on a boiling water bath for 30-40 minutes to complete precipitation of tannins. Cooled, filtered through an accurately

weighed filter. The filter cake was washed with purified water until a negative reaction to the copper ferrocyanide solution and dried to constant weight at a temperature 100-105°C.

Filter with the precipitate burned; ash was wetted with concentrated nitric acid and was calcined at the same temperature to constant weight.

The content of tannins in the sample was determined by the difference between the weight of tannate copper after drying filter with precipitate and the weight of copper oxide after calcination.

Determining the optimum extraction temperature. For the next step it was necessary to consider the effect of temperature on the extraction process of extracting the maximum amount of tannin from the investigated tobacco dust waste. For this purpose 6 samples of 1g. were filled with 10 ml. 80% ethanol. The first sample was left at room temperature for 24 hours and rest had been heated for 1 hour. Second heated at 40°C, and the third – at 50°C, and the fourth – at 60°C, the fifth – at 70°C, and a sixth – at the reflux at 80°C. Then the quantitative content of tannins was determined. Results are presented as a histogram (Figure 2).

Determining the optimal extraction time. For this 5 samples of 1g. were filled with 80% ethanol. The first sample was heated on a water bath at reflux at 80°C for 1 hour, the second – for 2 hours, the third for 3 hours, the fourth for 4 hours and the fifth for 5 hours. Then the tannins content was quantified. Results are presented as a histogram (Figure 3).

Determining the optimal mode of extraction. An important indicator for an exhaustive set of extraction of BAS medicinal plants is the number of extractions. Raw material maintains a portion of extractant on its surface and between pieces because of its absorbent retention ability within the cells. In order to determine the multiplicity of extraction 4 samples of 1g were filled with 10 ml 80% ethanol. The first and third sample was left at room temperature for 24 hours. Then all four samples heated at 80°C in two modes: samples number 1 and 2 were continuously heated for 4 hours; the solvent of samples 3 and 4 was decanted every hour and a new portion of ethanol was added. Then the tannins content was quantified. Results are presented as a histogram (Figure 4).

Selection of the optimal precipitant of tannins. For this purpose four samples of extract obtained from the previous steps were poured with equal volume of saturated solutions of various salts: sample 1 – lead acetate, sample 2 – barium chloride, sample 3 – calcium chloride, sample 4 – ferrous sulfate. Then samples were left in the refrigerator for 2 hours after

which the tannins content was quantified. Results are presented as a histogram (Figure 5).

Determination of the optimum ratio of the extract to precipitant. Equal amounts of the extract obtained in previous steps were poured with different amounts of lead acetate: first extract – 20% of the amount of the precipitant (v/v), the second extract – 40% (v/v), third extract – 60% (v/v), fourth extract – 80 % (v/v) and the fifth extract with equal volume of precipitant. Samples were left in the refrigerator for 2 hours. Then the tannins content was quantified. All experiments were performed in parallel. Results are presented as a histogram (Figure 6).

Results and their discussion

Selection of the optimal extractant. Based on calculations made the optimum solvent which extracts the greatest number of hydrolysable tannins is 80% ethanol.

Table 1 – Selection of the optimum solvent

Mass (g)	Solvent	Tannins yield (%)
0.997	Ethanol 80%	6.5491
0.934	Dimethyl	0.8901
0.922	Ethanol 70%	0.6387
0.945	Ethanol 30%	0.4032
0.968	Ethanol 50%	0.3220
0.933	Ethyl acetate	0.1856
0.965	Dioxane	0.1077
0.961	50% dioxane	0.0937
0.941	2-Butanol	0.0368
0.934	Water	0.0185

Determination of the optimum ratio between raw material and the solvent (80% ethanol). According to data it is clear that using the selected solvent for extracting the most amount of tannins is possible when the ratio of raw materials to solvent is 1:10, whereas the change in this ratio upwards or downwards significantly reduces the yield.

Determining the optimum extraction temperature. As can be seen from the figure, the optimum extraction temperature is 80°C. At higher temperatures there is a volatilization of the solvent.

Determining the optimal extraction time. According to tannins yield with different extraction time it can be concluded that the optimum extraction time is 4 hours.

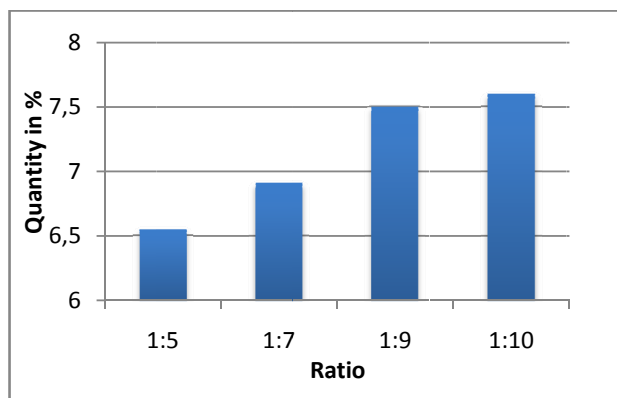


Figure 1 – Dependence of the tannins yield on the ratio of raw material to solvent

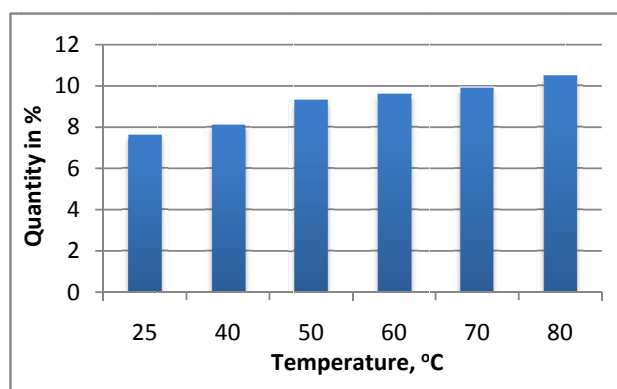


Figure 2 – Dependence of the tannins yield on the extraction temperature

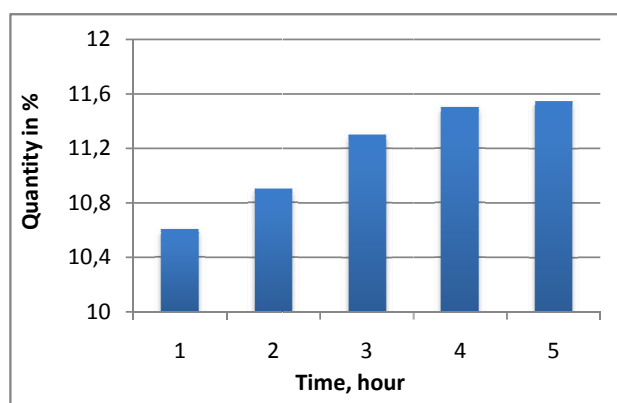


Figure 3 – Dependence of the tannins yield on the extraction time

Determining the optimal mode of extraction.

Based on the obtained data it can be concluded that the optimal extraction mode is a mode in which the raw material is continuously extracted for 4 hours without infusion.

Selection of the optimal precipitant of tannins.
According to obtained data the best tannin precipitant is lead acetate (II).

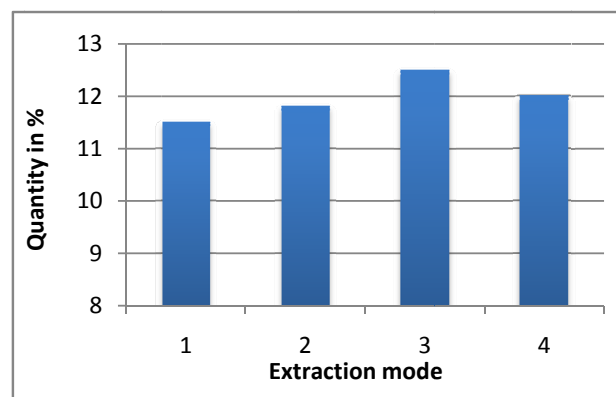


Figure 4 – Dependence of the tannins yield on the extraction mode (1-4x1h without infusion; 2-4x1h with infusion; 3-1x4h without infusion; 4-1x4h with infusion)

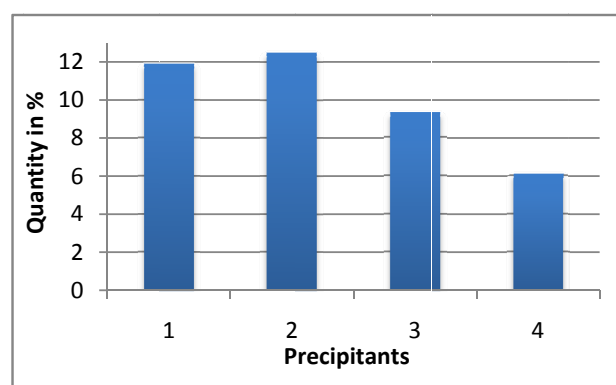


Figure 5 – Selection of the optimal precipitant of tannins from extract (1- barium chloride; 2- lead acetate II; 3- calcium chloride; 4- iron sulfate II)

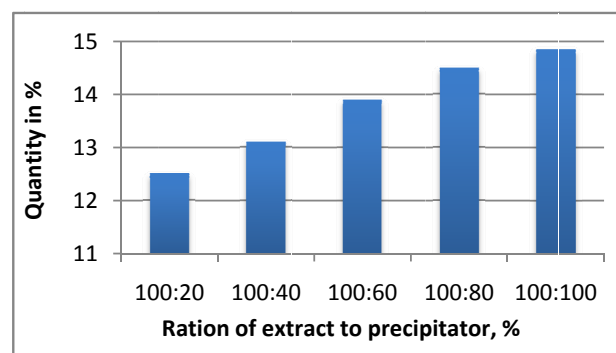


Figure 6 – Dependence of the tannins yield on the ratio of extract to precipitator.

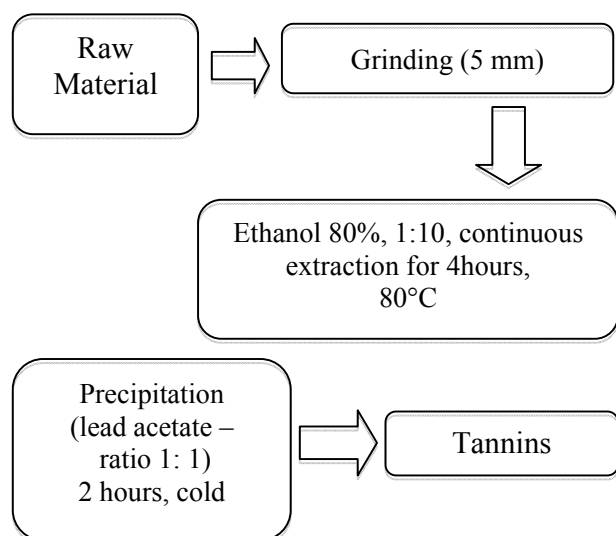


Figure 7 – Technological scheme of polysaccharides extraction from tobacco dust (*Nicotiana tabacum* L).

Determination of the optimum ratio of the extract to precipitator. Based on the data displayed in Figure 6 it is obvious that the optimal ratio of extract to precipitator is 1:1.

Technological scheme of extraction of tannins. Based on the analysis of the data we have found that the optimum parameters are:

1. Extraction of raw material with 80% ethyl alcohol
2. The ratio of raw material to solvent is 1:10
3. The extraction temperature 80°C
4. Extraction time is 4 hours
5. Extraction mode is continuous heating without a daily infusion for 4 hours.

Thus, the optimal technology scheme of tannins extraction is represented in block diagram in Figure 7.

Conclusion

In order to detect biological activity of a substance derived from tobacco dust waste of tobacco production qualitative and quantitative characteristics of the raw material were determined. Optimization of the technological parameters of extraction of tannins was carried out. Based on the data obtained the technological scheme of tannins extraction was developed. The determination of biological activity of substances will be established in the next work.

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