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Spectrophotometric Method for Quantitative Determination of the Amount of Flavonoids in Oil Extract

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Abstract
This paper presents the results of research on the development of a UV spectrophotometric method for the quantitative determination of total flavonoids in an oil extract obtained from a mixture of medicinal plant crudes. A method is proposed for extract sample preparation with saponification and transfer of the flavonoid fraction to 80% ethanol, followed by a complexing reaction with AlCl3 and photometric measurement at 415 nm. Total flavonoids were quantified based on rutin. The amount of flavonoids in oil extract samples varied within the range of 8.17-9.05 mg/g. The method can be used for quality control of the oil extract.

Keywords: extraction, oil extract, flavonoids, spectrophotometry, quantitative analysis, plant raw materials..

Introduction

It is known that Flavonoids are a large group of phenolic compounds of the plant world, which determine a number of biologically significant effects of multicomponent compositions, such as anti-inflammatory, hepatoprotective, antioxidant, antidiabetic, neuroprotective, etc. (1,2,3) In oil extracts, flavonoids are present in the lipophilic fraction (aglycone, co-extracted with terpene and phenolic components), or pass into the alcohol phase after saponification of the lipid base.

The work examines an oil extract obtained using a combined scheme, which consists of the enzymatic action of pre-treated with ethanol crushed medicinal plant material consisting of a mixture of Herba hyperici , Rosae fructus , Rhizomata Calami , Herba Tribuli terrestris , Glycyrrhiza glabra Radix followed by ultrasonic extraction in sunflower oil and clarification (4-5). For process control and comparability of various samples of the resulting oil extract, a reproducible, cost-effective

instrumental method for their quantitative evaluation is required. UV spectrophotometry with aluminum chloride is a generally accepted method for the quantitative determination of total flavonoids calculated as rutin (6), providing the necessary sensitivity and selectivity but, in our case, requiring proper sample preparation. The use of HPLC for the quantitative determination of flavonoids requires more complex sample preparation technology, carries a risk of column contamination, and requires a set of individual standards (rutin, quercetin, hyperoside, etc.).

Materials and Methods

The sample was an oil extract of a mixture of medicinal plant materials (St. John's wort, rose hips, calamus, Bidens sibirica , and licorice) taken in specific ratios. Fractions with particle sizes of 1-2 mm for flowers and 2-3 mm for herbs, fruits, and bark were used for extraction.

Sample preparation of oil extract for analysis was carried out as follows. First, it was necessary to completely free the flavonoid fraction from the oil base and transfer it to an alcohol-water medium suitable for subsequent complexation with $AlCl_3$ and spectroscopy at 415 nm. The preparation principle is based on the mild saponification of triacylglycerides with a KOH solution in 96% ethanol at 55–65° C for 30–40 minutes. The experimentally determined optimal conditions were a temperature of 60 ° C and a time of 35 minutes. This results in the formation of ethanol-soluble potassium salts of fatty acids and glycerol, while the flavonoids are transferred to the alcohol phase. Further neutralization to a slightly acidic pH range of 5.0–5.5 provides conditions for the flavonoid complex with aluminum chloride.

Complexation was carried out as follows. 1.000 g (accurately weighed) of the oil extract was weighed into a flask, and 10.0 ml of a 2% (wt.) KOH solution in 96% ethanol was added. The mixture was kept in a water bath at 60 ° C for 35 minutes with occasional shaking. The process was carried out in dark conditions. After saponification was complete, the solution was cooled to 20-25 ° C, and 1 M HCl was added with stirring until a pH of 5.0 was achieved. It should be noted that when stirring, HCl should be added slowly, in small portions, avoiding the formation of localized areas with excess acid. The resulting solution was quantitatively transferred to a 25.0 ml volumetric flask, rinsing the flask walls with 80% ethanol, and bringing the solution to the mark with the same solvent. Additionally, the obtained solution was centrifuged for 5 min at 3000 rpm for better clarification. 1.0 ml (accurately weighed) was taken from the obtained extract and placed in a 10 ml

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measuring flask, 1.0 ml of 2% $AlCl_{3\ solution}$ in ethanol and 0.5 ml of 1 M potassium acetate were added, after which it was brought to the mark with 80% ethanol, kept for 30 min. at 25 ^{0} C, excluding exposure to light and the optical density was measured at 415 nm. Relative to the comparison solution, prepared identically, but without aluminum chloride. Quantitative assessment was carried out by calibration. Calibration was performed on standard rutin solutions (5-50 mg / l), in this range there was a linear relationship A = aC + β; where in our case a - 0.0139 and β = 0.004; The content of total flavonoids in the extracts was expressed in mg/g of oil extract from the calibration, taking into account the weight and volumes:

$$X = \frac{D_{sample} - D_{ref}}{a} * \frac{V_{first} * V_{sample}}{V_{aluminum} * w_0}$$

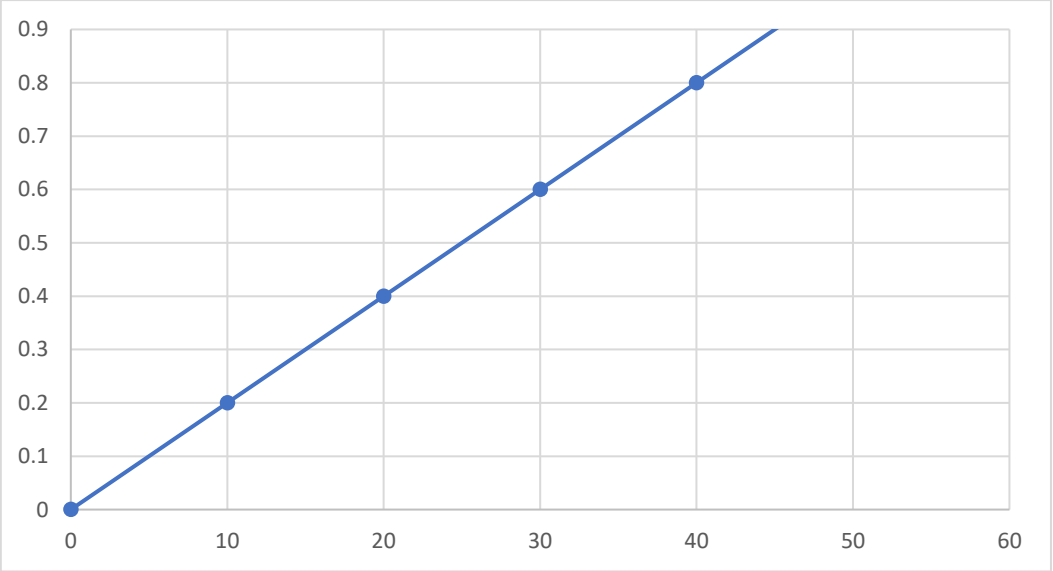

Fig. 1. Calibration curve of rutin in the range of 5-50 mg/l.

Where X is the sum of flavonoids; D_{sample} and $D_{reference}$ are the optical densities of the sample and reference solution at 415 nm; $V_{ektract}$ is the volume of the extract after saponification (1); V_{last} is the final volume of the measured solution in the cuvette; is the weight of the oil extract = 1.000 g. The optical density of the samples and the standard solution was measured on a META w_0S H spectrophotometer.

Oil extract samples were obtained using the following technology. Prior to extraction, the crushed medicinal plant material was fermented with cellolase and pectinase enzymes at specific concentrations, as well as a mixture of the two. The total enzyme concentration did not exceed 1% of the dry weight of the plant material. Fermentation was carried out at 45 $^{\circ}$C and pH 5.0 for 2 hours. After fermentation, the raw materials were dried for ultrasonic extraction in sunflower oil. The process

hydromodulus was 1:10, the temperature was 40 °C, and the extraction time was up to 30 minutes. After filtration and clarification of the samples, the total flavonoid content was determined. The oil extract samples differed in the technological parameters of the extract production process. In particular, Table 1 presents the results of studying the total flavonoid content of samples obtained using one and a mixture of enzymes, and Table 2 presents the results for samples for which the extraction time varied in the range of 10–30 minutes.

Table 1. The effect of the amount and nature of the enzyme on the yield of total flavonoids from plant materials.

N	Enzyme, %	Extra time, min	Total flavonoids, mg/g
1	Cellulose - 0.5	15	7.85±0.05
2	Pectinase – 0.5	15	7.93±0.06
3	Cellulose+Pectinase 0.25+0.25	15	8.19±0.05
4	Cellulose+Pectinase 0.5+0.5	15	8.43±0.06

Table 2. Results of the study of the influence of extraction time on the yield of flavonoids.

No.	Enzyme, %	Extra time, min	Total flavonoids, mg/g
1	Cellulose+Pectinase 0.5+0.5	10	8.13±0.06
2	Cellulose+Pectinase 0.5+0.5	15	8.41±0.05
3	Cellulose+Pectinase 0.5+0.5	20	8.49±0.05
4	Cellulose+Pectinase 0.5+0.5	25	8.62±0.06
5	Cellulose+Pectinase 0.5+0.5	30	9.24±0.05

The results presented in Tables 1 and 2 demonstrate, firstly, that the proposed UV spectrophotometric method for quantitative determination of total flavonoids in oil extracts is sufficiently technologically feasible, yields reproducible results, and can be recommended for routine analysis when developing optimal oil extract technology. Secondly, the results indicate that fermentation with a mixture of cellolase and pectinase enzymes in specific ratios significantly increases the yield of target biologically active substances in the extract and allows for a significant reduction in extraction time.

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