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STUDYING THE POLYPHENOLIC STRUCTURE OF *LAURUS NOBILIS L.* LEAVES

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Abstract:

In work research results of the polyphenols chemical composition of Laurus nobilis L leaves are presented. Bay laurel is a known food plant having also wide range of important pharmacological properties such as: hypoglycemic, anti-inflammatory, antiepileptic and antioxidant. Using a method of a highly effective liquid chromatography in the gradient mode of elution, the structure of flavonoids of a plant which includes 12 components which are glycosides of quercetine and kaempferol is established. The technique of quantitative definition of flavonoids in raw materials of bay laurel in terms of hyperoside, consisting in application of a method of absolute graduation is developed. The maintenance of hyperoside in raw materials made 0.211%±0.01.

Keywords: *bay laurel, highly effective liquid chromatography, flavonoids, hyperoside, method of absolute graduation*

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INTRODUCTION:

Bay laurel – that is *Laurus nobilis L.*, is the plant belonging to the *Laureaceae family*, which is widely used as aromatic. Plant leaves also find broad application as medicinal in traditional medicine [1]. Use them as hypoglycemic cure for diabetes, for treatment of fungal and bacterial infections, in therapy of gastrointestinal diseases [2-5]. Also anti-inflammatory, antiepileptic and antioxidant properties of a plant are known [6-9].

It is considered to be that biologically active components of a plant causing their pharmacological effect are essential oils. The majority of researches of bay laurel are devoted to studying of this class of connections. It is established that leaves of bay laurel contain more than 80 components generally monocyclic monoterpenes, and dominating are 1,8-tsineol, alpha terpineol acetate and terpinen-4-ol [10, 11].

However, in medicine generally aqueous-alcoholic extraction from leaves in which the maintenance of terpenoid small are applied. Therefore, it is important to emphasize that the pharmacological role of essential oils of laurels is strongly exaggerated. Usual components of aqueous-alcoholic extraction from vegetable raw materials are polyphenols, first of all flavonoids which, apparently, should be considered the main carriers of biological activity of bay laurel. There is a number of the works confirming antioxidant activity of a plant [12]. It is shown that water - ethanol extract (70%) of bay laurel had the expressed antioxidant properties caused by presence of flavonoids, such as kaempferol, kaempferol-3-ramnopiranozid, and kaempferol-3,7-diramnopiranozid, luteolin and routines [9, 13, 14].

Nevertheless, the flavonoid structure of leaves of bay laurel is studied fragmentary and demands specification. Therefore, **the purpose** of the real research was the research of composition of polyphenols of leaves of laurels of a question of standardization of these raw materials, noble for the decision.

The circle of tasks of realization of the planned purpose included definition of component composition of polyphenols of leaves *of L. nobilis L.*, and also development of a way of standardization of the studied raw materials.

RESEARCH OBJECTS AND METHODS:

As the studied object the sample of leaves of bay laurel, collected in the territory of Krasnodar Krai was taken. For extraction of the sum of biologically active agents' alcohol ethyl 70% was used. Extraction was carried out by percolation method in system from three diffusers. Frequency rate of insisting in each diffuser made 12 hours. United retrieved was distilled at a temperature of 4 °C within 3 days, after filtered via the Blue Film filter.

For studying of the chemical composition of the received extraction the Reverse phase highly efficient liquid chromatography method was used.

Chromatographic researches were conducted on the chromatographic device *of Agilent Technologies 1200 Infinity* of production of the USA with automatic *Agilent 1200* sampler, a vacuum microdecontaminator, the gradient pump and the thermostat of the same series. Electronic ranges of absorption registered by means of the spectro-photo-metric detector with a diode matrix *of the Agilent 1200 series* (range of lengths of waves from 190 to 950 nanometers, a ditch with a length of optical way of 10 mm; of 13 mkl), a scanning step - 2 nanometers.

For registration and processing of spectral data and chromatogram used the software *of "Agilent ChemStation"*.

For preparation of mobile phases used the following solvents: water super-pure (for a liquid chromatography), alcohol ethyl, formic acid.

Identification of components was carried out on coincidence of times of keeping of the analyzed substances with C recorded in similar experimental conditions and by results of diode and matrix detecting.

The sum of polyphenolic components of bay laurel was subjected to chromatographic division in the following conditions developed by us earlier [15]:

mobile phase: (A) - 1% water solution of formic acid, (B) - alcohol ethyl in the gradient mode of elution;

column: *Supelco Ascentis express C182.7μм × 100 mm × 4.6mm*.

speed of a mobile phase - 0,5 ml/min.;

column +35 temperature ° C;

volume entered tests 5 μl.

The structure of a mobile phase was programmed in the conditions specified in tab. 1.

Table 1: Conditions for the gradient elution of polyphenol compounds *of Laurus nobilis L.* leaves

Time, min	A,%	Б,%
0	90	10
10	80	20
20	70	30
30	50	50
40	10	90

Detecting was carried out: for proanthocyanidins 280 nanometers of flavone 336 nanometers, flavonol 360 nanometers, oxycinnamon acids 310 and 325 of nanometer.

Identification of components was carried out on coincidence of times of keeping of the analyzed substances with CO recorded in similar experimental conditions and by results of diode and matrix detecting.

The relative maintenance of individual flavonoids, oxycinnamon acids determined as the relation of the area of chromatographic peak and the sum of the areas of peaks of all identified flavonoids, oxycinnamon acids and anthocyanin by a formula 1.

$$Xi = \frac{Si \times 100}{\sum S},$$

where Si - average value of the area of peak of a component on sum chromatogram;

∑S - average value of the sum of all areas of peaks on chromatogram.

Control of chromatographic system suitability. The Chromatographic system can be considered suitable if the following conditions are reached:

- The efficiency of chromatographic system calculated on peaks on chromatogram has to make not less than 5000 theoretical plates;
- The coefficient of asymmetry of peaks has to be less than 2;
- Division coefficient not less than 1.5.

RESULTS AND DISCUSSION:

Chromatogram and interpretation of results of detection of component structure *flavonoidovl. nobilisL.* are presented in fig. 1 and in tab. 2.

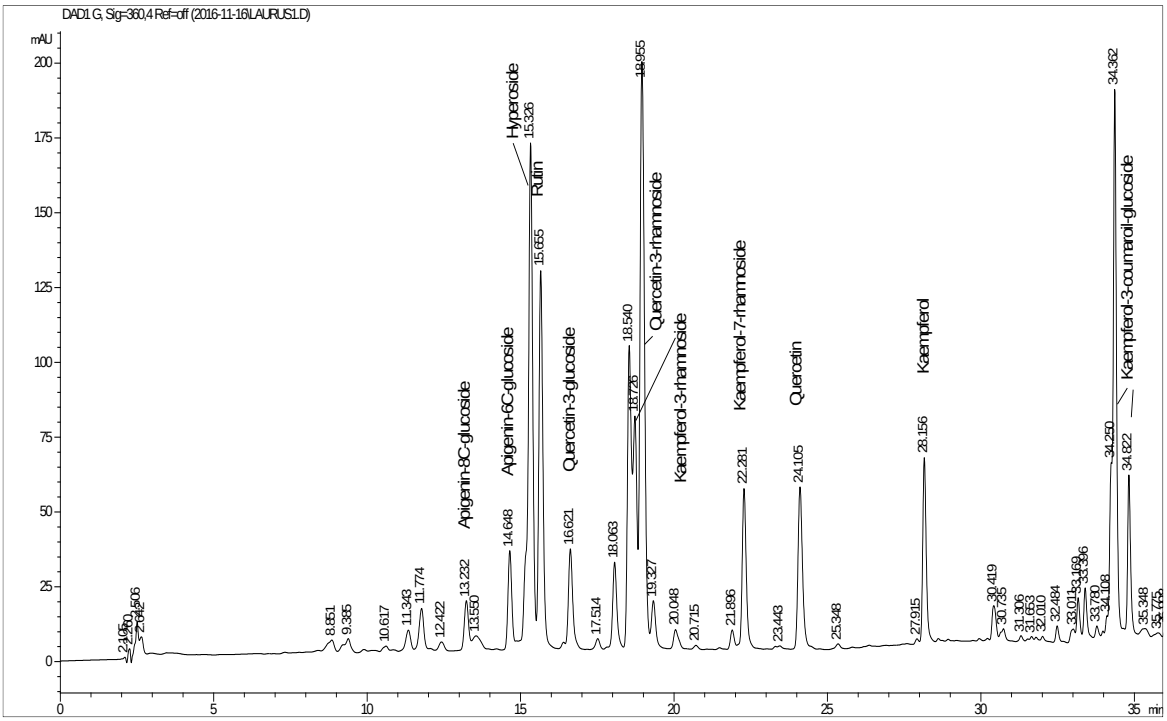
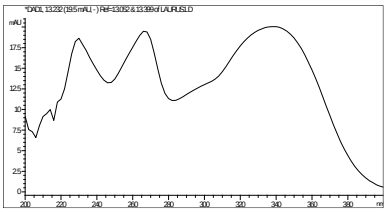
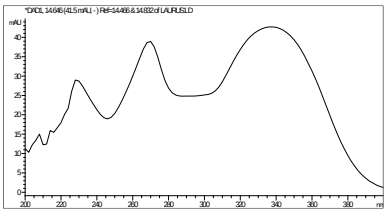


Fig. 1: Chromatogram of spirit extraction from *L.nobilisL* leaves.

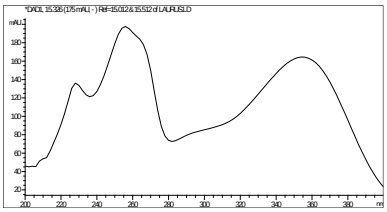
Table 2: The results of the detection of flavinoid components of L. nobilis L.



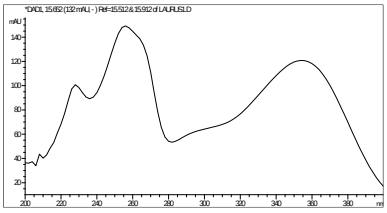
Apigenin-8-C-glucoside



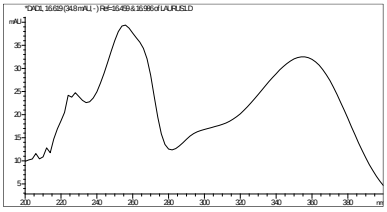
Apigenin-6-C-glucoside



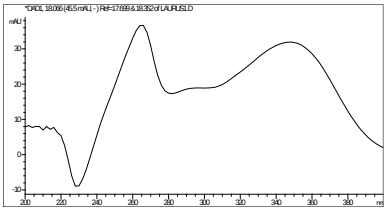
Hyperoside



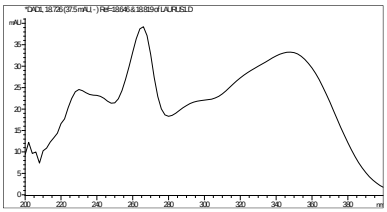
Rutin



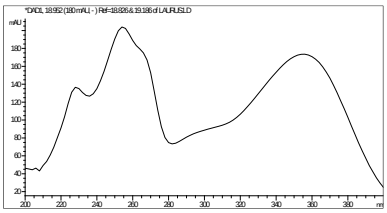
Quercetin-3-glucoside



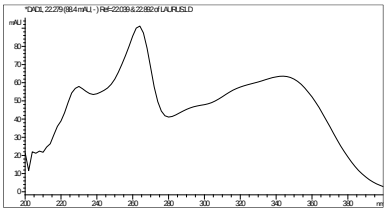
Not identified



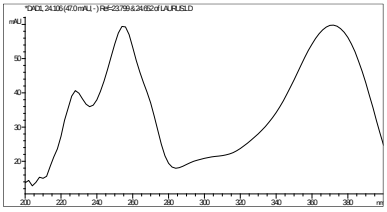
Kaempferol-3-rhamnoside



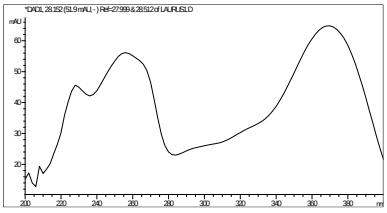
Quercetin-3-rhamnoside



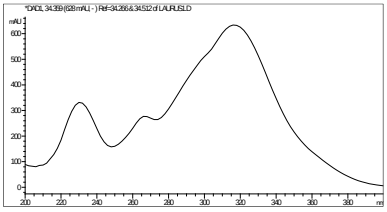
Kaempferol-7-rhamnoside



Quercetin



Kaempferol



Kaempferol-3-cumaroyl-glucoside

As the results given in tab. 2show, the calculated criteria of suitability (N> 5000, $_{to\ Rs}$> 1.5, $_{Tf}$ <2) generally, correspond to the reviewed values. Thus, the used chromatographic system can be recognized suitable for definition of biologically active *L. nobilis L* connections.

For check of suitability of the used chromatographic system the criteria of suitability presented in tab. 3 were defined.

Table 3: Indicators of the suitability of the chromatographic system for the determination of bioactive compounds of *L. nobilis L*.

t_R	S	N	HETP	R_s	T_f	W_b
13.323	173.78	50294	1.98×10^{-4}	2.98	1.01	0.1389
14.648	367.22	68209	1.4×10^{-4}	3.53	0.9	0.1320
15.326	1575.2	67464	1.48×10^{-4}	2.95	1.22	0.1389
15.655	1019.56	74852	1.3×10^{-4}	1.41	0.74	0.1347
16.621	301.4	76249	1.3×10^{-4}	4.11	0.74	0.1417
18.726	174.9	194281	5.1×10^{-5}	1.0	0.74	0.1
18.955	1570.5	93730	1.0×10^{-5}	1.1	0.7	0.1457
22.281	470.7	150894	6.6×10^{-5}	1.74	0.79	0.135
24.105	568.6	128422	7.7×10^{-5}	1.81	0.73	0.1583
28.156	476.0	311826	3.2×10^{-5}	1.29	0.76	0.1187
34.363	3583.57	750832	1.33×10^{-5}	1.69	0.75	0.093
34.823	1091.71	702566	1.4×10^{-4}	2.83	0.72	0.0978

tR-absolute retention time, S - peak area, N - number of theoretical plates, HETP - height equivalent to the theoretical plate, Rs-peak separation factor, $_{Tf}$ - coefficient of asymmetry, $_{Wb}$ - peak width at baseline.

In fig. 2 values of the areas of peaks of the components received during chromatogram making of biologically active agents in an object are shown.

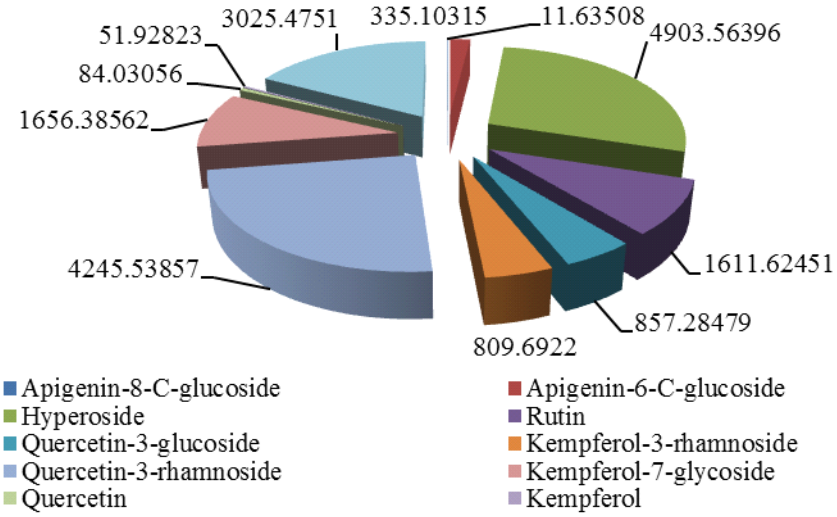


Fig. 2: Values of flavonoid peak areas in *L. nobilis L*. leaves

As the area of peak is directly proportional to its contents, on these indicators it is possible to draw a conclusion on quantity of each component as a part of an object.

By results of calculation of the relation of the area of chromatographic peak to the sum of the areas of all peaks percentage distribution of each flavonoid in leaves *of L. nobilisL* is made. presented in fig. 3).

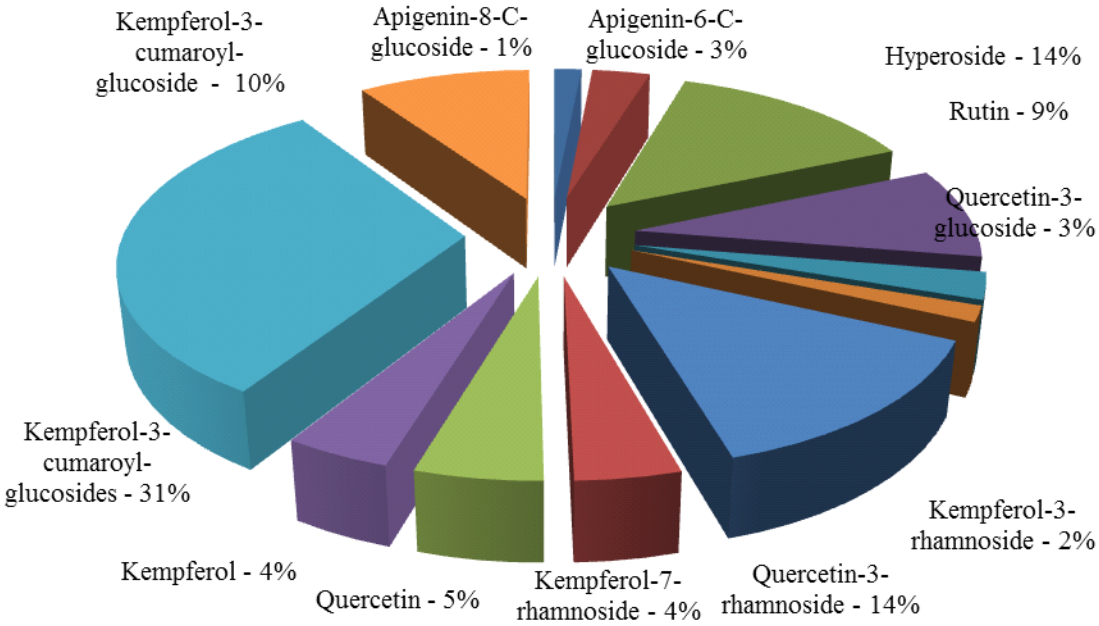


Fig. 3: Composition and percentage distribution of flavonoids in *L. nobilis L*. leaves

The data submitted in fig. 3 demonstrate that the dominating components in leaves *of L. nobilis L.* are a depsydikaempferol with p-coumaric acid and hyperoside.

For the purpose of receiving the more objective assessment of *L. nobilisL.* flavonoids structure we've carried out acid hydrolysis of spirit extraction. Acid hydrolysis was carried out by 2 M solution of acid hydrochloric when heating with the return refrigerator during 1.5 h. After hydrolysis mix was cooled to room temperature and centrifuged on *the EppendorfAG centrifuge* at 13.2×1000 rpm within 5 minutes. Nadosadochny liquid further subjected a hromatografirovaniya in the conditions given above. Results of a hromatografirovaniye of products of hydrolysis of spirit extraction from *L.nobilisL.* are provided on fig. 4.

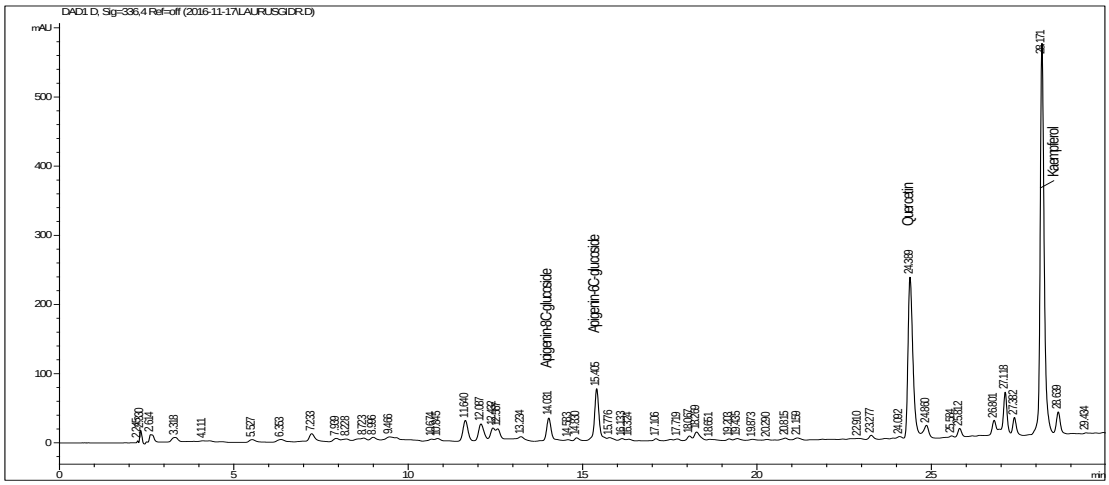
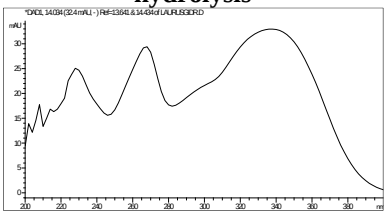


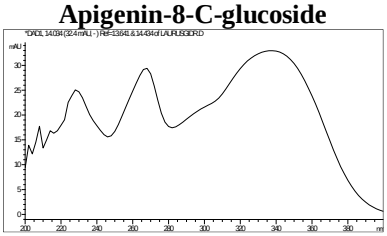
Fig. 4: Chromatogram and UV-profiles of products of acid hydrolysis of alcohol extract from a *Laurus nobilis L*. leaves

Component structure of products of hydrolysis of the flavonoid *L. nobilis L complex*. it is presented in tab. 4.

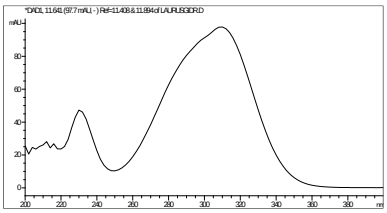
Table 4: The results of the detection of the flavinoid components of L. *nobilisL*. after acid hydrolysis



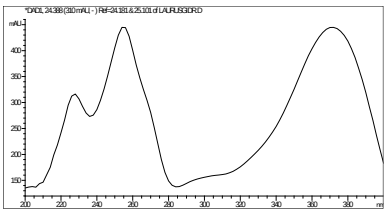
Apigenin-8-C-glucoside



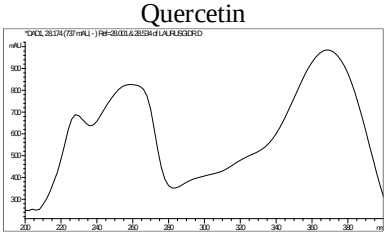
Apigenin-6-C-glucoside



p-cumaric acid



Quercetin



Kaempferol

Results of acid hydrolysis of the flavinoid *L. nobilisL* complex. showed that the main aglikona are кверцетин and kaempferol. At hydrolysis acid which in a plant is present at a type of a depsid with kaempferoly is released p-kumarovaya.

As one of the dominating components of the studied sample is hyperoside, further standardization of raw materials of leaves of laurels was carried out in terms of this component. The analysis was carried out by method of absolute graduation. For this purpose, the calibration schedule FROM hyperoside was received previously.

NOTE. 1. Preparation of solution of a standard sample of hyperoside. About 80 mg (an exact hinge plate) *of Gfugiperozid's* (series No. 201202) *SOFSO* placed in a measured flask with a capacity of 25.0 ml, added 10.0 ml of alcohol ethyl 95%, mixed, brought solution volume the same solvent to a tag and again mixed.

For creation of the calibration schedule of 0.08 g (an analytical hinge plate) of the FSO of GFU of hyperoside placed in a measured flask with a capacity of 100 ml, added 20 ml of alcohol ethyl 95%, carefully shook up before full dissolution and brought to a tag the same solvent (solution A).

From the received solution A further prepared a series of calibration solutions consisting of 6 samples. For this purpose, a pipette transferred solution A in volumes to each of 6 measured flasks with a capacity of 25 ml: 2.0; 4.0; 6.0; 8.0; 10.0, 12.5 ml, contents of flasks carefully mixed and brought alcohol ethyl 95% to a tag (solutions B).

Further entered into the chromatograph on 1 µl the received calibration solutions, including solution A also was registered by their areas of peaks. By results built the schedule of dependence of the area of peak on amount of the entered substance. The received calibration schedule is submitted in the drawing.

The provided calibration schedule demonstrates that on a piece of 0.064-2.048 mg/ml there is a rectilinear dependence between the areas of peaks solution calibration and their concentration in the specified range of concentration (fig. 5).

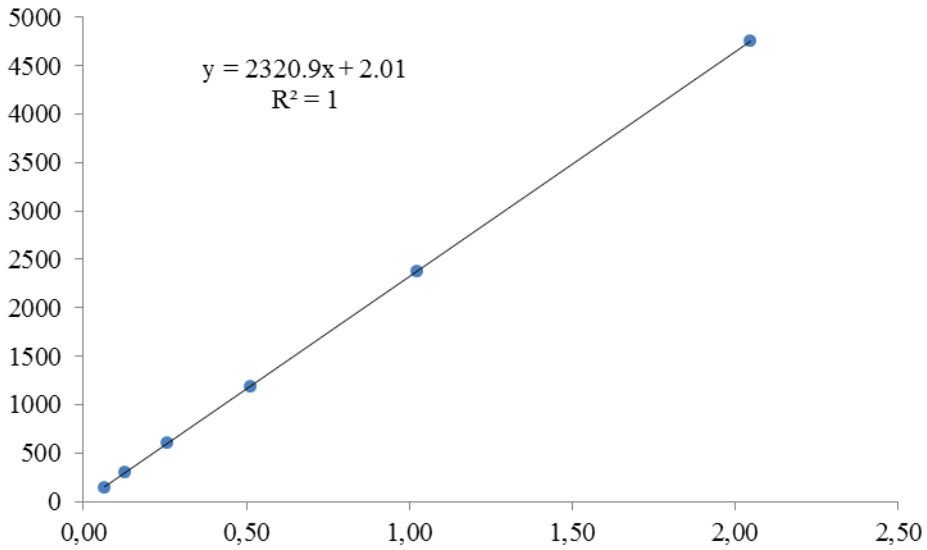


Fig. 5: A calibration plot of the area of the hyperoside SS peak against concentration

Results of calculations of maintenance of hyperoside in leaves of laurels of the calculated equation of regression, noble with use, showed that its contents made 0.211%±0.01.

SUMMARY:

Thus, during the real research the flavinoid structure of leaves of bay laurel is established. Presence of 12 flavonoids which are glycosides of a quercetine and kaempferol is shown. The fact that in the polyphenolic *L. nobilis L complex* attracts attention. practically otsustvut oxycinnamon acids in a free look, however the dominating component is депсид kaempferola with p-kumarovoy acid that apparently it is possible to consider a characteristic sign of polyphenols of the specified plant. The way of standardization of raw materials of leaves of bay laurel - a method of absolute graduation in terms of hyperoside is offered.

CONCLUSIONS:

1. The highly efficient liquid chromatography method established structure of flavonoids of leaves of bay laurel which includes 12 components which are glycosides of quercetine and kaempferol.
2. The technique of quantitative definition of flavonoids in raw materials of bay laurel in terms of hyperoside is developed. The maintenance of hyperoside in raw materials made 0.211%±0.01.

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