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COMPARATIVE STUDY AND MODELLING OF RUTIN EXTRACTION FROM HYPERICI HERBA AND HIPPOPHAES FRUCTUS

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The study on Rutin extraction from autochthonous *Hyperici herba* and *Hippophaës fructus* with methanol, ethanol and 1-propanol at different concentrations, temperatures and duration indicated that the most efficient solvent is 1-propanol, its optimum concentration being determined by the amount and type of the natural compounds from the two plants.
Using the statistical analyzis and a factorial experiment of second order, two mathematical correlations between the Rutin extraction degree and the main parameters influencing the process (1-propanol concentration, temperature, duration) have been established. For both extraction systems, the considered variables control the extraction process in a 99.2 – 99.5 % extent, the solvent concentration exhibiting the most important influence, especially for Rutin extraction from *Hippophaës fructus*.

Keywords: rutin, extraction, methanol, ethanol, 1-propanol, statistical analyzis

Introduction

Flavonoids have been generally considered as secondary metabolites, compounds not absolutely essential to the life of individual cells of plants or even to the plant as a whole. These compounds are found in the higher plants like angiosperms, gymnosperms, but also in some green algae, in the bryophytes, in all tracheophytes and in some insects (flavonoids are currently involved in attracting pollination vectors) [1]. These compounds could assume several roles as subtle mediators of complex processes, from plant growth and development until sparing of easily oxidizable metabolites, broadly effective enzyme inhibitors, and intervention like pesticides or light screens. Both of the mentioned characteristics and the reported efficiency of some flavonoids in improving capillary strength and controlling erythrocyte aggregation are of pharmacological interest [1-6].

The structures of these constituents are derived from flavone (2-phenylbenzopyran) or, sometimes, isoflavone (3-phenylbenzopyran). Depending on the oxidation extent, as well as on the position and nature of the various substitutes, the flavonoids could be flavonosides, biflavonosides, chalcones, aurones, isoflavones, anthocyanosides and 3-, 4-flavonols [6-8]. The presence of active phenolic and carbonyl groups in their molecule determines the variety of pharmacological properties. Among them, the maintaining or restoring of normal capillary resistance could be underlined, this property is claimed by Rutin.

Rutin is one of the most used flavonoids in the pharmaceutical practice, being an active compound in capillary tonic and vein tonic medicines, which is included in the complex therapy of toxicosis, thrombosis, and atherosclerosis. From chemical point of view, Rutin is *4h-1-benzopyran-4-one,3-[[6-0-(6~deoxy-α-l-mannopyranozyl)-β-d~glucopyrano - zyl]oxy] - 2 - (3,4~dihydroxyphenyl) - 5,7 - dihydroxy*, or *tetrahydroxyflavonol-5,7,3',4'-ramnoglucozide*, a solid, yellow and crystalline substance [7,8].

The most common combinations of Rutin are with Ascorbic acid and Dipyridamol. The medical effectiveness of Rutin consists in beneficial effects to the capillaries by:

a) chelating metals and thus sparing ascorbate from oxidation,
b) prolonging epinephrine action by inhibiting *o-methyltransferase*,
c) stimulating the pituitary-adrenal axis, and

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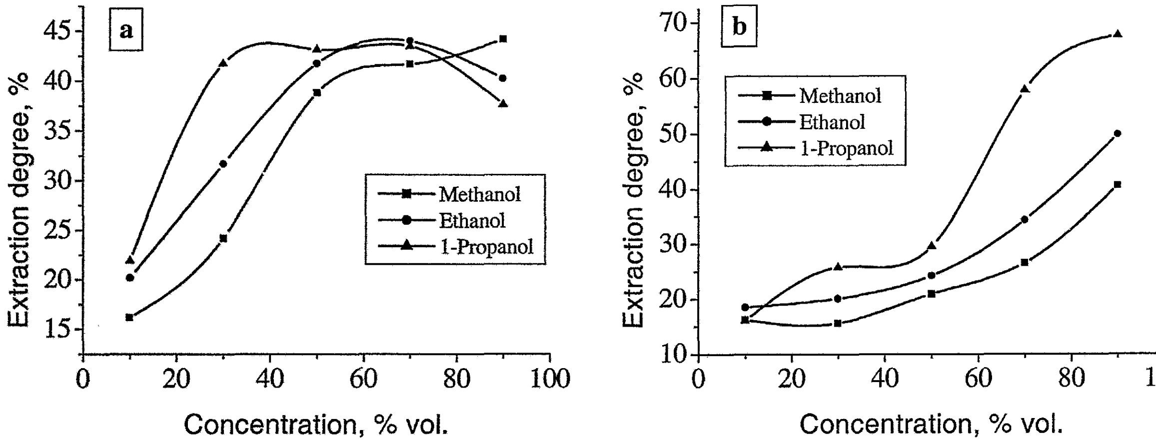

Fig.1 Influence of nature and solvent concentration on Rutin extraction degree from *Hyperici herba* (a) and *Hippophaës fructus* (b)

d) reducing the aggregation of erythrocytes and in retarding the loss of fluids from the capillary and lymphatic system under stress condition.

Rutin can be found in a proportion of 12-20 % in the flower buds of *Sophora japonica*, the classically raw material for industrial production. In smaller amounts, Rutin can be also found in *Fagopyri herba* (5-8 %), *Polygoni hydropiperis herba* (3.5 %), *Hyperici herba* and *Hippophaes fructus* [1,3,7,8].

Because the *Sophora japonica* is not an available raw material, the aim of this paper is the study on Rutin extraction from the last two raw vegetable materials, which are autochthonous and easily accessible.

The experiments were carried out in two steps. The former one consists on a comparatively study of Rutin extraction from *Hyperici herba* and *Hippophaes fructus*, respectively. For this purpose, methanol, ethanol and 1-propanol have been used as solvents, the separation being performed at different alcohol concentrations, temperatures and extraction time.

In second step of experiments, using the statistical analyzis, respectively the factorial experiment of second order, a mathematical model that describs the Rutin extraction from *Hyperici herba* and *Hippophaes fructus* with the most efficient solvent was established. The proposed model takes into account the cumulated influences of solvent concentration, temperature and the duration of extraction process on the Rutin extraction degree.

Experimental Part

The Rutin extraction was carried out in a glass vessel of 0.3 l volume, provided with jacket heated by water maintained at the prescribed temperature by means of a thermostat, back-flow condenser and two blade stirrers rotated at 300 rpm.

Rutin was extracted from *Hyperici herba* and *Hippophaës fructus*. Each extraction has been carried out using 1g dried and finely cutted material and 50 ml solvent. In the first step of experiments three alcohols were used as solvent: methanol, ethanol and 1-propanol. The solvent concentration was varied between 10 and 90 % vol.. The extraction temperature was maintained in the domain of 20 – 60 °C for methanol, respectively, 20 – 70 °C for ethanol and 1-propanol. The process duration was of 5 – 60 minutes.

In the second step of the experiments, the correlation between Rutin extraction yield and the main factors influencing the extraction process (solvent concentration, temperature and duration) has been established by means of the results obtained using the adequate experimental matrix.

The extraction yield, Y, was calculated as the ratio between the Rutin concentration in the extract phase and the total amount of Rutin in the vegetable material. The total amount of Rutin for *Hyperici herba* and *Hippophaës fructus* has been determined by total Rutin extraction with methanol in a Soxhlet extractor during 4 hours. The Rutin dosage in the extract phase was made by spectrophotometric method [8].

Results and Discussion

Rutin extraction from Hyperici herba and Hippophaës fructus

The analyzis of the effect of solvent nature and concentration on Rutin extraction from the two vegetable materials indicated that in both cases 1-propanol is the solvent that offers the highest extraction degree (*Fig.1*).

But, as it can be observed from *Fig.1*, the optimum concentration of 1-propanol depends on the type of used plant. Thus, the maximum extraction yield of Rutin from *Hyperici herba* was reached for a solvent concentration of 50 % vol., respectively 90 % vol. for extraction from *Hippophaës fructus*. This difference could be the result of the difference between the nature and amount of the natural compounds from the two plants, and consequently of the different capacity of

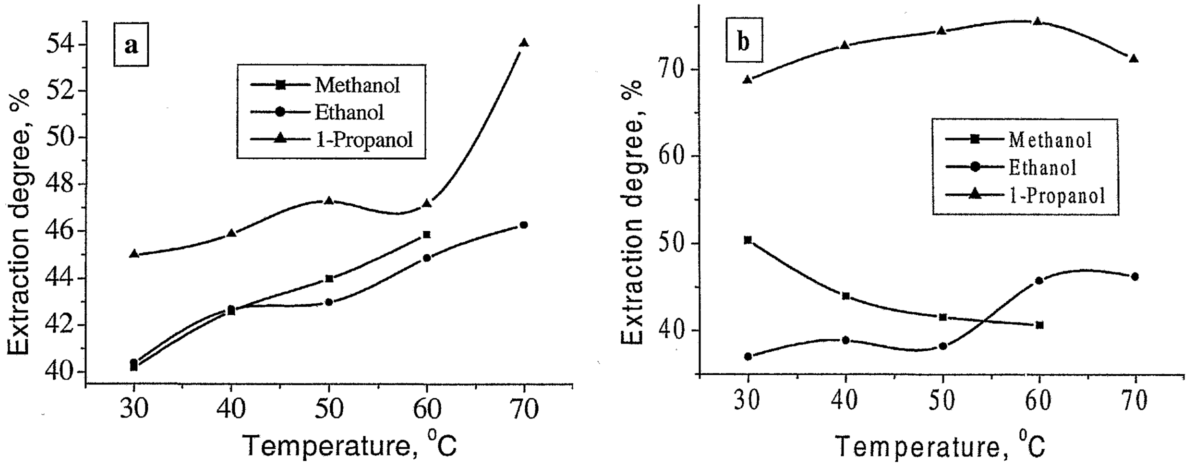

Fig.2 Influence of temperature on Rutin extraction degree from *Hyperici herba* (a) and *Hippophaës fructus* (b)

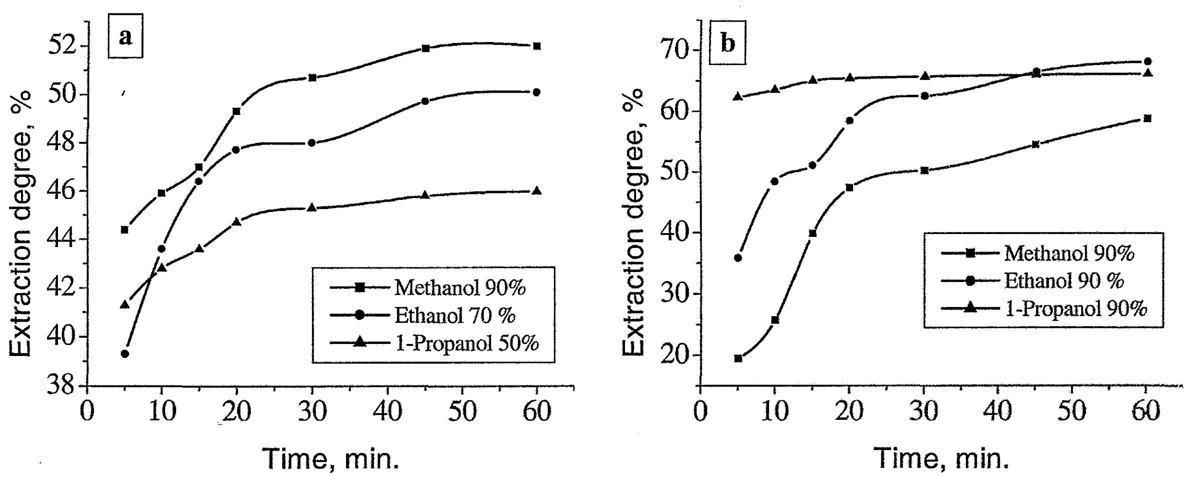

Fig.3 Influence of process duration on Rutin extraction degree from *Hyperici herba* (a) and *Hippophaës fructus* (b)

solvent to wet and soak the vegetable material. Owing to the high content of hydrophobic compounds in *Hippophaës fructus* (12-14 % wt. glycerides of oleic, palmitic, linolic and linoleic acids) the wetting and the soaking of the vegetable material, which allow the osmosis and diffusion of the active compound from solid phase to solvent, are possible only with concentrated 1-propanol. This conclusion is sustained by the fact that, contrarily to the extraction from *Hyperici herba*, the maximum Rutin extraction yield from *Hippophaës fructus* is reached in a concentration level of 90 % vol. for all used solvents, the presence of water reducing the extraction efficiency.

The study on temperature effect was carried out for the solvents concentration values that allow to reach the maximum extraction yield and indicates an increase of extraction yield with temperature increase (*Fig.2*).

As in earlier experiments, the extraction with 1-propanol was the most efficiently, too.

Using the previous data, the following extraction conditions have been chosen for analyzing the influence of process duration:

- *Hyperici herba*:
  - methanol 90 % vol., 60 ^{0}C
  - ethanol 70 % vol., 70 ^{0}C
  - 1-propanol 50 % vol., 70 ^{0}C
- *Hippophaës fructus*:
  - methanol 90 % vol., 60 ^{0}C
  - ethanol 90 % vol., 60 ^{0}C
  - 1-propanol 90 % vol., 60 ^{0}C

From *Fig.3* it can be observed that the Rutin extraction degree significantly increases in the time domain of 0 - 20 minutes, indifferent of the studied extraction system, then the increase becoms slower.

The extraction of Rutin from *Hippophaës fructus* with 90 % vol. 1-propanol is the exception, because the extraction degree reaches a high value after 5 minutes from the process beginning, remaining then at a constant level. As it was above mentioned, this variation can be explained by the high capacity of 1-propanol to wet and soak the vegetable material, thus intensifying the Rutin diffusion to solvent phase.

Modelling of Rutin extraction from Hyperici herba and Hippophaës fructus

The mathematical model that describes the influences of solvent concentration, temperature and extraction

Table 1 The limits and coding of process variables for Rutin extraction

Variable	Code	Variable level -1	0	+1	Step
Hyperici herba					
Solvent concentration, % vol.	x_1	10	30	50	20
Temperature, ^{0}C	x_2	30	50	70	20
Duration, min.	x_3	5	10	15	5
Hippophaes fructus					
Solvent concentration, % vol.	x_1	10	50	90	40
Temperature, ^{0}C	x_2	20	40	60	20
Duration, min.	x_3	5	10	15	5

Table 2 The experimental matrix

No. exp.	x_1	x_2	x_3
1.	-1	-1	-1
2.	1	-1	-1
3.	-1	1	-1
4.	1	1	-1
5.	-1	-1	1
6.	1	-1	1
7.	-1	1	1
8.	1	1	1
9.	-1	0	0
10.	0	0	0
11.	0	-1	0
12.	0	1	0
13.	0	0	-1
14.	0	0	1
15.	0	0	0
16.	0	0	0
17.	0	0	0

duration on Rutin extraction efficiency was established by statistical analyzis. In this purpose, a factorial experiment of second order has been used. Thus, the real values of the process variables were chosen arbitrarily, their limits and coding are given in *Table 1*.

In order to settle the correlation between the Rutin extraction degree and the above mentioned parameters the following model of polynomial equation type is proposed:

$$Y = b_0 + b_1 \cdot x_1 + b_2 \cdot x_2 + b_3 \cdot x_3 + \\ + b_{12} \cdot x_1 \cdot x_2 + b_{13} \cdot x_1 \cdot x_3 + b_{23} \cdot x_2 \cdot x_3 + \\ + b_{11} \cdot x_1^2 + b_{22} \cdot x_2^2 + b_{33} \cdot x_3^2 \quad (1)$$

where b_0,..., b_{33} are the regression coefficients.

The plan of the factorial experiment of second order is given in *Table 2*.

Because the highest Rutin extraction yield was obtained for 1-propanol, both the extraction from *Hyperici herba* and *Hippophaës fructus* were carried out using this solvent. By means of the obtained data, the

Table 3 The values of regression coefficients for Rutin extraction from *Hyperici herba*

Regression coefficient	Value
b_0	46.93
b_1	11.67
b_2	3.58
b_3	1.15
b_{12}	1.11
b_{13}	0.3875
b_{23}	0.0625
b_{11}	- 8.07
b_{22}	- 0.65
b_{33}	- 2.19

regression coefficients b_j were calculated using the following relations [9]:

$$b_0 = \overline{Y}, \quad b_j = \frac{\sum_{i=1}^{15} x_{ji} Y_i}{\sum_{i=1}^{15} x_{ji}^2}, \quad b_{jk} = \frac{\sum_{i=1}^{15} x_{ji} x_{ki} Y_i}{\sum_{i=1}^{15} x_{ji}^2 x_{ki}^2},$$
$$b_{jj} = \frac{\sum_{i=1}^{15} x'_{ji} Y_i}{\sum_{i=1}^{15} \left(x'_{ji}\right)^2}, \quad x'_{ji} = x_{ji}^2 - \frac{1}{15} \cdot \sum_{i=1}^{15} x_{ji}^2 \quad (2)$$

i = 1...15 number of experiments, j = 1...3 number of variables.

Initially the Rutin extraction from *Hyperici herba* was mathematically modelled. The obtained values of regression coefficients are listed in *Table 3*.

For checking the normal results obtained in the program center the Q test was used. Thus, the calculated Q value is [9]:

$$Q = \frac{|a_1 - a_2|}{A} = 0.67 \quad (3)$$

where:

a_1 - the uncertain value (47.1 %);
a_2 - the closest to the uncertain value (46.9 %);
A - the amplitude (difference between the most distant values, 0.3).

For a certain threshold of 0.05, Q = 0.77 was found in literature [9]. Since the calculated value of 0.66 is lower than the tabulated one, it could be concluded that the uncertain value of 47.1 % is also a normal value. Consequently, all of the three obtained values for Rutin extraction yields were taken in calculation.

Hence, the regression equation can be written as:

$$Y = 46.93 + 11.67 \cdot x_1 + 3.58 \cdot x_2 + \\ + 1.15 \cdot x_3 + 1.11 \cdot x_1 \cdot x_2 + 0.39 \cdot x_1 \cdot x_3 + \\ + 0.062 \cdot x_2 \cdot x_3 - 8.07 \cdot x_1^2 - 0.65 \cdot x_2^2 - 2.19 \cdot x_3^2 \quad (4)$$

The experimental and calculated values of the Rutin extraction yields from *Hyperici herba* were tabulated in *Table 4*.

Table 4 The experimental, Y_{exp}, and calculated values, Y_{calc}, for Rutin extraction yield from *Hyperici herba*

No. exp.	y_{exp}, %	y_{calc}, %
1.	21.6	21.2
2.	42.1	41.82
3.	26	25.6
4.	51	50.8
5.	22.9	22.9
6.	45	44.8
7.	27.6	28
8.	54.1	54
9.	23.6	27.2
10.	46.2	50.5
11.	42.8	42.7
12.	51.5	49.9
13.	43.3	43.6
14.	45.9	45.89
15.	46.9	
16.	47.1	46.93
17.	46.8	

The limits between which these values, calculated with the regression equation, oscillate around the experimental value are determined with the relation [9]:

$$Y_{calc_i} = Y_{\exp_i} \pm t \cdot S_{Yx}, \% \quad (5)$$

The standard deviation S_{Yx}^2 was calculated using the following relationship [9]:

$$S_{Yx}^2 = \frac{\sum_{i=1}^{8}\left(Y_{\exp_i} - Y_{calc_i}\right)^2}{n-(k+1)} = 0{,}202 \quad (6)$$

where n is the number of experiments and k the number of variables taken into account.

The t values are to be found in the tables for Student distribution [9], for a confidence threshold of 0.05 and 15 experiments, namely:

$$t = 2.131 \quad \text{and} \quad Y_{calc\,i} = Y_{\exp_i} \pm 0.95, \% \quad (7)$$

The individual influence of the factors under consideration is estimated by means of the value of the correlation coefficient, r_{Yx} [9]:

$$r_{Yx_i} = \frac{\sum_{i=1}^{8}\left[x_i \cdot \left(Y_i - \overline{Y}\right)\right]}{\sqrt{\sum_{i=1}^{8} x_i^2 \cdot \sum_{i=1}^{8}\left(Y_i - \overline{Y}\right)^2}} \quad (8)$$

which describes the nature of dependence between the process variables and the extraction yield. The determination coefficient, which represents the square of correlation coefficient, indicates the fraction of Rutin extraction yield that can be explained by variable x_i variation. In this case, the calculated values of determination coefficient are:

$$r_{Yx_1}^2 = 0.898 \qquad r_{Yx_2}^2 = 0.083 \qquad r_{Yx_3}^2 = 0.011$$

Table 5 The values of regression coefficients for Rutin extraction from *Hippophaes fructus*

Regression coefficient	Value
b_0	24.18
b_1	26.15
b_2	3.10
b_3	0.68
b_{12}	1.90
b_{13}	0.35
b_{23}	0.225
b_{11}	11.40
b_{22}	3.43
b_{33}	4.16

Table 6 The experimental, Y_{exp}, and calculated values, Y_{calc}, for Rutin extraction yield from *Hippophaës fructus*

No. exp.	y_{exp}, %	y_{calc}, %
1.	15.5	15.7
2.	63.3	63.5
3.	18.1	17.8
4.	72.8	73.0
5.	16.1	15.9
6.	64.6	64.8
7.	18.9	18.8
8.	75.7	75.6
9.	17.7	16.4
10.	71.4	68.7
11.	28.9	26.5
12.	33.9	32.7
13	32	30.7
14	33.2	33.02
15	24.3	
16	24.1	24.18
17	24.15	

These values suggest that the considered parameters influence the efficiency of Rutin extraction from *Hyperici herba* in a 99.2 % extent, the solvent concentration is the most important factor. The rest of 0.8 % can be attributed to the effect of other factors, namely: ratio between alcohol and plant quantity, mixing intensity.

The mathematical modeling of Rutin extraction process from *Hippophaës fructus* with 1-propanol was similarly established, using the previous statistical analyzis method. Therefore, the real values of the process variables and their limits and coding are given in *Table 1* and the experimental matrix in *Table 2*.

The regression coefficients b_j have been calculated using the relations (2), their values are given in *Table 5*.

For these experiments, Q = 0.77 was found, this value is inferior to those given in literature for a certain threshold of 0.05 (Q = 0.77) [9]. Consequently, it was confirmed the accuracy of the used experimental method.

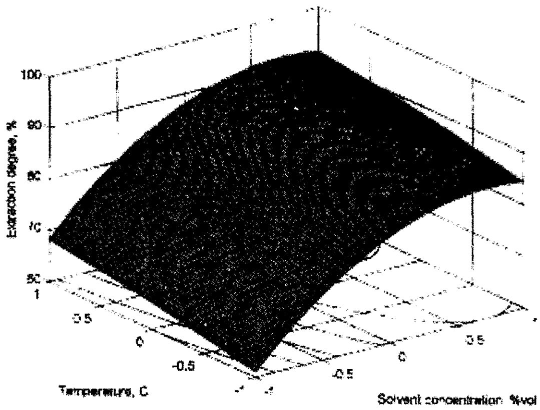

Fig.4 Surface given by *Eq.(4)* plotted for $x_3 = 0$

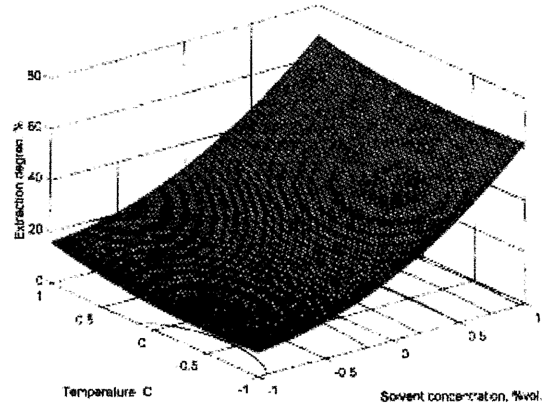

Fig.5 Surface given by *Eq.(9)* plotted for $x_3 = 0$

Thus, the correlation between Rutin extraction yield from *Hippophaës fructus* and the main parameters that influence the process (solvent concentration, temperature and extraction duration) can be written as follows:

$$\begin{aligned} Y = {} & 24.18 + 26.15 \cdot x_1 + 3.10 \cdot x_2 + \\ & + 0.68 \cdot x_3 + 1.90 \cdot x_1 \cdot x_2 + 0.35 \cdot x_1 \cdot x_3 + \\ & + 0.225 \cdot x_2 \cdot x_3 + 11.40 \cdot x_1^2 + 3.43 \cdot x_2^2 + 4.16 \cdot x_3^2 \end{aligned} \quad (9)$$

The experimental and calculated values for Rutin extraction yield from *Hippophaës fructus* were tabulated in *Table 6*.

The limits, between the calculated values oscillate around the experimental value, are:

$$Y_{calci} = Y_{\exp_i} \pm 0.593, \% \quad (10)$$

the standard deviation S_{Yx}^2 is 0.0775

The values of determination coefficient for the considered extraction system are:

$r_{Yx_1}^2 = 0.980$ $r_{Yx_2}^2 = 0.014$ $r_{Yx_3}^2 = 6 \cdot 10^{-4}$

In this case, the considered parameters influence the efficiency of Rutin extraction from *Hippophaës fructus* in a 99.46 % extent, the solvent concentration exhibiting a stronger influence compared with the extraction from *Hyperici herba*. The rest of 0.54 % could be attributed to the effect of the secondary factors earlier mentioned.

The graphical representation of the obtained regression *Eqs.(4)* and *(9)* are given in *Figs.4* and *5*, for a constant level of process duration (x_3).

Conclusions

The experimental results of the study on Rutin extraction from *Hyperici herba* and *Hippophaës fructus* indicated that the most efficient solvent is 1-propanol, the amount and type of the natural compounds from the two plants determining the concentration of used solvent.

By means of the statistical analyzis and using a factorial experiment of second order, the Rutin extraction with 1-propanol from the two plants was modeled. Thus, two mathematical correlations between the extraction degree and the main parameters influencing the Rutin extraction (solvent concentration, temperature, duration) have been established. For both cases, the considered variables control the extraction process in a 99.2 – 99.5 % extent the solvent concentration exhibiting the most important influence, especially for Rutin extraction from *Hippophaës fructus*.

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