RESEARCHES ON QUALITY OF NETTLE (*URTICA DIOICA*) SCREENING PROCESS IN ORDER TO OBTAIN BIOACTIVE EXTRACTS

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In the paper, there are presented both the results of experimental researches on sorting of four dimensional groups of nettle herb, harvested from spontaneous flora dried and chopped in order to obtain bioactive extracts and the evaluation of sifting process by assessing the screening degree through the 2nd degree polynomial functions. Determinations have been made in laboratory by means of a separation system of Rietch type and a classifier of cut plants with vibrating plane sieves. The following parameters of sieve were analyzed: oscillation frequency, flow rate and tilting angle. It was found that for obtaining nettle extracts rich in bioactive substances, the fragments size must be framed between 3.16 and 6.3 mm sorted on sieves with holes size over 3,16 mm or below 6,3 mm, at 45 Hz frequency, for a flow rate of 30 kg/h and a sieve angle of 13.33 degrees. Results are very important for medicinal plant processors in order to assure an appropriate processing of nettle herb and obtain high quality phyto-therapeutic products.

Keywords: bioactive extracts, herb, nettle, plane sieve, screening

1. Introduction

Medicinal plant varieties, due to the complexity of contained bioactive substances, may have multiple effects on human body. Knowing the bioactive substances specific to each plant is a main attribute in assessing the therapeutic value and method of extraction of these substances, so that the process could be as efficient as possible [1].

Quantity of plant bioactive substances depends on the ecological factors, species habitat, crop technology, biological value of a cultivar (population, species, variety, hybrid, etc.), plant vegetative organ (root, stem leaves, flowers, herb), that may be used as entire plant, chopped plant or powder, and also on processing methods [2].

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Urtica dioica (nettle) is one of the most popular and cosmopolitan plants (present in Europe, Africa, Asia, America), whose dietary and therapeutic benefits have been known since ancient times. The therapeutic activity of nettle extracts is related to the presence of phenolic compounds (mainly cafeoil-malic acid, chlorogenic acid, ferulic acid, rutin, isoquercitrin and astragalin) and to their antioxidant activity. In the paper [21] the results show that young nettle leaves have the highest content of polyphenols and ascorbic acid. In the paper [22] is shown that the leaves of *Urtica dioica* are richer in phenolic compounds, sterols and carotenoids.

Nettle contains proteic substances with a large quantity of amino acids, carbohydrates, amines, sterols, ketones, volatile oil, fat substances, sitosterols, acetic acid, formic acid, pantothenic acid, vitamins (A, B2, C, K), clorophyl, salts of Ca, Mg, Fe, Si, P and others. In nettle hairs there is an irritating substance made of formic acid, one enzyme and one toxalbumin, which harms the skin.[10].

In the paper [11] one new benzene derivative along with known constituents b-amyrin, b-sitosterol, stigmasterol and oleanolic acid were isolated from *Urtica dioica*. Utility of nettle (*Urtica dioica*) in layer diets as a natural yellow colorant for egg yolk is shown in the paper [12]. Another study proves nettle fibres have very interesting tensile properties and could also be suitable as reinforcing components in composite materials [13].

The use of nettle in the cosmetic sector is well known, several commercial products being currently present in the market, e.g. soaps, shampoo and lotions to clean skin [14].

The technological processing of medicinal plants for obtaining vegetal extracts comprises the technological operations of chopping the plants at a pre-established size, separating the cut plants in dimensional fractions by the refusal method according to sieve holes and extraction of bioactive substance in different solvents, during different periods of time suitable to fraction dimensions, solvent-vegetal product ratio, etc.[3].

Conditioning of medicinal plants is made of several operations able to ensure an added value to the end product. Therefore, the vegetal matter has to comply with criteria related to allowable maximum impurities and foreign bodies content, humidity, according to Pharmacopoeia [6,17].

Harvested raw vegetal matter may be used both fresh and dried. When it is processed in fresh state, the conditioning operations should be performed as soon as possible for avoiding fermentation and biological degradation of vegetal matter.

Screening is the operation of separation in several fractions [3,4] according to certain criteria such as size, weight, colour, components of a mixture of vegetal products (grains, fruits, herbs, roots, etc.), in order to obtain uniform fractions [5].

Paper [7] presents experimental researches on technological processing of medicinal and aromatic plants for obtaining essence teas, juices, teas, drawing up the following conclusions:

- *machine for cutting plants*: optimum cutting length of 4 to 12 mm;
- *tilted belt conveyor*: optimum speed setting of 0.05□ to 0.57 m.s^{-1};
- *classifier of chopped plants*: optimum tilting angle of sieves at 13°;
- extraction level between 15 and 24% using the *percolator* [7].

In paper [8], the dimensional characteristics of shepherd thyme, extraction of active substances from four fractions, results of sorting process, are presented. It was recommended to screen the shepherd thyme fragments on sieves with holes diameters of 5 mm, 3.15 mm, and 2.15 mm. It has been noticed that the maximum of active substances (17.67 %) was extracted in ethanol from the group whose average size was less than 2 mm [8].

Paper [9] presents experimental researches of lavender herb related to screening of dried chopped plant fragments into 4 sorts. After analyzing the data, it was found that: sort I contains only inflorescences and is mainly designed to extract essential oils; sort II contains inflorescences and stems and may be used for teas when packed in paper bags; sorts III and IV contain only stems and are considered as waste.

On one hand, this paper aims to present the nettle dimensional characteristics and three different-solvent extraction of bioactive substances coming from four dimensional fractions (sorts) obtained, and on the other hand, the experimental researches made on a dimensional classifier with flat vibrating sieves designed to identify the optimum work necessary to obtain the desired sort with a maximum content of bioactive substances.

2. Materials and methods

Nettle plants *(Urtica dioica)* used in experiments were identified and harvested from spontaneous flora according to morphological and biological characteristics [15,16] of the species. Herb was naturally dried in the shadow up to storing humidity (13 %, at most), cleaned of foreign bodies (inorganic materials or other plants, damaged parts) according to provisions from Pharmacopoeia [6,17], then it was chopped in bulk using a medicinal plants chopper of TIMATIC type, equipped with guillotine cutting system by a to-and-fro movement of the mobile knife set at 6 mm size.

In order to determin the dimensional characteristics of chopped vegetal matter, five samples for each plant have been analyzed. For each sample, 120g of vegetal matter was weighed, then was sieved with a Rietsch AS 200 type classifier, at 50 mm amplitude for 5 minutes. The quantity of vegetal matter on each sieve is formed of the totality of fragments with smaller dimensions than the size of holes of the sieve above the considered one, and with bigger dimensions than the holes of considered sieve.

After weighing the vegetal matter on each sieve, the average of five samples has been made and size groups have been established. Further, 20 g of vegetal matter from each sieved fraction were weighed. In each sample were added one after another, 250 ml ethanol of 96%, respectively water and ether for extraction of bioactive substances. Samples were manually agitated for 10 minutes up to their homogenization and to the moment when the vegetal product is imbibed in solvent, then, they were put for 120 minutes on water bath at 50^0C and 100 rpm bottle's rotation speed, in order to perform a continuous agitation and achieve rapid maceration. The obtained macerates were filtered with filter paper and the vegetal matter was dried in the oven for 3 hours at 105^0C.

Then, the vegetal extracts were concentrated for all the appropriate groups, samples were weighed and the quantity of bioactive substances extracted from each fraction was determined, identifying the fraction with maximum yield.

Following the dimensional analysis of vegetal fragments [19] and based on distribution of dimensional sorts (figure 1), the fraction containing maximum of bioactive substances extracted was established and sieves holes size was chosen (8.00 mm, 6.15 mm and 3.15 mm) [18]. Experimental researches were performed using the dimensional sorter of cut plants (figure 2), fed by means of a tilted belt conveyor, that discharges the vegetal matter in classifier funnel centre and from here to its upper sieve, where the proper screening is made by refusal method.

Fig. 1. Distribution of size groups of nettle

Working parameters chosen for assessing the screening degree of equipment used during tests were: sieve tilting angle ($\alpha = 12.08^0$; 13.33^0; 14.07^0), oscillation frequency adjusted by the frequency converter (f =50 Hz; 47.5 Hz; 45 Hz) and feed rate (30 kg/h; 45 kg/h; 60 kg/h).


Fig. 2 – Sorter of cut plants

The mass M of the sorter flow (Q_{alim}) was uniformly distributed on the conveyor surface and was adjusted according to relation 1, knowing that the belt has a length L of 6 meters and conveyor has a speed v of 0.04 m/s (Table 1).

$$Q_{a\lim} = \frac{M}{L} \cdot v \text{, [kg/s]} \tag{1}$$

Table 1

Adjustment of sorter flow

Material mass, M [kg]	Belt length, L [m]	Belt speed, v [m/s]	Flow rate set, Q_{alim} [kg/s]
1.25	6	0.04	0.0083
1.875			0.0125
2.5			0.0166

An experimental determination was performed as it follows:
- one box for collecting the vegetal fragments was mounted at each of the four exhaust openings of sieves;
- a mixture of chopped vegetal fragments was fed on conveyor's belt;
- first, the sorter was put into operation, then the conveyor belt and sieves were supplied with vegetal matter, letting it to go along the sieves length up to the discharge areas; then, the material flew uniformly through the four exhausting openings;
- chronometer was put into operation and covers of vegetal fragments collecting boxes were drawn;
- chronometer was stopped after 30 seconds and the vegetal matter suitable to a certain variety, from each box was weighed.

The sieve supplying rate (Q_{exp}) were determined by relation 2:

$$Q_{\exp} = \frac{M_t}{t} \text{, [kg/s]} \tag{2}$$

where: M_t is total mass of material sorted by sieves;
t is during an experiment.

Interpretation of outcome was made for the desired Sort II, by representing functions with multiple variables, situation when the dependent variable (percentage of Sort II) depends, at the same time, on several independent variables, x_1 respectively x_2, replaced one after another, so that: x_1= f, x_2 = α; then, x_1= Q_{exp}, x_2 = α, then, x_1= Q_{exp}, x_2 = f, resulting in one polynomial function of second degree with two variables, having the general form shown in relation 3 [20]:

$$f_{(x_1,x_2)} = a_0 + a_1 \cdot x_1 + a_2 \cdot x_2 + a_3 \cdot x_1^2 + a_4 \cdot x_1 \cdot x_2 + a_5 \cdot x_2^2 \tag{3}$$

In order to process the experimental data, an experimental fragmented testing program was chosen, for 15 tests out of the total of 27 experiments, within Mathcad program, given by values of independent and dependent variables for each experiment**.**

3. Results and discussion

Mass and percentage values obtained after sieving are shown in table 2.

Table 2

Nettle fragments separated in size fractions

Sample No.	Limits of nettle dimensional fractions [mm]							
	< 3.15		*3.16-6.3*		*6.31-8.00*		*8.01-10.0*	
	[g]	[%]	[g]	[%]	[g]	[%]	[g]	[%]
1	13.09	10.91	75.15	62.63	20.23	16.86	11.7	9.75
2	13.28	11.07	75.34	62.78	19.86	16.55	11.33	9.44
3	13.48	11.23	74.58	62.15	20.42	17.02	11.14	9.28
4	13.67	11.39	74.77	62.31	20.6	17.17	10.96	9.13
5	13.86	11.55	74.96	62.47	20.05	16.71	11.52	9.6
Samples average	13.48	11.23	74.96	62.47	20.23	16.86	11.33	9.44

Results obtained following the fragments dimensional analysis were ploted in figure 3 by representing the variation curves of screening, impurities removing and vegetal matter distribution as percentages according to sieve mesh size.

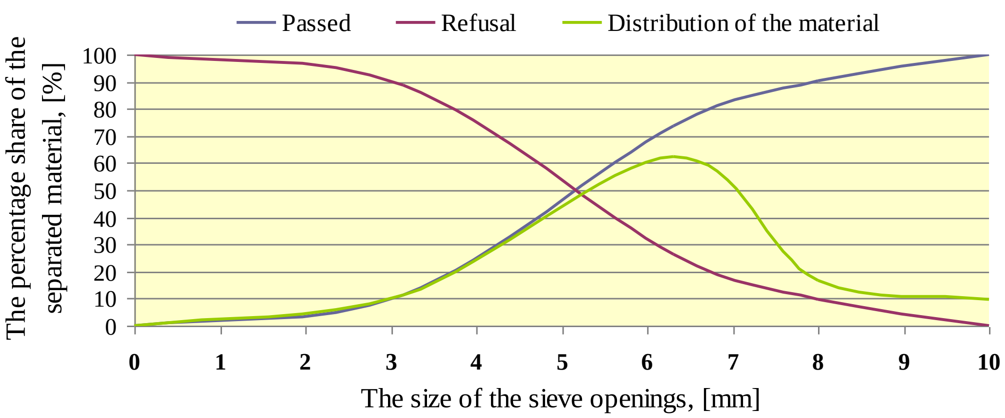


Fig. 3 – Curves of variation of percentages of screening and vegetal matter distribution as percentages according to sieve mesh size

These curves establish in percentage the distribution of vegetal matter separated from the first till the last sieve of vibrating classifier and can be used for choosing the suitable sieves allowing to separate the fragments appropriate to vegetal matter desired, for obtaining high quality extracts and determining the flow rates of screening product and impurities removal.

Percentage of substances extracted in three solvents was calculated for each fraction and is shown in figure 4.

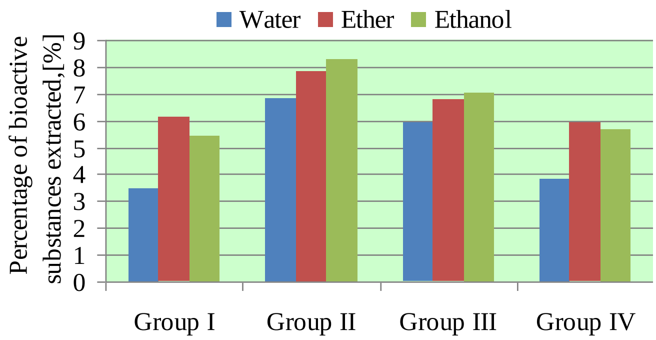


Fig. 4 – Percentage of substances extracted in three solvents for four *nettle* size groups

Values of experimental data characterizing the sorting process by sorter on 4 size groups, are presented in table 3.

Table 3

Experimental results regarding the screening of nettle fragments

Flow rate adjusted Q_{alim} [kg/s]	Frequency, f [Hz]	Sieve angle, α [^{0}]	Sorting time, t [s]	Mass Group 1 [kg]	Mass Group 2 [kg]	Mass Group 3 [kg]	Mass Group 4 [kg]	Total mass [kg]	Experimental flow, Q_{exp} [kg/s]
0.0166	50	12.08	30	0.055	0.275	0.094	0.074	0.498	0.0166
		13.33	30	0.068	0.247	0.094	0.065	0.475	0.0158
		14.70	30	0.046	0.274	0.072	0.068	0.459	0.0153
	47.5	12.08	30	0.073	0.280	0.061	0.049	0.463	0.0154
		13.33	30	0.067	0.273	0.089	0.057	0.486	0.0162
		14.70	30	0.051	0.293	0.067	0.087	0.498	0.0166
	45	12.08	30	0.078	0.253	0.049	0.037	0.418	0.0139
		13.33	30	0.062	0.211	0.077	0.041	0.391	0.0130
		14.70	30	0.047	0.254	0.046	0.051	0.398	0.0133
0.0125	50	12.08	30	0.037	0.220	0.071	0.021	0.350	0.0117
		13.33	30	0.047	0.182	0.066	0.052	0.348	0.0116
		14.70	30	0.044	0.224	0.076	0.031	0.375	0.0125
	47.5	12.08	30	0.055	0.222	0.049	0.049	0.374	0.0125
		13.33	30	0.057	0.172	0.045	0.037	0.311	0.0104
		14.70	30	0.039	0.206	0.060	0.039	0.345	0.0115
	45	12.08	30	0.054	0.195	0.037	0.035	0.322	0.0107
		13.33	30	0.058	0.195	0.048	0.035	0.336	0.0112
		14.70	30	0.044	0.195	0.039	0.021	0.300	0.0100
0.0083	50	12.08	30	0.038	0.149	0.050	0.011	0.249	0.0083
		13.33	30	0.043	0.144	0.044	0.019	0.250	0.0083
		14.70	30	0.035	0.149	0.044	0.020	0.249	0.0083
	47.5	12.08	30	0.047	0.158	0.032	0.004	0.241	0.0080
		13.33	30	0.040	0.131	0.035	0.022	0.228	0.0076
		14.70	30	0.046	0.149	0.035	0.013	0.243	0.0081
	45	12.08	30	0.046	0.140	0.029	0.012	0.227	0.0076
		13.33	30	0.048	0.117	0.023	0.017	0.205	0.0068
		14.70	30	0.039	0.120	0.023	0.007	0.190	0.0063

The significance and and sizes of mass groups from table 1 are the following:

- mass of group 1 represents the screening of the lower sieve of the sorter with sizes framed between 0.1 and 3.15 mm;
- mass of group 2 is made of sieved matter from middle sieve and refuse matter from lower sieve and comprises vegetal fragments framed between 3.16 and 6.15 mm;
- mass of group 3 is made of big sieve screened product and middle sieve refuse product, with vegetal fragments framed between 6.16 and 8.00 mm;
- mass of group 4 represents the big sieve refuse product with size of vegetal fragments bigger than 8.00 mm.

Sorting level of vegetal fragments (S_f) is expressed in percentages (%) and is defined as the ratio between the quantity of fragments from each collecting box (group I, group II, group III, group IV) and quantity of fragments from the four collecting boxes.

Using the experimental data coming from the *Nettle* fragments sorting, for a constant value of group II, the screening degree (Sf) variation was calculated in percentages.

Sorting degree according to oscillation frequency (x1=f) and sieve slope angle (x2=α), was represented in figure 5. Values of experimental data were correlated with polynomial function resulting function coefficients shown in relation 4 and a correlation coefficient of **R=0.924.**

$$f_{(f,\alpha)} = 560{,}435 - 0{,}075 \cdot f - 67{,}934 \cdot \alpha + 2\times10^{-5} \cdot f^2 - 2{,}557\times10^{-4} \cdot f \cdot \alpha + 2{,}573 \cdot \alpha^2 \quad (4)$$

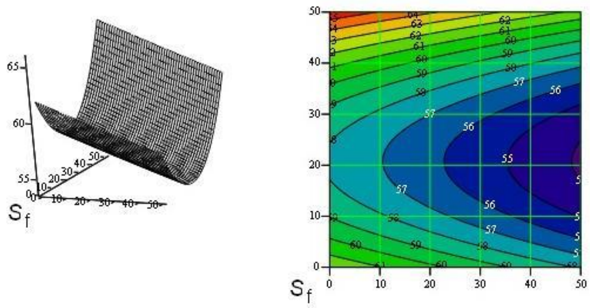


Fig. 5 – Variation of sorting degree (S_f) depending to oscillation frequency and sieve slope angle for group II of *Nettle*

Sorting degree according to experimental flow rate ($x_1=Q_{exp}$) and sieve slope angle ($x_2=\alpha$), was represented in figure 6. Values of experimental data were correlated with polynomial function resulting function coefficients shown in relation 5 and a correlation coefficient of **R=0.820.**

$$f_{(Q_{exp},\alpha)} = 541{,}482 - 2{,}769 \cdot Q_{exp} - 70{,}612 \cdot \alpha + 0{,}018 \cdot Q_{exp}^{\ 2} + 0{,}156 \cdot Q_{exp} \cdot \alpha + 2{,}594 \cdot \alpha^2 \quad (5)$$

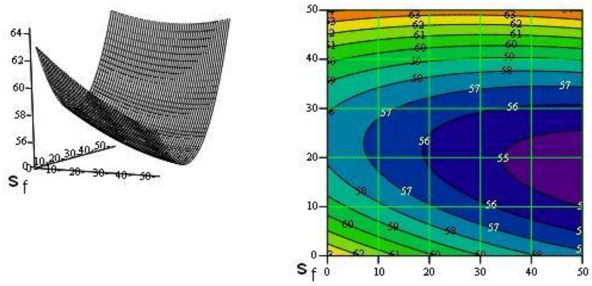


Fig. 6 – Variation of sorting degree (S_f) depending to experimental flow rate and sieve slope angle for group II of *Nettle*

Sorting degree according to experimental flow rate (x_1=Q_{exp}) and oscillation frequency (x_2=f) was represented in figure 7. Values of experimental data were correlated with polynomial function resulting function coefficients shown in relation 6 and a correlation coefficient of **R=0.683.**

$$f_{(Q_{exp},f)} = 661{,}295 + 2{,}988 \cdot Q_{exp} - 1{,}266 \cdot f + 0{,}083 \cdot Q_{exp}^{\ 2} - 5{,}34 \times 10^{-3} \cdot Q_{exp} \cdot f + 6{,}772 \cdot 10^{-4} \cdot f^{2} \quad (6)$$

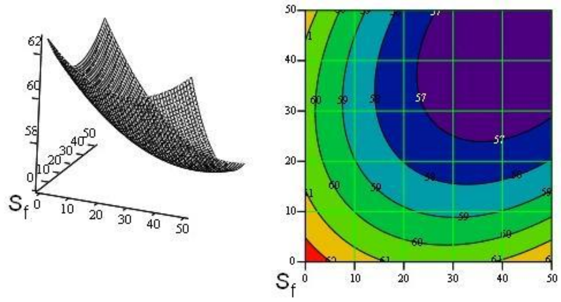


Fig. 7 – Variation of sorting degree (S_f) depending to experimental flow rate and oscillation frequency for group II of *Nettle*

In figure 8 are comparatively represented the experimental values and sorting degree (S_f) values calculated, for each experiment depending on two parameters.

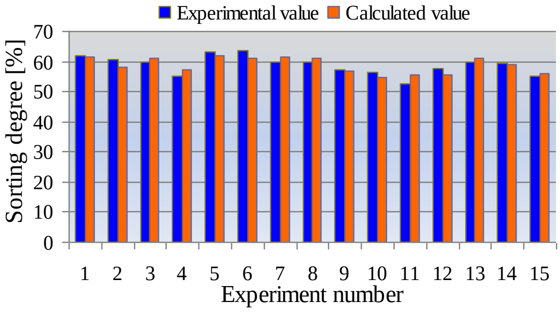
For nettle screening degree of group II depending on oscillation frequency and sieve tilting angle (fig.8 a), it has been found that differences between the experimental values and the calculated ones are very little (0.02 %) for average tilting angle of sieve, α =13.33^0 and frequency of 45 Hz and differences are big (5.04%) for small angle (12.08^0) and average frequency 47.5 Hz.

Differences between the experimental values and the calculated ones, related to experimental flow rate and sieve tilting angle, (fig. 8 b), were:

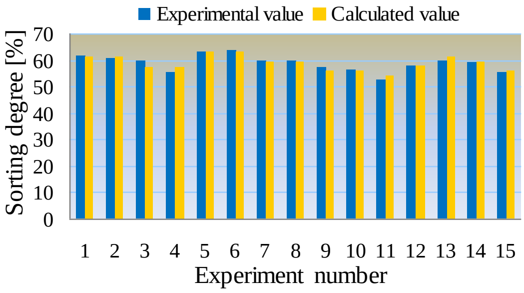
- non important for small flow rate (0.0083 kg/s) and small tilting angle (12.08^0) or average angle (13.33^0) of the sieve;

- important, difference of 6,16 %, for sieve average flow rate (0.0125 kg/s) and sieve average angle (13.33^0).

The screening degree in comparison with experimental flow rate (fig.8 c) has indicated non important differences for the small flow rate (0.0083 kg/s) and small frequency 45 Hz.



a)



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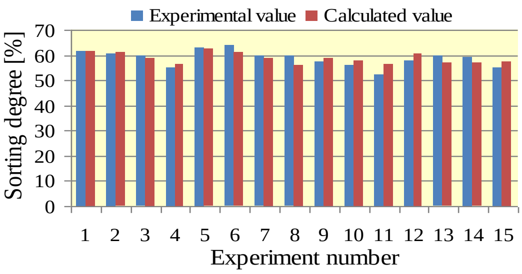

c)

Fig. 8 Content in percentages of group II of *Nettle*, for experimental and theoretical values depending on a) oscillation frequency and sieve slope angle; b) experimental flow rate and sieve slope angle; c) experimental flow rate and oscillation frequency

4. Conclusions

Results of experimental researches obtained with plant classifier equipped with flat vibrating sieves have eventually enabled to find data and values by means of which the quality of sorting process could be studied.

Following the variation curves analysis of percentages related to sifting, refusal and vegetal matter distribution on sieves according to sieve holes size, certain recommendations could be made for medicinal plant fragments screening. It has found that nettle can be appropriately separated on sieves with holes diameters between 5 and 7 mm.

For group II with fragments size between 3.16 and 6.3 mm, the biggest quantity of bioactive substances has been extracted, namely, 8.31 % in ethanol. The quantity of bioactive compounds for this sort was made of p-coumaric acid, at a concentration of 73.27 %.

After analyzing the obtained experimental values and the values calculated as a second degree polynomial function with two variables, in order to obtain an optimum efficiency of dimensional classifier for nettle group II, the following aspects were noticed:

- depending on the oscillation frequency (f) and sieve tilting angle (α), the optimum is obtained for a tilting angle of 13.33^0 and a frequency of 45 Hz;
- depending on experimental flow rate (Q_{exp}) and sieve tilting angle (α) the optimum is a small flow rate of 0.0083 kg/s and a tilting angle of 12.08^0 or 13.33^0;
- depending on experimental flow rate (Q_{exp}) and frequency (f) the optimum efficiency is obtained at a small flow rate of 0.0083 kg/s and a frequency of 45 Hz;

In order to obtain the nettle group II, the following optimum parameters have been identified: sieve tilting angle of 12.08^0, frequency of 45 Hz and experimental flow rate of 0.0083 kg/s.

Taking into account that *nettle* bioactive substances are comprised in *nettle* herb (stalk with leaves and flowers), in order to evaluate the sorting and product quality resulted after the extraction, it was aimed to perform a comprehensive screening for the desired sort (group II) and obtain a big quantity of bioactive compounds.

In the context of current concern and increased interest in Romania for natural remedies based on plants, dimensional fraction screening of medicinal plants represents an important premise for obtaining high quality phyto-therapeutic products.

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