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# COMPARISON, ARTIFICIAL NEURAL NETWORK MODELING AND GENETIC ALGORITHM OPTIMIZATION OF THE RESINOID AND POTASSIUM YIELDS FROM WHITE LADY'S BEDSTRAW (*Galium mollugo* L.) BY CONVENTIONAL, REFLUX AND ULTRASOUND-ASSISTED AQUEOUS--ETHANOLIC EXTRACTION

*In this work, the yields of resinoid and potassium were obtained from aerial parts of white lady's bedstraw (Galium mollugo L.) by maceration, reflux extraction and ultrasound-assisted extraction using aqueous ethanol solutions as solvents. The main goal was to define the influence of the extraction technique and the ethanol concentration on the resinoid and potassium yields. The resinoid and potassium yields were determined by the solvent evaporation from the liquid extracts to constant weight and the AAS emission method, respectively. The dependence of resinoid and potassium yields on the ethanol concentration was described by linear and quadratic polynomial models, respectively. The best potassium extraction selectivity of 0.077 g K/g of dry extract was achieved by maceration at the ethanol concentrations of 10 g/100 g. The artificial neural network (ANN) was successfully applied to estimate the resinoid and potassium yields based on the ethanol concentration in the extracting solvent and the time duration for all three extraction techniques employed. The response surface methodology was also used to present the dependence of ANN results on the operating factors. The extraction process was optimized using the ANN model coupled with genetic algorithm. The maximum predicted resinoid and potassium yields of 30.4 and 1.67 g/100 g of dry plant were obtained by the ultrasonic extraction (80 min) using the 10 g/100 g aqueous ethanol solution.*

*Keywords: artificial neural networks; genetic algorithm; Galium mollugo; lady's bedstraw; maceration; reflux extraction; ultrasound-assisted extraction.*

Plants, their extracts or pure components isolated from them are used in various industries such as pharmaceutical, cosmetic, food, etc. The use of plants and their extracts in the form of para-pharmaceuticals and dietary products is growing every year. The plant extracts are used in treatment of specific diseases, but they can also be used preventively to preserve normal function of the organism [1].

Various organic molecules with pharmacological activities have been isolated from the plants, such as: alkaloids, glycosides, terpenoids, phenolic compounds (phenolic acids, flavonoids, catechins, tannins) and essential oils (mixtures of volatile compounds from the class of mono- and sesquiterpenes) [1]. The plant materials are a source of not only organic compounds, but also mineral matters. It is known that Mg, Mn and Zn minerals are found in cinnamon, parsley and black tea [2], while Ca, Mg

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and K minerals are observed in lemon [3] and lady's bedstraw [4]. Although minerals could be used for the prevention of different disorders caused by their lack in the human organism such as hypokalemia caused by the lack of potassium [5], it is hard to explain the insufficient knowledge on their contents in plant extracts. Although potassium ions do not participate in the chemical structure formation, they have more important roles such as the electric neutralization of anion groups, the control of membrane polarization and the osmosis regulation [6,7], the maintenance of the osmotic pressure of the cell contents against the cell wall (turgor) [7] and the activation of many enzymes of the photosynthesis process [8]. Although plant materials can be directly used as a source of minerals for the human organism, the use of extracts with their augmented contents can be more effective in many cases, compared to the use of the origin plant material.

Comprehensive studies of the methods for recovering extractive substances and individual bioactive components from a great number of various plants are in progress. The attention of investigators has been focused on the yield, chemical composition and biological activity of the extracts and the process kinetics, which have a vital influence on the decision for industrial application of an extraction process. Ultrasound-assisted extraction, representing a maceration process performed in the presence of ultrasound, is more and more used to obtain plant extracts [9]. The effect of ultrasound on the extraction of bioactive substances from plant materials is attributed to the cavitation phenomena inducing the destruction of cell walls, hydration of the plant material, reduction of plant particle size and the acceleration of mass transfer of extractive substances [10,11]. A positive effect of ultrasound has been established in extraction of pharmacologically active substances from many plants [4,12-14].

In the last decade, there has been a progressive growth of using various mathematical methods for describing extraction processes. The suggested mathematical models mostly relate to the description of the kinetics of maceration and ultrasound-assisted extraction of extractive substances from plants [4,9,15]. More recently, the optimization of ultrasound-assisted extraction has been increasingly optimized by the response surface methodology (RSM) [16,17] and the artificial neural network (ANN) method [18,19]. ANNs enable modeling by using a computer that learns from examples, through iterations, without the need of previous knowledge of the relationships among the process parameters. The increased importance of ANNs arises from their possibility to parallel process of data despite their components are independent of each other. This has been used as an advantage for non-linear (non-parameter) modeling and optimizing separation processes in various fields of chemical engineering [20-23]. The limitation of ANNs in process optimization can be overcome by their combination with genetic algorithms (GAs). GA has been proved to be an ideal technique to solve diverse optimization problems in biochemical engineering [18,24,25].

The plants from the genus Galium are well-used in traditional medicine as diuretics, choleretics, against diarrhea [26], sedatives or spasmolytics, as well as for the treatment of skin and rheumatic diseases [4]. While yellow lady's bedstraw (Galium verum) has been extensively studied chemically [26-30], there are a few data on the chemical composition of white lady's bedstraw (*Galium mollugo*) [4,31]. Various techniques, including conventional (maceration), reflux and ultrasound-assisted extraction, have already been investigated for recovering extractive substances from the *Galium* species. Maceration is the main extraction technique applied in one to four days at different temperatures (from the room to boiling temperature) and plant material-to-solvent ratios (from 1:1 to 1:20 g/mL), while a relatively new ultrasound-assisted extraction has been used in a few experiments for obtaining extracts from *G. mollugo* aerial parts [4]. A literature survey on the extraction of extractive substances from *Galium* species is given in Table 1. Of these species, the extraction and extractive substances of *G. verum* have been the most frequently studied. So far, methanol, pure or as aqueous solutions, have been mostly used to isolate the extractive substances from *Galium* species [29,32-35], although some other solvents such as an aqueous solution of ethanol [36,37], hexane, clorophorm [32], dichloromethane and acetone [37] have been also employed. The yield of extractive substances depends on the extraction technique, the type of plant material, the type of solvent, the plant material-to-solvent ratio, the operating temperature and the time duration of the extraction.

Although potassium belongs to pharmacologically active substances [1], there have been only a few studies on its content in lady's bedstraw, which is an herbaceous perennial plant with yellow or white flowers. Yellow lady's bedstraw (*G. verum*) is proven to contain essential oils [28], phenol [29], flavonoids [29,30] and various types of anthraquinone [27,37], and it has antioxidant properties [29]. The content of flavonoids in white lady's bedstraw is three times less than that of yellow lady's bedstraw [30]. White lady's

*Table 1. A literature survey on the yield of dry extract from Galium species*

| Plant material | Origin of plant material | Extraction technique | Solvent | Plant material to solvent ratio g/mL | Tempe-rature °C | Duration time, h | Dry extract yield g/100 g dry plant | Ref. |
|---|---|---|---|---|---|---|---|---|
| *G. mollugo* | Serbia | Maceration | Ethanol | 1:20 | 25 | 24 | 23.06-10,35 | This |
| Aerial parts | (Mt. Vlasina) | Reflux extraction | (10-90 %) | | B.p. | 4 | 26.86-10.05 | work |
| Blooming | | Ultrasound-assisted extraction | | | 25-40 | 1.3 | 27.78-10.96 | |
| *G. mollugo* | Serbia | Maceration | Ethanol | 1:20 | 25 | 24 | 16.80 | [4] |
| Aerial parts | (Mt. Vlasina) | Reflux extraction | (50 vol %) | | B.p. | | 17.60 | |
| Blooming | | Ultrasound-assisted extraction | | | 25-40 | | 17.05 | |
| *G. verum* | Serbia | Maceration | Methanol (80%) | 1:1 | Room | 24 | 5.69 | [29] |
| Aerial parts | (Veternik) | | | | | 48 | 7.18 | |
| Blooming | Serbia | | | | | 24 | 5.67 | |
| | (Mt. Zlatar) | | | | | 48 | 7.21 | |
| *G. verum* | Iran | Soxhlet extraction | Methanol | na | B.p. | 96 | 9.17 | [26] |
| Aerial parts | (Ardabil) | | | | | | | |
| July 2009 | | | | | | | | |
| *G. mexicanum* | Mexico | Sequential maceration | Hexane | na | Room | 48 | 0.50 | [32] |
| Aerial parts | (San Jose del Pacifico) | | Cloroform | | | | 0.47 | |
| | | | Methanol | | | | 15.62 | |
| *G. rivale* | Bulgaria | Maceration | Methanol (twice) | na | Room | na | 7.03 | [33] |
| Aerial parts | (Struma valley) | | | | | | 10.00 | |
| July 1992 | Bulgaria | | | | | | | |
| August 1994 | (Mt. Slavyanka) | | | | | | | |
| *G. tortumense* | Turkey | Maceration | Methanol (three times) | 1:4 | 40 | na | 19.78 | [33] |
| Aerial parts | (Erzurum) | | | | | | | |
| July 2002. | | | | | | | | |
| *G. verum* | Turkey | Maceration | Methanol | 1:3 | 40 | na | 19.60 | [35] |
| Aerial parts | (Altınyayla) | | | | | | | |
| June 2001. | | | | | | | | |
| *G. aparine* | Turkey | Percolation | Ethanol | 1:15 | Room | na | 2.32 | [36] |
| Aerial parts | (AnkaraÇankırı) | (several times) | (80 %) | | | | | |
| June 1997 | | | | | | | | |
| *G. schmidii* | Italy (Sardinia) | Maceration | Dichloromethane | na | Room | 72 | 1.38 | [37] |
| Aerial parts | | | Acetone | | | | 0.66 | |
| May 2004 | | | Ethanol | | | | 0.52 | |
| *G. glaucophyllum* | | | Dichloromethane | | | | 1.36 | |
| Aerial parts | | | Acetone | | | | 0.44 | |
| May 2004 | | | Ethanol | | | | 0.60 | |

bedstraw contains some minerals, too. Recently, it has been reported that the content of potassium in the aerial parts of white lady's bedstraw is 1.734 g/100 g [4], and approximately the same extraction degrees (about 80%) were achieved by conventional, reflux and ultrasound-assisted extraction using an aqueous solution of ethanol (50 vol.%).

In this work, the yields of resinoid (total ethanol extract) and minerals (potassium) in ethanol extracts of white lady's bedstraw (*G. mollugo* L.), obtained by conventional, reflux and ultrasound-assisted extraction, were studied. As extracting solvent, the aqueous ethanol solutions of different concentration (from 10 to 90 g/100 g) were employed. The scopes of this work were to establish the effect of extraction techniques and conditions on the yield of resinoid and potassium, to define a mathematical model describing the relationships of the resinoid and potassium yields on the ethanol concentration in the aqueous solution, to predict the resinoid and potassium yields based on the ethanol concentration in the extracting solvent and the extraction time for all three extraction techniques

using an ANN. Also, the ANN was combined with a GA to optimize the ethanol concentration and the extraction time. The content of other minerals, except potassium, were found to be below of the detection limits.

## EXPERIMENTAL

### Plant raw material

The aerial parts of white lady's bedstraw (*G. mollugo* L.) were collected at the foot of the mountain Kruševica, south Serbia. The plant material was dried in a shadow at ambient temperature and ground by a mill (Alpina) immediately before the extraction. The average particle diameter of 0.75 mm was determined by a sieve analysis of the ground plant material. Ethanol (96 vol.%) was from Zorka Pharma (Šabac, Serbia).

### Plant material extraction

The extraction of extractive substances from the plant material was carried out using aqueous ethanol solutions of the following ethanol concentrations: 10, 20, 30, 40, 50, 60, 80 and 90 g/100 g, in the suspension, at the plant material-solvent ratio of 1:20 g/mL. Three extraction techniques were used: maceration, reflux extraction and ultrasound-assisted extraction.

Ground plant material (5 g) was put in Erlenmeyer flasks, and aqueous ethanol solutions (100 mL) were poured. The plant material was macerated for 24 h at a constant temperature (25 °C). For the reflux extraction, each Erlenmeyer flask was equipped with a reflux condenser, and the extraction was carried out by the procedure for the maceration, providing that it was performed at the solvent boiling temperature (between 80 and 90 °C) for 240 min. For the ultrasound-assisted extraction, the Erlenmeyer flasks containing the plant material and the solvent were placed in an ultrasonic bath (EI, Niš, Serbia, model USK6, power 120 W, frequency 40 kHz) for 80 min; it was observed that the operating temperature was increased from 25 to 40 °C from the beginning to the end of the extraction process. Each experiment was repeated twice. By a preliminary study on the kinetics of the extraction, it had been established that the maximum yields of resinoid and potassium were obtained in 24 h, 240 min and 80 min by employing the maceration, reflux extraction and ultrasound-assisted extraction.

### Resinoid yield

After the completion of an extraction batch, the liquid extract was separated from the plant residue by vacuum filtration. The solvent was evaporated under vacuum until a half-solid residue was obtained, which was then dried at 60 °C to constant weight. The dry residue represents the total ethanol extract known as resinoid.

### Potassium yield

The potassium content in the resinoid was determined by the AAS emission method (Thermo Electron Corporation, Series S14 AA).

### Mathematical models

The mathematical models connecting the resinoid and potassium yields with the concentration of ethanol solution were obtained using the least square method by help of a computer program (Origin Pro 8, demo version). The RSM method was applied by using Matlab 7.8 (demo version). The determination coefficient ($R$) and the root-mean-square error ($RMSE$) of the function were used for evaluating the statistical significance of the models obtained.

### ANN Method

The experimental data on either the resinoid or potassium yield obtained by three extraction techniques using the ethanol solution of different concentrations were used for training the ANN. For each technique employed, the extraction time was fixed and corresponded to the time needed to reach a maximum extraction yield. The ANNs used were composed of three layers: input, hidden, and output (Figure 1). The ethanol concentration of the extracting solvent (10-90 g/100g) and the extraction time (1440, 240 and 80 min) were used as the input variables, so the input layer consisted of two neurons. The output layer consisted of a single neuron because the output value was either the resinoid or potassium yields obtained by all three extraction techniques (44 experimental data). Neurons in the input layer receive information on the ethanol concentration and the extraction time, while the output layer generates the output value of resinoid or potassium yield. The number of hidden neurons was determined by a heuristic procedure consisted of testing a different number of neurons until the RMSE of the output data was minimized. For network training, the back-propagation algorithm was used for the supervised learning. The ANN was simulated by the Neural Network Toolbox™ Software realized in the Matlab program package. The main characteristics of the ANN model are presented in Table 2.

### Genetic algorithm

The input space of the developed ANN model was optimized to search for the optimal operating

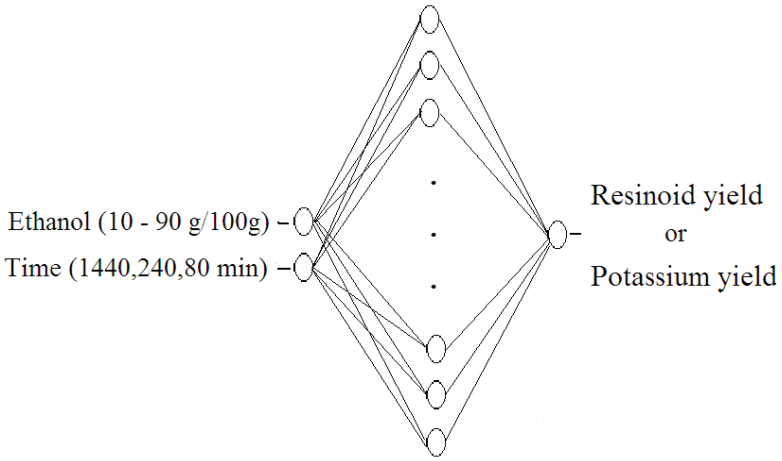


*Figure 1. ANN Architecture.*

conditions for extracting the resinoid and potassium from the aerial parts of white lady's bedstraw by using GA [18,24]. The input vector of the ANN model's independent variables became the decision variable for the GA, which performed the optimization through repeating a simple loop until convergence [24]. Solution populations (chromosomes), were initialized, fitness was computed based on the objective function and the best chromosomes were selected. Then, genetic propagation of the selected parent chromosomes was performed using genetic operators (crossover and mutation) to create the new population of chromosomes. The objective function used to maximize the resinoid yield in the ranges of extraction variables applied in the experiment is as follows:

$$\text{Maximize } z = f(x, y) \tag{1}$$

where $z$ is the resinoid or potassium yield (ANN or experimental values), $x$ is the ethanol concentration, and $y$ is the extraction time.

*Table 2. The training parameters of ANN model for the all three extraction techniques*

| Property | Value/Comment |
|---|---|
| Algorithm | Levenberg-Marquardt back-propagation |
| Minimised error function | RMSE |
| Learning | Supervised |
| Input layer | No transfer function is used |
| Hidden layer | Hyperbolic tangent transfer function |
| Output layer | Hyperbolic tangent transfer function |
| Number of experimental data | 44 |
| Number of input neurons | 2[a] |
| Number of hidden neurons | 20 |
| Number of output neurons | 1[b] |

[a]Ethanol concentration (10-90 g/100g), extraction time (1440, 240 and 80 min); [b]resinoid or potassium yield

## RESULTS AND DISCUSSION

### Analysis of the yields of resinoid and potassium

The influence of solvent polarity on the resinoid and potassium yield from lady's bedstraw was studied at various concentrations of aqueous ethanol solution. The resinoid yield obtained by various extraction techniques was shown to depend on the ethanol concentration in the extracting solvent. Figure 2, illustrating the dependence of the resinoid yield on the ethanol concentration, shows that regardless of the extraction technique used, the resinoid yield decreased with the increase of the ethanol concentration. With increasing ethanol concentration, the solvent polarity decreased, reducing solubility of the polar substances and thus affecting the resinoid yield. For the solutions with

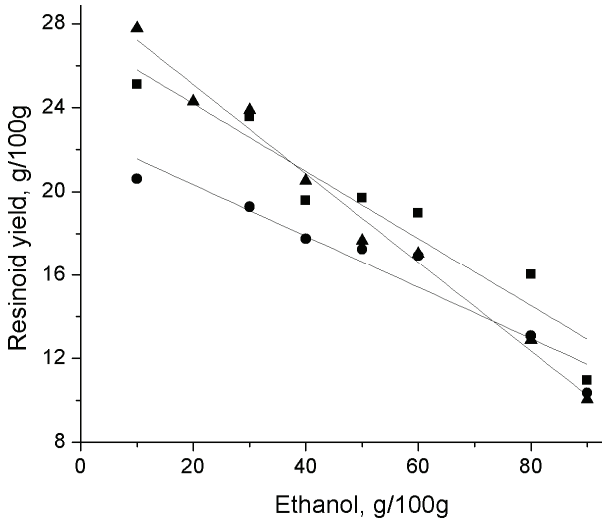


*Figure 2. Dependence of resinoid yield on the ethanol concentration in the extracting solvent (maceration - ●, ultrasonic extraction - ▲, reflux extraction - ■). Each point is presented as the average experimental value.*

ethanol concentration lower than 40 g/100 g, the extraction technique had some influence on the resinoid yield, and higher yields were obtained by the reflux and ultrasound-assisted extraction than by the maceration extraction.

The type of the resinoid yield dependence on the ethanol concentration indicates that it could be described by the linear model:

$$z = p_0 + p_1 x \quad (2)$$

where $z$ is the resinoid yield (mg/100 g of dry plant material), and $x$ is the ethanol concentration (g/100 g). Figure 3 illustrates the fitting of Eq. (2) to the experimental data. The values of $p_0$ and $p_1$ coefficients of Eq. (2), $R^2$ and RMSE are given in Table 3. The linear fit, apart from having a high statistically significant $R^2$-value, simultaneously gave a low value of *RMSE*.

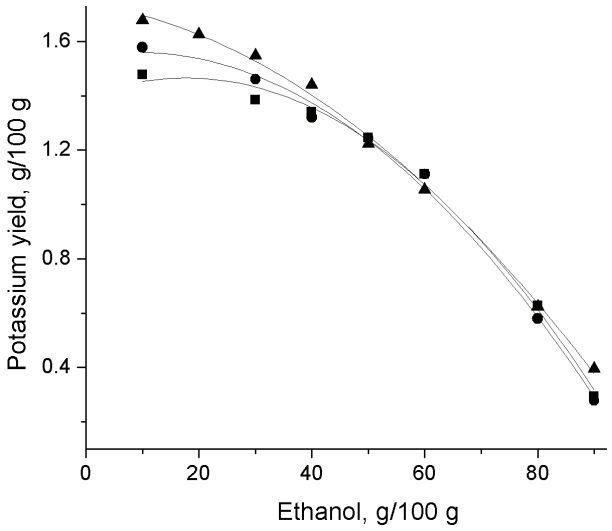


*Figure 3. Dependence of potassium yield on the ethanol concentration in the extracting solvent (symbols are the same as in Figure 2). Each point is presented as the average experimental value.*

*Table 3. Values of coefficients of Eq. (2) (values of coefficients±standard error), $R^2$ and RMSE*

| Extraction technique | $p_0$ | $p_1$ | $R^2$ | *RMSE* |
|---|---|---|---|---|
| Maceration | 22.79±0.87 | -0.122±0.016 | 0.912 | 1.05 |
| Reflux extraction | 27.41±1.25 | -0.161±0.022 | 0.899 | 1.49 |
| Ultrasonic extraction | 29.35±0.56 | -0.212±0.010 | 0.984 | 0.77 |

Figure 2 shows that the dependence of the resinoid yield on the ethanol concentration has a maximum for each extraction technique used. Maximum values of the resinoid yield obtained by maceration, reflux extraction and ultrasound-assisted extraction were calculated from the corresponding fitting equations to be 21.6±0.3, 25.8±0.2 and 27.2±0.5 g/100 g of dry plant material and were achieved at the ethanol concentration of 10 g/100 g. The relative percentage deviation between corresponding predicted and experimental values of the resinoid yield (20.6, 25.1 and 27.8 g/100 g obtained by maceration, reflux extraction and ultrasound-assisted extraction, respectively) using the ethanol solution having the concentration of 10 g/100 g were ±4.8, ±2.7 and ±2.2%, respectively. By increasing the ethanol concentration, the solvent polarity and thereby the solubility of organic compounds and inorganic salts decreased, which influenced the resinoid yield. The data presented in Table 1 show that the yields of extractive substances obtained from aerial parts of *Galium* species differed to each other in a complex way. This was attributed to the facts the extracts were obtained from plant materials originated from various localities, probably with different contents of extractable substances, using different extraction techniques, solvents, operating conditions and process duration times.

The potassium yield obtained by different extraction techniques depended on the ethanol concentration. Figure 3, illustrating the dependence of the potassium yield on the ethanol concentration, shows that regardless of the extraction technique used, the potassium yield decreased with increasing the ethanol concentration, at first slowly (to concentration of about 40 g/100 g), and then faster. The extraction technique affected the potassium yield only within the concentration range lower than 40 g/100 g, where the potassium yield was somewhat higher in the reflux extraction than in the ultrasound-assisted extraction and the maceration.

The dependence of potassium yield on the ethanol concentration was successfully described by the quadratic model:

$$z = p_0 + p_1 x + p_2 x^2 \quad (3)$$

where $z$ is the potassium yield (g/100 g of dry plant material), and $x$ is the ethanol concentration (g/100 g). Figure 3 illustrates the fitting Eq. (3) to the experimental data. The values of $p_0$, $p_1$ and $p_2$ coefficients of Eq. (3), $R$ and *RMSE* are given in Table 4. The results of the fitting of Eq. (3) show that the quadratic model, apart from having a high statistically significant $R$ value, simultaneously gives a low *RMSE* value.

Figure 3 shows that the dependence of the potassium yield on the ethanol concentration has a maximum independently of the type of extraction technique used. Maximum values of the potassium

yield obtained by maceration, ultrasound-assisted extraction and reflux extraction were calculated from the corresponding fitting equations to be 1.56±0.08, 1.70± ±0.01, and 1.47±0.07 g/100 g of dry plant, and were achieved at ethanol concentrations of 10, 18 and 10 g/100 g, respectively. The relative percentage deviation between the predicted and the experimental (1.58, 1.68 and 1.43 g/100g) potassium yields for the same ethanol concentrations were ±1.3, ±1.2 and ±2.8%, respectively. The greatest potassium yield of 1.56 g/100 g of dry plant (corresponding to the extraction degree of 90%) achieved using an aqueous ethanol solution (10 g/100 g) was somewhat higher than that obtained using the 50 vol.% aqueous ethanol solution (1.39 g/100 g corresponding to the extraction degree of 80%). These yields of potassium from *G. mollugo* are approximately the same as the potassium content in different plant materials such as *Artemisia* sp. collected in Pakistan (from 1.14 to 1.84 g/100 g) [38] but much greater than the potassium content of Solenostemma argel leaves from Sudan (0.54 g/100 g) [39].

*Table 4. Values of coefficients of Eq. (3) (values of coefficients±standard error), $R^2$ and RMSE*

| Extraction technique | $p_0$ | $p_1\times10^3$ | $p_2$ | $R^2$ | *RMSE* |
|---|---|---|---|---|---|
| Maceration | 1.54±0.06 | 3.47±0.25 | -1.93±0.01 | 0.993 | 0.04 |
| Reflux extraction | 1.39±0.05 | 7.81±0.23 | -2.20±0.01 | 0.993 | 0.04 |
| Ultrasonic extraction | 1.74±0.04 | -3.07±0.16 | -1.34±0.01 | 0.996 | 0.03 |

The potassium extraction selectivity (g K/g of dry extract) for different extraction techniques depended on the ethanol concentration. Figure 4, illustrating the dependence of the potassium selectivity on the ethanol concentration, shows that the potassium selectivity stayed constant (maceration) or increased (ultrasound-assisted and reflux extraction) with increasing the ethanol concentration up to the concentration of about 40 g/100 g, and then decreased rapidly. The highest potassium selectivities were achieved by the macration. Thus, the extraction temperature and ultrasound affected negatively the potassium selectivity.

The dependence of potassium selectivity on the ethanol concentration was successfully described by the quadratic model (Eq. (3)), where $z$ is the potassium selectivity (g K/g of dry extract). Figure 4 illustrates the fitting Eq. (3) to the experimental data. The values of $p_0$, $p_1$ and $p_2$, coefficients of Eq. (3), $R^2$ and *RMSE* are given in Table 5. The results of the fitting of Eq. (3) show that the quadratic model, apart from having a high statistically significant $R^2$-value, simultaneously gives a low *RMSE* value.

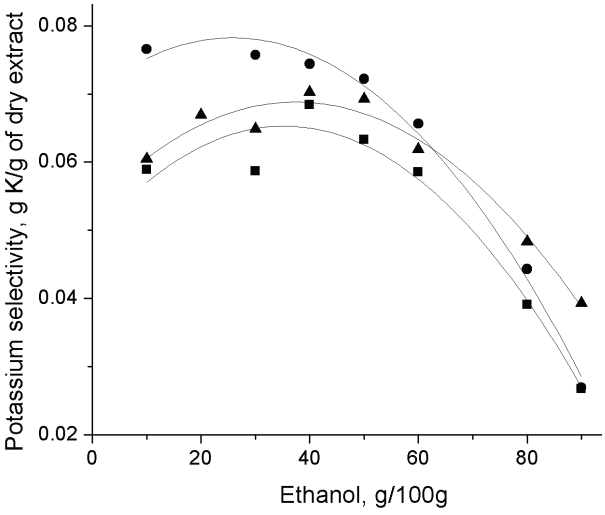


*Figure 4. Dependence of potassium selectivity on the ethanol concentration in the extracting solvent (symbols are the same as in Figure 2). Each point is presented as the average experimental value.*

*Table 5. Values of coefficients of Eq. (4) (values of coefficients±standard error, $R^2$ and RMSE*

| Extraction technique | $p_0$ | $p_1\times10^4$ | $p_2\times10^5$ | $R^2$ | *RMSE* $\times10^3$ |
|---|---|---|---|---|---|
| Maceration | 0.071±0.003 | 6.29±1.28 | -1.21±0.12 | 0.988 | 3.24 |
| Reflux extraction | 0.049±0.005 | 9.07±2.30 | -1.28±0.21 | 0.937 | 3.33 |
| Ultrasonic extraction | 0.053±0.003 | 8.19±1.29 | -1.09±0.12 | 0.961 | 2.56 |

Figure 4 shows that the dependence of the potassium selectivity on the ethanol concentration has a maximum for each extraction technique employed. Maximum values of the potassium selectivity obtained by maceration, ultrasound-assisted extraction and reflux extraction were calculated from the corresponding fitting equations to be 0.077±0.008, 0.069±0.003, and 0.065±0.005 g K/g of dry extract, was achieved at ethanol concentrations of 10, 40 and 45 g/100 g, respectively. The relative percentage deviation between the predicted and the experimental selectivity (0.076, 0.070 and 0.068 g/100g) for the same ethanol concentrations were ±0.7, ±1.7 and ±4.9%, respectively.

## ANN-GA Method

### *Development of ANN model*

Two ANNs were trained for the all three extraction techniques, whereby one gave the result for the resinoid yield (ANN1), and the other for the potassium

yield (ANN2). The resinoid or potassium yield was predicted by the corresponding ANN with the LM algorithm consisted of one output and two input layer neurons (Figure 1). The optimum number of hidden neurons was found to be 20. The hidden layer of 20 neurons was chosen because it gives the minimum *RMSE* between the corresponding ANN and experimental values of the resinoid or potassium yield, as can be seen in Figure 5, where the RMSE is shown as a function of the number of neurons in the hidden layer. The two ANNs were successfully trained as concluded from their high R value (0.988 and 0.999 for resinoid and potassium, respectively) and small RMSE (1.22 and 0.03 for resinoid and potassium, respectively).

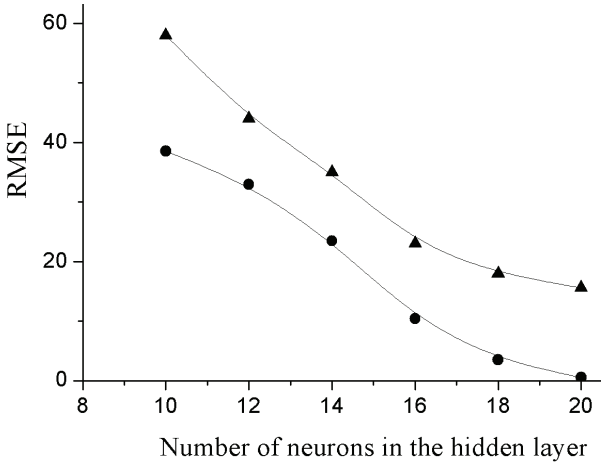


*Figure 5. RMSM as a function of the number of neurons in the hidden layer of the ANN applied (resinoid - ●, potassium - ▲).*

*Testing of developed ANN model*

To examine the possibilities of ANN application for calculating the resinoid or potassium yield achieved by different extraction techniques, the operation of the networks (ANN1 or ANN2, respectively) were checked. To check the ANN model, average values of resinoid or potassium yields for each individual extraction technique was tested. The trained networks were tested using average values of the resinoid and potassium yields for maceration (date set of seven average values), reflux extraction (date set of seven average values) and ultrasound-assisted extraction (date set of eight average values).

The experimental values of the resinoid and potassium yields are compared to the results of the corresponding ANNs for each extraction technique in Figure 6. The ANN results are highly correlated with the experimental results of the three extraction techniques, the *R* values being in the range from 0.959 to 0.992 and from 0.984 to 0.996 for the resinoid and potassium, respectively. Testing the ANN1 for the resinoid yield calculation, the RMSE ranging from 1.22 to 2.49 was obtained, while the testing the ANN2 (for calculating the potassium yield) gave a *RMSE* values ranging from 0.01 to 0.08. The small *RMSE* values and the high *R* values show that the trained networks have been successfully tested for individual extraction techniques.

*RSM applied to ANN results*

How the two ANNs connected their resulting data on the resinoid and potassium yields with the ethanol concentration and the extraction time was also determined using the RSM. In this way, a mathematical model describing the ANN operation for resinoid (linear dependence) and potassium (non-linear dependence) yields was derived in the form of the following polynomial model:

$$z = p_{00} + p_{10}x + p_{01}y + p_{20}x^2 + p_{02}y^2 + \\ + p_{30}x^3 + p_{21}x^2y + p_{12}xy^2 \quad (4)$$

where $z$ is the resinoid yield (g/100 g) or the potassium yield (g/100 g), $x$ is the ethanol concentration in the extracting (10–90 g/100 g), and $y$ is the extraction time (1440, 240 and 80 min). In Figure 7, the results of the RSM applied to the ANNs for the resinoid and potassium yields, Eq. (4), are shown. For this equation, the values of coefficients of Eq. (4) were determined (Table 6). The RSM results show a high *R* value and a low *RMSE* value, so that the ANN operation for resinoid and potassium yields can be described by Eq. (4).

The RSM applied to the experimental results gives the same polynomial model (Eq. (4), Table 6). The RSM results for experimental values show a high *R* value and a low *RMSE* value (Table 6).

*GA-based optimization*

The maximum predicted values of the resinoid and potassium yield were found through the optimization by the ANN-GA model. The values of GA-specific parameters used in the optimization simulations were population size, number of generations, crossover rate and mutation probability. The default values population size, number of generations, crossover rate and mutation probability were 20, 100, 0.8 and 0.01, respectively.

The maximum predicted resinoid yield was 30.4 g/100 g of dry plant and corresponded to the following reaction conditions: the ethanol concentrations of 10 g/100 g and the ultrasonic extraction time of 80 min. The relative percent deviation between the optimum resinoid yield and the yields obtained experimentally

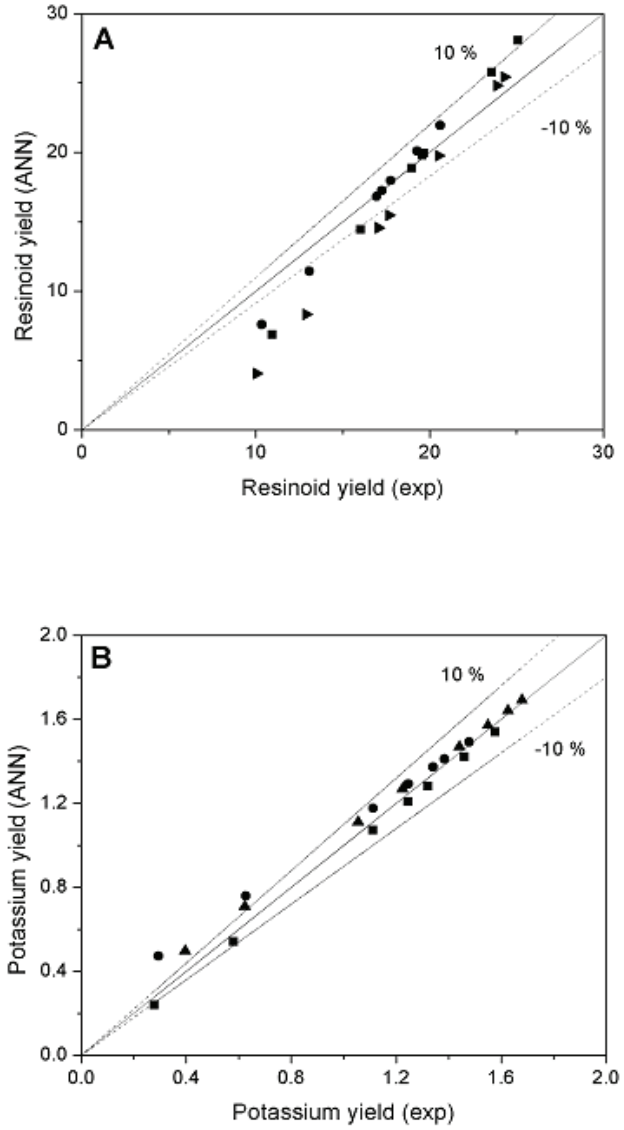


*Figure 6. Comparison of experimental results with ANN results: A) resinoid yield, and B) potassium yield (symbols are the same as in Figure 2).*

(27.8 g/100g) for the same extraction conditions was ±9.3%.

The maximum predicted potassium yield was 1.67 g/100 g of dry plant and corresponded to the following extraction conditions: the ethanol concentration of 10 g/100 g and the ultrasonic extraction time of 80 min. The relative percent deviation between the optimum potassium yield and the yields obtained experimentally (1.68 g/100 g) for the same extraction conditions was ±0.3%.

The maximum experimental values of the resinoid and potassium yields were found through the optimization by the GA. The maximum experimental resinoid and potassium yields of 27.5 and 1.67 g/100 g of dry plant were obtained by the ultrasonic extraction (80 min) using the 10 g/100 g aqueous ethanol solution. The relative percentage deviation the optimum resinoid yields from the yields obtained experimentally for the same extraction conditions was lower than for the ANN model (±1.1%). However, no difference between optimum and experimental potassium yields was observed for the same extraction conditions.

## CONCLUSIONS

The effect of solvent polarity on the yields of resinoid and potassium from aerial parts of white lady's

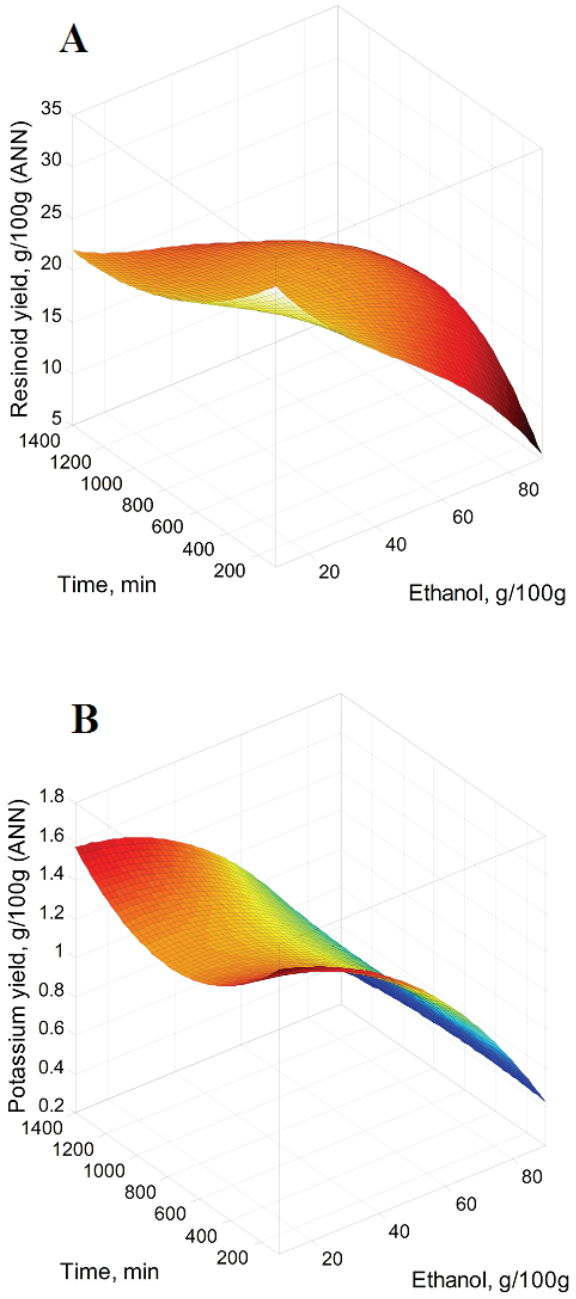


*Figure 7. RSM graph for the ANN data on the resinoid (A) and potassium (B) yield as a function of the ethanol concentration and the extraction time.*

*Table 6. Values of coefficients of Eq. (5) (values of coefficients±standard error), $R^2$ and RMSE*

| Coefficients | Experimental | | ANN | |
|---|---|---|---|---|
| | Resinoid | Potassium | Resinoid | Potassium |
| $p_{00}$ | 39.3±2.7 | 1.8±0.1 | 32.2±1.7 | 1.8±0.3 |
| $p_{10}$ | -0.631±0.174 | -0.005±0.001 | -0.421±0.086 | -0.005±0.002 |
| $p_{01}\times10^{2}$ | -2.5±0.1 | -0.1±0.03 | -1.7±0.2 | -0.2±0.03 |
| $p_{20}\times10^{3}$ | 6.8±0.3 | -0.05±0.01 | 4.6±0.1 | -0.06±0.03 |
| $p_{11}\times10^{4}$ | 7.3±2.4 | 0.17±0.06 | 4.9±2.2 | 0.19±0.03 |
| $p_{02}\times10^{5}$ | 1.0±0.8 | 0.08±0.02 | 0.7±0.5 | 0.09±0.02 |
| $p_{30}\times10^{5}$ | -4.8±2.5 | -0.07±0.01 | -3.2±0.5 | -0.08±0.02 |

*Table 6. Continued*

| Coefficients | Experimental | | ANN | |
|---|---|---|---|---|
| | Resinoid | Potassium | Resinoid | Potassium |
| $p_{21}\times10^6$ | -1.2±0.9 | -0.02±0.001 | -0.8±0.7 | -0.02±0.001 |
| $p_{12}\times10^7$ | -3.4±1.5 | -0.1±0.04 | -2.3±1.3 | -0.1±0.04 |
| $R^2$ | 0.961 | 0.991 | 0.960 | 0.990 |
| *RMSE* | 1.45 | 0.04 | 0.96 | 0.05 |

bedstraw, regardless of the type of the extraction technique, are expected to be described by different polynomial models, due to differences in solubility of the total soluble (ballast) substances and the potassium salts present in raw plant materials. The resinoid (total extract including organics) and potassium (determining the non-polar substances) yields are described by different types of correlation, namely by the quadratic and linear model, respectively, for all three extraction techniques employed. However, the successfully-tested ANNs can be used to find a uniform model describing the dependence of the yield of resinoid or potassium on both the ethanol concentration in the extracting solvent and the time of extraction for the three extraction techniques used. Based on ANN model, the extraction process was optimized by using the GA. The optimum ethanol concentration and extraction time found by the ANN-GA techniques was 10 g/100 g and 80 min (ultrasonic extraction). The same optimum conditions were obtained by the GA applied to the experimental values.

This study indicates that ANN could be successfully applied for examining the influence of other operational conditions (plant material-to-solvent ratio, breakup of plant material, various types of solvents, different parts of plant material), which will be done soon. Furthermore, future investigations should be directed toward the establishment of a unique ANN that would enable calculation of yields of various pharmacologically active substances from various plant materials and under different extraction conditions. However, prior to that, an additional, systematic study is needed for a plant material and an extraction technique to elucidate the complex relationships of the yields of extractive substances and minerals on the most important extraction operating conditions.

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NAUČNI RAD

# POREĐENJE, MODELOVANJE VEŠTAČKOM NEURONSKOM MREŽOM I OPTIMIZACIJA GENETIČKIM ALGORITMOM PRINOSA REZINOIDA I KALIJUMA IZ IVANJSKOG CVEĆA (*Galium mollugo* L.) DOBIJENIH KONVENCIONALNOM, REFLUKS I ULTRAZVUČNOM VODENO-ETANOLNOM EKSTRAKCIJOM

*U radu je prikazan prinos rezinoida i kalijuma dobijenim iz nadzemnih delova belog ivanjskog cveća (Galium mollugo L.) konvencionalnom (maceracijom), refluks i ultrazvučnom vodeno-etanolnom ekstrakcijom. Glavni cilj je bio da se definiše uticaj ekstrakcionih tehnika i koncentracije etanola na prinos rezinoida i kalijuma. Prinos rezinoida, odnosno kalijuma određen je isparavanjem rastvarača iz tečnih ekstrakata, odnosno metodom atomske apsorpcione spektrometrije. Zavisnost prinosa rezinoida i kalijuma, kao i selektivnosti kalijuma od koncentracije etanola matematički je opisano linearnim, kvadratnim polinomnim modelom. Najveća selektivnost kalijuma postiže se maceracijom i iznosi 0,077 g K/g suvog ekstrakta. Veštačka neuronska mreža (VNM) je uspešno primenjena za predviđanje prinosa rezinoida i kalijuma na osnovu koncentracije etanola pri ekstrakciji rastvaračem i vremenskog trajaja za sve tri primenjene tehnike ekstrakcije. Metodom odziva površine predstavljena je zavisnost rezultata VNM od operativnih uslova. Proces ekstrakcije je optimizovan primenom genetičkog algoritma (GA) na rezultate VNM. Određeno je da optimalna koncentracija etanola iznosi 10 g/100 g, pri kojoj se postiže maksimalni prinos rezinoida od 30.4 g/100 g i kalijuma od 1.67 g/100 g ultrazvučnom ekstrakcijom od 80 min. Relativno procentno odstupanje optimalnih prinosa od eksperimentalno dobijenih prinosa za isto vreme iznosi ±9.3% ,odnosno ±0.3%.*

*Ključne reči: veštačka neuronska mreža; genetički algoritam; Galium mollugo; ivanjsko cveće; maceracija; refluks ekstrakcija; ultrazvučna ekstrakcija.*