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Research Article

# Determination of the optimum extraction regime of reducing compounds and flavonoids of *Primula denticulata* Smith leaves by a dispersion analysis

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## Abstract

Herbal medicines are widely used in the complex treatment of various diseases. Therefore, theoretical and practical interest is the in-depth study of drumstick primrose (*Primula denticulata* Smith). The study aimed to determine the optimal extraction mode of flavonoids and reducing compounds of drumstick primrose leaves. The concentration of ethanol, the ratio of raw materials and extractant, and extraction method were studied by dispersion analysis. This allowed reducing the number of experiments from 64 to 16. To obtain the alcohol extract of drumstick primrose leaves with the highest content of reducing compounds and flavonoids, found that maceration is the optimal method of extraction, the ratio of raw materials to extractant should be 1 to 5 and 40% ethanol is the most appropriate extractant.

## Keywords

*Primula denticulata* Smith, dispersion analysis, flavonoids, reducing compounds

## Introduction

During many centuries, plants have been used not only as a source of nutrition but also in the struggle with diseases (Shakya 2016; Stoiko and Kurylo 2018). The increased demand for modern phytopreparations and the tendency towards their wider use in medical practice is not accidental, because herbal medicines have many undoubted advantages. Phytopreparations are widely used in the complex treatment of various diseases. Many studies confirm that plant products are potential agents because of the absence of unwanted side effects (Jagetia and Rajanikant 2003; Singh et al. 2014a) and high tolerability regardless of the age of patients (Slobodianiuk et al. 2019; Kurylo et al. 2020).

It is estimated that 80% of the world's population living in the developing world relies on herbal medicinal products as a primary source of healthcare. A traditional medical practice that involves the use of herbs is viewed as an integral part of the culture in those communities (Ong et al. 2005; Ekor 2013).

Theoretical and practical interest leads to an in-depth study of drumstick primrose (*Primula denticulata* Smith).

*Primula denticulata* Smith, commonly known as drumstick primrose , belongs to family *Primulaceae* (Singh et al. 2014a, 2014b). The genus *Primula* L. is one of the largest genera in the family *Primulaceae* which consists of over 400 species of both annual and perennial herb belonging to 6 subgenera and 37 sections plants distributed in cold and temperate regions of the Northern hemisphere and in tropical mountains (Colombo et al. 2014; Singh et al. 2014b; Liu et al. 2016). *Primula denticulata* Smith is a popular species cultivated worldwide for ornamental purpose and which is now mainly used only in folk medicine (Sinichenko et al. 2018).

The plant contains various active ingredients (secondary plant metabolites) as alkaloids, phenolics, flavonoids, tannins, cardiac glycosides, terpenes, saponins, steroids, coumarins and carbohydrates (Aslam et al. 2015; Bhat et al. 2015; Marchyshyn and Sinichenko 2016).

The chemical profiling of *Primula denticulata* Smith showed the presence of 5-hydroxyflavone 5,8-dihydroxyflavone, 2'-hydroxyflavone, and 5,2'-dihydroxyflavone (Vetschera et al. 2009). Flavonoids have anti-inflammatory, antimicrobial, antioxidant, antineoplastic, hepatoprotective, choleretic and antiulcer activities (Skakun and Stepanova 1988; Narayana et al. 2001). Flavonoids of primula possess strong cytostatic properties even at low concentrations (Tokalov et al. 2004).

The HPLC determined the qualitative composition and quantitative content of glycosides of flavonols – rutin, isoquercitrin, hyperoside and aglycones: flavons – luteolin, apigenin; flavonols – kaempferol (Marchyshyn and Sinichenko 2016).

In the literature sources, there is evidence of the healing properties of *Primula denticulata* Smith.

Ethanolic extract of *Primula denticulata* Smith shows wound healing, antioxidant, antibacterial, antidiabetic activities (Singh et al. 2014a; Bhatt et al. 2016) and plays an important role in the intonation of oxidative stress (Aslam et al. 2015).

In the world practice plant extracts are widely used as curative and preventive nutrition in the production of specialized food products (Venugopal and Liu 2012; Popov et al. 2017).

The aim of the study was to determine the optimal extraction mode of flavonoids and reducing compounds of *Primula denticulata* Smith leaves.

Therefore, the influence of the extraction method, the nature of the extractant, the ratio of raw materials to extractant were studied. These factors have the greatest impact on the process of extraction of biologically active substances (BAS) from the studied raw materials.

During planning an experiment, mathematical methods were used both at the stage of processing the results, and the preliminary stage of experimentation, so-called stage of the experiment plan formation (Rozycki and Synoradzki 2003; Stoiko and Kurylo 2018).

# Material and method

## Plant materials

Leaves of the *Primula denticulata* Smith were collected in Western Ukraine, Tysmenetsk district, Ivano-Frankivsk region (49°01'18.2"N, 24°40'34.4"E), during a mass flowering period in 2018. The raw material was authenticated by prof. Svitlana Marchyshyn (TNMU, Ternopil, Ukraine). A voucher specimen no. 239 is kept in the Department of Pharmacognosy and Medical Botany, TNMU, Ternopil, Ukraine.

## Analytical equipment

Spectrophotometer UV/VIS Lambda 25 (Perkin Elmer, USA) was used. A 1-cm quartz cell was used.

## Standards

Rutin and pyrogallol were used as standards. Rutin (analytical grade 94% purity) and pyrogallol (analytical grade ≥98% purity) were obtained from Sigma-Aldrich. All other reagents were of the highest purity available.

For the planning of the experiment we used one of the dispersion analysis plans – 4×4 Latin square of the third order (Gao 2005; Hroshovyi et al. 2008; Rushing et al. 2013). This made the reduction of the number of experiments possible. In a full factorial experiment $4^3$ (three factors at four levels) N = 64 experiments would have had to be performed. In the Latin square the number of experiments is reduced by 4 times and N = 16 (Bolboaca et al. 2009). So, it was obligatory to implement 16 experimental series and obtain the necessary information about the influence of each parameter under study on the extraction process of the drumstick primrose leaves. Each researched factor (Popa et al. 2016) (the type of extractant, the ratio of raw materials to extractant, extraction method) was studied on four levels (Table 1).

**Table 1.** List of technological factors studied during extraction of *Primula denticulata* Smith.

| Factor | Level of factor |
|---|---|
| A – the type of extractant | $a_1$ – 20% ethanol |
| | $a_2$ – 40% ethanol |
| | $a_3$ – 50% ethanol |
| | $a_4$ – 70% ethanol |
| B – the ratio of raw materials to extractant | $b_1$ – 1 : 5 |
| | $b_2$ – 1 : 8 |
| | $b_3$ – 1 : 10 |
| | $b_4$ – 1 : 12 |
| C – extraction method | $c_1$ – maceration |
| | $c_2$ – maceration with stirring |
| | $c_3$ – remaceration |
| | $c_4$ – ultrasonic extraction |

The matrix of experiment planning and research results are given in Table 2.

The leaves of the *Primula denticulata* Smith were ground and mixed with extractant (20%, 40%, 50%, and 70% ethanol). Maceration, maceration with stirring, remaceration and ultrasonic extraction were used as methods of extraction.

During maceration and maceration with stirring, the raw material filled with the extractant was being infused for seven days. The resulting extracts were drained,

**Table 2.** The matrix of experiment planning and results of extraction of flavonoids and reducing compounds of *Primula denticulata* Smith.

| Series No. | A | B | C | Content of flavonoids, mg/g | Content of reducing compounds, mg/g |
|---|---|---|---|---|---|
| 1 | $a_1$ | $b_1$ | $c_1$ | 0.93 | 0.84 |
| 2 | $a_1$ | $b_2$ | $c_2$ | 0.26 | 0.74 |
| 3 | $a_1$ | $b_3$ | $c_4$ | 0.22 | 0.71 |
| 4 | $a_1$ | $b_4$ | $c_3$ | 0.14 | 0.63 |
| 5 | $a_2$ | $b_1$ | $c_2$ | 0.99 | 1.08 |
| 6 | $a_2$ | $b_2$ | $c_1$ | 0.51 | 0.98 |
| 7 | $a_2$ | $b_3$ | $c_3$ | 0.37 | 0.80 |
| 8 | $a_2$ | $b_4$ | $c_4$ | 0.32 | 0.91 |
| 9 | $a_3$ | $b_1$ | $c_3$ | 0.68 | 0.96 |
| 10 | $a_3$ | $b_2$ | $c_4$ | 0.45 | 0.85 |
| 11 | $a_3$ | $b_3$ | $c_1$ | 0.54 | 0.87 |
| 12 | $a_3$ | $b_4$ | $c_2$ | 0.48 | 0.80 |
| 13 | $a_4$ | $b_1$ | $c_4$ | 0.57 | 0.81 |
| 14 | $a_4$ | $b_2$ | $c_3$ | 0.73 | 0.91 |
| 15 | $a_4$ | $b_3$ | $c_2$ | 0.82 | 0.88 |
| 16 | $a_4$ | $b_4$ | $c_1$ | 0.72 | 0.82 |

the residue of plant raw materials was washed with an extractant. The extracts were then combined and filtered through a paper filter. The difference between these methods is in the periodic mixing of raw materials with the extractant.

During remaceration, the amount of extractant was divided into four portions and each portion was being infused with the raw material within 24 hours.

The device «Ultratone» with a frequency of ultrasonic waves 50 Hz was used as a source of ultrasound during ultrasonic extraction (Vasenda et al. 2018). Extraction was carried out for 4 hours. The extracts were cleaned by filtration.

The evaluation criterium was the content of the sum of flavonoids and reducing compounds , the quantitative determination of which was carried out by the method of spectrophotometry.

The content of flavonoids was determined by this method.

*Test solution*. Aliquot of the obtained alcohol extract is placed into a 25 ml volumetric flask, added 10 ml of alcohol (70% (vol/vol)), 2.0 ml of 3% alcohol (70% (vol/vol)) solution of aluminum chloride, added alcohol (70% (vol/vol)) to the mark and it is mixed.

*Compensatory solution*. Aliqout of the obtained alcohol extract is placed into a 25 ml volumetric flask and added alcohol (70% (vol/vol)) to the mark and it is mixed.

*Standard sample solution of rutin*. 0.05 g (exact weight) of the standard sample of rutin is placed in a 100 ml volumetric flask, then 70 ml of alcohol (70% (vol/vol) are added, dissolved and added alcohol (70% (vol/vol)) to the mark and stirred.

*Comparison solution.* 1.0 ml of the standard sample solution of rutin is placed in a 25 ml volumetric flask, added 2.0 ml of 3% alcohol (70% (vol/vol)) aluminum chloride solution, and added alcohol (70% (vol/vol)) to the mark and stirred.

*Compensatory solution*. 1.0 ml of the standard sample solution of rutin is placed in a 25 ml volumetric flask and added alcohol (70% (vol/vol)) to the mark and stirred.

The optical density of the test solution and the comparison solution are measured 45 min after preparation at wavelength 408 nm relatively to the compensatory solutions for each one respectively.

The content of the sum of flavonoids in liquid extracts (X) in mg /g and in terms of rutin is calculated by the formula:

$$x = \frac{A \times m_0 \times 10000}{A_0 \times m_a},$$

where: A – the optical density of the test solution;

$A_0$ – the optical density of the comparison solution;

$m_0$ – the mass of the standard sample of rutin, in grams;

$m_a$ – the mass of aliquot of the extract taken for analysis, in grams (Vronska 2015).

The content of reducing compounds was determined by spectrophotometric method (The State Pharmacopoeia of Ukraine 2015; NIST/SEMATECH 2013).

*Initial solution*. The aliquot of the obtained alcohol extracti is placed in a 25 ml volumetric flask and added waterto the mark, stirred and, if necessary, filtered.

*Tested solution*. 2.0 ml of the initial solution is placed in a 25 ml volumetric flask, 1.0 ml of phosphorus-molybdenum-tungsten reagent and 10.0 ml of water are added and added solution of 290 g/l sodium carbonate to the mark, stirred.

*Standard solution*. 50.0 mg of pyrogallol is placed in a 100 ml volumetric flask and added water to the mark, stirred. 5.0 ml of the obtained solution is placed in a 100 ml volumetric flask and added water to the mark, stirred.

*Comparison solution*. 2.0 ml of a standard solution of pyrogallol is placed in a 25 ml volumetric flask, 1.0 ml of phosphorus-molybdenum-tungsten reagent and 10.0 ml of water are added and added solution of 290 g/l sodium carbonate to the mark, mixed. 30 minutes later, the optical density of the tested solutions and the comparison solution are measured at wavelength 760 nm, using water as a compensatory solution.

The content of reducing compounds in the liquid extract (X) in mg/g and in terms of pyrogallol is calculated by the formula:

$$x = \frac{A \times m_0 \times 25000}{A_0 \times m_a}$$

where: A – the optical density of the test solution;

$A_0$ – the optical density of the comparison solution;

$m_0$ – the mass of the standard sample of pyrogallol, in grams;

$m_a$ – the mass of aliquot of the extract taken for analysis, in grams (Vronska 2015).

The results were undergonethe dispersion analysis. The data were interpreted using the method of 4×4 Latin squares (Microsoft Office Excel, 2010), which allows us to conduct statistical processing of research results quickly.

## Results and discussion

Regression or dispersion analysis is used to establish the optimal mode of extraction of plant raw materials and obtain extract with the highest content of BAS. These analyses make it possible to reduce the number of experiments.

The regression analysis was used in development of optimal technology of alcohol extract *Centaurium erythraea* Rafn. with the highest BAS content. As a result of studies, ethanol concentration and the ratio of raw materials to extractant were determined which are 69% and 1 to 5 respectively (Stoiko and Kurylo 2018). The optimal composition of tablets of *Pyrola rotundifolia* L. extract was determined by the regression analysis too (Darzuli et al. 2019).

Dispersion analysis was used to study the influence of technological parameters (extraction method, extractant concentration, degree of grinding of plant raw materials) on the extraction of BAS of walnut membranes. It is determined that the best method to obtain an extract with a high content of BAS is maceration, it is advisable to use 35% ethanol as an extractant, the degree of grinding of the raw material should be 0.5 mm (Vasenda et al. 2018).

For determination of the optimum extraction regime of flavonoids and reducing compounds of drumstick primrose leaves dispersion analysis method was also used.

In Figures 1, 2, the influence of the extractant nature on the extraction of flavonoids and reducing compounds from the leaves of *Primula denticulata* Smith is presented. The maximum amount of flavonoids was extracted using 70% and 40% ethanol, which was 0.71 mg/g and 0.55 mg/g, respectively. The smallest amount of research substances was extracted with 20% ethanol (0.39 mg/g).

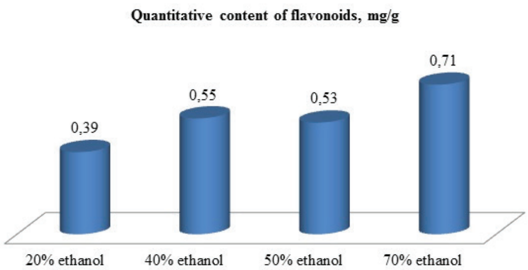


**Figure 1.** Influence of extractant nature on the extraction of flavonoids from *Primula denticulata* Smith leaves.

During extraction of reducing compounds (Figure 2), it is advisable to use 40% and 50% ethanol, which extracts the largest amount of these BAS. When extracted by 40% and 50% ethanol was obtained, 0.94 mg/g and 0.87 mg/g of reducing compounds, respectively.

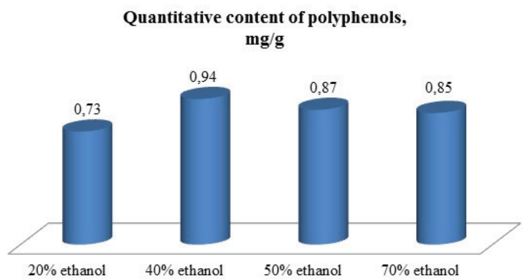


**Figure 2.** Influence of the extractant nature on the extraction of reducing compounds from *Primula denticulata* Smith leaves.

The influence of ratio of raw material to extractant on the flavonoid extraction (Figure 3) illustrates a number of advantages: b1 >> b2 ≥ b3> b4. At the ratio of raw material to extractant 1:5 we obtained an extract with the optimal amount of the studied substances, their quantitative content was 1.92 times more than with the use of the maximum amount of ethanol (the ratio of the raw material to extractant 1:12).

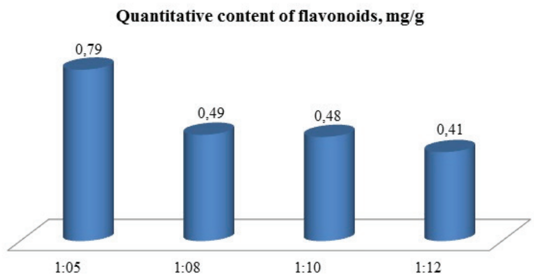


**Figure 3.** Influence of the ratio of the raw materials to extractant on the flavonoids extraction from the *Primula denticulata* Smith leaves.

A similar result was obtained by the extraction of reducing compounds (Figure 4). This allowed reducing the cost of extractant that is quite positive in obtaining extraction drugs.

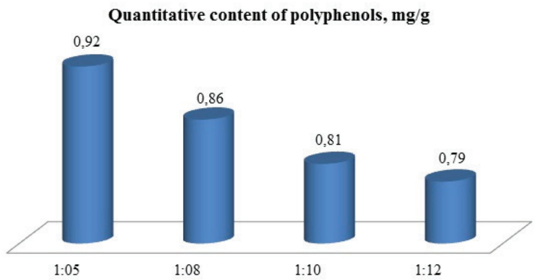


**Figure 4.** Influence of the ratio of raw materials to extractant on the extraction of reducing compounds from the *Primula denticulata* Smith leaves.

The dependence of the degree of extraction of flavonoids and reducing compounds from *Primula denticulata* Smith leaves on the method of extraction is shown in Figures 5, 6. The maximum amount of flavonoids and reducing compounds was obtained during maceration. Used

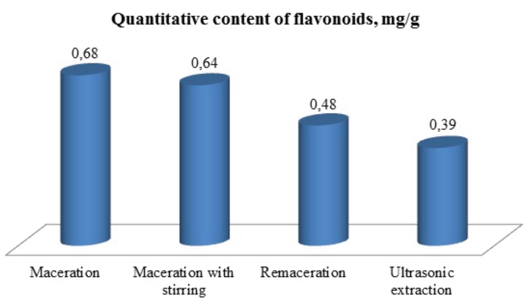


**Figure 5.** Effect of the extraction method on the extraction of flavonoids from *Primula denticulata* Smith leaves.

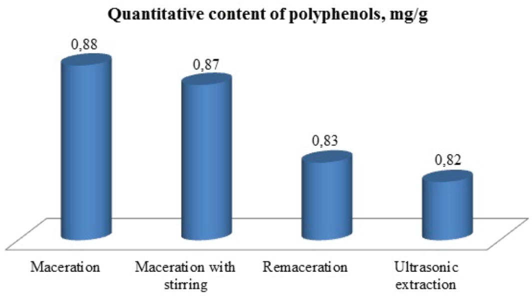


**Figure 6.** Effect of the extraction method on the extraction of reducing compounds from *Primula denticulata* Smith leaves.

this method, the content of flavonoids that were extracted from the investigated raw materials was 0.68 mg/g and reducing compounds was 0,88 mg/g.

The quantitative content of the studied substances of extracts that were obtained by maceration with stirring became slightly inferior. The smallest amount of flavonoids and reducing compounds is extracted by ultrasonic extraction.

## Conclusion

The optimal extraction regime of reducing compounds and flavonoids was determined by the dispersion analysis. After analyzing the experimental data, it can be argued that the optimal extraction of flavonoids and reducing compounds of *Primula denticulata* Smith leaves, was reached when we used maceration as the extraction method, 40% ethanol as the most appropriate extractant and correlation of raw materials to extractant 1:5.

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