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ESSENTIAL OIL CONTENT IN FLOWERS FROM *ROSA CENTIFOLIA* HARVESTED IN DIFFERENT PHENOPHASES

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Abstract

Rosa centifolia L. with common name cabbage rose (*Rosaceae*) is perennial plant that is known as hundred-leaved rose. It is a complex hybrid rose, bred from *Rosa gallica* L., *Rosa moschata* Herrm., *Rosa canina* L., and *Rosa damascene* Mill. The essential oils and extracts are high-value natural products. They are indispensable in fine perfumery and cosmetics, as food additives and aromatherapy agents. The therapeutic use of the rose flower is recommended for mild inflammations of the mucous membrane of the oral cavity and pharynx, and for the treatment of mild skin inflammations. The aim of this research was to determine of essential oil content in fresh and dried flowers of two varieties of *Rosa centifolia* L. in different phenophases of flowering.

Material for the research was essential oil content in samples of fresh and dried flowers (*Rosae flos*) of two varieties of *Rosa centifolia* L. in three phenophases of flowering, collected in 2020 and 2021 growing seasons, in the area of Malé Leváre (Malacky district, Slovakia). The determination of essential oils was performed by steam distillation according to the requirements of the European Pharmacopoeia (10th Edition). The obtained data were evaluated statistically by using multifactor analysis of variance in the program Statistica, ver. 10.0. Significant differences were evaluated at the 0.05% significance level using the Fisher LSD test.

The content of essential oil in fresh flowers of the light variety, expressed as volume-weight percentage, were in defined phenophases F1, F2 and F3 as: 0.48%, 0.40%, and 0.40% in 2020, and as: 0.53%, 0.47%, and 0.44% in 2021. Significantly the highest essential oil content (3.04 mg/kg in average) was observed in F1 phase - the growth stag of floral bud with coiled petals and tight-fitting sepals. By drying the flower samples, the essential oil content decreased significantly, in most samples by more than two thirds compared to the fresh plant material (1.20 mg/kg in average in dry matter, 3.86 mg/kg in average in fresh matter). There was significant difference between light and dark variety (2.00 ml/kg in average in dark variety, 3.06 ml/kg in average in light variety).

The research results show that the content of essential oil in rose flowers (*Rosae aetheroleum*) depends on the investigated genotype, growing environment, harvest date and agro-climatic conditions. Our research was important for the selection of a selected genotype of the genus Rosa for the growing conditions of the warm agro-climatic region of Slovakia.

Key words: *Cabbage rose, Yield, Essential oil, Distillation*.

1. Introduction

The genus Rosa is a large taxon consisting of many species and thousands of cultivars, particularly due to extensive spontaneous hybridization that generates new speciation (Koopman *et al.*, [1]; Tomljenovic and Pejić [2]). The subgenus Rosa comprises about 180 species characterised by a variety of colours and scents (Tomljenovic and Pejić [2]; Younis *et al.*, [3]). These

woody perennials are cultivated for multiple purposes, such as cut flowers, outdoor and indoor plants, and fragrance and flavouring in many industries (perfume, personal care and food sectors mainly) (Hassanein [4]; Dubois *et al.*, [5]).

Cabbage rose (*Rosa centifolia* L.) is a perennial plant that is commonly known as hundred-leaved rose. It is a complex hybrid, the origin of which is derived from several historical roses, especially *Rosa damascene* (Mill.), *Rosa gallica* (L.), *Rosa moschata* (Herrm.), and *Rosa canina* (L.) (Khosh-Khui [6]; Caissard *et al.*, [7]). According to the World Federation of Rose Societies, it advises to the group of old garden roses. These types of historical roses existed before the 1867, which represents the imaginary milestone of the beginning of the era of modern roses (Richter and Proll [8]).

It is of Asiatic origin, cultivated in Bulgaria (region Kazanlak), Turkey (region Isparta), Morocco, Uzbekistan, France, and Italy (Plainfossé *et al.*, [9]; Richter and Proll [8]). Cabbage rose grows as a plant, shrub, or bush, 1.2 - 1.5 m tall, with flowers characterised by numerous thin, overlapping, highly scented petals, generally light pink, more rarely white to purple (Martínez *et al.*, [10]). To do so, *Rosa centifolia*'s globular flowers, which only bloom for a few days at the end of May. The flowering period lasts until mid-June in our geographical conditions. It blooms only once a year (Větvička [11]). Flowers are carefully hand-picked in the early morning and could be distilled (production of floral water) or extracted while still fresh (Dubois *et al.*, [5]).

The rose flower and its medicinal use are stated in the official document of the European Medicines Agency entitled Community herbal monograph on *Rosa gallica* L., *Rosa centifolia* L., *Rosa damascena* Mill. flos. Document primarily includes data on the therapeutic use of the rose flower in the form of specified preparations and defines its therapeutic indications (EMA [12]). According to the current edition of the French Pharmacopoeia, the dried rose petals and flower buds make up the herbal drug of the rose (ANSM [13]).

The therapeutic use of the rose flower (*Rosae flos*) is recommended for mild inflammations of the mucous membrane of the oral cavity and pharynx, and for the treatment of mild skin inflammations. Many other potential biological effects of rosehip have been confirmed, among them mainly anti-inflammatory, antibacterial or anti-ulcer, for which its ingredients - flavonoids, tannins, carotenoids, phenolic acids and silica - are responsible. Thanks to its medicinal effects, it has also found application in folk medicine and pharmacy (Richter and Proll [8]).

Rosa centifolia fragrance, often described as clear and sweet, with slight notes of honey, is widely used in the perfume industry, and in personal care products under several forms, i.e., rose water, concrete and absolute (Dubois *et al.*, [5]). It is the one famous among oil producing species of roses in the world (Lawrence, [14]). This oil is semisolid, pale, yellow, and very expensive (Baydar and Baydar [15]). Pharmacological activities of rose oil have been evaluated by several in vitro and in vivo studies (Maleev *et al.*, [16]; Boskabady *et al.*, [17]) which demonstrated its effects on the central nervous system (CNS) including hypnotic, anti-convulsant, anti-depressant, anti-anxiety, analgesic activities as well as alleviation of morphine withdrawal signs (Mohebitabar *et al.*, [18]; Abbasi Maleki *et al.*, [19]; De Almeida *et al.*, [20]; Naziroglu *et al.*, [21]; and Boskabady *et al.*, [22]). Rose oil has revealed wide spectrum of antibacterial and antifungal properties against some pathogens including *Bacillus cereus*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Penicillium notatum*, *Aspergillus niger* and *Candida albicans* (Eris and Ulusoy [23]; Shohayeb *et al.*, [24]; Uniyal *et al.*, [25]; and Mohebitabar *et al.* [18]).

Cabbage rose has also found its application in aromatherapy. After inhalation or topical application, on the basis of several studies, analgesic, antidepressant and anxiolytic effects, improvement of sexual dysfunction after the use of selective serotonin reuptake inhibitors (SSRI-SD) or reduction of cortisol levels have been attributed to the oil of rose flowers (Mohebitabar *et al.*, [18]). Köse *et al.*, [26], mentioned inhalation of rose oil showed protective effects against damages caused by exposure to formaldehyde in male reproductive system.

The aim of this research was to determine essential oil content in samples of fresh and dried flowers (*Rosae flos*) of two varieties of *Rosa centifolia* L. in three phenophases of flowering, collected in 2020 and 2021 growing seasons, in the area of Malé Leváre (Malacky district, Slovakia).

2. Materials and Methods

A field experiment was carried out at locality Malé Leváre in the district of Malacky, Slovakia (N 48.50472688339842, E 16.96839233423379). Geographically, locality is located in the south-western Slovakia, in Záhorská nížina (Záhorská lowland), about 40 km north-west of Bratislava - the capital city of Slovakia (Figure 1). Malé Leváre is a border village, the Morava and Rudava rivers and the Lakšár stream flow through. The experimental locality has flat character. The altitude is 153 metres above sea level (Malé Leváre [27]), with an average annual air temperature of 12 ^{0}C, an average winter air temperature of 2 ^{0}C, an average

summer air temperature of 22.6 ^{0}C and an average annual rainfall of 520 mm (SHMÚ [28]).

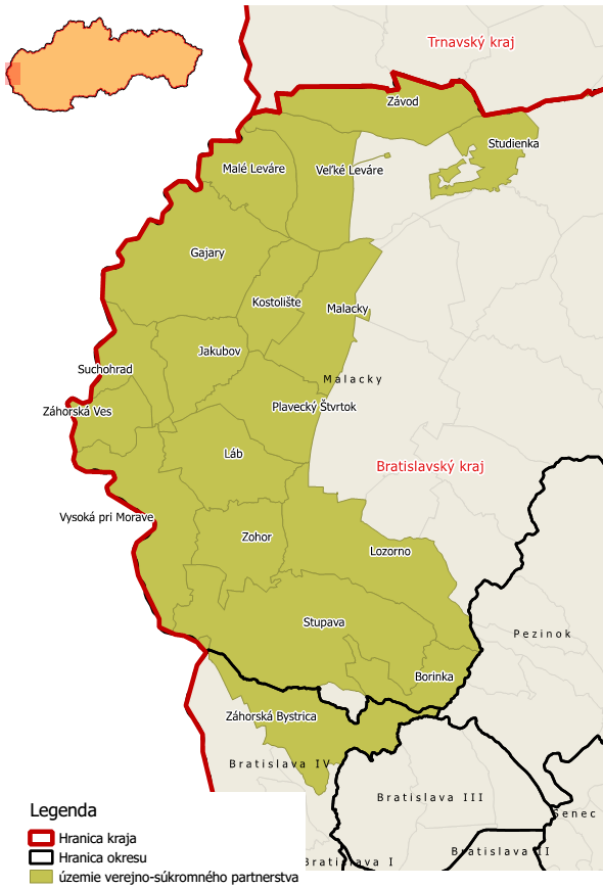

Figure 1. Location of experimental locality Malé Leváre in the district of Malacky (MAS Dolné Záhorie [29])

The plant material represented flowers of two varieties of cabbage rose - "light variety" and "dark variety". Cabbage rose bushes were planted at regular intervals in a sunny area, with support for growth, regularly watered. Hand-picking was done at the peak of the flowering period in two consecutive growing years, on June 3, 2020 and on June 9, 2021. Flower samples were taken from mother plants between 09.00 a.m. and 11.00 a.m. During harvest in June 2020 was partly cloudy weather with a temperature of 17 ^{0}C. Harvest in June 2021 was in sunny weather at a temperature of 22 ^{0}C. According to Slovak Hydrometeorological Institute (SHMÚ [28]) the air temperature during the growing season in 2021 was 5 ^{0}C higher on average compared to 2020.

Whole flowers were collected - with the flower bed and part of the flower stem. Three flower phenophases were distinguished and separated in both varieties of the roses:
- phenophase 1 (F1): closed flower bud with tightly fitting sepals,
- phenophase 2 (F2): opening flower bud with sepals diverging from corolla petals,
- phenophase 3 (F3): fully open flower with drooping sepals and visible stamens in the centre of the flower.

Post-harvest treatment of plant material: the whole flowers in the fresh state were finely chopped, and in a weight of 50 g steam distilled with 500 ml of distilled water for 4 hours in a distillation apparatus. The process for determining the content of essential oil in herbal drugs was carried out according to the requirements of the European Pharmacopoeia, Chapter 2.8.12. Essential oils in herbal drugs (European Pharmacopoeia [30]).

The determined volumes of essential oil obtained from individual flower samples were expressed as the volume of essential oil in millilitres per kilogram (mL/kg) of original plant material after subtracting the volume of xylene used.

Within each experiment, data were subjected to multifactor analysis of variance (ANOVA) in programme Statistica, version 10.0. Treatment means were compared using Fisher's least significant difference (LSD) test at $P < 0.05$.

3. Results and Discussion

In Table 1 are presented obtained results which show that significantly the highest average essential oil content was determined in fresh matter during F1 phase (closed flower bud) in light variety. The essential oil content for light variety in F1 phase was 4.83 mL/kg in 2020 and 5.28 mL/kg in 2021, respectively. The essential oil content in dark variety was 4.26 mL/kg in 2020 and 4.09 mL/kg in 2021, in the growth phase of a closed flower bud with rolled corolla petals and tightly fitting sepals. There was a significant difference between light and dark variety in fresh matter - the light variety contained more essential oil compared to the dark variety. It is known that individual types of roses, as well as varieties and cultivars of the same species, differ in their essential oil content and composition. No specific study dealt with the comparison of the content of essential oil of different varieties of cabbage rose so far. Farooq *et al.*, [31], compared several landscape varieties of Damask rose. They found that the individual varieties differed in terms of both morphological characteristics and essential oil content.

According to results of Gochev *et al.*, [32], and Kovatcheva *et al.*, [33], silica obtained by steam distillation is with a relatively high proportion of oxidized monoterpenes and saturated aliphatic hydrocarbons, but with a low content of phenylethyl alcohol, which is retained in the distillation liquid due to its solubility in water.

Table 1. Essential oil content in cabbage rose in 2020 and 2021

Parameters		Essential oil content (mL/kg)			
		Light variety		Dark variety	
		2020	2021	2020	2021
Fresh matter	F1	4.83 a	5.28 a	4.26 a	4.09 a
	F2	4.00 b	4.67 b	3.85 b	3.15 b
	F3	3.96 b	4.37 b	2.57 c	1.24 c
Dry matter	F1	2.10 c	1.74 c	1.11 d	0.88 d
	F2	1.95 c	1.17 d	0.95 d	0.63 d
	F3	1.70 d	0.89 e	0.89 d	0.33 e

Legend: F1, F2, F3 - growth phenophases of flowering, F1 - closed flower bud; F2 - opening flower; F3 - fully open flower values in column followed by different letters are significantly different at $P \leq 0.05$.

In the case of comparing the content of essential oil in fresh and dry matter, that the drying of the herbal drug resulted in a significant decrease in the content of essential oil in all compared pairs of samples. The average percentage decrease in essential oil content due to drying was -54.95% (2020) and -73.88% (2021) in flowers of the light variety, and -71.54% (2020) and -77.29% (2021) in the dark variety, respectively. Our results of the content of essential oil determined in fresh and dried flowers are in agreement with the results of Iranian scientists. Honarvar *et al*., [34], compared the content of essential oil in the flowers of musk rose (*Rosa moschata* Herrm.) in the fresh matter and in the flowers dried in the shade in the air at room temperature. The essential oil content, determined by a combination of steam distillation and *n*-hexane extraction, was 0.36% (w/w, %) in fresh flowers, 0.14% (w/w, %) in dried flowers. The content of essential oil in the dried drug was thus more than half lower - decrease of -61.11%. Honarvar *et al*., [35], observed changes in the content and composition of essential oil isolated by hydro-distillation from dried Damask rose flowers, too. The process of drying flowers resulted in a significant decrease in essential oil content, from 0.15 - 0.41% (w/w, %) in fresh flowers to 0.09 - 0.17% (w/w, %) in dried flowers. The authors considered the main reason for this difference to be the high proportion of volatile components in the total content of essential oil, which are released into the air during the drying process.

Figure 2 shows the trend of decreasing essential oil content in fresh matter across the phenophases of the light and dark rose variety for both growing seasons. Decrease was more pronounced in the case of the dark variety. We observed significantly the largest percentage decrease in essential oil content in the light variety between the F1 and F2 phenophases, by -17.18% in 2020, and by -11.55% in 2021, respectively. There was a significant decrease of essential oil content in dark variety between the F2 and F3 phenophases by -33.25% in 2020, and by -60.63% in 2021. The lowest essential oil content was observed in flowers in the third phenophase, when the roses were in full bloom - fully open flowers (F3).

Most of scientific articles dealing with the issue of changes in essential oil content across the phenophases in roses agree that rose flowers, regardless of their specific species, have the highest essential oil content at the fully open flower stage (Xihan *et al*., [36]).

Several authors (Dobreva *et al*., [37]; Chaudhary and Kumar [38]) stated that the peak of essential oil accumulation in rose flowers, regardless of their specific species, occurs at the stage of full bloom. Dobreva *et al*., [37], who obtained essential oil from fresh flowers of white rose (*Rosa alba* L.) in six flowering phenophases confirmed the highest essential oil

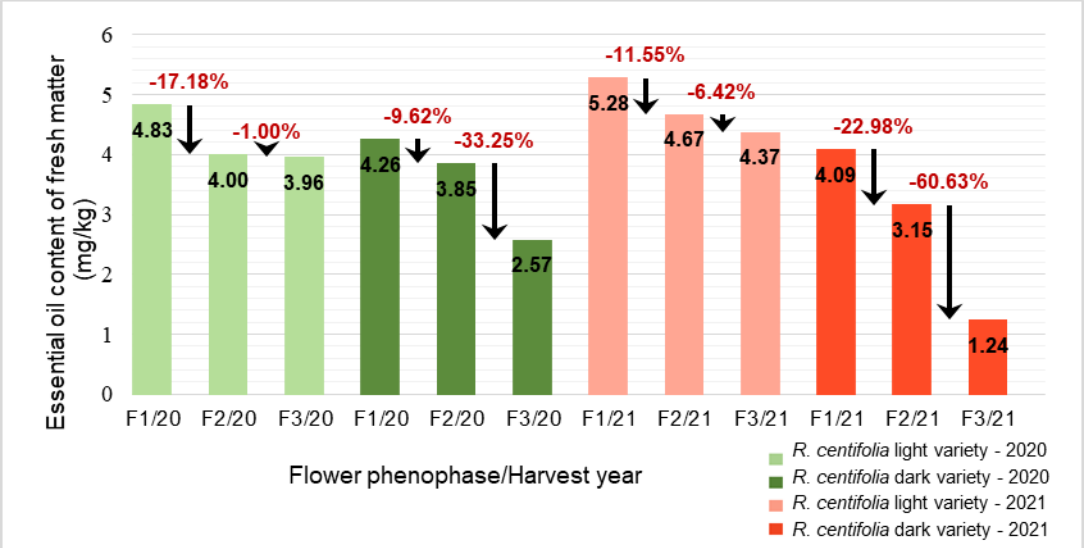

Figure 2. Essential oil content of fresh matter in the three flowering phenophases in 2020 and 2021

content in mid-blooming flowers. They reported that the highest yield of essential oil, obtained by steam distillation in a Clevenger-type still, was observed at the half-open flower stage with visible stamens. According to these authors and results, the white rose, as a representative of roses with a lower essential oil content, had the highest essential oil content in the flowers before they fully open. Species with a higher content of essential oil, which include e.g. Damask rose and Gallic rose contain the most essential oil in the growth phenophase of fully open flowers. Xihan *et al.*, [36] stated that rose flowers, regardless of their specific species, have the highest essential oil content in the fully open flower stage.

The results of our experiment are not in agreement with another authors. The difference in the phenophase shift could be caused by several factors.

Li *et al.*, [39], observations led to the conclusion that the synthesis and accumulation of essential oil components, and therefore the content of essential oil in the flowers of the same rose species and its composition, are influenced by: 1.) Internal factors of the plant (genotype, age of the plant or its developmental stage and degree of maturity, or growth phase within the vegetation period), 2.) Factors of the external environment (soil composition, amount of light, environmental temperature, sufficient water) and especially 3.) The geographical location and climatic conditions in which the mother plant grows. A certain circadian rhythm was also observed within the essential oil content of the plant.

The peak of essential oil accumulation in cabbage rose flowers is officially assumed in the phase of full opening flowers, while subsequently the essential oil in aging flowers decreases (Yaniv and Dudai [40]). The values of the essential oil content across the phenophases determined by us contradict this statement - in our experiments we observed the most essential oil in the flower phase of pucks. We consider the weather conditions at the time of harvest to be the main factor. The weather and the picking time window within the day has a significant impact on the essential oil content of rose flowers. The most suitable conditions are those when the presence of morning dew, high relative humidity and low temperature (10 - 15 ^{0}C) is recorded. For this reason, it is recommended to collect flowers intended for essential oil distillation in the early morning hours, from 04.00 a.m. till 10.00 a.m. After this time, due to the increasing temperature of the environment, there is a significant loss of the volatile components of the essential oil from the petals of the open flowers. However, it is not correct to fixate on only part of the day - the current state of the weather and its effect on the loss of essential oil from flowers must be taken into account every time (Yaniv and Dudai [40]). The results of Younis *et al.*, [41], and Chaudhary and Kumar [38], confirmed that early harvesting leads to a higher yield of essential oil from the flowers of various roses. The amount of essential oil in rose flowers from the morning harvest was almost twice as high as in those from the evening harvest. Differences in the ratio of individual content components were also visible - flowers from the morning harvest mainly contained more citronellal, methyl eugenol and geraniol.

According to Xihan *et al.*, [36], the harvest period within the flowering season has an effect on the content of essential oils in rose flowers. The essential oil content in flowers is not constant, but changes during the flowering season. It is the highest in flowers harvested at the beginning of the flowering season, relatively high in the final period of the flowering season (when there are fewer flowers on the plants), and the lowest in the stage peak flowering, i.e. in the middle of the flowering season (when there are the most flowers on the plants). Essential oil content was negatively correlated with flower production by mother plants. These results confirm the relationship between the secondary and primary metabolism of plants - when the flower yield is high together with the primary metabolism, the secondary metabolism is reduced, due to which less essential oil is formed.

Baydar and Baydar [15], showed that the highest essential oil content in Damask rose was at the beginning of the flowering season and decreased over time until the end of the flowering season. The authors attribute this decrease in essential oil content to increasing air temperatures in the given period of observation, as it is known that high temperatures cause the volatile components of the essential oil to evaporate from the trichrome of the petals of rose petals.

4. Conclusions

- The main goal of the work was to determine the content of essential oil in the three phenological phases of the flowers of cabbage rose (*Rosa centifolia* L.) by the pharmacopoeia method of determining the content of essential oil in herbal drugs, by the steam distillation.
- The results of the experiment show that the peak of essential oil content in both varieties observed by us can be considered the first of the observed phenophases. The F1 phase at the beginning of flowering, when the flower buds of the rose centipede are closed.
- Our findings differ from those of other researchers, which we attribute mainly to weather conditions and a later harvest time window, which likely resulted in losses of essential oil from the corolla petals of open

flowers. In case of further verification of the results obtained in 2020 and 2021, we recommend continuing the research in the next growing seasons.

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