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Enhancement of concentration of coumarins and essential oil in extract from *Ruta graveolens*

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Introduction

Ruta graveolens is a traditional medicinal plant, the positive healing effects on human body are known for centuries. It belongs to the family Rutaceae, the *Ruta* genus includes about 60 species. It is considered a perennial subshrub. Originally it is from southern Europe, where it grows abundantly. In the Czech Republic it is mainly cultivated for its medicinal properties. Among the most common medicinal effects is lowering of blood pressure, and it is used against intestinal pain and headaches. Many substances are produced as so-called secondary metabolites by *Ruta graveolens*. It means, these substances have no effect on growth process, development and reproduction of plant. The family Rutaceae contains exclusively acridones and furoquinoline derivatives, which exhibit numerous physiological activities. Rue is a valuable natural source of alkaloids, too [1].

Essential oils – a mixture of many lipophilic metabolites – are stored in special plant tissues. From the chemical point of view it is a mixture of terpene and phenylpropanoid derivatives. Essential oils are used in food and perfumery industry and also in the production of pharmaceuticals and insecticides. They are volatile, insoluble in water, and oily substances. Several plant families are known to contain more essential oils than it is common, and these include the Rutaceae. The main representative of aromatic oil of *Ruta graveolens* is keton 2-undecanone. This substance is primarily used in insect repellents [2].

Ruta graveolens was shown as the richest in furanocoumarin substances out of *Ruta* genus. The content of furanocoumarins in rue depends on its origin (where, when and under which conditions it is grown) and different parts of rue. The most valuable substances of *Ruta graveolens* belonging to furanocoumarins and coumarins are xanthotoxin, bergapten, psoralen and rutamarin. These substances increase skin sensitivity to light and the subsequent UV irradiation is used to heal skin problems. Xanthotoxin is known especially for its antitumor effects, bergapten can be used against inflammation or pain. Rutamarin exhibits antidiabetic activity [3].

Among the traditional methods used for obtaining essential oils belongs steam distillation. Other substances are isolated from plants usually by extraction. In addition to

conventional solvents, supercritical CO₂ is used more and more frequently. Advantages of supercritical CO₂ extraction process are mainly faster operation, lower environmental impact and the absence of residual solvent in the extract. Properties of supercritical CO₂ are highly variable with temperature and pressure and therefore there is the possibility of adjusting them as needed.

The goal of this work is to examine possible methods of enrichment of valuable substances in CO₂ extracts of rue and compare their results.

Experimental part

The supercritical CO₂ extraction was performed at pressures 12 to 30 MPa and temperatures from 35 to 80 °C. The density of supercritical carbon dioxide under these conditions is in the interval from 719 to 911 kg/m³. The pressure is reduced to atmospheric pressure in a micrometer valve and then the solution is precipitated in the separator. A further increase in pressure could decrease the percentage of target substances due to the increased amount of other, less soluble co-extracted substances. In addition, the Soxhlet extraction was performed using *n*-hexane and ethanol in order to determine the content of substances in the plant.

Three basic methods were selected for the enrichment in controlled substances. The first set of experiments was performed by fractionation in time. Samples were collected gradually in vials and the changing composition was monitored during the run. The vials with extract were cooled in bath with dry ice and ethanol to reduce the escape of volatile substances with gaseous CO₂.

The second method with additional separator is often used to separate waxes from essential oil in the extract. The experimental set-up includes two separators. The pressure and temperature in the first separator are adjusted to enable precipitation of less soluble components of extract while the others remain in the solution and pass to the second separator. The conditions in the first separator were chosen as follows: the pressure of 8 MPa and the temperature of -8, 40 and 60 °C.

The last investigated method is the adsorption on silica gel under high pressure, with the adsorber connected between the extractor and the separator. The first fraction containing the extract components that were not adsorbed was collected in the separator during the extraction. Adsorption is performing together with the extraction, in this step is obtained the first fraction, which doesn't adsorb. The second fraction was obtained by desorption with pure CO₂ at elevated pressure. Finally, the third fraction was desorbed with CO₂ modified with ethanol. The conditions in the extractor were: pressure 12 and 15 MPa, temperature 35 and 40 °C. The pressure in the adsorber was equal to the pressure in the extractor and the temperature ranged from 35 to 50 °C. The conditions were selected as 28 MPa and 40 °C for the desorption with pure CO₂, and 30 MPa and 40 °C for the desorption with the modified solvent. The concentrations of modifier in supercritical CO₂ were 2,3% and 5%.

The extract samples were analyzed with help of gas chromatography equipped with FID and using the method of internal standard. The concentration of components were evaluated as the ratio of peak areas.

Results and discussion

The extraction rate was initially high because the substances freely occurring at the surface of plant material were dissolved. Later the extraction was slowed down. The extract samples were collected to keep the size of samples approximately equal.

As an example of the experiment with fractionation in time, the run performed under the condition (12 MPa, 40 °C) was selected and the results are shown in Tab. 1.

Tab.1: Concentrations achieved using the method of fractionation in time

Substances	Fraction 1 [%]	Fraction 2 [%]	Fraction 3 [%]	Fraction 4 [%]
rutamarin	5,95	28,65	27,57	17,85
2-undecanone	39,48	1,80	1,18	1,70
psoralen	1,52	5,89	5,72	6,42
xanthotoxin	1,38	7,83	7,68	8,47
bergapten	1,12	7,05	7,11	8,63
hentriacontan	0,00	11,33	12,12	21,26
n-tritriacontan	1,23	5,34	5,63	12,29

The fractionation was more effective under these conditions than at higher pressures. Essential oil was extracted at the outset, waxes were mainly accumulated in the last fraction.

In the second method with additional separator, the influence of conditions in the first separator on the composition of extract fractions was observed. It was shown, that higher concentration and yield of 2-undecanone was achieved with this method than with the help of fractionation in time. An example of results of the run under conditions (12 MPa, 40 °C) in extractor and (8 MPa, 60 °C) in separator is shown in Tab. 2.

Tab.2: Concentrations and yields achieved using the method of additional separator

	1. separator	2. separator

Substance	yield [mg/g plant]	concent. [%]	yield [mg/g plant]	concent. [%]
rutamarin	1,34	25,08	0,02	1,99
2-undecanone	0,19	3,65	0,65	74,41
psoralen	0,35	6,54	0,01	0,98
xanthotoxin	0,40	7,49	0,01	0,70
bergapten	0,36	6,70	0,00	0,29
hentriacontan	0,73	13,69	0,00	0,07
n-tritriacontan	0,49	9,12	0,00	0,00

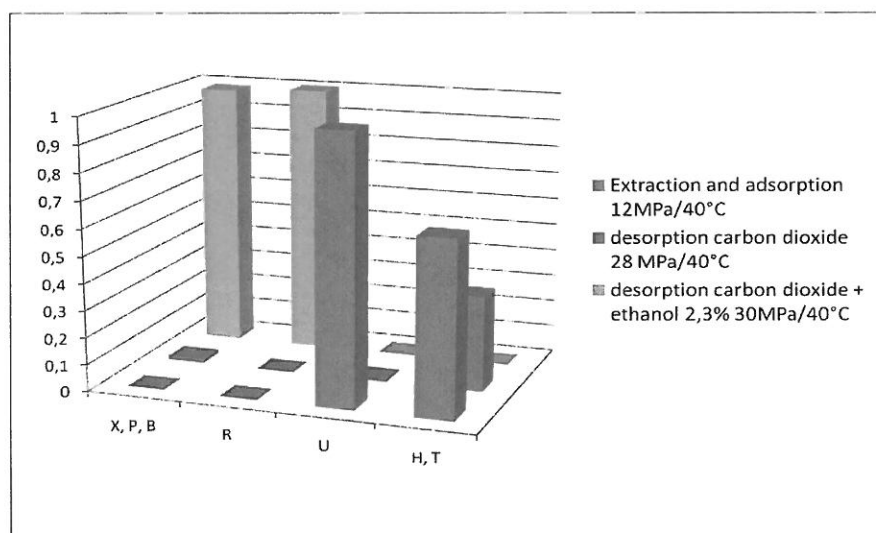
The yields of essential oil obtained using both methods of fractionation were by about 30% lower compared to the Soxhlet extraction.

The last fractionation method was the adsorption on silica gel. The major component of essential oil, 2-undecanone, was almost not adsorbed and its main part was extracted directly into the vials. The polar furanocoumarins were adsorbed well on silica gel, since it is a polar sorbent. Thus the largest share of coumarins including rutamarin was obtained through the subsequent desorption with ethanol modifier. The other monitored substances were the main representatives of waxes – hentriacontan and *n*-tritriacontan, whose main part was obtained together with 2-undecanone in the first fraction [Fig. 1].

Fig.1: Fractionation of monitored substances in adsorption experiments.

X = Xanthotoxin, P = Psoralen, B = Bergapten, R = Rutamarin,

U = 2-undecanone, H = hentriacontane, T= *n*-tritriacontane



The yield of extracted rutamarin was under the condition (15 MPa, 35 °C) compared with the yield obtained by Soxhlet extraction. Rutamarin was from all controlled substances best concentrated in one fraction. The largest number yield of 2-undecanone, was collected in the first fraction of the extraction under conditions (15 MPa, 35 °C). An example of results of experimental run under the condition (12 MPa, 40 °C, ratio solvent to feed 15,32 g/g) for adsorption, (28 MPa, 40 °C, 15,32 g/g) for desorption by pure CO₂, (30 MPa, 40 °C, 15,32 g/g) with modifier 2,3 % ethanol is shown in Tab. 3.

Tab.3: Concentrations and yields achieved using the adsorption method

Substances	1. fraction		2. fraction		3. fraction	
	yield [mg/g plant]	concent. [%]	yield [mg/g plant]	concent. [%]	yield [mg/g plant]	concent. [%]
rutamarin	0,00	0,00	0,01	4,15	0,62	33,61
2-undecanone	2,37	56,61	0,01	2,17	0,00	0,05
psoralen	0,00	0,00	0,01	5,34	0,23	12,37
xanthotoxin	0,00	0,00	0,01	3,56	0,25	13,57
bergapten	0,00	0,00	0,04	15,61	0,2	10,99
hentriacontan	0,33	7,98	0,01	3,31	0,00	0,00
n-tritriacontan	0,21	4,97	0,01	3,54	0,00	0,00

During the adsorption at high value solvent to feed the furanocoumarins are desorbed, too. Out of the substances detectable by GC, the seven components listed in the tables represent 70% on the average.

Conclusion

Ruta graveolens was extracted under the conditions (12 – 30 MPa, 35 – 40 °C).

Three methods for on-line fractionation of monitored components of rue have been examined:

fractionation in time, method with additional separator, adsorption method. The adsorption experiment was found to be the most effective. The furanocoumarins were successfully separated from essential oil and waxes by the adsorption on silica gel (30 MPa, 40 °C, 5% ethanol).

Acknowledgements

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