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CONTRIBUTION TO INVESTIGATION OF ST. JOHN´S WORT HERB EXTRACTS *HYPERICUM PERFORATUM L. SSP. ANGUSTIFOLIUM*, OBTAINED BY HIGH PRESSURE EXTRACTION WITH CO_2

UDC:582.824

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Abstract. *The extracts of St. John´s wort Hypericum perforatum L .ssp. angustifolium from the new locality, Sobina, (the surroundings of Vranje, South Serbia, Yugoslavia) obtained by high pressure extraction with CO_2, exert antimicrobial action, where the extract obtained under higher pressure exerted stronger action. The extracts exerted the best antimicrobial action on Candida albicans fungi. The practical application of HPE (High Pressure Extraction) for obtaining extracts of St. John´s wort herb suitable for producing parapharmaceutical preparations will depend on techno-economic analysis, which will compare them, in terms of quality and price, with the extracts obtained by classic procedures.*

INTRODUCTION

Although Hannay and Hogarth established diluting power of compressed gases back in 1879 [1], technical application of high pressure extraction (HPE) is of a recent date; by the end of 70s a decaffeinication plant was put into operation, and the production of hop extracts by HPE started in 1982.

The essential difference between HPE and conventional extraction methods lies in use of special solvents which require high pressure and specific temperature conditions to keep them in liquid or supercritical state. Under normal conditions these solvents are

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gases [2]. Due to its suitable physical parameters (t_c=31°C, p_c=7,38 MPa), its inert nature as well as its physiological harmlessness, CO_2 is a suitable extraction agent for use in food and pharmaceutical industry. From chemical engineering standpoint, the most important advantage of using gaseous solvents is that their properties can be changed in wide range of pressure and temperature changes [3]. As additional components considerably widen the range of changes, multicomponent gases are becoming increasingly important in HPE application [4].

St.John's wort herb *Hypericum perforatum L.* is known and often used plant species which during recent years takes central place on West European market. At the Sobina locality (surroundings of Vranje, South Serbia, Yugoslavia), we have discovered a new habitat of *Hypericum perforatum L.* subspecies *ssp.angustifolium*, which has not been investigated so far. The aim of this study was to investigate the antimicrobial properties of mentioned plant species extracts, obtained with CO_2 under high pressure, and to get a better insight into their pharmacological value.

HPE WITH CO_2

Extractions with CO_2 are mainly carried out under supercritical conditions, i.e., temperature and pressure are above the critical values. It is also possible to work with liquid gases under the critical temperature, while pressure may be either supercritical or subcritical. Such conditions have been described as subcritical (Fig. 1).

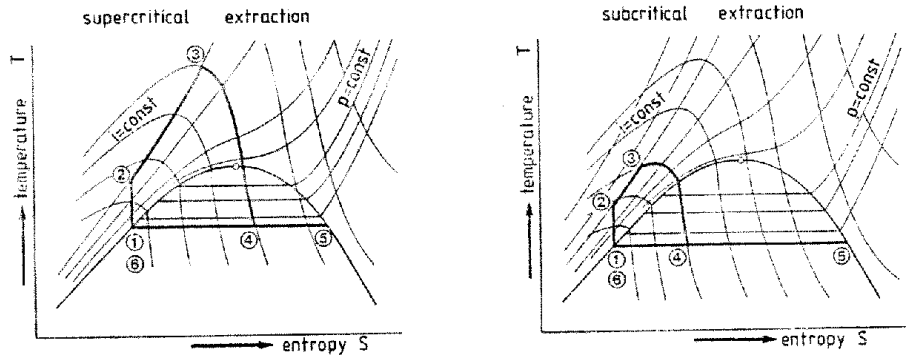

Fig. 1. HPE extraction in TS diagram; 1.condensed gas 2.compressed liquefied gas, 3.extraction conditions, 4.separations condition, 5.vaporised gas, 6.conensed gas

Table 1 shows the most characteristic extraction parameters: mean viscosity, density and diffusion coefficient in gaseous, liquid and supercritical states. In supercritical state viscosity is similar or slightly higher than in gaseous state, and considerably lower than in liquid state. In supercritical state, diffusion coefficient is between the values for gaseous and liquid state.

When a supercritical fluid is used as a solvent for extraction, it is possible to separate multicomponent mixture by using both differences: in volatility of components (i.e., significant distillation characteristics) and in specific interactions between mixture

components and supercritical fluid (i.e., significant characteristics of liquid extraction) [5].

Table 1. Physicochemical Properties of Gases, Liquids and Supercritical Fluids Phases

	Gas	Supercritical fluid	Liquid
Density [g/cm^3]	10^{-3}	0,3	1
Diffusion coefficient [cm^2/s]	10^{-1}	$10^{-3} – 10^{-4}$	10^{-5}
Viscosity [g/cm s]	10^{-4}	$10^{-4} – 10^{-3}$	10^{-2}

In relation to traditional separation techniques, HPE offers the following advantages:
– selective extraction by pressure, temperature and physical properties optimization
– the obtained products do not contain solvents
– an easy treatment of natural substances which are often sensitive to heat
– good solubility of nonvolatile components
– high quality of raw extracts.

HPE is primarily used in food and pharmaceutical industry (e.g., decaffeinization of coffee and tea, and extraction of hop and spices). In more recent days, along with the application in extraction of consumer goods, HPE is also applied in extraction, but to a lesser extent, of expensive, special chemicals and pharmaceutical products (e.g., extraction of carotenoids).

Fig. 2 shows the development of natural substances extraction process as well as comparison of HPE methods with conventional extraction processes. The decision as to which extraction method the priority will be given in practical use will depend on the results of comparison of extracted products quality and process expenses [2].

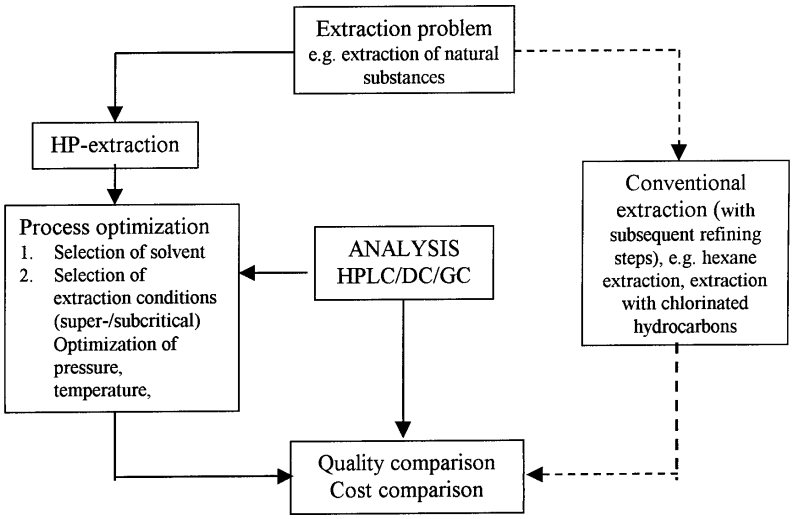

Fig. 2. Choice of extraction process

MATERIALS AND METHODS

St. John´s wort herb *Hypericum perforatum L.*, gathered on June 19, 1998 at the Sobina locality, on mountain Krstilovica sides (surroundings of Vranje, South Serbia, Yugoslavia) has been used in this study. The plant material was dried at room temperature, in thin layer 5-7 cm, up to moisture content below 11 %, and then milled on an electric mill and sieved through the sieve of 1 mm mesh.

The high pressure extraction with CO_2 was carried out on high pressure extraction plant (HPEP) Nova - Swiss (Fig. 3), under the following conditions:

1. p = 100 bar, t = 40^0C, τ = 2 h
2. p = 300 bar, t = 40^0C, τ = 2 h.

The mass of analyzed drug extracts was 50,0 g each, flow rate of CO_2 97,725 dm^3/h (at 20^0C), while the conditions in separator were p = 15 ± bar and t = 22 ± 1^0C.

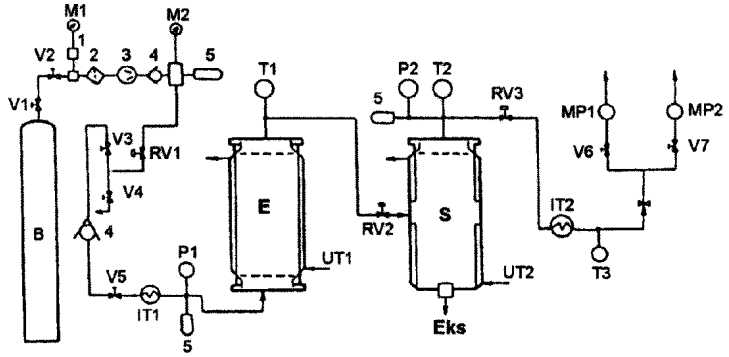

Fig. 3. Scheme of the high pressure extraction plant (HPEP) - NOVA - Swiss.

1. Measuring connector; 2. Filter; 3. Compressor with diaphragm; 4. Control valve; 5. Safety valve; B-bottle with carbon dioxide; V-valve; M-manometer; RV-regulating valve; IT-heat exchanger; P-pressure gauge; E-extractor (V = 200 cm^3); T-thermometer; UT-Ultrathermostate; S-Separator (V = 200 cm^3); Eks-extract; MP-flow meter.

The analyses of general qualities parameters of plant material were carried out according to the methods of Ph. Jug. IV (6).

Antimicrobial activity of ethanolic solutions of extracts of the mentioned herb species (C=0,01 g/cm^3) was tested by application of diffusion method on cellulose disc, as prescribed in Ph. Jug. IV, using the following 10 test cultures: *Candida albicans 24493 ATCC*, *Aspergillus niger*, *Escherichia coli 95*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Sarcina lutea 9341*, *Klebsiella pneumoniae*, *Bacillus subtilis S* and *Staphylococcus aureus 6538 ATCC.*

RESULTS AND DISCUSSION

In Flora of SR Serbia (7) it is said that *Hypericum perforatum L., ssp. angustifolium* is represented in mountain belt in thin oak woods and on dry meadows of Šumadija (the

surroundings of Kragujevac, Rudnik) and on serpentines in West Serbia (surroundings of Kraljevo, region of Tara mountain), so the region of Krstilovica mountain, i.e., the Sobina locality, which we have discovered, represents the new find of this subspecies.

Table 2 shows the results of investigation of the drug quality general parameters, which on the whole meet the requirements of Ph. Jug. IV.

Table 2. Results of investigation of drug quality general parameters

Parameter	Content [%]
Essential oil	0,2
Moisture	10,61
Ash	3,528
Sand	0,258

Table 3 shows the yields of St. John's wort herb *Hypericum perforatum ssp. angustifolium* by high pressure extraction with CO_2.

Table 3. Yields of St.John's *Hypericum perforatum L. ssp.angustifolium* by high pressure extraction with CO_2

No. of extract	Conditions of the extraction (p,t,τ)	Yield in g. per kilo of the drug
1.	p = 100 bar; t = 40°C; τ = 2 h	16,019
2.	p = 300 bar; t = 40°C; τ = 2 h	28,575

As we have found, in our previous investigation [8], that yields of dry extracts obtained by maceration (drug:solvent ratio = 1:10) with occasional stirring, during 24 h of the investigated plant material with extractants of different polarity, vary from 2,6 % with petroleum ether to 12,1 % with methanol (in relation to dry drug), we can conclude that the yields of high pressure extraction with CO_2 are smaller. It could also be seen from the Table 3 that the increase of CO_2 pressure leads to increased extracts yield.

The extracts obtained with CO_2 under high pressure were dissolved in ethanol (C = 0,01 g/cm^3), and their antimicrobial activity was tested by means of diffusion method on cellulose disc, as prescribed in Ph. Jug. IV (Table 4).

Table 4. Inhibition zones of ethanolic solutions of extracts of *Hypericum perforatum L. spp. angustifolium*, obtained by high pressure extraction with CO_2, expressed in mm

Microorganisms	Extract 1	Extract 2
Candida albicans 24493 ATCC	23,9	25,0
Aspergillus niger	14,4	20,2
Saccharomyces cerevisiae	–	–
Escherichia coli 95	–	14,8
Salmonella enteritidis	–	14,6
Pseudomonas aeruginosa	14,8	15,9
Sarcina lutea 9341	16,0	18,2
Klebsiella pneumoniae	14,0	16,0
Bacillus subtilis S	14,4	15,8
Staphylococcus aureus 6538 ATCC	14,1	15,0

The extract obtained by extraction with CO_2 under higher pressure (the extract 2) showed better antimicrobial action. The extracts exerted the best antimicrobial action on *Candida albicans* fungi (Fig. 4), while the culture *Saccharomyces cerevisiae* was resistant to both extracts.

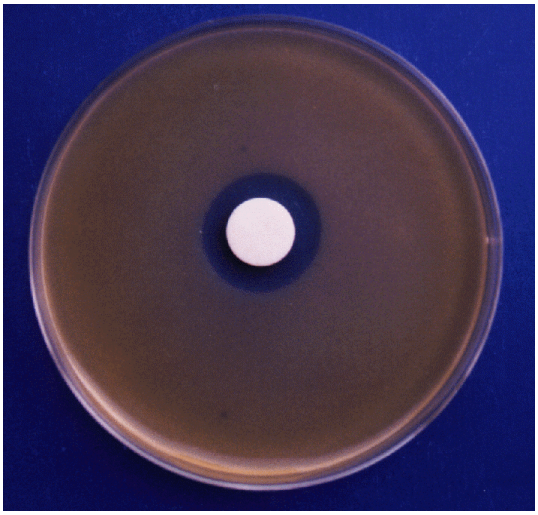

Fig. 4. The action of the extract 2. on *Candida albicans*

The extract 2 exerted somewhat weaker antimicrobial action than the ethanolic extract of the investigated plant species obtained by maceration. However, in comparison with antimicrobial action of petroleum ether macerate, which exerted the best action out of ten tested extracts of different polarity [9], antimicrobial action of extracts 1 and 2 was significantly weaker.

However, by changing the extraction conditions (pressure and temperature) HPE process could be optimized and that will be the subject of our further investigations. The final decision on using HPE for producing parapharmaceutical preparations will be made on the basis of economic analysis and comparison of quality of the obtained extracts with the quality of extracts obtained by conventional extraction processes.

CONCLUSION

1. The yields of dry extracts *Hypericum perforatum L. ssp. angustifolium* obtained by high pressure extraction with CO_2 were smaller in relation to the yields of dry extracts obtained by maceration with extractants of different polarity.

2. Ethanolic solutions of thus obtained dry extracts ($C = 0{,}01\ g/cm^3$) exerted the antimicrobial action on all examinated test cultures exept *Saccharomyces cerevisiae*, where the extract obtained under higher pressure exerted stronger action.

3. By changing the extraction conditions (pressure and temperature) it is possible to optimize HPE process, and the practical application of this method may be taken into consideration if the obtained extracts proved competitive (in terms of quality and price)

with the extracts obtained by conventional extraction processes. It is obvious that from the ecological point of view CO_2, as the extractant, is at advantage.

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PRILOG ISPITIVANJU EKSTRAKATA KANTARIONA *HYPERICUM PERFORATUM L. SSP. ANGUSTIFOLIUM* DOBIJENIH EKSTRAKCIJOM SA CO_2 POD VISOKIM PRITISKOM

Andrija Šmelcerović, Siniša Đorđević, Branislav Gudžić, Milovan Dimić, Žika Lepojević

Ekstrakti kantariona Hypericum perforatum L. ssp. angustifolium sa novog lokaliteta Sobina (okolina Vranja, južna Srbija, Jugoslavija), dobijeni ekstrakcijom sa CO_2 pod visokim pritiskom, pokazuju antimikrobno dejstvo, s' tim što ekstrakt dobijen pod višim pritiskom pokazuje jače dejstvo. Najbolje antimikrobno dejstvo ekstrakti pokazuju na gljivici Candida albicans. Praktična primena HPE (High Pressure Extraction), za dobijanje ekstrakata kantariona pogodnih za izradu parafarmaceutskih preparata zavisiće od tehno-ekonomske analize, u kojoj će se uporediti, u pogledu kvaliteta i cene, sa ekstraktima dobijenim klasičnim postupcima.