Extraction of Calendula using supercritical CO_2

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1 Introduction

Calendula officinalis is a annual or perennial herbaceous plants in the daisy family Asteraceae, native to the Mediterranean region. The plant is 30-50 cm high and attracts attention by its yellow to orange florescence in June to October. Especially the flowers of the plant are important for cosmetical and pharmaceutical applications. The paper presents the benefits and performance extracting dried calendula flowers using supercritical CO_2.

2 Summary

Pharmaceutical significant ingredients of the flowers are especially triterpene faradiol-esters and carotenoids. Traditionally these components are extracted using a classical maceration process with solvent mixtures of water and ethanol. The extracts are intermixed to e.g. creams and distinguish by their anti-inflammatory properties and support of the natural wound healing.

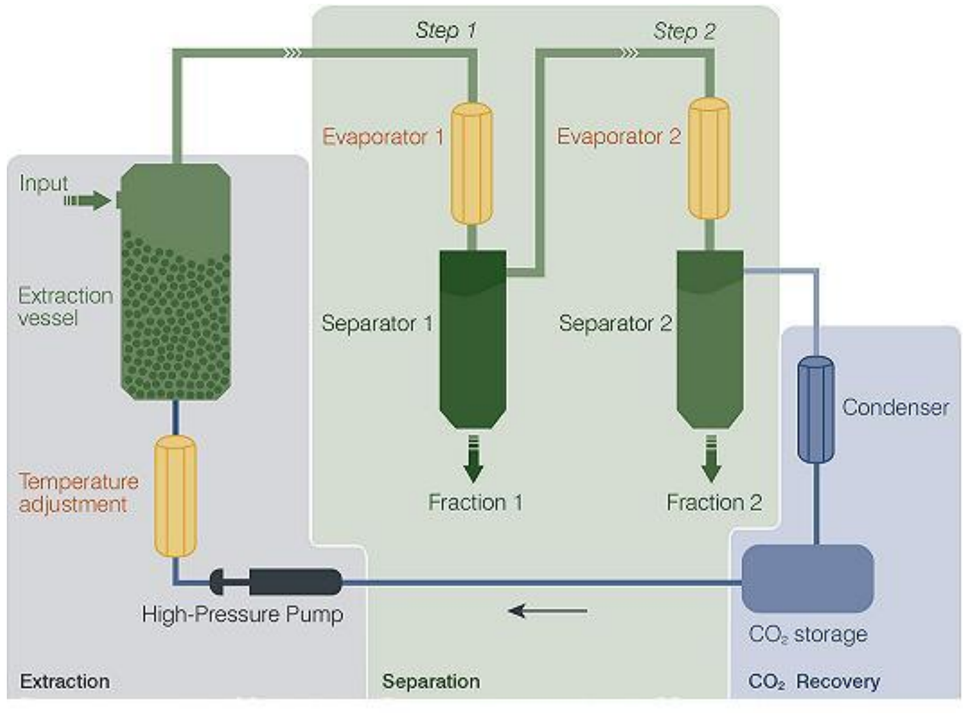

Fig. 1: Technical chart of a CO_2 extraction plant for calendula

2.1 Extraction

In collaborations with the company UNIFARCO and the University of Padova NATECO$_2$ performed a CO_2 extraction process of dried *Calendula* flowers. By optimized preparation of the material and adjusted parameters high yields of extracts and active substances could be attained.

The dried flowers have been milled with a hammer mill to a particles size of < 2 mm. The extraction process was operated at 70 °C and > 600 bar. At the first separation step the pressure was reduced to 250 bar before decompression to the pressure of the storage tank (60-70 bar) at the second separator was performed (fig. 1).

The total yield of extracts was approx. 8%. In the first fraction 1,5% and in the second separator 6,5% of oleoresin precipitated. Quantitative analyses of active compounds in the extracts were obtained by HPLC-MS measurements.

3 Qualitative and quantitative analysis

3.1 Triterpene ester determinations

Qualitative and quantitative analysis of triterpene ester fractions were obtained by HPLC-APCI-MS and HPLC-ELSD. The main active constituents of the extracts were the faradiol, arnidiol and calenduladiol ester of palmitic, lauric and myristic acid. Triterpene esters are detected as positive $[M+H-H_2O]^+$ ions. Their amounts in the extracts of fraction A (separator 1), B (separator 2) and in the total extract are reported in table 1.

The total content of triterpene esters in the CO_2-extracts is remarkably high if we compare it to extracts obtained by maceration in aqueous ethanol with a final amount of total triterpene esters of 0.01 -0.06 % (tab. 2). MS/MS fragmentation allowed to identify the different constituents (fig 2).

Tab. 1: Amount of the various triterpene esters in CO_2- extracts

		% Fraction A Separator 1	% Fraction B Separator 2	% total extract
Lauric	Calenduladiol	0,051 ± 0.009	0,042 ± 0.003	0,059 ± 0.003
	Arnidiol	0,135 ± 0.010	0,098 ± 0.009	0,131 ± 0.006
	Faradiol	0,750 ± 0.060	0,594 ± 0.002	0,758 ± 0.016
Myrsistic	Calenduladiol	0,363 ± 0.010	0,248 ± 0.009	0,293 ± 0.010
	Arnidiol	0,876 ± 0.010	0,683 ± 0.009	0,809 ± 0.007
	Faradiol	3,122 ± 0.092	2,086 ± 0.011	2,525 ± 0.014
Palmitic	Calenduladiol	0,196 ± 0.010	0,121 ± 0.009	0,128 ± 0.009
	Arnidiol	0,516 ± 0.042	0,364 ± 0.010	0,448 ± 0.010
	Faradiol	2,013 ± 0.091	1,164 ± 0.010	1,285 ± 0.040
% Total		8,020 ± 0.091	5,400 ± 0.010	**6,440 ± 0.013**

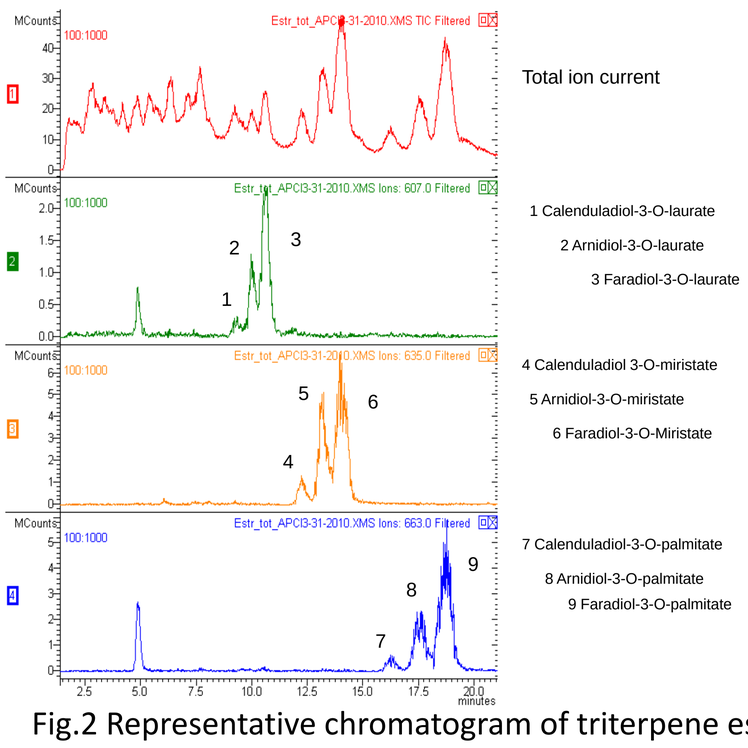

Fig.2 Representative chromatogram of triterpene esters

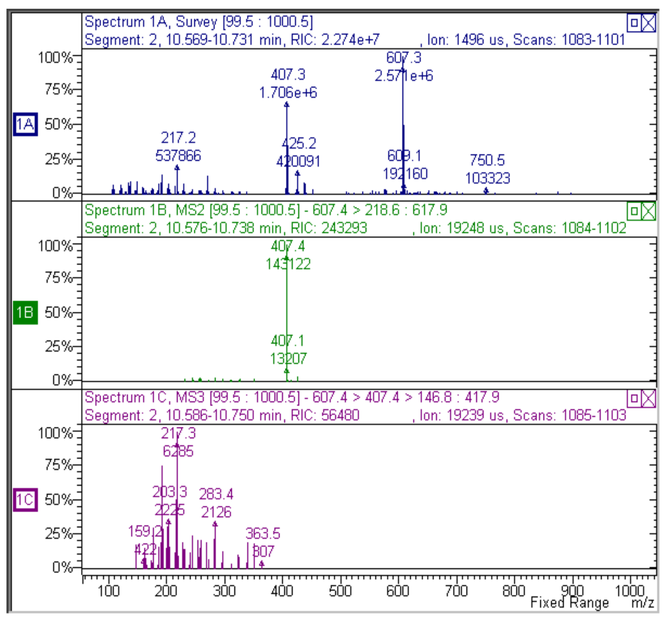

Fig.3 MS/MS of faradiol-3-O-laurate

Tab. 2: Extracts from maceration compared to CO_2-etxraction

Compound	Relative amount		
	Maceration sample 1	**CO_2**	Maceration sample 2
Calenduladiol-3-O-laurate	0	**0,059**	0,0013 ± 0.0001
Arnidiol-3-O-laurate	0	**0,145**	0,0028 ± 0.0001
Faradiol-3-O-laurate	0,0094 ± 0.0001	**0,787**	0,0014 ± 0.0001
Calenduladiol-3-O-miristate	0,0035 ± 0.0001	**0,293**	0,0014 ± 0.0001
Arnidiol-3-O-miristate	0,0087 ± 0.0001	**0,809**	0,0029 ± 0.0001
Faradiol-3-O-miristate	0,0289 ± 0.0005	**2,530**	0,0008 ± 0.0001
Calenduladiol-3-O-Palmitate	0,0013 ± 0.0001	**0,132**	0,0010 ± 0.0001
Arnidiol-3-O-Palmitate	0,0031 ± 0.0001	**0,455**	0
Faradiol-3-O-Palmitate	0,0116 ± 0.0008	**1,285**	0
Total triterpene amount	0,0665 ± 0.0001	**6,440**	0,0116 ± 0.0001

Comparison of CO_2 extract with samples obtained by maceration (ethanol 50 %) clearly show the efficiency of the extraction of triterpene esters with this techniques (tab. 2; fig. 4).

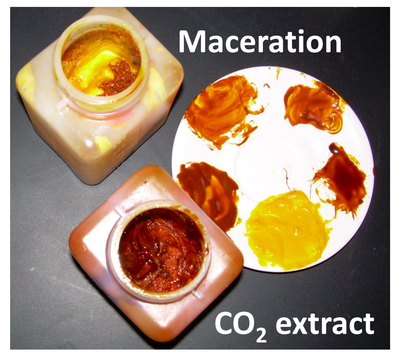

Fig.4: Samples of extracts

3.2 Efficiency of the extracts in cosmetic applications

Extracts were used to prepare cosmetic with lenitive and antiinflammatory properties. In vitro tests on models of artificial skin showed the antiinflammatory effects of cosmetic preparations containing the extracts.