1st International Congress on Analytical Chemistry, Electrochemistry and Separation Techniques
October 15th-16th, 2022

CARUM CARVI SEEDS ESSENTIAL OIL FROM CONVENTIONAL TO GREEN EXTRACTION: CHIRAL HPLC SEPARATION OF ITS ENANTIOMERS

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Introduction

The medicinal plants and essential oils from aromatic are unlimited source of new "lead compounds" especially, single enantiomers which are relatively inexpensive chiral compounds and have recently received a particular attention for exhibiting antioxidant and anticancer properties.

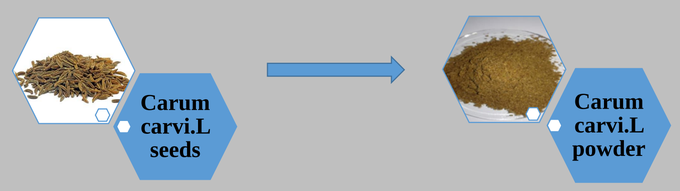
Carum Carvi L or caraway seeds, which belongs to the Apiaceae family, is one of the earliest cultivated herbs in Asia Africa and Europe[1]. The principle content of caraway extract is (S)-(+)- carvone (50–60%) and (R)-(–)-limonene (30-40%)[2]. Moreover, Caraway species contain from 1 to 6% of essential oils that give the caraway its characteristic aroma[3].

Objective

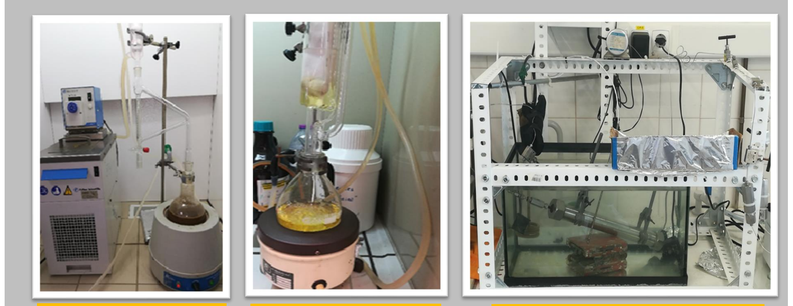
Our work was focused on the Carum carvi.L plant in order to extract a bioactive compounds such as monoterpene essential oils content (S)-Carvone and (R)-Limonene. Our aim was maximized the yield of the major compounds of the EOs, different techniques were employed named a Clevenger hydrodistillation (HD), Soxhlet extraction (SE), and supercritical fluid extraction (SFE). Beside, we have optimized the chromatographic conditions chiral HPLC to separate the enantiomeric essential oil mixtures.

Materials and Methods

1 Pre-treatment Extraction



2 Essential oil Extraction



Hydrodistillation (HD) | Soxhlet extraction (SE) | Supercritical fluid extraction (SFE)

Figure1. Pre-treatment and essential oil extraction procedures.

Table 1. The extraction conditions of Carum carvi volatile oil.

Parameter	HD	SE	SFE (grinded)	SFE (seeds)
Ratio of 1:10 (w/v)	100 g	25 g	75 g	75 g
Solvent	Water	Hexane	Carbone dioxide	Carbone dioxide
Temperature (°C)	100	68.5	35	35
Pressure (bar)	-	-	125	125
Time (hrs)	3	3 to 6	2	2

3 Chemical components analysis

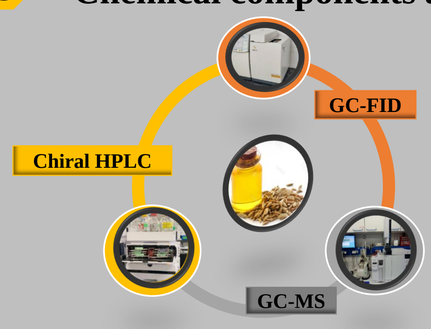


Figure2. Essential oil analysis by GC-FID, GC-MS and Chiral HPLC

Results and Discussion

1 Identification of essential oils extracted

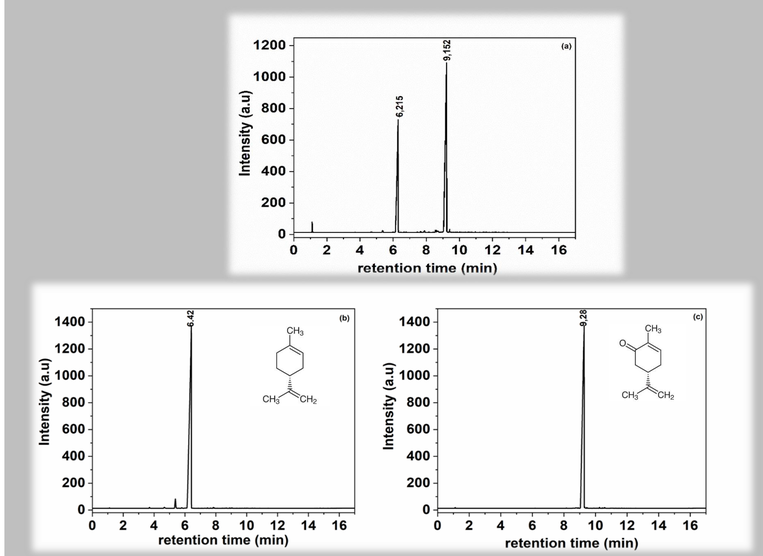


Figure 3. (a) GC-FID analysis of the essential oil (b) (R)-limonene and (c) (S)-carvone

- GC-FID analysis showed the presence of two major picks corresponding to (R)-limonene at retention time 6.215 min and (S)-carvone at retention time of 9.152 min, respectively compared with the standard solutions of (S)-(+)-Cravone and (R)-(+)-Limonene were injected in the same conditions analysis (Fig 3).

Table 2. The GC-MS and GC-FID results of the chemical identification of all of the extracts

	GC-FID GC-MS											
	Hydrodistillation (HD)		Soxhlet (SE)		Supercritical Fluid Liquid (SFE)				Etalon Standard			
					Powder		Seeds		R-Limonene		S-Carvone	
	RT (min)	Area (%)	RT (min)	Area (%)	RT (min)	Area (%)	RT (min)	Area (%)	RT (min)	Area (%)	RT (min)	Area (%)
α-Pinene	3.63	0.02	0.95	0.2	3.64	0.02	6.09	4.23	3.70	0.15	6.17	0.01
Sabinene	4.62	0.08	1.08	11.2	4.58	0.04	9.01	34.46	4.66	0.25	8.15	0.04
β-Myrcene	5.28	0.32	1.10	4.2	5.29	0.32	14.75	5.04	5.38		8.55	0.05
Octanal	5.92	0.11	5.28	0.3	5.93	0.02	15.73	19.02	5.59	0.02	8.90	0.28
P-Cymene	6.21	36.52	6.13	35.1	6.23	31.8	15.81	32.14	5.79	0.14	9.28	98.82
Limonene	6.40	0.01	6.60	0.0	6.41	0.01	15.95	0.49	6.01	0.10	9.45	0.13
β-Cis-ocimene	6.60	0.05	6.72	0.1	6.61	0.05	15.97	0.73	6.41	96.66	9.67	0.02
γ-Terpinene	6.72	0.14	7.62	0.1	6.72	0.05	16.54	0.24	7.08	0.01	9.90	0.04
β-Linalool	7.40	0.04	8.60	0.3	7.41	0.03	16.70	0.33	7.31	0.02	10.01	0.02
Trans-p-mentha-2,8-dienol	7.60	0.08	8.87	0.3	7.61	0.1	19.24	3.3	7.45	0.05	10.12	0.04
-	7.85	0.17	9.05	37.3	7.78	0.24			7.66	0.03	10.25	0.13
P-mentha-8-en-2-ol	8.11	0.08	9.34	0.2	8.37	0.02			7.83	0.18	10.59	0.20
Dihydrocarvone	8.56	0.60	10.02	0.1	8.50	0.55			8.40	0.02	10.97	0.02
Trans-p-mentha-6,8-dien-2-ol	8.77	0.02	10.93	0.1	8.95	0.15			8.68	0.05	10.06	0.04
Dihydrocarveol	8.94	0.17	14.77	1.2	9.16	42.98			8.90	0.11	11.26	0.02
Carvone	9.15	59.92	15.75	4.3	10.01	0.17			9.87	0.02	11.35	0.04
1-Cyclohexane-1-carbaxeldehyde	9.35	0.52	15.83	4.8	10.71	0.03			9.99	0.03	11.66	0.05
β-Elemene	9.54	0.26	17.04	0.2	10.93	0.06			10.38	0.02	11.81	0.02
Trans-caryophyllene	9.70	0.06			11.42	0.13			14.76	0.02	12.10	0.02
-	10.11	0.04			12.34	0.12			14.96	0.01	17.06	0.01
-	10.71	0.09			12.66	0.1						
-	10.92	0.06			13.46	0.12						
	11.41	0.08			13.76	0.09						
	11.54	0.04			14.12	0.04						
	12.34	0.15			14.51	0.02						
	12.63	0.08			14.61	0.09						
	12.85	0.03			14.82	1.85						
	13.09	0.06			15.08	0.05						
	14.03	0.01			15.6	0.07						
	14.75	0.04			15.88	9.48						
					15.93	8.59						
					16.70	0.05						
					16.79	0.09						
					16.90	0.07						
					17.05	0.14						
						Total		100.00				

2 Effect of extraction technique on the chemical composition of the volatile oil

Table 3. Comparison the main major components of essential oil .

Procedure	HD	SE	SEF	
			All Seeds	Grinded seeds
Oil aspect	Pale yellow	Greenish-brown	Brownish-yellow	Brownish-yellow
Yield (%)	**3.85 ± 0.7**	2.92 ± 0.5	1.37 ± 0.5	1.37 ± 0.5
(S)-carvone (%)	**59,53**	32,99	38,62	40,09
(R)-limonene (%)	**39,134**	23,03	2,65	29,61

- The best results remains the HD present a higher yield 3.85 % than the other two processes SE and SFE.

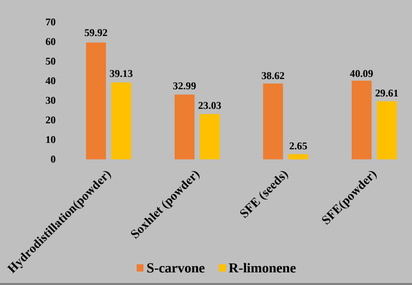


Figure 4. Comparison the main major components of Eos.

- HD recovered a higher percent of (S)-carvone (59.53 %) and (39.14 %) of limonene that were also highly present in SFE extracts of integral seeds (Fig 4).

3 Chiral HPLC separation

Table 4. Chromatographic conditions optimized for a reverse and normal phase HPLC.

Condition	Reverse Phase (RP-HPLC)	Normal Phase (Chiral HPLC)
Column	C18 column (30mm × 4.6 mm × 5µm)	Chiralpake IB Column cellulose tris(3,5-dimethylphenyl-carbamate (250mm ×4.6mm × 5µm)
Mobile phase	Acetonitrile/water(70:30 v/v, HPLC grade)	Hexane/isopropanol(60:40 v/v, HPLC grade)
Flowrate	1 ml/min	0.5 ml/min
Wavelength	304 nm	220 nm
Injection	20 µl	5 µl

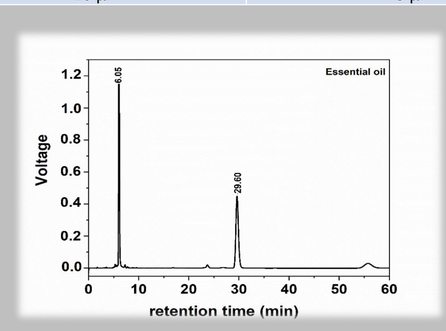


Figure 5. Chromatogram of Eos analysis in RP-HPLC

- RP-HPLC results showed two peaks corresponding to (S)-carvone appeared first at 6,05 min and (R)-limonene at 29,60 min, respectively. The separation was dependent on their polarity.

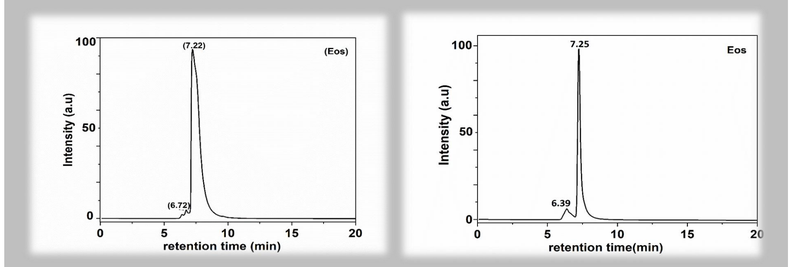


Figure 5. Chromatogram of Eos separation in chiral HPLC at wavelength detection 304 nm (left) and 220 nm (right)

- The chiral HPLC results showed a fail separation of Eos performed under wavelength 304 nm, However, a good separation of Eos with high resolution at the detection wavelength 220 nm.

Conclusion

- All extraction techniques have provided good results and the best results remain that HD present a higher percent of oil recovery.
- The powerful of SFE Carbone dioxide to extract directly the essential oil from the seeds without crushing or prior treatment performed before the extraction compared to HD and SE techniques.
- The chiral HPLC method was suitable to applied for the routine analysis in quality control laboratories.

References

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Acknowledgement

This work was supported by the Faculty of Chemistry of University of Science and Technology Houari *Boumediene*.We are thankful to NOVA University of Lisbon (FCT/UNL) for the financial support from Erasmus+ EU international credit mobility 2017–2019.