SUPPLEMENTARY MATERIAL

Comparison Between Several Techniques Of Olive Tree Bark Extraction (Tunisian Chemlali Variety)

Aimen Issaoui ^a, Hatem Ksibi ^{b,*}, Mohamed Ksibi ^{a,c}

^a LEEE, ENIS, P. Box W, Sfax 3038, Tunisia

^b Sfax University, IPEIS, P. Box 1172, Sfax 3018, Tunisia

^c Sfax University, ISBS, P. Box 1175, Sfax 3038, Tunisia

* Corresponding author: hatem.ksibi@ipeis.rnu.tn

Abstract:

In order to better understand the chemical composition of the olive tree bark of Tunisian chemlali variety (*Olea Europaea* cv. “Chemlali”), this material was extracted within different ways. Compositions of extracts were carried out at best-selected conditions for each technique, and characterized using HPLC, LC/MS, and GC-MS techniques. Analysis conducts to an important variety of high carbon number compounds such as aliphatic compounds as nanocosane and heptacosane, and molecules with high added value (VAT) which can be classified as follows: diterpenes as phytol, triterpenes as squalene, and also esters as Benzyl cinnamate. Hydrodistillation at high pressure seems the very common method to get a wide variety of compounds, the results are better than the ones obtained using SFE and solvent extraction.

Keywords:

Olive tree bark, Extraction, Hydrodistillation, Supercritical Fluid, Polyphenols, Chemical Composition

1. Experimental Section

1.1. Plant material

Bark tree of the “Chemlali” variety was collected in autumn 2013, grown in the region of Sfax (Tunisia). “Chemlali” is one of two main varieties in Tunisia. It covers almost 60% of the areas planted with olive and gives more than two-thirds of the oil production. The samples are always taken from the same tree, weighed 200g, and calibrated in order to compose a uniform batch of sheets which are formed for the extraction of phenolic compounds. These vegetable matrices have been dried in the shadow at room temperature (25°C), for 8 days. The obtained samples were gotten into the laboratory and dusted carefully, and then ground into small pieces (1-3 mm). The powder was vacuum packed until used. The material was comminuted before utilization.

1.2. Used Extraction Techniques

In literature, there are mainly three techniques for vegetable materials extraction: solvent extraction (SE); supercritical fluid extraction (SFE) and hydrodistillation extraction (HDE). These different extraction methods can affect the quality, aroma, and also appearance of oils, making it crucial to know the difference between them.

The solvent extraction of olive tree bark was conducted according to a protocol conventionally used for phenolic. Therefore the powder thus obtained is taken up with methanol-water (80:20, v/v). The solution is placed in a mechanical shaker along the night to stir at room temperature and at a speed of 200 rpm. The extract was filtered through a paper filter, and then evaporated to dryness and the residue is taken up in methanol. The refinement of phenolic compounds was conducted within two steps. First, after the removal of methanol in vacuo the crude extract at a temperature below 40°C, the aqueous layer was partially defatted by extraction with hexane. This aqueous phase is extracted three times with ethyl acetate (v/v 2:1). The organic extracts are then combined and concentrated by rotary evaporator to dryness to eliminate ethyl acetate. The residue is taken up in 5 ml of methanol to identify different constituents of the extract and also to find out total phenolics and antioxidant activity.

High-pressure hydrodistillation, 90 bars in this study, was used with this vegetable material where essential oils are difficult to extract. However, due to the high temperatures used, the risks of decomposition and degradation are even higher. Each sample (500 g) of the fresh and air-dried tree bark was subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus. The volatile components obtained after trapping in diethyl- ether were dried under anhydrous sodium sulfate, evaporated and concentrated under a mild stream of N₂ and stored at 4°C until verified and chemically analyzed.

The supercritical fluid extraction (SFE) was accomplished by using experimental pilot of University of Monserrato (Cagliari, Italy). Supercritical CO₂ extractions were performed in the laboratory apparatus equipped with a 400 cc extraction vessel, which operated in a single-pass mode by expanding CO₂ through the fixed bed charged with olive tree bark powder.

Let mention that the present study was conducted following several techniques of analysis mainly high-performance liquid chromatography (HPLC), liquid chromatography coupled to mass spectrometry (LC/MS), and Gas chromatography coupled to mass spectrometry (GC-MS).

2. Tables

Table S1 : Yield (%) phenolic compounds in **MeOH** of olive leaves and bark of Chemlali variety.

Matrices	yield (%)
leaves	6,77
Tree bark	10,33

Table S2 : anthocyanins content of the studied substances.

Matrices	leaves	Tree bark powder
Quantity (mg/kg)	63	981

Table S3 : DPPH Test Results (mg / ml)

Matrices	IC ₅₀ (mg/ml)
Olive leaves (MeOH)	29,46
Olive bark (MeOH)	14,84

Table S4 : Concentration and retention time of the phenolic compound extracted from the bark of olive .

Formula	Designation	Tr (mn)	Area	Concentration g/l
C₈H₁₀O₃	Hydroxytyrosol	12.667	5.53398	0.02830
C₈H₁₀O₂	Tyrosol	15.333	3.2075	0.01640
C₈H₈O₃	p-hydroxyphenylacetic Acid	18.450	2.47578	0.01266
C₈H₁₀O₄	vanillic Acid	22.467	39.6141	0.20264
C₉H₈O₄	caffeic acid	23.867	16.8255	0.08607
C₉H₈O₃	coumaric acid	28.133	7.19673	0.03681
C₈H₈O₃	Vanillin	32.100	18.9514	0.09694
C₁₀H₁₀O₄	ferulic acid	35.917	1.4299	0.00731
C₂₅H₃₂O₁₃	Oleuropein	36.467	755.866	3.866

Table S5 : Concentration and retention time of phenolic compounds extracted from olive bark by steam distillation .

R_{IK}	Tr (mn)	Compound	Formula	% Aera	Concentration g/l
1763	36,398	Benzyl benzoate	C14H12O2	4,9857	0,506
2086	46,925	Benzyl cinnamate	C16H14O2	14,3786	1,459
2112	47,699	Phytol	C20H40O	1,9463	0,1985
2300	53,080	Tricosane	C23H48	3,9559	0,4015
2403	55,869	Cinnamyl cinnamate	C18H16O2	15,7601	1,5996
2499	58,383	Pentacosane	C25H52	8,5644	0,8692
2700	63,402	Heptacosane	C27H56	13,0438	1,3239

Table S6 : Concentration and retention time of the phenolic compound extracted from the bark of olive supercritical CO2 P=90bar.

R_{IK}	Tr(min)	Compound	Formula	% Area
2100	47,336	Heneicosane	C ₂₁ H ₄₄	1,1436
2110	47,695	Phytol	C ₂₀ H ₄₀ O	2,1998
	48,941	N.I.		2,738
2300	53,080	Tricosane	C ₂₃ H ₄₈	6,0442
2350	54,436	4,8,12,16-Tetramethyl-heptadecan -4-olide	C ₂₁ H ₄₀ O ₂	0,9915
2400	55,782	Tetracosane	C ₂₄ H ₅₀	0,8949
2395	55,861	Cinnamyl cinnamate		1,9329
2500	58,396	Pentacosane	C ₂₅ H ₅₂	18,8583
2547	59,550	N.I.		1,588
	60,770	9-Hexacosene	C ₂₆ H ₅₂	0,9237
2600	60,892	Hexacosane	C ₂₆ H ₅₄	1,4989
2700	63,428	Heptacosane	C ₂₇ H ₅₆	32,5034
2828	67,354	Squalene	C ₃₀ H ₅₀	10,6882
2845	68,136	N.I.		2,3857
2867	68,980	N.I.		1,787
2900	70,234	Nonacosane	C ₂₀ H ₆₀	5,894
2919	71,157	N.I.		2,6623
3100	81,330	Untriacontane	C ₃₁ H ₆₄	5,2656

Table S7 : Concentration and retention time of the phenolic compound extracted from the bark of olive supercritical CO₂ P=250bar.

R _{IK}	Tr (mn)	Compound	Formula	% Area	Concentration mg/ml
2699	63,397	Heptacosane	C ₂₇ H ₅₆	5,4675	0,5546
2897	70,121	Nonacosane	C ₂₉ H ₆₀	4,0181	0,4078
3099	81,277	Untriacontane	C ₃₁ H ₆₄	19,6574	1,9952

3. Figures

Figure S1: Olive tree bark

Figure S2: GC -MS chromatogram of the extract of olive tree bark by steam distillation.

Figure S3- Comparison of Hydrodistillation-Supercritical fluid Extraction processes