National University of Science and Technology POLITEHNICA Bucharest

Doctoral School of Chemical Engineering and Biotechnologies

PhD Thesis

Obtaining and characterizing new products derived from medicinal mushrooms or from hydrosol-type residues.

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CONTEXT AND STRUCTURE: MOTIVATION FOR THE RESEARCH AND THESIS ORGANIZATION

This thesis is the result of a close and fruitful collaboration between HOFIGAL Export-Import S.A. and the Politehnica University of Bucharest (UPB), demonstrating the synergistic potential of partnerships between industry and academia. This endeavor was made possible, in particular, by the effective coordination of PhD students employed at HOFIGAL by renowned professors and researchers from UPB, who provided advanced scientific expertise and essential methodological guidance for the project's development.

HOFIGAL Export-Import S.A. has an active and dynamic research department that has achieved valuable results through its involvement in numerous applied research projects, such as PED (Demonstrative Experimental Projects), PTE (Experimental Technological Projects), and EUREKA, carried out in partnership with national research and development institutes (INCDs) and prestigious universities. These initiatives have enabled the exploration of innovative fields, contributing to the transfer of knowledge and technologies to the pharmaceutical and nutraceutical industries. The research topics addressed within these projects cover situations and needs identified as essential for HOFIGAL's development, such as optimizing the extraction processes of bioactive compounds from natural sources, evaluating the biological activity of plant and fungal extracts, or developing functional products with therapeutic potential, thereby ensuring the alignment of research with the company's strategic objectives.

Through this collaboration, the thesis not only strengthens the scientific foundation of HOFIGAL's research activities but also opens new perspectives for applying the results in production, promoting sustainable innovation in the fields of health and nutrition.

This doctoral thesis integrates two distinct research themes, each driven by specific objectives and aligned with the innovative needs of the pharmaceutical and nutraceutical industries within the context of HOFIGAL Export-Import S.A.'s activities. The first theme stems from a deep personal ambition focused on studying and valorizing medicinal mushrooms, such as *Pleurotus ostreatus* and *Lentinula edodes*, for the development of dietary supplements. This direction was inspired by the absence of a dedicated range of medicinal mushroom-based products in HOFIGAL's portfolio, providing an opportunity to explore their bioactive potential—including glucan content, antioxidant, antiproliferative, and antimicrobial activity—to create innovative solutions for preventive and therapeutic health.

The second theme addresses the valorization of hydrosols obtained as by-products from the extraction of volatile oils from medicinal plants, transforming these residues into a valuable resource with multiple applications. By evaluating their composition and bioactive properties, the research proposes uses in agriculture (as natural plant protection agents), ecology (to promote sustainable practices and waste reduction), and dietary supplements (as functional ingredients with antioxidant and immunomodulatory effects). Both themes underscore a commitment to sustainability and innovation, contributing to the expansion of HOFIGAL's expertise and the development of eco-friendly and effective products.

EXPERIMENTAL STUDY 1

1. Objectives of the Study

The first theme addressed in the doctoral thesis had the following objectives:

- Physicochemical characterization of the *Pleurotus ostreatus* species, both from a commercially acquired sample obtained through cultivation and from a sample collected from the spontaneous flora of Romania, as well as a sample of *Armillaria mellea* collected from the spontaneous flora, to evaluate their potential for gastronomy and the dietary supplement industry.
- Conducting a comparative study regarding the content of total carbohydrates, proteins, lipids, and the chromatographic profile of fatty acids in two wild mushrooms collected from spontaneous flora (*Pleurotus ostreatus* and *Armillaria mellea*) and a commercially cultivated *Pleurotus ostreatus* sample.
- Investigating the influence of extraction parameters in classical extraction, such as solvent, time, temperature, and particle size, on the ratio of α-glucans and β-glucans in lyophilized extracts of *Pleurotus ostreatus*.
- Studying the applications in colon cancer of *Pleurotus ostreatus* and *Lentinula edodes* powders, as well as preparations obtained through ethanol precipitation of liquid extracts derived from ultrasound-assisted extraction, by in vitro testing of antiproliferative properties on NCTC cells (normal fibroblast cell line), HT29 (colon tumor epithelial cell line), and CaCo2 (colon tumor epithelial cell line), antimicrobial inhibitory properties against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Enterobacter cloacae*, *Citrobacter freundii*, *Candida albicans*, and *Candida parapsilosis*, as well as testing their antioxidant capacity using the CUPRAC method.

2. Materials and Methods Used

2.1. Medicinal Mushrooms

The raw materials for *Pleurotus ostreatus* commercial (CPO) and *Lentinula edodes* were purchased from the local market. Wild *Armillaria mellea* (WAM) was collected from the Comana

Forest, Giurgiu County, in southern Romania, while wild *Pleurotus ostreatus* (WPO) was gathered from the Bușteni area, Prahova County. Additionally, a standardized extract containing 30% polysaccharides from HUNAN HUAKANG BIOTECH INC. (China) was tested in the experiments. The raw mushrooms were dried at 38°C using a Biovita-DEH600D dryer. Subsequently, the dried raw material was ground using a Retsch laboratory knife mill (model Grindomix GM 200).

2.4. Methodology Used to Assess the Influence of Extraction Parameters on the Ratio of α- and β-Glucans in Lyophilized Extracts of *Pleurotus ostreatus*

Influence of Solvent

To investigate whether the solvent affects the amount of β-glucans extracted from *P. ostreatus* powder, four solvent variants were compared: 1) H_2O; 2) 25% EtOH in H_2O (v/v); 3) 50% EtOH in H_2O (v/v); 4) 75% EtOH in H_2O (v/v). The extracts were obtained following the same protocol. A total of 3 g of dried *P. ostreatus* commercial powder was weighed using a Partner AS 310 analytical balance, and 90 mL of solvent was added. The mixture was left to macerate using a magnetic stirrer. After one hour, the samples were filtered using a Vacuubrand PC600 system and qualitative MN 617 we filter paper from MACHEREY-NAGEL. The supernatant was frozen at -80°C in an ARCTIKO ultra-low temperature freezer and then lyophilized using a Labogene CoolSafe touch 100-9.

Influence of Temperature

Temperature was another parameter studied in the process of obtaining extracts with high β-glucan content from *P. ostreatus*. The extraction was performed following the same protocol used to study the influence of solvent, at temperatures of 25°C, 50°C, and 70°C, using H_2O and a 75% EtOH in H_2O (v/v) solution as extraction solvents. After filtration, the supernatant was frozen at -80°C and lyophilized.

Extraction Time

To determine the optimal extraction time, four extracts were obtained. For their preparation, 3 g of *P. ostreatus* powder (weighed with an analytical balance) and 90 mL of solvent (H_2O) were subjected to mechanical stirring for 0.5 h, 1 h, 1.5 h, and 2 h at a temperature of 25°C. After extraction, the samples were filtered using the Vacuubrand PC600 system and qualitative MN 617 we filter paper from MACHEREY-NAGEL. The supernatant was then frozen at -80°C and lyophilized.

Particle Size

The dried *P. ostreatus* powder was separated using a Retsch analytical sieve shaker (model AS 200 Control) equipped with a 315 µm sieve into two fractions. Using the same protocol, 3 g of the sample with 90 mL of solvent (H_2O) and extraction with mechanical stirring for 1 hour at 25°C, followed by lyophilization, the influence of particle size was studied. Fractions larger than 315 µm

and fractions smaller than 315 μm were analyzed for the amount of extract obtained and the concentrations of β- and α-glucans in the extracts.

2.5. Obtaining Dry Extracts from *P. ostreatus* and *Lentinula edodes* by Ethanol Precipitation from Aqueous Extracts Obtained via Ultrasound-Assisted Extraction

The powder of *Pleurotus ostreatus* and *Lentinula edodes* was weighed using a Partner AS 310 analytical balance. A total of 50 g of the powders was placed in a 2000 mL flat-bottom flask, to which 500 mL of distilled H_2O was added. The flask was placed in an Elmasonic P180 ultrasonic bath, and the mixture was treated for 30 minutes in pulse mode, using a frequency of 80 kHz and 100% power, with periodic stirring. During the extraction, the water temperature in the bath increased from 20°C to 33°C. After 30 minutes, the aqueous extract was filtered using a Vacuubrand PC600 pump. Then, 1000 mL of EtOH was added to the aqueous extract. The mixture was stirred for 10 minutes and subsequently left to settle in a separatory funnel until complete sedimentation of the suspended particles. The precipitate was selectively collected and centrifuged using an Ortoalresa centrifuge at 9000 rpm for 3 minutes. The sediment was separated and dried at 40°C.

3. RESULTS AND DISCUSSION

3.2.2. Results on the Influence of Solvent

In the present study, we observed that the solvent significantly influences the composition of the lyophilized extracts obtained. As demonstrated in Figures 1.29 and 1.30, aqueous extracts exhibited notably higher concentrations of α-glucans compared to ethanolic extracts, while higher levels of β-glucans were extracted in ethanolic solutions. This phenomenon can be attributed to the hydrophilic nature of α-glucans, which enhances their solubility in aqueous solvents. These findings are of paramount importance for the development of selective extraction methods targeting either α-glucans or β-glucans, given their significant structural differences and the potential variation in their therapeutic effects depending on the intended application.

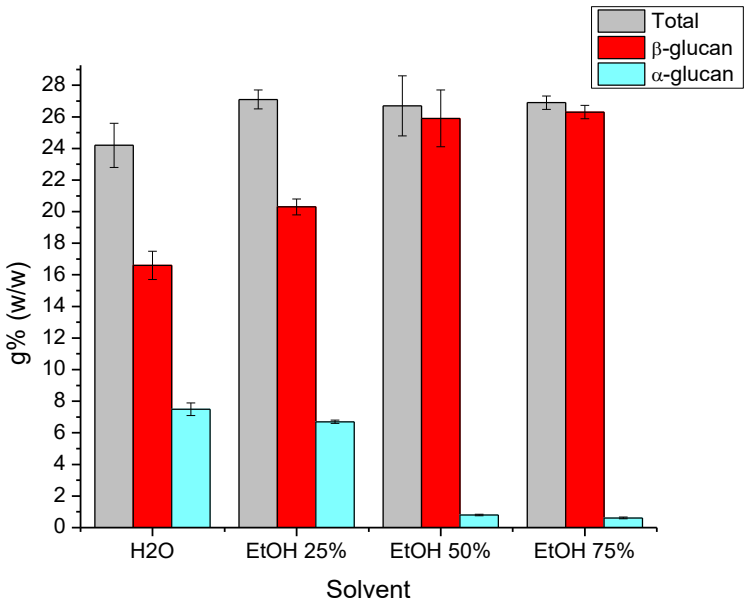


Figure 1.29. Distribution of α-, β-, and Total Glucans in Lyophilized Pleurotus ostreatus Extracts Obtained in Different Solvents

The solvent-dependent extraction of glucans observed in this study corroborates the findings of Pérez-Bassart et al. (2023) [1], who employed a sequential extraction protocol (cold and hot aqueous extractions followed by alkaline extractions) on mushrooms such as Pleurotus ostreatus, Lentinula edodes, and Grifola frondosa. Their work revealed that water-soluble β-glucans were predominantly extracted in the aqueous fractions, particularly from P. ostreatus, while recalcitrant β-glucans from G. frondosa remained in the residue. Similarly, the aqueous extracts obtained in the present thesis study favored the extraction of α-glucans, suggesting that water effectively solubilizes these polysaccharides, likely due to their linear α-1,4 or α-1,3 linkages, which are structurally less complex than the branched β-1,3/1,6 linkages of β-glucans [1]. The higher β-glucan content in the ethanolic extracts from our study may reflect the reduced solubility of β-glucans in ethanol, allowing for their selective precipitation or retention, a phenomenon also observed in the ethanolic purification steps by Shaheen et al. (2021) [2], where ethanol precipitation enriched the β-glucan content following alkaline extraction.

3.2.4. Results on the Influence of Extraction Time

The observed change in the β-glucan to α-glucan ratio during prolonged magnetic stirring at 25°C, followed by lyophilization, as shown in Figure 1.31, suggests that processing conditions can influence extraction. The presented results indicate that, while differences between extractions of 0.5 h and 1 h were relatively minor, a linear trend emerged in the alteration of glucan composition in the lyophilized extracts, characterized by an increase in the α-glucan content relative to the β-glucan content. This trend became much more pronounced in extracts obtained after 1.5 and 2 hours of extraction. These findings suggest that the duration of extraction significantly affects the structure, solubility, or detectability of these polysaccharides.

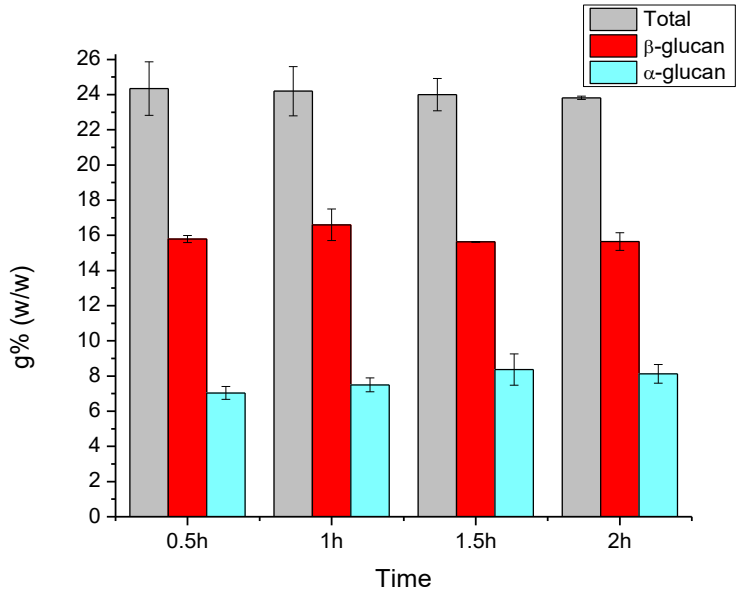


Figure 1.31. Distribution of α-, β-, and Total Glucans in Lyophilized Pleurotus ostreatus Extracts Obtained Using H_2O as Solvent and Different Extraction Times

A plausible explanation for the observed decrease in β-glucan content is the degradation of these polysaccharides under prolonged mechanical stirring. β-Glucans, characterized by β-1,3 and β-1,6 glycosidic linkages, are susceptible to chain cleavage when subjected to shear forces in aqueous environments. Such mechanical stress can fragment high molecular weight β-glucans into smaller oligosaccharides or glucose units. This phenomenon has been documented in cereal β-glucans, where Tosh et al. (2004) [3] reported that mechanical processing reduced the molecular weight of β-1,3/1,4-glucans from oats, altering their functional properties and detectability [3]. Although fungal β-glucans differ in linkage patterns, their branched structures may be similarly vulnerable to shear, particularly during extended stirring. The slight reduction in β-glucan content observed in our study supports this hypothesis, suggesting that prolonged stirring compromises the integrity of β-glucans.

The increase in α-glucan content may reflect improved extraction efficiency over time. α-Glucans, often present as starch-like polysaccharides with α-1,4 and α-1,6 linkages, are highly hydrophilic and readily solubilize in aqueous systems. Prolonged stirring likely enhances the disruption of the fungal cell matrix, facilitating the release of α-glucans that are tightly bound or less accessible during shorter extraction periods. Wood (2010) noted that aqueous extraction of β-glucans from cereals is time-dependent, with extended processing improving the yield of soluble polysaccharides [4].

3.3.1. Results on Obtaining Preparations by Ethanol Precipitation

The extracts were obtained by ultrasonication using a frequency of 80 kHz for half an hour, followed by filtration. The aqueous extract from *Pleurotus ostreatus* had a yellow color, while the extract from *Lentinula edodes* was reddish-brown, both extracts being very clear after filtration. Immediately upon the addition of EtOH, a precipitate began to form in the mixture in the form of crystalline flakes, as can be observed in Figures 1.33 and 1.34.



Figure 1.33. Precipitate Formed Following the Addition of EtOH to the Liquid Extract of *Pleurotus ostreatus*

After separating the sediment using a separatory funnel, the precipitate was centrifuged, the supernatant was removed, and the sediment was dried at 40 °C. The resulting preparations were analyzed for the content of total glucans, alpha-glucans, and beta-glucans, alongside the raw materials and a standardized extract with 30% polysaccharides of commercial origin from China, with the results presented in Table 1.4.

Table 1.4. Content of α-, β-, and Total Glucans in Samples P1 - Pleurotus ostreatus, P2 - Precipitate Obtained from Pleurotus ostreatus Extract, P3 - Lentinula edodes, P4 - Precipitate Obtained from Lentinula edodes Extract, P5 - Commercial Standardized Extract with 30% Polysaccharides

Sample	α-glucani g/100g	β-glucani	Total glucan
P1	4,28±0,12	36,1±0,57	40,3±0,69
P2	19,18±0,59	2,39±0,68	21,58±0,09
P3	2,87±0,70	34,88±2,67	37,75±3,37
P4	19,88±0,44	2,16±2,34	22,05±1,89
P5	49,97±0,35	1,68±0,56	51,65±0,21

3.3.2. Results on In Vitro Testing of Mushroom Extract Samples on Normal NCTC Fibroblast Cells and HT-29 and CaCo2 Colon Tumor Cells Using the MTT Method

The antiproliferative results on HT-29 cells, correlated with the glucan content of the samples, reveal a complex relationship between polysaccharide composition and biological activity, highlighting the predominant role of β-glucans in selective anticancer effects, as well as the potential contribution of α-glucans in purified fractions. Studies indicate that aqueous polysaccharide extracts from *Pleurotus ostreatus* reduce HT-29 cell viability through selective oxidative stress, even when α-glucans predominate, suggesting synergy with other components.

The more moderate results for *Pleurotus ostreatus* powder, with an HT-29 cell viability of 74.82% at 1000 µg/mL after 48 hours, can be attributed to its mixed composition, where β-glucans are present but diluted by other matrix components, leading to lower efficiency compared to purified fractions. Nevertheless, the antiproliferative effect remains significant, supported by the antioxidant and anti-inflammatory properties of mushrooms, which inhibit tumor growth without inducing general cytotoxicity.

Table 1.5. Antiproliferative Activity of P1 - *Pleurotus ostreatus* Powder, P2 - Precipitate Obtained from *Pleurotus ostreatus* Extract, P3 - *Lentinula edodes* Powder, P4 - Precipitate Obtained from *Lentinula edodes* Extract, and P5 - Commercial Standardized Extract with 30% Polysaccharides

Sample	Concentration	NCTC		HT29		CaCo2	
		24h	48h	24h	48h	24h	48h
1. *P. Ostreatus* powdered	50µg/mL	98.55	101.14	100.76	90.59	97.60	92.70
	100µg/mL	99.73	101.81	103.67	95.66	101.48	96.86
	300µg/mL	107.63	101.99	101.84	101.24	100.55	99.18
	500µg/mL	111.35	103.91	99.77	89.63	97.41	89.49
	800µg/mL	102.36	102.95	99.16	87.10	97.04	83.41
	1000µg/mL	90.83	82.95	99.08	74.82	96.67	80.68
	1500µg/mL	62.76	30.48	94.18	69.80	95.84	78.09
	2000µg/mL	35.15	29.04	71.05	51.66	92.52	74.40
2. *P. Ostreatus* EtOH precipitate	50µg/mL	106.81	100.90	112.63	121.01	99.26	95.56
	100µg/mL	107.72	103.85	120.52	126.14	104.06	101.64
	300µg/mL	108.45	107.65	128.33	120.84	113.94	104.78
	500µg/mL	101.36	116.75	119.83	117.01	113.57	107.64
	800µg/mL	100.82	101.32	118.99	116.84	119.85	98.43
	1000µg/mL	100.18	98.25	114.16	113.18	118.93	97.61
	1500µg/mL	96.55	96.14	111.79	97.01	101.85	95.49
	2000µg/mL	89.37	80.00	99.69	90.48	95.01	85.73
3. *Lentinula Edodes* powdered	50µg/mL	98.44	87.56	100.08	107.61	95.40	90.15
	100µg/mL	95.50	83.67	105.10	108.72	95.91	93.57
	300µg/mL	87.23	80.62	105.18	107.27	116.46	90.77
	500µg/mL	50.36	39.12	104.63	102.81	112.70	86.25
	800µg/mL	49.84	28.56	103.92	85.71	108.18	63.82
	1000µg/mL	36.81	27.00	99.29	73.16	105.73	53.49
	1500µg/mL	29.42	26.17	76.77	50.29	90.08	37.89
	2000µg/mL	26.44	25.44	54.94	28.10	71.57	29.21
	50µg/mL	113.68	102.57	105.65	111.97	99.39	91.38
	100µg/mL	106.79	101.23	106.91	104.84	113.60	92.00

4. *Lentinula Edodes EtOH precipitate*	**300µg/mL**	**103.86**	**95.07**	**107.77**	**103.39**	**125.56**	**94.80**
	500µg/mL	**100.92**	**86.91**	**111.62**	**101.65**	**137.52**	**90.63**
	800µg/mL	**98.07**	**81.20**	**106.28**	**90.50**	**160.53**	**75.85**
	1000µg/mL	**90.54**	**80.30**	**101.88**	**86.77**	**153.78**	**74.69**
	1500µg/mL	**84.94**	**61.89**	**68.68**	**49.90**	**146.11**	**49.32**
	2000µg/mL	**31.77**	**30.14**	**38.54**	**28.78**	**117.38**	**45.55**
5. *P. Ostreatus 30%* Polysaccharides Extract	**50µg/mL**	**99.56**	**101.83**	**101.15**	**95.82**	**105.83**	**104.49**
	100µg/mL	**100.78**	**104.41**	**102.88**	**100.74**	**111.45**	**108.73**
	300µg/mL	**101.82**	**99.05**	**103.6**	**101.64**	**124.45**	**109.32**
	500µg/mL	**109.20**	**95.02**	**103.75**	**102.95**	**123.31**	**110.73**
	800µg/mL	**102.60**	**91.49**	**117.17**	**103.77**	**121.85**	**107.07**
	1000µg/mL	**100.26**	**85.62**	**110.37**	**104.09**	**112.90**	**101.00**
	1500µg/mL	**98.18**	**82.60**	**91.07**	**87.96**	**104.79**	**83.44**
	2000µg/mL	**94.62**	**80.40**	**65.99**	**55.69**	**85.44**	**65.56**

3.3.3.2. Quantitative Results Regarding Antimicrobial Activity

The quantitative test results allowed for the determination of Minimum Inhibitory Concentrations (MIC) and Minimum Inhibitory Concentrations for Adhesion to Inert Substrates (CMIA) for each tested sample, with tested concentrations starting at 5 mg/mL. These values are included in Table 1.7.

The quantitative results of the antimicrobial tests, which determined the MIC and CMIA values, highlight the selective potential of the precipitate obtained through ultrasound-assisted aqueous extraction and ethanol precipitation from *Pleurotus ostreatus* (sample P2). This sample exhibited MIC and CMIA values below 5 mg/mL for all tested strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* 145 – clinical strain, *Salmonella typhi* ATCC 14028, *Enterobacter cloacae* 5479 – clinical strain, *Citrobacter freundii* 22 – clinical strain, *Candida albicans* ATCC 10231, and *Candida parapsilosis* ATCC 22019), confirming the qualitative observations of clear inhibition zones. This broad-spectrum efficacy against Gram-positive and Gram-negative bacteria, as well as fungi, suggests a multifactorial action, likely mediated by bioactive compounds concentrated through the extraction process, which disrupt microbial cell walls and inhibit biofilm formation [5].

Table 1.7. MIC and CMIA Values (mg/mL) Determined for Each Tested Microbial Strain for P1 – *Pleurotus ostreatus*, P2 – Precipitate Obtained from *Pleurotus ostreatus* Extract, P3 – *Lentinula edodes*, P4 – Precipitate Obtained from *Lentinula edodes* Extract, P5 – Commercial Standardized Extract with 30% Polysaccharides

Bacterial strains	**CMI and CMIA values expressed mg/ml**									
	P1		**P2**		**P3**		**P4**		**P5**	
	CMI	**CMIA**	**CMI**	**CMIA**	**CMI**	**CMIA**	**CMI**	**CMIA**	**CMI**	**CMIA**
S. aureus ATCC 25923	>5	>5	2.5	2.5	>5	>5	>5	5	>5	5
E. coli ATCC 25922	>5	>5	1.25	0.625	>5	>5	5	5	>5	>5
K.pneumonia – clinical strain	5	5	0.625	0.3125	>5	>5	5	2,5	5	5
S. typhi ATCC 14028	>5	>5	1.25	1.25	>5	>5	5	5	>5	>5
E. cloacea 5479 – clinical strain	>5	>5	2.5	2.5	>5	>5	>5	5	5	5
C.freundii 22 – clinical strain	>5	>5	1.25	0.625	>5	>5	>5	>5	5	5
C.albicans ATCC 10231	5	5	1.25	0.625	>5	>5	5	5	>5	>5
C.parapsilosis ATCC 22019	5	5	1.25	0.625	>5	>5	5	5	>5	>5

Similar studies have reported low MIC values (below 5 mg/mL) for extracts of *P. ostreatus* against *E. coli*, attributed to polysaccharides and phenols that induce oxidative stress and inhibit adhesion [6].

Correlating with the glucan composition, the precipitate P2 (21.58 g/100g total glucans, with 19.18 g/100g α-glucans and 2.39 g/100g β-glucans) benefits from selective fractionation, where α-glucans improve solubility and bioavailability, allowing residual β-glucans to interact effectively with microbial membranes. β-glucans in *P. ostreatus.* They are known for antifungal and antibacterial properties, reducing adhesion by inhibiting cell wall synthesis enzymes [7].

B. EXPERIMENTAL STUDY 2

1. Research Objectives

The second theme addressed in the doctoral thesis had the following objectives:

- Characterization of hydrosols from sage (*Salvia officinalis*), lavender (*Lavandula angustifolia*), and rosemary (*Rosmarinus officinalis*) in terms of total polyphenol content, flavone content, their profile obtained via HPLC-DAD, volatile compounds via GC-MS, and their antioxidant capacity using various spectrophotometric methods.
- Determination of the acetylcholinesterase (AChE) inhibition capacity of sage, lavender, and rosemary hydrosols for their potential use as biopesticides in organic agriculture technologies.

- Obtaining and characterizing fennel seed hydrosols through hydrodistillation and steam distillation.
- Determination of the antimicrobial activity of the two fennel hydrosols against *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 9027) strains.

2. Materials and Methods

2.1. Plant Material

Sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), and lavender (*Lavandula angustifolia*) were harvested from organic crops of HOFIGAL Export-Import, and their aerial parts were air-dried at 25°C until constant weight and then ground. Fennel seeds (*Foeniculum vulgare* L.) were harvested from organic crops of HOFIGAL Export-Import S.A., Romania. They were dried, ground, and stored in a cool, dry place until the extraction process.

3. Results and Discussion

3.1. Characterization of Sage, Lavender, and Rosemary Hydrosols and Evaluation of Their Potential as Biopesticides

3.1.2. Results Regarding the Chemical Composition of Sage, Lavender, and Rosemary Hydrosols

The results of the GC-MS analysis of the hydrosols and the percentage of the main volatile compounds are detailed in Table 2.1. For sage hydrosol, camphor was the dominant component, but thujone and isothujone were also identified. For rosemary hydrosol, six volatile compounds were identified, with camphor, verbenone, and eucalyptol (1,8-cineole) being the most abundant. Lavender hydrosol exhibited a wide range of volatile compounds, particularly linalool and its derivatives, coumarin, α-terpineol, and caryophyllene oxide. These findings suggest that hydrosols obtained via steam distillation contain cyclic monoterpenes and their oxygenated derivatives, known for superior biological activity compared to acyclic monoterpenes [8]. The structure of volatile compounds in the examined hydrosols was comparable to that of their corresponding essential oils, although the levels and distribution of these components were lower (data not included).

The polyphenolic compounds identified and quantified by high-performance liquid chromatography (HPLC-DAD) for sage, rosemary, and lavender hydrosols are recorded in Table 2.2. Rosmarinic acid, characteristic of the Lamiaceae family, was present in all hydrosols, with a notable predominance in sage and rosemary hydrosols. Caffeic and ferulic acids were found in all samples, but in higher concentrations in sage hydrosol. Among flavonoids, quercetin, rutin, luteolin, and glycosylated derivatives of quercetin (isoquercetin) and kaempferol (astragalin) were detected in varying proportions. Cinaroside, a glycosylated derivative of luteolin, was identified exclusively in sage hydrosol.

Table 2.1. Composition of volatile compounds determined by GC-MS in sage, lavender, and rosemary hydrosols

Volatile compound	Aria %		
	Sage	Lavender	Rosemary
Camphene	-	-	4,20
Eucalyptol (1,8-Cineole)	-	-	15,42
Thujone	15,02	-	-
Isothujone	3,41	-	-
Camphor	81,57	-	37,52
Linalool Oxide	-	6,85	-
Trans-Linalool Oxide	-	5,97	-
Linalool	-	29,03	2,07
Linalyl Acetate	-	6,47	-
Verbenone	-	-	34,80
Borneol	-	-	6,01
4-Terpinen-ol	-	2,51	-
Cryptone	-	4,22	-
α-terpineol	-	14,21	-
Geraniol	-	2,62	-
Caryophyllene Oxide	-	9,83	-
Cuminol	-	2,82	-
Coumarin	-	15,47	-

Regarding the total polyphenol content (TPC) and total flavonoid content (TFC) for sage, rosemary, and lavender hydrosols, the analyses indicated that sage hydrosol had the highest polyphenol levels, followed by rosemary and lavender (Table 2.2). A similar trend was observed for flavonoid content, with sage hydrosol having a TFC value approximately twice that of rosemary and lavender hydrosols.

Table 2.2. Polyphenolic acids and flavonoids compounds determined by the HPLC-DAD method in sage, lavender, and rosemary hydrosols

Compound	Concentration µg/g d.w.		
	Sage	Sage	Sage
Gallic Acid	-	-	-
Chlorogenic Acid	-	-	-
Caffeic Acid	7,08±0,02	4,80±0,01	5,35±0,01
Ferulic Acid	10,70±0,02	4,89±0,02	0,86±0,01
Rosmarinic Acid	23,29±0,03	5,15±0,02	25,56±0,04
Rutin	5,66±0,01	-	8,32±0,02
Quercetin-3-Glucoside	2,77±0,01	3,17±0,01	6,85±0,02
Luteolin-7-Glucoside	2,95±0,01	-	-
Kaempferol-3-Glucoside	8,63±0,02	11,16±0,02	6,52±0,02
Quercetin	16,05±0,03	-	16,33±0,03
Luteolin	-	7,40±0,02	7,60±0,02
Kaempferol	-	-	-
TPC & TFC Results			
TPC (µg EAC/g d.w.)	325,49±17,05	216,36±13,84	267,11±19,70
TFC (µg EAC/g d.w.)	59,32±0,64	26,55±0,24	29,28±1,93

3.1.3. Results Regarding the Antioxidant Activity of Sage, Lavender, and Rosemary Hydrosols

In this research, the antioxidant properties of the hydrosols were investigated using four methods, with results detailed in Table 2.3. In the TEAC and CUPRAC tests, sage hydrosol exhibited the highest antioxidant activity, followed by rosemary, both significantly ($p < 0.05$) superior to lavender. In the DPPH test, sage and rosemary hydrosols showed comparable antioxidant capacity, superior to that of lavender hydrosol. Regarding the superoxide anion neutralization test, sage hydrosol demonstrated the highest inhibition efficiency, while lavender and rosemary hydrosols had similar but significantly ($p < 0.05$) lower performance.

Table 2.3. Results of the antioxidant activity of sage, lavender, and rosemary hydrosols using four different methods

Antioxidant Activity	Sage hydrosol	Lavander hydrosol	Rosemary hydrosol
TEAC µM ET/g	2.09 ± 0.07	1.17 ± 0.03	1.84 ± 0.09
CUPRAC µM ET/g	4.33 ± 0.23	2.77 ± 0.23	3.73 ± 0.17
DDPH inhibition µM ET/g	11.77 ± 0.63	9.67 ± 0.50	11.80 ± 0.59
Superoxid inhibition µM ET/g	3.22 ± 0.15	2.57 ± 0.12	2.62 ± 0.03
FRAP µM ET/g	0.54 ± 0.03	0.10 ± 0.01	0.14 ± 0.01

Similarly, sage hydrosol proved to be the most effective Fe2+ ion-binding agent, followed by rosemary and lavender hydrosols, which exhibited comparable levels. However, the data on metal chelating capacity suggested a lower antioxidant activity.

3.1.4. Results Regarding the Acetylcholinesterase Inhibition Capacity of Sage, Lavender, and Rosemary Hydrosols

The in vitro acetylcholinesterase (AChE) inhibition capacity in the presence of the studied hydrosols is illustrated in Figure 2.4. The data indicated that all hydrosols inhibited enzymatic activity in a manner proportional to the tested concentration. Rosemary hydrosol showed a rapid increase in AChE inhibition, from 10.65% at 2.5 mg/mL to 42.48% at 5 mg/mL. Within the same concentration range, sage and lavender hydrosols exhibited increases in AChE inhibition of 160% and 130%, respectively. At the 5 mg/mL level, rosemary hydrosol demonstrated significantly superior performance ($p < 0.05$) in inhibiting AChE compared to sage and lavender hydrosols. Its efficacy in blocking AChE was comparable to that of quercetin, used as a reference, suggesting a potential role as a neurological agent against pests. The IC50 values followed the same trend, with rosemary proving more effective (6.23 mg/mL), followed by sage (7.48 mg/mL) and lavender (15.08 mg/mL).

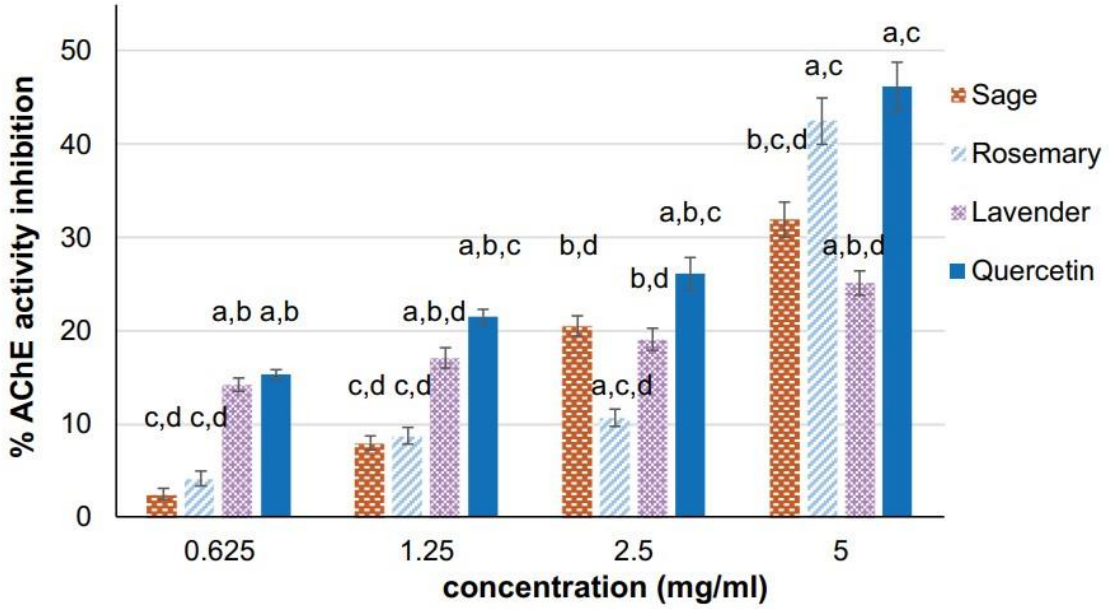


Figure 2.4. In vitro acetylcholinesterase (AChE) inhibition capacity of sage, rosemary, and lavender hydrosols. Values are expressed as mean ± standard deviation (SD) (n = 3).

GENERAL CONCLUSIONS

The purpose of this thesis is to valorize medicinal mushrooms and hydrosols from medicinal plants, utilizing residues from volatile oil extraction, for the development of dietary supplements and agricultural applications, thereby contributing to sustainable innovation and promoting a circular bioeconomy through resource reuse.

The conclusions that can be drawn based on the experiments and results obtained are:

- The studied mushroom species from Romania's wild flora and those cultivated industrially (*Pleurotus ostreatus* and *Armillaria mellea*) contain proteins, carbohydrates, lipids, and essential fatty acids, with the obtained values being consistent with the specialized literature and highlighting their potential for the dietary supplement industry.
- There are compositional differences between wild and industrially cultivated *Pleurotus ostreatus* regarding protein and carbohydrate content, with the industrially cultivated species being richer in carbohydrates and the wild species richer in proteins. These results indicate the need for standardizing raw materials for use in the dietary supplement industry.
- The extraction of glucans from *Pleurotus ostreatus* can be influenced by the studied extraction parameters, namely solvent, time, temperature, and particle size.
- For the extraction of β-glucans, it is recommended to use a solvent with a high EtOH concentration to enhance extraction efficiency, while using water as a solvent results in

precipitates dominated by α-glucans. Higher temperatures facilitate the extraction of β-glucans in lyophilized extracts from Pleurotus ostreatus. Prolonged extraction time in water at 25°C leads to larger quantities of lyophilized extract but does not significantly alter the α- and β-glucan ratio in the lyophilized extracts. Using a particle size fraction smaller than 300 μm results in significantly larger quantities of lyophilized extract but does not affect the α- and β-glucan ratio.

- Ethanol precipitation of aqueous extracts from Pleurotus ostreatus and Lentinula edodes, obtained through ultrasound-assisted extraction, yields preparations rich in α-glucans with only trace amounts of β-glucans.
- Both Pleurotus ostreatus powder and the commercially standardized 30% polysaccharide extract from Pleurotus ostreatus exhibited antiproliferative activity on the HT-29 cell line while maintaining a non-cytotoxic profile for normal NCTC cells.
- The precipitate obtained from the aqueous extract of *Lentinula edodes* and the commercially standardized 30% polysaccharide extract from *Pleurotus ostreatus* exhibited an antiproliferative effect on the CaCo2 cell line while maintaining a non-cytotoxic profile for normal NCTC cells.
- The precipitate obtained from the aqueous extract of *Pleurotus ostreatus* showed inhibitory effects against Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Klebsiella pneumoniae 145 (clinical strain), Salmonella typhi ATCC 14028, Enterobacter cloacae 5479 (clinical strain), Citrobacter freundii 22 (clinical strain), Candida albicans ATCC 10231, and Candida parapsilosis ATCC 22019, with MIC and MBC values lower than 5 mg/mL.
- All tested samples, including Pleurotus ostreatus and Lentinula edodes powders and precipitates from their liquid extracts, exhibited antioxidant activity, but with lower values compared to the commercially standardized 30% polysaccharide extract.
- Lavender, rosemary, and sage hydrosols demonstrated the ability to inhibit acetylcholinesterase.
- Fennel hydrosols exhibited antimicrobial activity.

PROSPECTS

The results obtained in this thesis, focused on the extraction and evaluation of the bioactivity of polysaccharides from medicinal mushrooms, open promising perspectives for the development of innovative products in the fields of health, nutrition, and the cosmetic industry.

Current limitations, such as selective extraction reducing β-glucan content in precipitates, suggest future directions: optimizing ultrasound-assisted extraction (UAE) with higher temperatures or alkaline solvents for improved yields, obtaining purified fractions of α-glucans and β-glucans, and conducting further in vitro studies to precisely identify which components are responsible for these effects and to investigate their inhibition mechanisms.

From a therapeutic perspective, the selective antiproliferative effects on colorectal cancer cell lines (HT29 and CaCo2) suggest applications in adjuvant oncology. These results, correlated with high antioxidant activity, indicate a role in reducing oxidative stress and inflammation associated with colorectal cancer (CRC), where β-glucans modulate the immune response, and α-glucans enhance absorption. Prospects include formulating targeted dietary supplements for CRC prevention, potentially integrated into functional diets, with future clinical studies to validate in vivo efficacy.

The antimicrobial activity, limited to the P. ostreatus precipitate against strains such as E. coli, K. pneumoniae, and C. albicans, highlights its potential as a natural alternative to antibiotics, particularly against microorganisms associated with gut dysbiosis in CRC. This could be leveraged in probiotic or antimicrobial products to promote gut health and reduce antimicrobial resistance.

In the context of a circular bioeconomy, the valorization of medicinal mushrooms—often underutilized in agriculture—enables the reuse of residual biomass, minimizing waste and generating value-added products, aligning with EU sustainability goals.

The results concerning hydrosols open new perspectives in organic agriculture. The next step involves field testing hydrosols as treatments and proposing their use on an industrial scale. These results could also be commercialized as a product aimed at reducing losses in organic agriculture caused by pests.

Integrating products derived from these results could expand the company's portfolio, offering eco-friendly solutions for preventive health. Overall, these findings position medicinal mushrooms and hydrosols as key resources in the bioeconomy, with significant economic and societal potential, paving the way for multidisciplinary innovations.

ORIGINAL CONTRIBUTIONS

The elements of originality identified as a result of this research are:

- Conducting a comparative study on the composition of commercially available Pleurotus ostreatus versus a specimen of Pleurotus ostreatus collected from Romania's wild flora.
- Performing a study on the influence of extraction parameters on the α- and β-glucan ratio in lyophilized extracts from Pleurotus ostreatus.
- Establishing protocols for obtaining medicinal mushroom-based preparations with antiproliferative and antimicrobial effects for applications in CRC therapy.
- Testing medicinal mushroom preparations with high α-glucan content and traces of β-glucans.
- Conducting a study on the applications of medicinal plant hydrosols in organic agriculture and the dietary supplement industry.

DISSEMINATION OF RESULTS

PUBLISHED ARTICLES **ISI**

1. A. Gaspar-Pintiliescu, E. Mihai, T. Ciucan, A. F. Popescu, C. Luntraru, **J. Tomescu**, O. Craciunescu , *Antioxidant and acetylcholinesterase inhibition capacity of hyrosols from lamiaceae plants for biopesticide use: role of phenolics*, INTERNATIONAL JOURNAL OF FOOD PROPERTIES, *25*(1), 981–993. **2022**, (**IF – 1.8, Q2**) – ISSN: 1094-2912, WOS: 000796075500001
2. A. Gaspar-Pintiliescu, A.-M. Perlipceanu, E. Mihai, T. Coican, R. E. Anton, A. F. Popescu, **J. A. Tomescu,** O. Craciunescu, , *Free radicals scavenging, acetylhydrolase inhibition and antimicrobial activity of aromatic plant hydrolate mixtures*, Journal of Food Measurement and Characterization, 16, 3589–3596. **2022** (**IF – 3.95, Q2**) – ISSN: 1934-7081, WOS: 000795297800001
3. D. Gheorghita, A. Robu, A. Antoniac, I. Antoniac , L. M. Ditu , A.-D. Raiciu**, J. Tomescu,** E. Grosu and A. Saceleanu , *In Vitro Antibacterial Activity of Some Plant Essential Oils against Four Different Microbial Strains*, Applied Sciences.; 22(10):434. **2022**, (**IF – 2.8, Q2**) – ISSN: 2076-3417, WOS: 000862108400001
4. C.M. Luntraru, L. Apostol, O.B. Oprea, M. Neagu, A.F. Popescu, **J.A. Tomescu**, M. Multescu, I.E. Susman, L. Gaceu, *Reclaim and Valorization of Sea Buckthorn (Hippophae rhamnoides) By-Product: Antioxidant Activity and Chemical Characterizati*, FOODS, 11(3), 462. **2022**, (**IF – 5.5, Q1**) – ISSN: 2304-8158, WOS: 000756896300001
5. G. Petrisor, L. Motelica, R. D. Trusca, A.-L. Mîrt, G. Vasilievici, **J.-A. Tomescu**, C. Manea, A. S. Dumbrava, V. M. Corbu, I. Gheorghe-Barbu , D. Ficai, O.-C. Oprea, B.-S.Vasile, A. Ficai and A. D. Raiciu, 2024, *The Antimicrobial Potency of Mesoporous Silica Nanoparticles Loaded with Melissa officinalis Extract,* Pharmaceutics, **2024**, 16, 525. (**IF – 4.9, Q1**) – ISSN: 1999-4923, WOS: 001201159100001
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7. M. Neagu, C. Luntraru, A, Bîra, **Tomescu J**., N. Ionescu, Stan R., 2021, *Bioactive phenolic compounds from white willow (salix alba) bark, leaves and branches*, University Politehnica of Bucharest Scientific Bulletin Series B-Chemistry and Materials Science , 83(3), 41-50, (**IF –, Q4**) – ISSN: 1454-2331, WOS: 000692192600004
8. B. Dangnon, D. Dah-Nouvlessounon, S. M. Ismaël Hoteyi, H. Sina, **J. A. Tomescu**, K. J.-M. Akpo, M. M. Sangare-Oumar, A. Adjanohoun, O. O. Babalola, E.Vamanu , "*Gastroprotective, Antioxidant, Anti-Inflammatory, And Toxicological Evaluation Of Stem Bark Extracts Of Vitellaria paradoxa And Parkia biglobosa*, Pharmaceuticals, **2025,** 18, 1184. https://doi.org/10.3390/ph18081184

9. **J. A. Tomescu**, V. Staicu, C. Luntraru, I. Calinescu*, "Comparative nutritional and fatty acid profiling of commercially cultivated and wild-harvested pleurotus ostreatus and armillaria mellea mushrooms", acceptat la publicare, U.P.B. Sci. Bull., Series B, **2025**

PUBLISHED ARTICLES BDI

1. E. Mihai, A. Gaspar-Pintiliescu, T. Ciucan, A.-M. Prelipcean, J. Tomescu, M. Neagu, O. Craciunescu, 2023, *Comparative study on the composition, antioxidant and antimicrobial activity of fennel hydrolates*, Scientific Bulletin. Series F. Biotechnologies, Vol. XXVII, No. 1, (**IF –, Q**) – ISSN: 2285-1364

2. V. Staicu, C. G. Chisega Negrila, J. A. Tomescu, M. Neagu, I. Calinescu, **2023**, *Separation and Purification of Plant Extracts by Removal of Chlorophyll, Xanthophylls and Pigments using Mineral Adsorbents in Liquid Chromatography (CLS)*, Current Trends in Engineering Science (CTES), 3(6),– ISSN: 2833-356X

3. V. Staicu, J. A. Tomescu, I. Calinescu, **2023**, *Environmental Protection and Superior Recovery of Vegetal Waste and Biomass, Major objectives in the Applied Research Activity*, Journal of biomedical research & environmental sciences,– ISSN: 2766-2276,

4. M. Andrei, A. Stefaniu, N. Ene, L. Pintilie, N. Radu, **A. J. Tomescu**, A. D. Raiciu, **2024**, *Multiple natural approaches of Salix alba*, Archives of Microbiology & Immunology, (8), 410-427

Conference Participations

1. Conference **NUTRICON**, Ohrid, Macedonia de Nord - Mihaela Neagu, Cristina-Mihaela Luntraru, Adriana Florina Popescu, **Justinian-Andrei Tomescu**, Livia Apostol, *Evaluation of the antioxidant capacity of sea-buckthorn (hippophae rhamnoides) berries by-product extracts for further valorization*.

2. Conference **SICHEM 2022**, București, România - **Justinian-Andrei Tomescu**, Livia Apostol, Theodor Trausan-Matu, Georgeta-Alexandru, Alexandru Suciu, Simona Craparescu, Mihaela Neagu, *Characterization of Valuable Nutraceutical Compounds from Sea Buckthorn and Blueberry Waste Extracts Destined for Functional Supplements Enrichment*

3. Conference **SICHEM 2022**, București, România - Vasile Staicu, **Tomescu Justinian-Andrei**, Theodor Trausan, and Calinescu Ioan, *Extraction, separation and dosage of ursolic acid from Rosmarinus officinalis using ultrasound.*

4. Conference **SICHEM 2022**, București, România - **Justinian-Andrei Tomescu**, Vasile Staicu, Theodor Trausan-Matu[1], Ioan Calinescu, and Christina-Marie Zalaru, *Time, temperature and solvent influence on polysaccharides content of p. Ostreatus extracts*

5. Conference **RICCCE 22**, București, România - Anca Daniela Raiciu**, Andrei Justinian Tomescu,** Cristina Manea, *Comparative Study on the Physico-chemical Characterization and Medical Properties of Volatile Oils of Cinnamon Bark & Cinnamon Leaf*

6. Conference **ULTRASONICS 2023**, Lisabona, Portugalia - **Justinian-Andrei Tomescu ,** Vasile Staicu, Simona Spînu, Alina Ortan, Daniela Ionescu, Theodor Trausan-Matu, Ioan Calinescu, 2023, *Practical applications and guidelines in the process of obtaining extracts rich in ursolic and oleanolic acid from Rosmarinus officinalis and Rosmarinus officinalis residue after the extraction of volatile oils using ultrasound and microwave assisted extractions*

7. Conference **XI INTERNATIONAL SIMPOSIUM FOOD TECHNOLOGY 2023**, Murcia, Spania - Livia Apostol, Mihaela Neagu, Cristina Manea, **Justinian Andrei Tomescu**, Mihai Bogdan Nicolcioiu, Nastasia Belc, *Recovering nutritional value from berries by-products*

Patent applications filed

1. CBI: a/2022/00228 - Suplimente alimentare recomandate pentru atenuarea simptomelor neplăcute ale menopauzei, procedeu de obținere si procedeu de stabilire a biosiguranței si eficacității biologice, Mihaela NEAGU, Cristina – Mihaela LUNTRARU, Alexandru SUCIU, **Justinian – Andrei TOMESCU**, Sevinci POP, Emilia MANOLE, Lucian ALBULESCU, Cristina TĂNASE , published BOPI 11/2023

2. CBI: A/00762/2023 ; 138836 A2-Produs de tip supliment alimentar pe baza de componente naturale ecologice si procedeu de obținere, Ionescu Daniela, Fierascu Radu Claudiu, Alina-Ruxandra-Eugenia Ortan, Buhaev Stefan, Fierascu Irina, Marcu-Spinu Simona, **Tomescu Justinian-Andrei**, Baroi Anda-Maria, Babeanu Narcisa, Trausan-Matu Theodor, Brazdis (Matei) Roxana Ioana, Fistos Toma; published BOPI nr. 05/2025

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