A HYBRID ULTRASOUND – MICROWAVE SYSTEM FOR PHYTOCOMPOUNDS EXTRACTION FROM *HEDERA HELIX* L.

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Emerging extraction technologies are promising approaches to address the drawbacks of the conventional process. This study presents a comparison between conventional extraction methods and the extraction of bioactive components from Hedera helix leaves using nonconventional techniques based on ultrasounds (UAE), microwaves (MAE), and a hybrid technology (ultrasonic and microwave extraction UMAE). Total phytocompounds content (polyphenols, saponins, and carbohydrates) and antioxidant capacity were determined. Advanced techniques like UAE, MAE, and their hybrid form UMAE, demonstrated significantly higher efficiency compared to conventional extraction. While both UAE and MAE are recognized as effective approaches, UMAE emerged as the most efficient method, yielding superior results in terms of extraction performance.

Keywords: microwave; ultrasound, ivy leaves, hybrid system, Principal component analysis.

1. Introduction

Hedera helix L., (ivy) is an perennial dioecious, arboreal climbing plant with yellow flowers, black fruits, and dark green leaves originating in Europe, America, and Asia [1; 2]. The ivy extracts are a promising source of bioactive compounds, including triterpene saponins (hederacoside C, hederacoside D, and

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α-hederin), polyphenolic acids (caffeic and chlorogenic acids), carbohydrates, flavonoids, and sterols [3; 4]. These compounds exhibit anti-inflammatory, antimicrobial, antioxidant, and cytotoxic activities, making ivy extracts highly therapeutic and valuable for pharmaceutical, cosmetic, and agricultural industries [5; 6].

Conventional extraction processes consist of maceration or classical heating (reflux and Soxhlet extraction), but these methods consume a lot of time, solvents and energy. Non-traditional techniques, such as ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE), are therefore more effective. The microwave radiation creates heat inside plant materials, disrupting cell membranes and releasing compounds rapidly. The solubility of the desired compounds in the selected solvent, the diffusion, and the partition coefficient all affect the extraction of the compounds of interest [7]. The plant materials are penetrated by microwaves that generate heat because of the polar molecules inside the materials [8]. When MAE takes place, the plant material adsorption capacity is greater than the solvent adsorption capacity [9]. As a result, the temperature of the vegetable material rises above that of the solvent, leading to an increased cell pressure and destruction of cell membranes, releasing compounds into the solvent [10]. Ultrasound assisted extraction (UAE) applies low-frequency sound waves to create cavitation bubbles [11]. These cavitation bubbles implodes and create micro-jets and high-speed collision conducting to disruption of plant tissues, enhancing solvent diffusion and compound release from plants [12-14]. Combining ultrasounds and microwaves radiation (MW+US) offers higher extraction rates, reduced time, and greater yields. Ultrasound enhances solvent penetration and disrupts cell walls, while microwaves accelerate heating and mass transfer, facilitating compound desorption [14-16]. The utilization of this advanced technologies results in enhanced efficiency in both energy consumption and economic viability [16].

The purpose of this research is to use a hybrid ultrasonic and microwave technique to extract valuable extracts from *Hedera helix* leaves in an environmentally friendly and effective method. To our knowledge, no studies have explored the combined use of microwave and ultrasound extraction for obtaining bioactive compounds from *Hedera helix* leaves.

2. Materials and Methods

Materials

Hedera helix leaves were harvested and collected in the summer of 2024 at Hofigal S.A., Romania. The leaves were dried in an airflow-heating oven at 60°C (final moisture content of 5.8%). Subsequently, the dried leaves were ground using an electric grinder and sieved to obtain a particle size of less than 315 μm.

The processed material was then portioned into 25 g aliquots, stored in plastic bags, and conserved at 4–5°C until the extraction procedures were conducted. To evaluate the total phytochemical content, the following reference standards were utilized: diosgenin, glucose, gallic acid, and Trolox, all obtained from Sigma-Aldrich Co, Romania. Ethanol, methanol, sulfuric acid, vanillin, Folin-Ciocalteu reagent, phenol, neocuproine, ammonium acetate, sodium carbonate, and copper chloride were purchased from Merck, Germany.

Procedures for phytocompounds extraction from Hedera helix leaves

The hybrid equipment utilized for the UMAE of phytocompounds from *Hedera helix* has been previously described by Calinescu et al. [16]. This system incorporates a ultrasound generator (Bandelin Sonopuls HD 4200, BANDELIN electronic GmbH & Co. KG, Berlin, Germany) alongside a microwave mono-mode applicator (Miniflow 200SS, SAIREM, Décines-Charpieu, France). The extraction hybrid reactor is partially immersed in the coupling fluid for US energy transfer. A jacket with circulating liquid is used to cool the coupling fluid and regulate its temperature. The constant conditions for all extractions were temperature of 50°C, solvent to plant ratio 1/20 (w/v), and water as extraction solvent. After each extraction, the plant material was separated by centrifugation for 5 min at 2500 rpm. The extraction mixture was collected and analyzed immediately. All experiments were carried out in triplicate in the same reactor, with the same stirring rate (800 rpm), at the same temperature (50°C), the US or MW generators were started differently.

❖ *The conventional heating extraction* (***Control***) was conducted in absence of US or MW, the temperature of 50°C was ensured by the thermal agent circulating through the mantle.

❖ *Ultrasound-Assisted Extraction* (***UAE***): During UAE, the microwave mono-mode applicator was deactivated, and only continuous sonication was applied. The amplitudes were set at 50% and 70%, corresponding to power outputs of 50.7 W and 56.9 W, respectively, delivered to the system.

❖ *Microwave-Assisted Extraction* (***MAE***): The MAE employed same equipment, but without the ultrasonic component. Microwave irradiation was applied at a constant power of 15 W for all extractions.

❖ *Combined Technology* (***UMAE***): The hybrid method involved the use of ultrasounds at 50% amplitude, applied in continuous mode, in combination with microwave irradiation at 15 W.

Phytochemical content analysis

The bioactive components were evaluated by colorimetric methods, using a Shimadzu UV mini-1240 UV/Vis Spectrophotometer, 115 VAC (Duisburg, Germany) and the absorbance was measured for all analysis in triplicate.

The total saponins content (TSC) was measured using an adapted version of the method described by Hiai et al. [17], which is based on the formation of chromogenic compounds through the interaction of saponins with vanillin and sulfuric acid. To begin, 0.5 mL of the extract was evaporated and redissolved in 10 mL of 80% methanol. Subsequently, 0.5 mL of this solution was combined with 0.5 mL of 8% vanillin solution and 5 mL of 72% sulfuric acid. The reaction mixture was heated at 60°C for 10 min, then rapidly cooled in an ice bath for 4 min. The absorbance was recorded at 544 nm. TSC was determined using a diosgenin standard curve (ranging from 40 to 550 mg/L) and the results were expressed in mg diosgenin equivalents per gram of dry matter (mg DE/g DM).

The total carbohydrates content (TCC) was assessed following a modified protocol based on the method of Varkhade et al. [18]. In this procedure, 1 mL of sample solution was mixed with 1 mL phenol (5% aqueous solution) and 5 mL of concentrated sulfuric acid (98%) in a reaction vial. The mixture was allowed to stand at room temperature for 10 min, followed by incubation in a water bath at 25–30°C for 20 min. The absorbance was measured at 490 nm. A glucose calibration curve (0.2 mg/mL standard solution, 20–140 µg/mL range) was used to quantify the results, which were expressed as mg glucose equivalents per gram of dry matter (mg GE/g DM).

The total polyphenols content (TPC) is based on the described method by Gavrila et al. [5]. Briefly, 0.5 mL of diluted extract (with a dilution factor of 20) was mixed with 5 mL of 10% Folin-Ciocâlteu reagent and stirred for 5 min to initiate the reaction. This was followed by the addition of 1.5 mL of 20% sodium carbonate decahydrate ($Na_2CO_3 \cdot 10H_2O$) and 3 mL of distilled water. The mixture was kept in the dark for 1 h at room temperature before measuring the absorbance at 760 nm. The results were calculated as mg gallic acid equivalents per gram of dry matter (mg GAE/g DM), based on a calibration curve using gallic acid solutions ranging from 1 to 5 mg/mL.

The antioxidant activity (AA) was assessed using the CUPRAC method as described by Özyürek et al.[19], with slight changes. The assay mixture consisted of 1 mL of 0.01 M copper (II) chloride solution, 1 mL of 0.0075 M neocuproine in ethanol, 1 mL of ammonium acetate buffer, and 1.1 mL of the diluted extract. The solution was incubated in the dark at room temperature for 30 min. Absorbance was read at 450 nm, and antioxidant capacity was expressed in µmol Trolox equivalents per gram of dry matter (µmol TE/g DM), using a calibration curve prepared with a Trolox standard.

Statistical Analysis

Every measurement was performed three times, and the results were presented as the mean value ± SD (standard deviation) for each of the three

samples. Multivariate principal component analysis (PCA) and univariate ANOVA were used to all of the results acquired at various process factor levels. Differences in the mean values of key components across multiple independent groups were assessed using Duncan's multiple range test at a 95% confidence level. Pearson's correlation coefficient (r) was calculated to evaluate linear associations between dependent variables [20; 21]. Principal component analysis (PCA) was conducted on the TSC, TCC, TPC, and antioxidant activity data to identify patterns and potential groupings based on the extraction methods. All statistical analyses were performed using XLSTAT software (version 2019.1, Addinsoft, New York, USA).

2. Results and discussions

a) Extraction of saponins and carbohydrates from Hedera helix leaves

One of the key parameters influencing the extraction process is time, as it significantly affects the target compounds yield. The concentration of phytocompounds in the extract increases until equilibrium is achieved, at which point mass transfer between the plant cells and the solvent reaches its maximum efficiency. Previous studies have demonstrated that extending the extraction time beyond 20 min is unnecessary, as prolonged exposure may result in the re-adsorption of the extracted compounds by the plant material, thereby reducing the overall yield of bioactive components [22]. In Fig. 1 are presented TSC (A) and TCC (B) of the extracts from *Hedera helix* leaves using ultrasounds, microwaves and a combination of them.

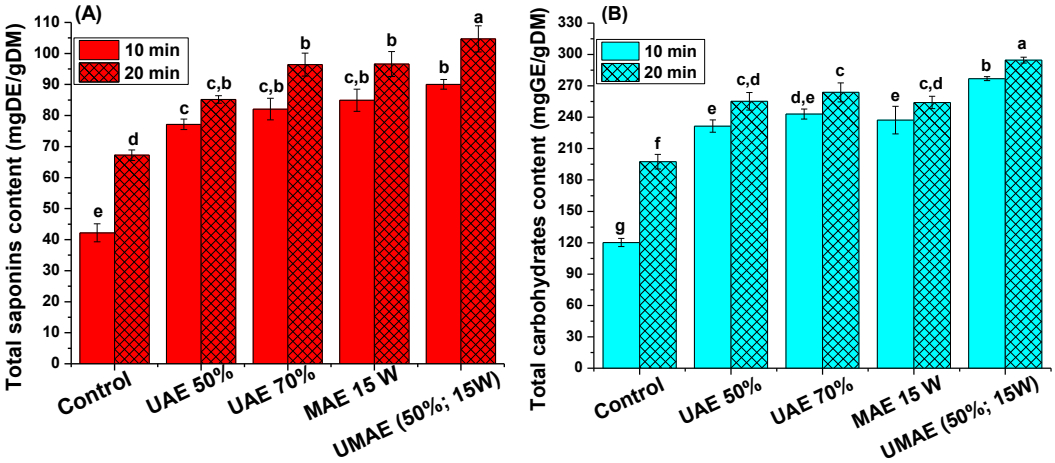

Fig. 1. The influence of extraction method on TSC (A) and TCC (B). The significantly difference between groups ($p < 0.05$, ANOVA and Duncan's post hoc t-tests) are highlighted by different letters (a–g).

As shown in Fig. 1, the conventional method yielded a significantly ($p < 0.5$) lower quantity of saponins, making it incomparable to the other methods. The MAE of saponins from ivy leaves is significantly influenced by several parameters, including extraction time, temperature, and microwave power. Among these parameters, the extraction time has a high impact on saponins extraction. However, prolonged extraction time combined with high temperatures in MAE can reduce the content of triterpenoid saponins, as they are known to decompose easily under such conditions [23]. The highest TPC is observed when the MAE is applied for 20 min (96.6 ± 4.02 mgDE/gDM). In UAE, applying longer extraction times increases the extraction rate of saponins. Additionally, ultrasonication power plays a crucial role, enhancing mass transfer even over short durations, because a shockwave damage is generated at the superficial layer between the solvent and the plant material [24]. The UAE tests were performed at two US amplitudes: 50% and 70% and a higher TSC is obtained increasing the ultrasonication power during a longer extraction time. For TSC the best results were obtained when the combined technology of ultrasound and microwave energy at 10 and 20 min (90.05 ± 1.57 mgDE/gDM and 104.70 ± 4.24 mgDE/gDM) was used.

Similary to the extraction of saponins, using a conventional method to extract carbohydrates is not the best option because as it can be seen, the results are not favorable. Higher TCC were obtained when the extractions were performed in the presence of microwaves and ultrasounds compared with control (254.1 ± 5.9 mgGE/gDM and 263.7 ± 9.6 mgGE/gDM). As shown in Fig. 1(B), UMAE technology makes a major difference in achieving the best results for TCC. The mechanical vibration generated during ultrasonication induces cavitation bubbles, which enhance cell rupture and improve compound release [25]. Meanwhile, microwave irradiation, combined with prolonged extraction time, accelerates the extraction of carbohydrate molecules from the plant matrix [26]. As a result, UMAE at 10 and 20 min yields significantly higher TCC values (276.9 ± 1.8 mgGE/gDM and 294.6 ± 2.8 mgGE/gDM, respectively) compared to other extraction techniques ($p < 0.5$), demonstrating that UMAE is the most effective method for extracting the interest compounds. The synergistic effect of the two methods combines the mechanical vibration and the internal heating leads to an increased extraction efficiency [25].

b) Extraction of polyphenols from Hedera helix leaves and antioxidant activity of the extracts

The choice of extraction technique significantly influences the efficiency of polyphenol recovery and the resulting antioxidant activity of plant extracts. Hybrid methods (UMAE) can optimize polyphenol yields and antioxidant activity by combining the benefits of cavitation and rapid heat transfer. Thus, selecting

appropriate extraction methods is crucial for maximizing the recovery of bioactive compounds and their functional properties.

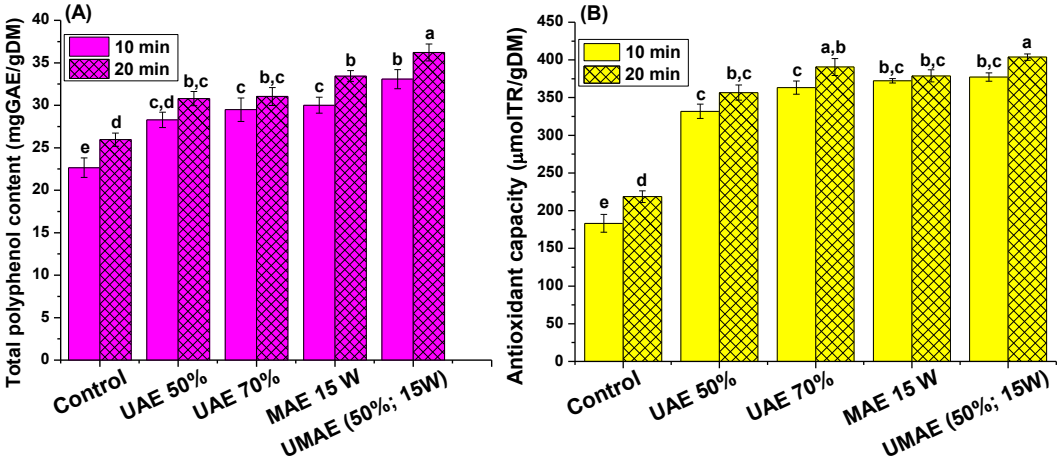

Fig. 2. The influence of extraction method on TPC (A) and antioxidant capacity (B). The different letters (a–e) show significant difference between groups ($p < 0.05$, ANOVA and Duncan's post hoc t-tests).

As illustrated in Fig. 2, extraction time plays a critical role as a key parameter in UAE. Conventional extraction methods consistently demonstrated the lowest efficacy across comparative studies. In contrast, UAE substantially enhances extraction performance, particularly when ultrasonication amplitude is increased from 50% to 70%, as evidenced by elevated phenolic compound concentrations and antioxidant capacity. Extending the extraction time from 10 to 20 min, along with a higher ultrasonication power from 50% to 70%, improves the migration of polyphenols from plant matrices into the solvent, resulting in a significantly higher phenolic content (31.03 ± 1.1 mgGAE/gDM, $p < 0.05$). However, a less extraction time (<20 min) diminishes ultrasound efficacy, as limited exposure impedes cell wall penetration and subsequent compound release. The results are similar to the ones obtained when MAE (33.44 ± 0.6 mgGAE/gDM) is applied, but UMAE makes a major difference in achieving a significantly higher TPC (36.22 ± 1.0 mgGAE/gDM, $p < 0.05$). Thus, prolonged extraction time and UMAE lead to a better mass transfer between solvent and plant molecules resulting in increased yields of TPC and improved antioxidant activity. [27]. The antioxidant activity was improved by MAE (378.79 ± 7.98 mmolTR/gDM) but the best results were obtained when using UMAE (403.78 ± 4.17 mmolTR/gDM).

Statistical analysis indicated that extraction time and extraction techniques highly influenced TPC and antioxidant capacity, with significantly higher results

for UMAE ($p < 0.05$). The hybrid UMAE approach synergizes the advantages of ultrasound and microwave technologies: cavitation (induced by ultrasound) disrupts cellular matrices, while microwave-derived rapid heat exchange accelerates mass transfer [28]. This dual mechanism enhances solvent permeation and bioactive compound recovery, establishing UMAE as a superior methodology for polyphenol extraction and antioxidant properties of extracts from ivy leaves. These results are showing the *Hedera Helix* is an important source of antioxidants that can be used in multiple industries – pharmaceuticals, cosmetics, and even in agriculture. Likewise, the UMAE is an efficient and promising nonconventional method to achieve the best quality extracts, reducing the extraction time.

Principal component analysis (PCA)

PCA was conducted to discover potential relationships between total phytocompounds content from *Hedera helix* leaves and the extraction methods – conventional and nonconventional extractions (UAE, MAE, and UMAE). Correlation analysis is a widely used approach to evaluate the interaction between phytochemicals and antioxidant properties in plant materials. For the multivariate analysis, the TPC, TSC, TCC, and AA of extracts obtained through different extraction methods were quantified. The first two principal components (PCs) explained 96.93% of the total variance, with PC1 accounting for 93.49% and PC2 contributing 3.45%. The factor loadings of the variables on the PC1-PC2 plane, along with their significance levels (bolded), are presented in Table 1.

Table 1

Factor loadings

	PC1	PC2
TSC	**0.980**	-0.033
TCC	**0.978**	0.022
TPC	**0.956**	-0.255
AA	**0.954**	0.267

The correlation matrix is displayed in Table 2. Correlation coefficients (r) with significant values are marked (* significant level at $p < 0.5$).

Table 2

Correlation matrix

Variables	TSC	TCC	TPC	AA
TSC	**1**			
TCC	0.954*	**1**		
TPC	0.924*	0.910*	**1**	
AA	0.911*	0.916*	0.863*	**1**

The factor scores for each extraction method, projected onto the PC1-PC2 plane, are detailed in Table 3.

Table 3

Factor scores

Method	Description	PC1	PC2	Method	Description	PC1	PC2
1	Conventional, 10 min	-4.746	0.032	6	UAE, Amplitude 70%, 20 min	1.241	0.200
		-4.228	-0.150			1.308	0.259
		-4.612	-0.349			0.921	0.397
2	Conventional, 20 min	-2.314	-0.192	7	MAE, 15 W, 10 min	-0.067	0.459
		-2.203	-0.641			0.564	0.177
		-2.299	-0.476			0.308	0.316
3	UAE, Amplitude 50%, 10 min	-0.495	0.576	8	MAE, 15 W, 20 min	1.236	-0.247
		-0.257	0.395			1.222	-0.340
		-0.351	0.505			1.422	-0.267
4	UAE, Amplitude 50%, 20 min	0.626	0.436	9	UMAE, Amplitude 70%, 15 W, 10 min	1.550	-0.336
		0.584	-0.080			1.140	-0.007
		0.404	0.079			1.171	-0.171
5	UAE, Amplitude 70%, 10 min	-0.039	0.348	10	UMAE, Amplitude 70%, 15 W, 20 min	2.629	-0.747
		0.109	0.505			2.633	-0.567
		0.279	0.176			2.263	-0.291

The PCA biplot of the *Hedera Helix* samples, illustrating the relationships between conventional extraction, UAE, MAE, UMAE, TSC, TCC, TPC, and AA is presented in Fig. 3.

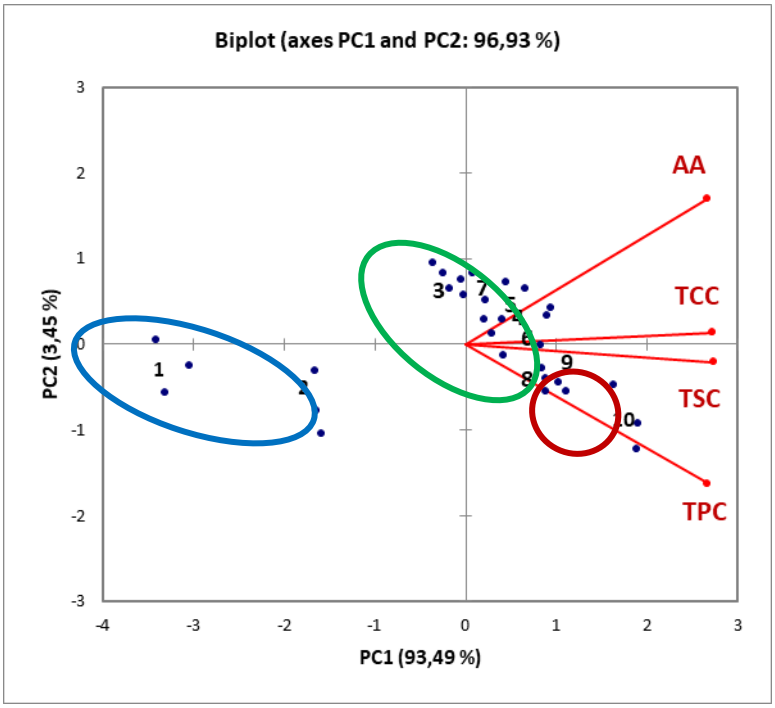

Fig. 3. The PCA biplot of the relationships between variables (TSC, TCC, TPC, and AA) and extraction methods (1–10)

As illustrated in Fig. 3 and supported by the factor loadings and scores detailed in Tables 2 and 3, correlations can be established between the extraction methods, their conditions, and the total bioactive compound content as well as antioxidant capacity. All varialbles (TPC, TSC, TCC, and AA) exhibit a strong positive correlation with PC1. Specifically, TSC exhibits a strong positive correlation with TCC (r = 0.954) and TPC (r = 0.924), as well as a positive correlation with AA (r = 0.911). The PCA biplot reveals three distinct clusters of extraction samples: Group 1–2 (highlighted by a blue ellipsoid), Group 3–9 (highlighted by a green ellipsoid), and Group 10 (highlighted by a red circle). Extracts obtained using methods 1–2 (blue ellipsoid) displayed a lower TSC, TCC, TPC, and AA compared to those obtained using methods 3–10, but the highest difference was between group 1-2 and group 10, discrimination along PC1. Overall, the hybrid UMAE technique was the most effective extraction method for all bioactive compounds studied and antioxidant capacity of ivy leaves extracts.

6. Conclusions

Advancements in extraction technologies and their combinations have shown considerable promise for isolating bioactive compounds from *Hedera helix* leaves. This study focused on determining the most effective approach for extracting bioactive compounds from ivy leaves. During all the experiments, the UAE, MAE, UMAE and a conventional method were investigated and the extraction time varied from 10 to 20 min. The study revealed that *Hedera Helix* leaves are valuable sources of phytochemicals associated with antioxidant activity. Compared to conventional extraction methods, advanced techniques such as UAE, MAE, and UMAE exhibited superior efficiency. Although UAE and MAE are promising green techniques, the most efficient extraction method that led to higher results was UMAE. Synergistic effects were observed when these technologies were combined, leading to enhanced yields of total bioactive compounds and antioxidant activity of the extracts. Specifically, hybrid approach – UMAE was identified as innovative strategy to optimize extraction performance. Furthermore, a strong positive correlation was established between saponins, carbohydrates, phenolic compounds, and antioxidant activity. The findings suggest that emerging extraction technologies, particularly when combined, can produce *Hedera Helix* leaves extracts with improved functional properties, making them suitable for future applications as pharmacological agents and dietary supplements.

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PN-III-P2-PED-2019-2118, project: Technologies for obtaining of natural products with immunostimulatory properties—"IMUNOSTIM" financed by contract: PED, no. 381PED/2020. The authors acknowledge the financial support received from the Competitiveness Operational Programme 2014-2020, Action 1.1.4: Attracting high-level personnel from abroad in order to enhance the RD capacity, project: P_37_471, „Ultrasonic/Microwave Nonconventional Techniques as new tools for nonchemical and chemical processes", financed by contract: 47/05.09.2016.

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