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DOCTORAL SCHOOL OF "CHEMICAL ENGINEERING AND BIOTECHNOLOGIES"
FIELD OF DOCTORAL RESEARCH: CHEMICAL ENGINEERING

DOCTORAL THESIS

Unconventional techniques used in obtaining food supplements

Tehnici neconvenționale utilizate în obținerea suplimentelor alimentare

Summary of the thesis

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OBJECTIVES AND STRUCTURE OF DOCTORAL THESIS

The objective of this thesis is to highlight and develop new methods for obtaining bioactive compounds from plants. A wide range of industry branches have a strong need for bioactive compounds, traditional extraction methods being outdated. Thus, the emphasis will be on the development and optimization of unconventional methods to improve extraction processes, but also to satisfy consumer needs.

The general objective of this work is to optimize the extraction of phytocompounds - saponins, carbohydrates, polyphenols, but also other compounds with antioxidant activity - from ivy (*Hedera helix*) and neem (*Azadirachta indica*) leaves, while also targeting to intensify the process through unconventional extraction techniques (microwave and/or ultrasound-assisted extraction).

The general objectives result in the specific objectives of this work, namely:

❖ Study of the influence of different parameters for selecting optimal values for extraction by unconventional techniques (influence of extraction time, temperature, plant/solvent ratio, solvent concentration, influence of ultrasonication power) from *Hedera helix* and *Azadirachta indica* leaves;

- ❖ Determination of the total content of bioactive compounds in *Hedera helix* and *Azadirachta indica* leaves: saponins, carbohydrates, polyphenols, but also determination of antioxidant activity;
- ❖ Influence of the type of extraction on the content of phytocompounds in the studied plants (microwave-assisted extraction, ultrasound-assisted extraction, conventional extraction, microwave-assisted extraction combined with ultrasound);
- ❖ Evaluation of the in vitro biocompatibility of extracts from *Hedera helix* leaves;
- ❖ Study on the in vitro cytotoxicity of ivy extracts;
- ❖ Energy considerations of the extraction processes;
- ❖ Statistical analysis of the main components in ivy and neem extracts.

The literature review in Part I consists of the first 3 chapters of this work. **Chapter 1** describes the extraction methods of active principles from plants. In this context, both the principles of conventional methods and the mechanisms and advantages of unconventional methods are presented, the best known, but also used in this work, being: microwave-assisted extraction, ultrasound-assisted extraction and the combined technology of the two methods.

Chapter 2 contains data from the literature on the intensification of extraction processes (using ultrasound and microwaves) and the basic principles of extraction methods, such as the use of alternative solvents, renewable resources, reducing energy consumption, studying and selecting optimal extraction parameters.

Chapter 3 provides a brief introduction to the complexity of food supplements, as they are found under a diverse range of products. The importance of food supplement packaging and the impact this has on consumer safety is also briefly presented.

Chapter 4 and **chapter 9**, respectively, cover the botanical aspects of the plants of interest, namely ivy or *Hedera helix* and neem. The most notable properties, as well as their major compounds, are also presented.

The experimental study begins in **chapter 5**, first describing the materials and methods used for the extraction processes of bioactive compounds, the extraction procedures and equipment, and the analysis methods of the extracts obtained. Further, the intensification of the extraction process of bioactive compounds from ivy leaves using ultrasound and the influences of different parameters on the extraction process will be discussed.

Chapter 6 presents the microwave-assisted extraction of bioactive compounds from *Hedera helix* leaves, in which the same parameters were studied. **Chapter 7** describes the study of microwave-assisted extraction technology in comparison with the study of ultrasound-assisted extraction technology and the impact on the content of phytocompounds in ivy, the statistical analysis of the composition of the extracts and the biological activity.

Chapter 8 presents the hybrid microwave-ultrasound extraction system with which the compounds of interest in ivy extracts could be quantified: saponins, carbohydrates, polyphenols, but also the antioxidant activity.

Chapter 10 describes the extraction of bioactive compounds from neem leaves, in which two extraction methods - the conventional and the ultrasound-assisted - are compared. The study of different extraction parameters such as extraction time, plant/solvent ratio, ethanol concentration, extraction temperature, but also the analysis of the main components is pursued.

Finally, the doctoral thesis presents the general conclusions, highlighting how the proposed objectives were achieved, as well as possible directions for research development.

EXTRACTION OF BIOACTIVE COMPOUNDS FROM *HEDERA HELIX* L.

6. ULTRASOUND-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM *HEDERA HELIX*

6.1. Extraction procedures and extraction equipment

The extraction of bioactive compounds was performed in a batch system consisting of a jacketed glass reactor. To maintain the temperature at the optimal value, water was recirculated between the jacket and the thermostat throughout the extractions. The application of ultrasound was performed with an ultrasound probe, Vibracell VCX750. The ultrasound probe was inserted into the extraction reactor, directly into the mixture.

6.2. Intensifying the extraction process of bioactive compounds from ivy leaves using ultrasound

The aim of this study is to evaluate the extraction parameters for the ultrasound-assisted extraction of saponins from *Hedera helix* leaves and to establish an extraction protocol for the main extracted hederasaponins.

6.2.1. Influence of ultrasound power

The TSC variation with the sonication power and the ultrasound amplitude are presented in **Figure 6.2**, and the main saponins analyzed by HPLC are shown in **Figure 6.3**.

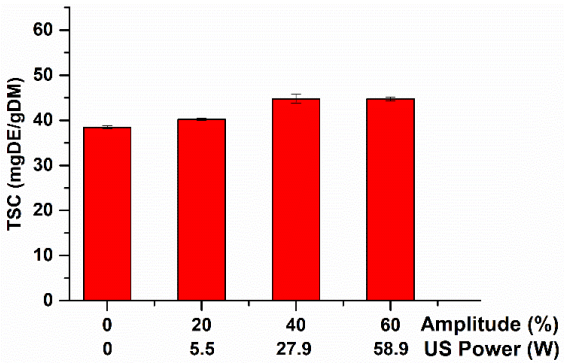


Figure 6.2. Influence of ultrasonic power on the total saponin content (TSC) of *Hedera helix* leaves extracts

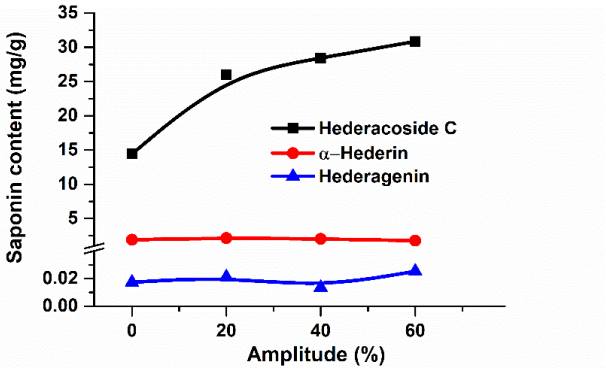


Figure 6.3. Influence of ultrasonic power on the main saponins of *Hedera helix* leaves extracts

As shown in **Figure 6.2**, the lowest content is obtained for the extraction of saponins by conventional method (38 mg DE/g DM). To the contrary, the highest total saponin content is obtained when the ultrasonic power is 27.9 W. The ethanolic extracts were analyzed by HPLC in order to determine the saponins content (hederacoside C, α-hederin, and hederagenin). As shown in **Figure 6.3** hederacoside C is the saponin extracted in the highest amount, followed by α-hederin.

6.2.2. Influence of temperature and extraction time

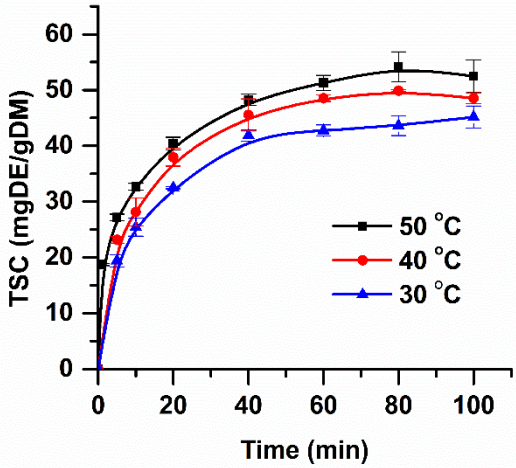


Figure 6.4. Influence of extraction time and temperatures on the TSC.

The amount of extracted saponins TSC at different temperatures (30, 40, and 50 °C) is shown in **Figure 6.4**. At low temperatures, the number of bubbles generated is small, but they collapse vio-lently, increasing the mass transfer between vegetal material and solvent [1]. At 30 °C, the use of acoustics leads to a lower amount of extracted saponins of 42.69 mg DE/g DM.

The content of bioactive components in the extracts was also evaluated by HPLC analysis. For all three temperatures used, only the extracts obtained at 60 min were analyzed. The results are shown in **Figure 6.5a**. However, since the highest TSC values were obtained at 50 °C and in order to assess the stability of key compounds over time, the main saponins were evaluated for all extraction times (see **Figure 6.5b**).

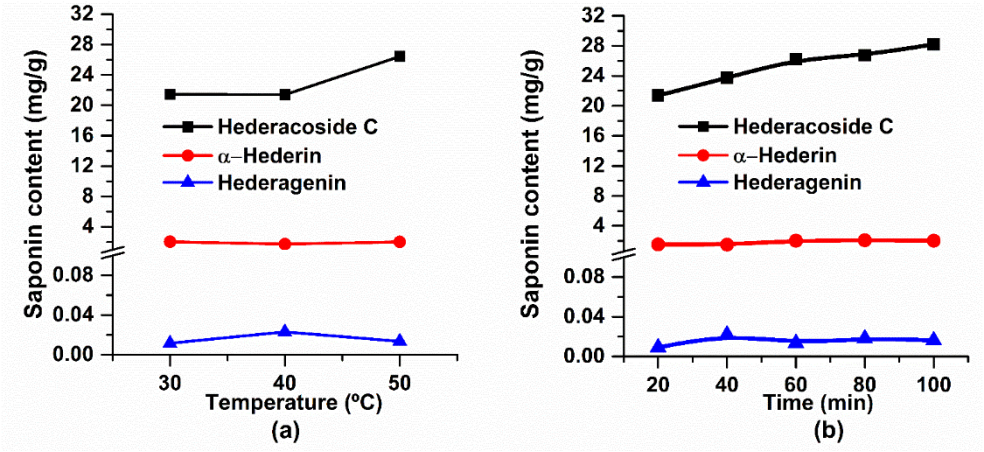


Figure 6.5. Influence of temperature (a) and extraction time (b) on the main saponins of the extracts

6.2.3. Influence of plant to solvent ratio

Solid to liquid ratio is a fundamental variable that can considerably affect the saponin extraction efficiency in the UAE process. In order to find the suitable solid to liquid ra-tio, this parameter was evaluated by varying the plant to solvent ratio from 1/10 to 1/30 (w/v), keeping the other variables constant (**Figure 6.6**).

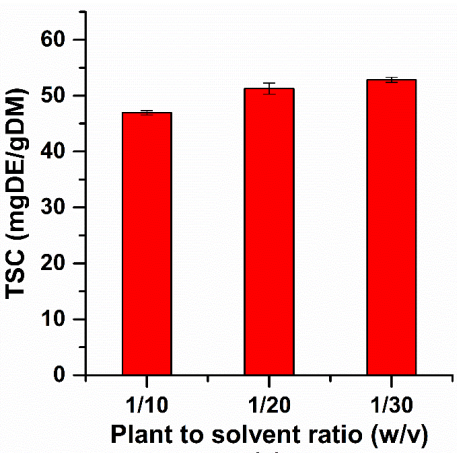


Figure 6.6. Influence of plant to solvent ratio on the TSC

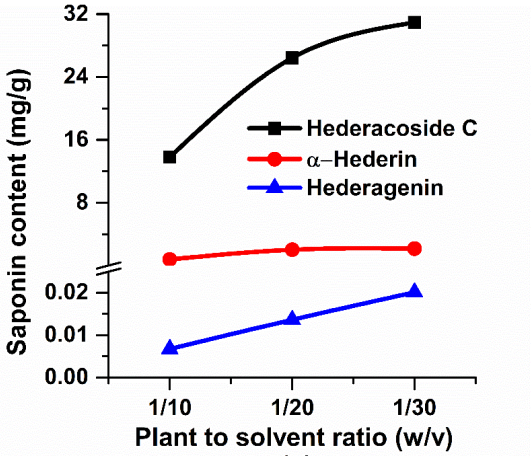


Figure 6.7. Influence of plant to solvent ratio on the on the main saponins of the extracts

6.2.4.The influence of ethanol concentration

Another important parameter for saponins extraction is the selection of solvent and the influence of the solvent concentration. As shown in **Figure 6.8**, it can be noticed that the highest TSC was obtained when using a mixture of 80% ethanol in water – 56.87 mg DE/g DM. According to ANOVA analysis, it was noted that TSC increased significantly with increasing of the ethanol concentration from 20% to 80% ($p<0.05$). When the solvent contacts the plant material, it will surround it nd a slow swelling process will begin [2].

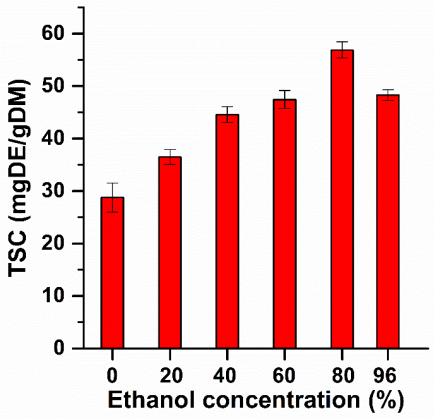


Figure 6.8. Influence of ethanol concentration on the TSC

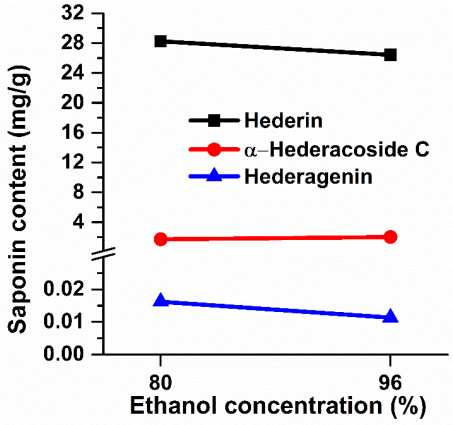


Figure 6.9. Influence of ethanol concentration on the main saponins of the extracts

The extracts obtained for a concentration of 80% ethanol in water were analyzed by HPLC and compared with the ones when a mixture of 96% ethanol in water was used. The results are shown in **Figure 6.9**. The highest content of hederacoside C was also ex-tracted at this ethanol concentration – 80%.

7. MICROWAVE-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM HEDERA HELIX LEAVES

7.1. Extraction procedures and equipment

In a microwave extraction reactor (Initiator, Biotage, **Figure 7.1A**) a 20 mL vial containing the appropriate amount of ivy and extraction solvent − ethanol of various concentrations is introduced. The extraction parameters are set: temperature, time, stirring speed, pre-stirring duration (30 sec), vial volume, etc.

5.1. Influența tipului de extracție

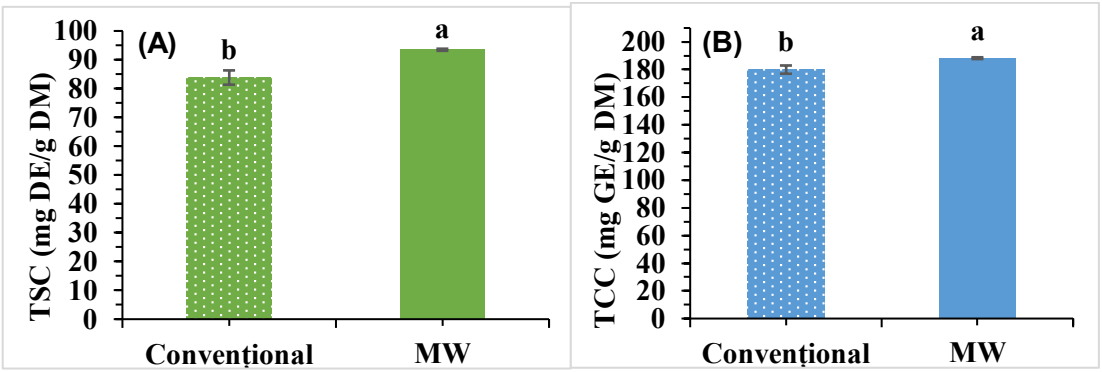


Figure 7.1. Influence of extraction type on total saponin content (TSC) **(A)** and on total carbohydrate content (TCC) **(B)**

Analyzing **Figures 7.1. – 7.2**. it can be seen that MAE led to significantly better results for all active principles studied and also in the case of antioxidant activity (ANOVA, $p < 0.05$).

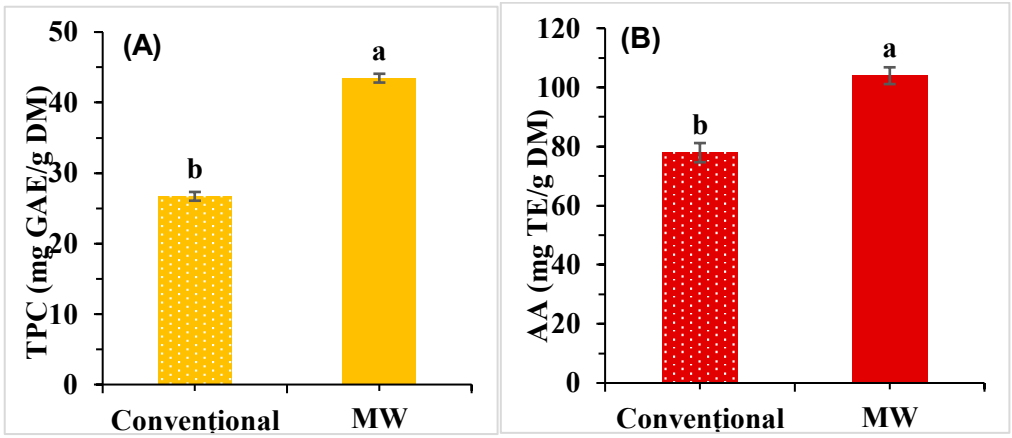


Figure 7.2. Influence of extraction type on total polyphenols content (TPC) **(A)** and on antioxidant activity (AA) **(B)**

7.3. Influence of extraction time

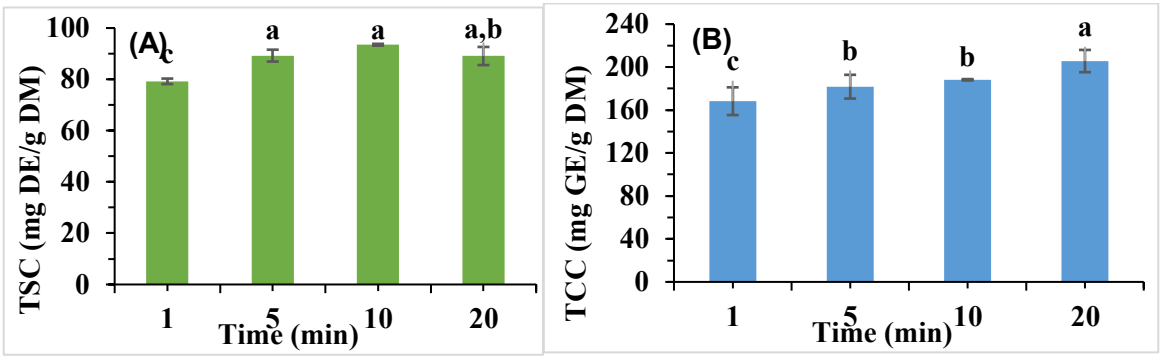


Figure 7.3. Influence of extraction time on total saponin content (TSC) **(A)** and on total carbohydrate content (TCC) **(B)**

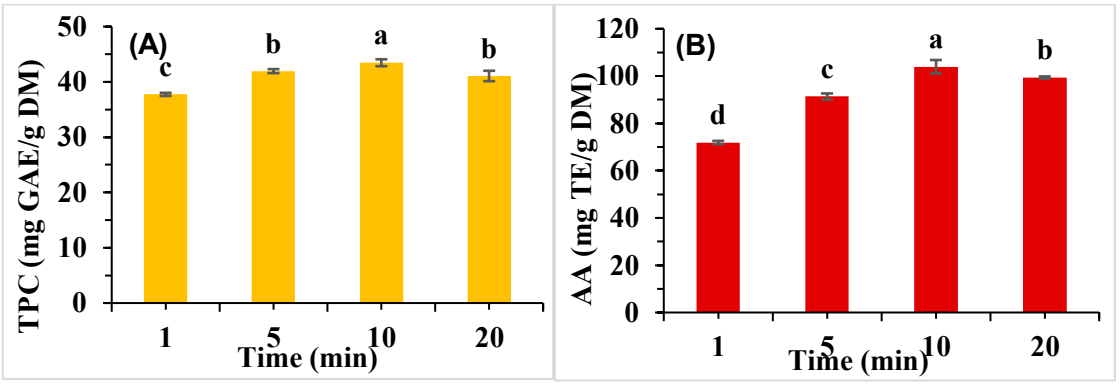


Figure 7.4. Influence of extraction time on total polyphenols content (TPC) **(A)** and on antioxidant activity (AA) **(B)**

As can be seen, the amount of saponins, polyphenols, and antioxidant activity of ivy extracts increased significantly ($p < 0.05$) over time, reaching maximum values.

7.4. Influence of extraction temperature

Another factor with a significant impact, especially in the case of thermolabile products, is the extraction temperature. [3].

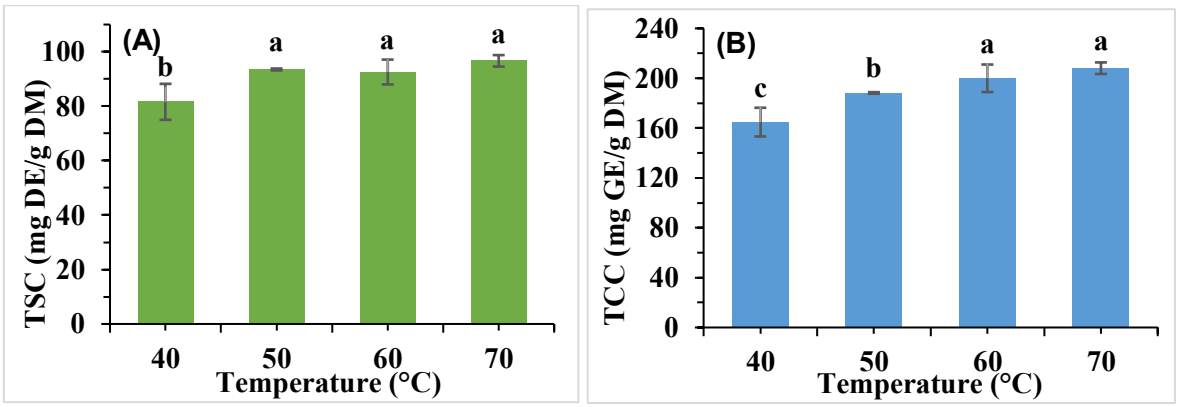


Figure 7.5. Influence of extraction temperature on total saponin content (TSC) **(A)** and on total carbohydrate content (TCC) **(B)**

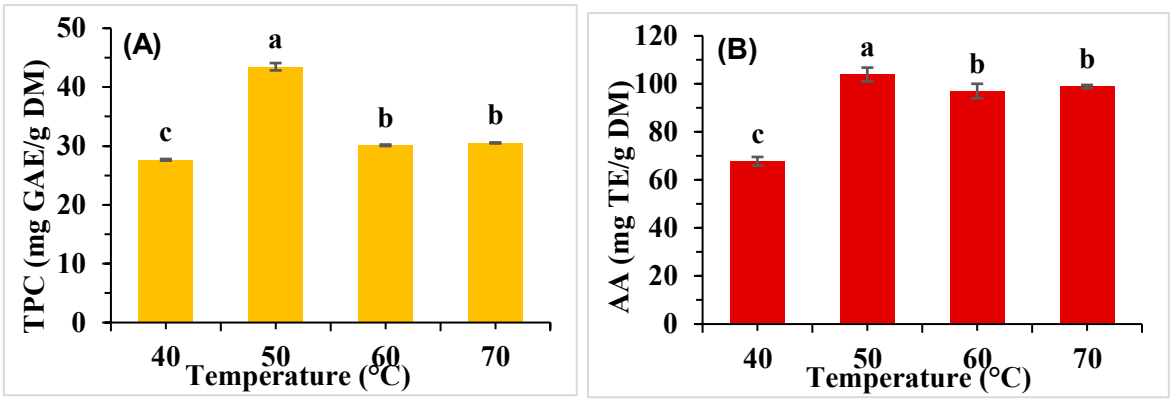


Figure 7.6. Influence of extraction temperature on total polyphenols content (TPC) **(A)** and on antioxidant activity (AA) **(B)**

Thus, in **Figures 7.5.-7.6.**, when the temperature is increased to 70°C, an increase in the content of saponins (with a slight decrease at 60°C) and carbohydrates can be observed up to the maximum values of 96.66 mg DE/g DM, respectively 208.05 mg GE/g DM. However, high temperatures can lead to the degradation of chemical compounds and, implicitly, to a decrease in the extraction efficiency. Considering the results obtained on the compounds of interest, it is concluded that the optimal extraction temperature is 50°C.

7.5. Influence of the plant to solvent ratio

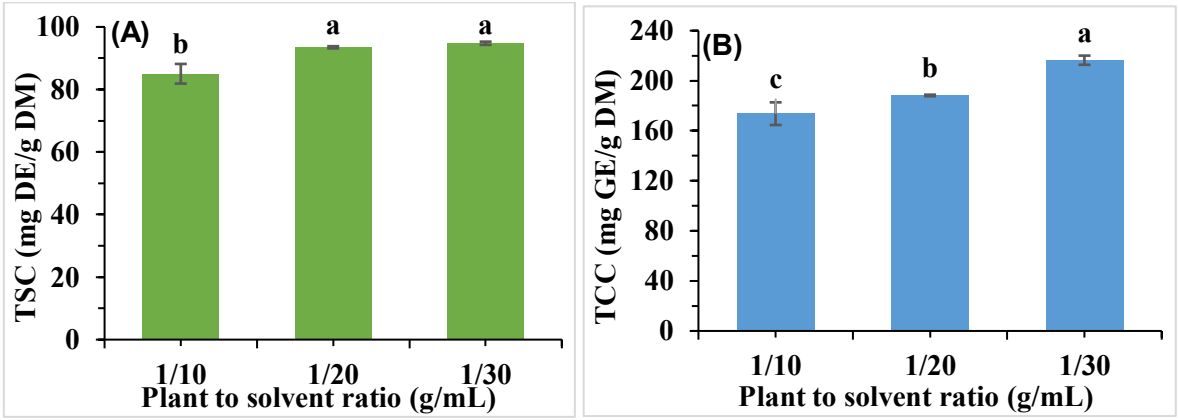


Figure 7.7. Influence of plant to solvent ratio on total saponin content (TSC) **(A)** and on total carbohydrate content (TCC) **(B)**

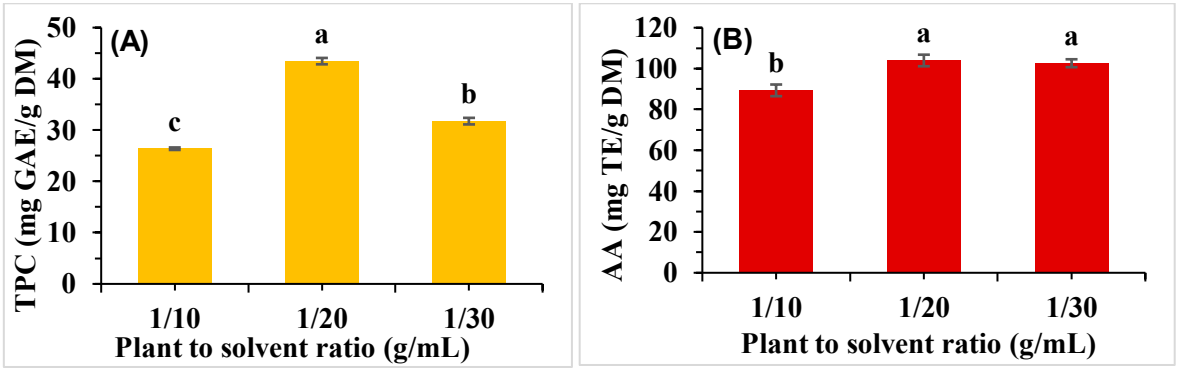


Figure 7.8. Influence of plant to solvent ratio on total polyphenols content (TPC) **(A)** and on antioxidant activity (AA) **(B)**

Another particularly important factor in the microwave-assisted extraction process is the plant to solvent ratio. In general, a larger volume of solvent can dissolve the active components more efficiently and lead to higher extraction yields.

7.6. Influence of ethanol concentration

Ethanol has alow toxicity and is used in large quantities in the extraction of compounds from plants. To improve the efficiency of microwave-assisted extraction, it is used in various aqueous concentrations (due to its high dielectric properties) [4].

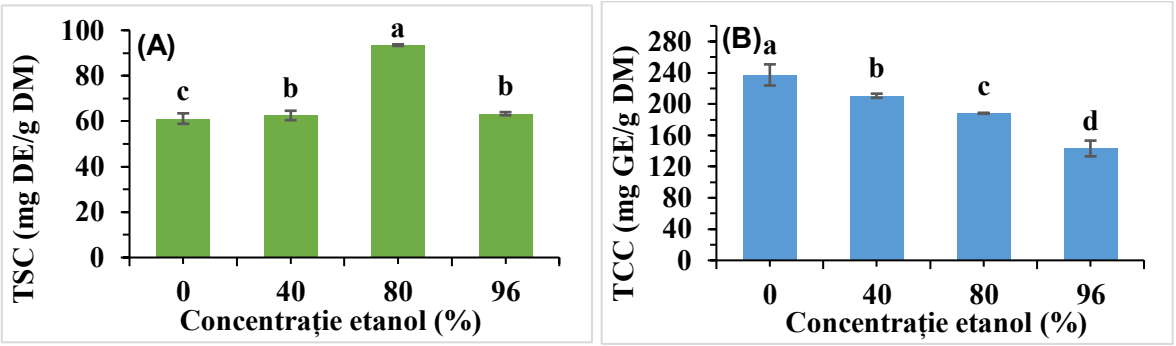


Figure 7.9. Influence of extraction time on total saponin content (TSC) **(A)** and on total carbohydrate content (TCC) **(B)**

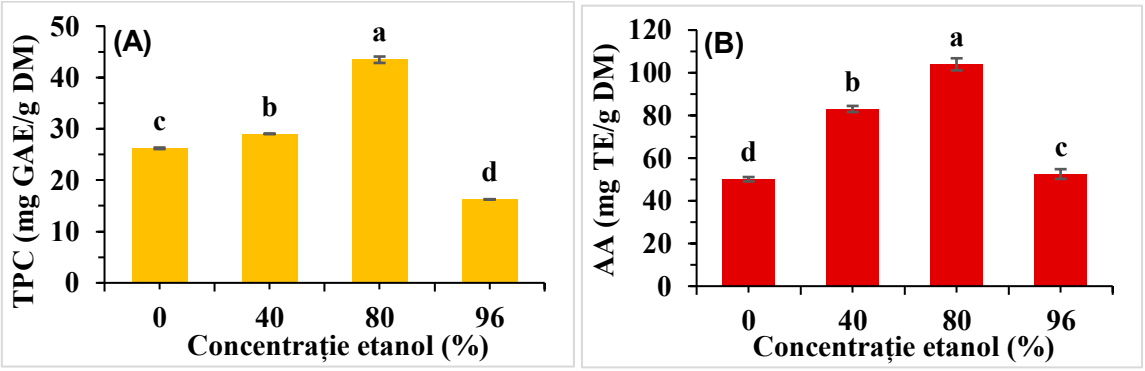


Figure 7.10. Influence of ethanol concentration on total polyphenols content (TPC) **(A)** and on antioxidant activity (AA) **(B)**

The total extracted amounts of bioactives, as well as the antioxidant capacity, were reported at different ethanol concentrations. The lowest values of the amounts of biocompounds were obtained when the extraction took place at an ethanol concentration of 0%, except for carbohydrates, which reached a maximum value.

8. COMPARATIVE STUDY ON THE EXTRACTION EFFICIENCY OF BIOACTIVE S FROM IVY USING DIFFERENT EXTRACTION TYPES

Next, the following extraction methods will be comparatively evaluated: CHE – conventional heating extraction method, UAE – ultrasound-assisted extraction method and MAE – microwave-assisted extraction method, as well as the most important process parameters.

8.1. Multiple successive extractions

In order to assess the efficiency of bioactive compounds extraction, information about the total amount of extractable phytochemicals in Hedera helix leaves is required [5].

Thus, discontinuous multiple successive extractions were performed using the best extraction conditions obtained for each extraction method. The total phytochemicals content obtained by multiple successive extractions, for each method, is shown in **Figure 8.1.**

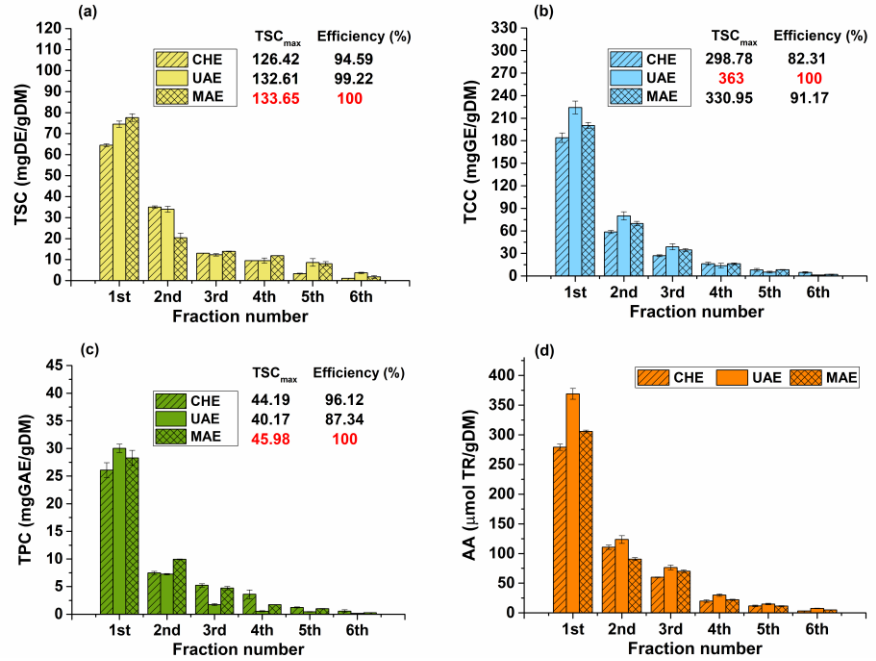


Figure 8.1. The phytochemical content of the multiple successive extractions: **(a)** total saponins content (TSC), **(b)** total carbohydrates content (TCC), **(c)** total phenolic content (TPC), and **(d)** antioxidant activity (AA).

8.2. Influence of extraction time

The extraction time is an important parameter that impacts the content of ex-tracted products. The amount of extracted bioactive phytochemicals increases over time until an equilibrium between the solubilized compounds by the solvent and the un-extracted constituents from the plant cells is reached. The effect of extraction time on the total content of bioactive compounds and antioxi-dant activity is shown in **Figure 8.2**. Each graph contains the maximum values of bioactives obtained during successive extraction.

Using ANOVA and Duncan's post hoc t-tests and considering the best extraction time of 10 min, the following findings are emerged: Saponins and polyphenols (**Figures 8.2A and C**) increases until 10 min ($p<0.05$) and with no significant differences observed by extending the extraction time to 20 min; carbohydrates (**Figure 8.2B**) increases significantly until 20 min; antioxidant activity (**Figure 8.2D**) is correlated with the phytocompounds content, at 10 min the highest activity being achieved..

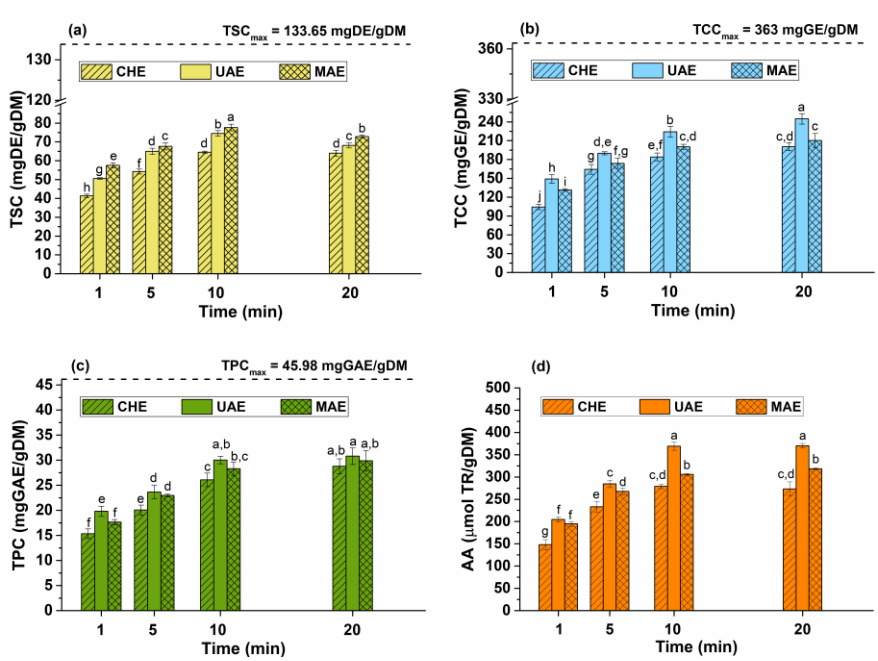


Figure 8.2. Influence of extraction time on the total content of bioactive compounds: **(a)** total saponins content (TSC), **(b)** total carbohydrates content (TCC), **(c)** total phenolic content (TPC), and **(d)** antioxidant activity (AA).

8.3. Influence of extraction temperature

The extraction temperature is another important factor, which may affect the sol-id-liquid extraction process. Due to limitations of the non-conventional methods, 40 and 50 °C were chosen to evaluate this parameter.

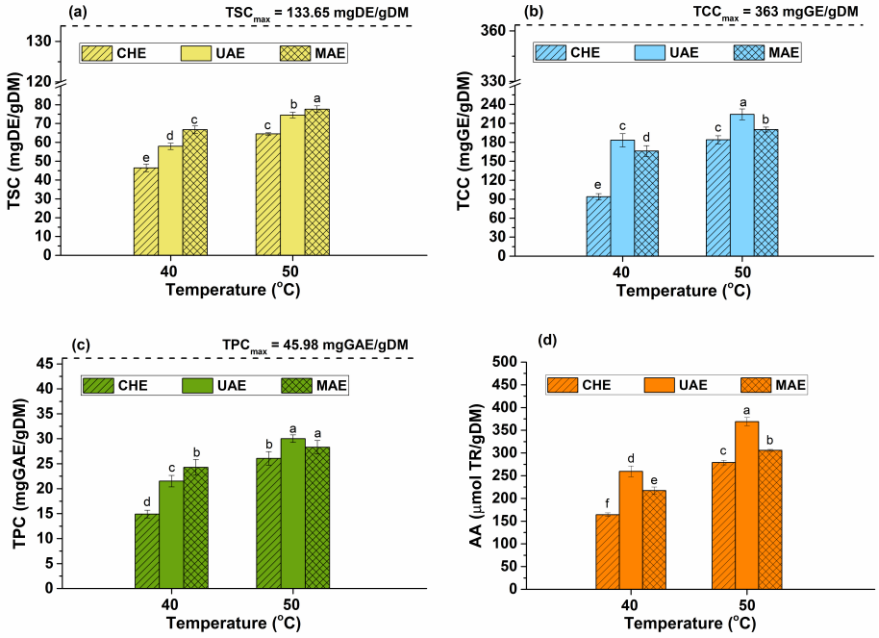


Figure 8.3. Influence of temperature on the total content of bioactive compounds: **(a)** total saponins content (TSC), **(b)** total carbohydrates content (TCC), **(c)** total phenolic content (TPC), and **(d)** antioxidant activity (AA).

According to **Figure 8.3**, the highest amount of all bioactive compounds was extracted at a temperature of 50 °C. Increasing temperature leads to an increase in solubility

of bioactive compounds and enhances the mass transfer rate between solvent and vegetal material matrix. The extraction efficiency of the studied phytochemicals is higher than 55 % for the non-conventional methods.

8.4. Influence of ethanol concentration

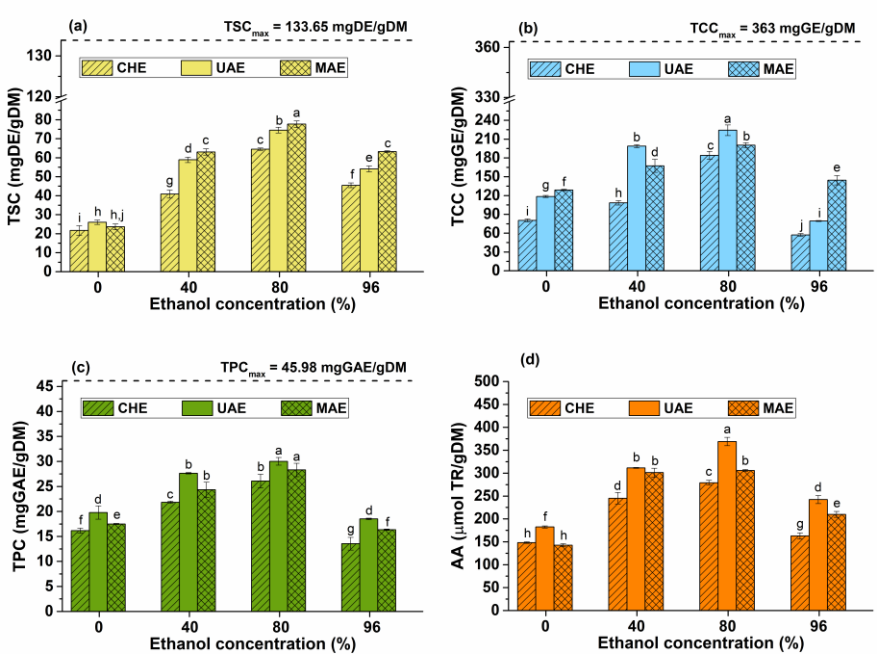


Figure 8.4. Influence of ethanol concentration on the total content of bioactive compounds: **(a)** total saponins content (TSC), **(b)** total carbohydrates content (TCC), **(c)** total phenolic content (TPC), and **(d)** antioxidant activity (AA).

To improve the extraction efficiency, ethanol is commonly used at different concentrations in water. For this study different concentrations were used. The influence of solvent concentration on the phytoconstituents content is shown in **Figure 8.4**. The total content of bioactive compounds increases until an ethanol concentration of 80% is reached.

8.5. Principal Component Analysis

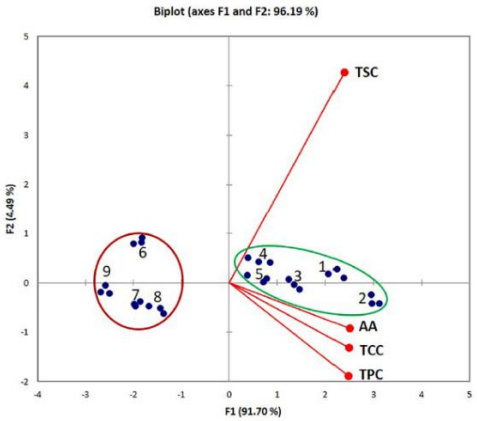


Figure 8.5. Projections of variables (TSC, TCC, TPC, and AA) and extraction methods (1–9) on the fac-tor-plane PC1–PC2.

Principal Component Analysis (PCA) was performed in order to study possible correlations between TSC, TCC, TPC, AA and the extraction methods (CHE, UAE, and

MAE) of these compounds from *Hedera helix* leaves. The bioactive compounds and antioxidant activity in the plane formed by the first two axes (PCs) explain 96.11% (eigenvalues >1) of the total variation, as well as 91.70% on the first axis and 4.41% on the second one.

From the information from **Figure 8.5**. it is possible to correlate the extraction method and extraction conditions with the total bioactive compounds content and antioxidant capacity.

8.6. Energy Considerations

During all experiments, the ultrasound and microwave powers were recorded. The power input for the heating plate, utilized for the conventional extraction, was meas-ured using a Wattmeter. These recorded powers were used to determine the total en-ergy introduced into the systems [6]. Further, the specific energy was calculated using the following equation:

$$E_s = E_{total}/m_{Phc}\ [kJ/g\ of\ phytocompounds], \quad (1)$$

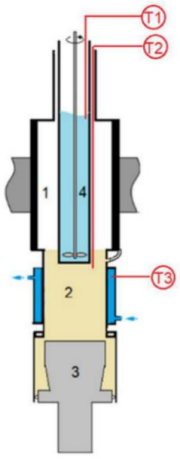
where E_s is the specific energy [kJ/g of phytocompounds], E_{total} is the total energy in-troduced into the system [kJ], and m_{Phc} is the total amount of phytocompounds (the sum of saponins, carbohydrates, and polyphenols) obtained by each extraction method [g].

Energy consumption for CHE is ranged between 360 and 378 kJ, while for MAE and UAE is ranged from 7.1 to 9.2 kJ and from 16.2 to 28 kJ, respectively. Although the lowest energy is consumed for extraction at a temperature of 40 °C, for both UAE and MAE, the lowest specific energy is achieved for the experiments performed at a temperature of 50 °C using an 80% ethanol concentration in water

9. EXTRACTION OF BIOACTIVE COMPOUNDS FROM HEDERA HELIX LEAVES IN A HYBRID MICROWAVE AND ULTRASOUND SYSTEM

9.2. Extraction equipment

Echipamentul hibrid utilizat pentru UMAE a fitocompușilor din *Hedera helix* a fost descris anterior de Calinescu și colab. [7] și este prezentat schematic în **Figure 9.1**.



1 – monomode microwave applicator,
2 – thermostated ultrasonic bath with coupling fluid,
3 – ultrasound converter,
4 – extraction reactor,
T1, T2, T3 – thermocouples.

Figure 9.1. Hybrid ultrasonic and microwave extraction equipment [7, 8]

This system incorporates a Bandelin Sonopuls HD 4200 ultrasound generator along with a Miniflow 200SS monomode microwave applicator.

9.3. Interpretation of results

9.3.1. 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 yield of the target compounds [9]. In **Figures 9.2**. şi **9.3** are presented TSC and TCC of the extracts from *Hedera helix* leaves using ultrasounds, microwaves and a combination of them.

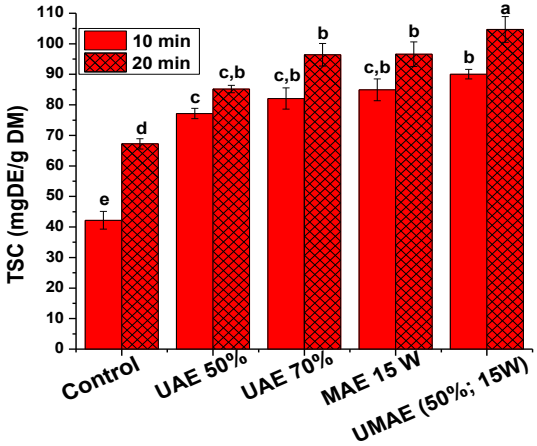


Figure 9.2. The influence of extraction method on TSC.

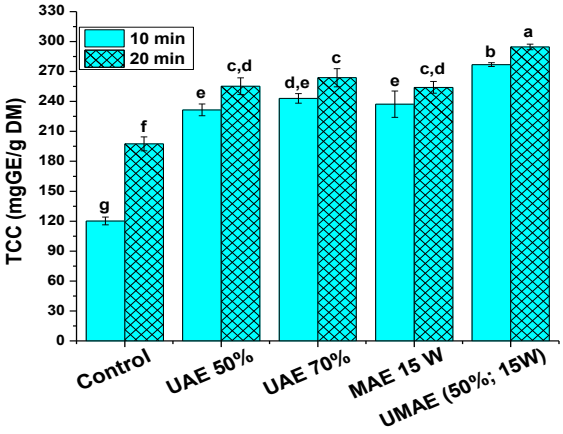


Figure 9.3. The influence of extraction method on TCC.

As shown in **Figure 9.2**., the conventional method yielded a significantly ($p < 0.5$) lower quantity of saponins. Similary to the extraction of saponins, using a conventional method to extract carbohydrates is not the best option because as it can be seen in **Figure 9.3**, the results are not favorable. Higher TCC were obtained when the extractions were performed in the presence of microwaves and ultrasounds

8.4.2. Extraction of polyphenols and their antioxidant activity obtained from *Hedera helix* leaves

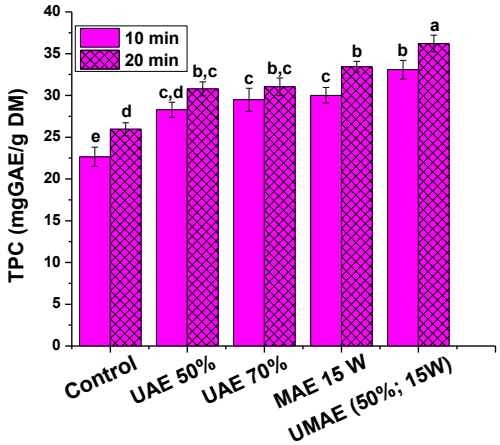


Figure 9.4. The influence of extraction method on TPC.

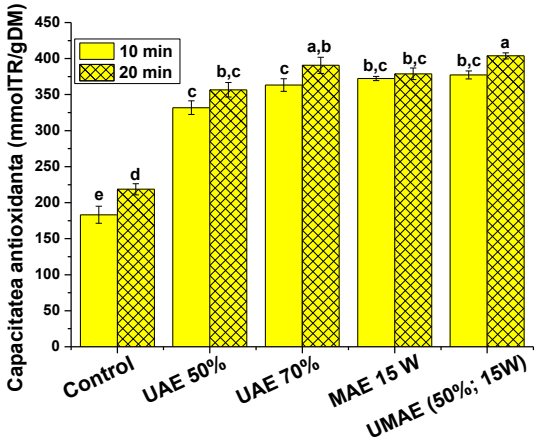


Figure 9.5. The influence of extraction method on antioxidant capacity.

UMAE technology can optimize polyphenol yields and antioxidant activity due to the synergistic action between cavitation and rapid heat transfer.

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 [10].

10. EXTRACTION OF BIOACTIVE COMPOUNDS FROM *AZADIRACHTA INDICA*

10.2. ULTRASOUND ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM NEEM LEAVES

10.2.3. Influence of extraction time

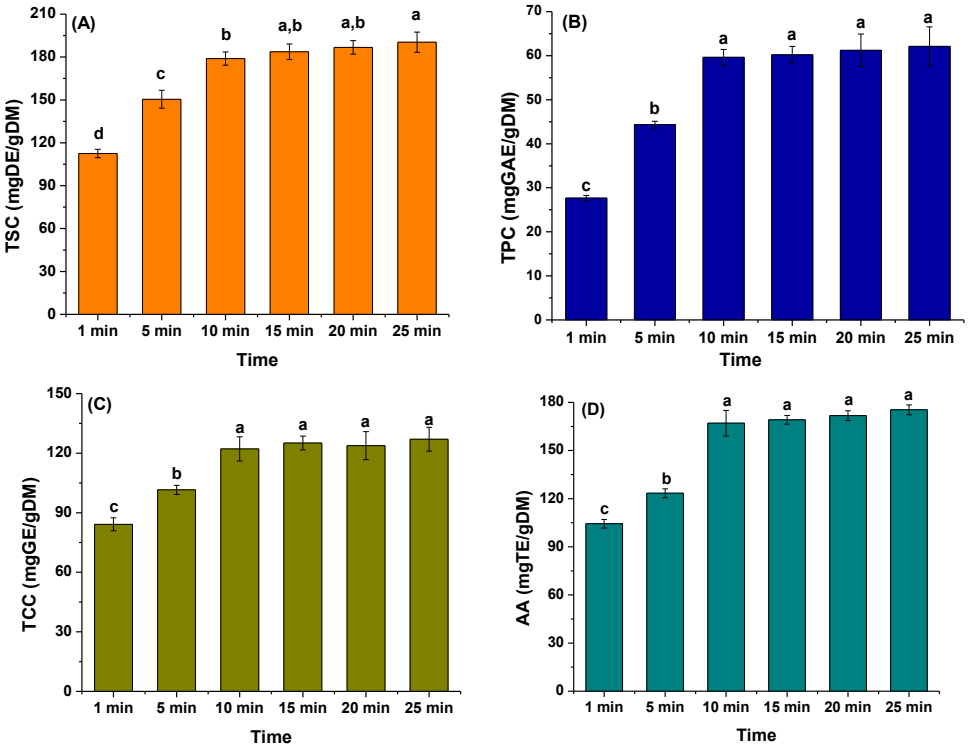


Figure 10.4. Influence of extraction time on the total content of bioactive compounds: A) TSC; B) TPC; C) TCC and D) AA

Figure 10.4. shows the extraction results obtained for different periods of time under the same reaction conditions. it can be seen that the amount of saponins and polyphenols, as well as the antioxidant activity of the neem extracts increased over time, reaching a maximum value of 182.2 mg DE/g DM, 59.6 mg GAE/g DM, and 167 mg TE/g DM at 10 min from the start of ex-traction, respectively, after which a slight decrease occurred at 15 min.

10.2.4. Influence of ultrasound power

The ultrasound power is an important factor because it indicates the amount of energy that is effectively transferred to the liquid.

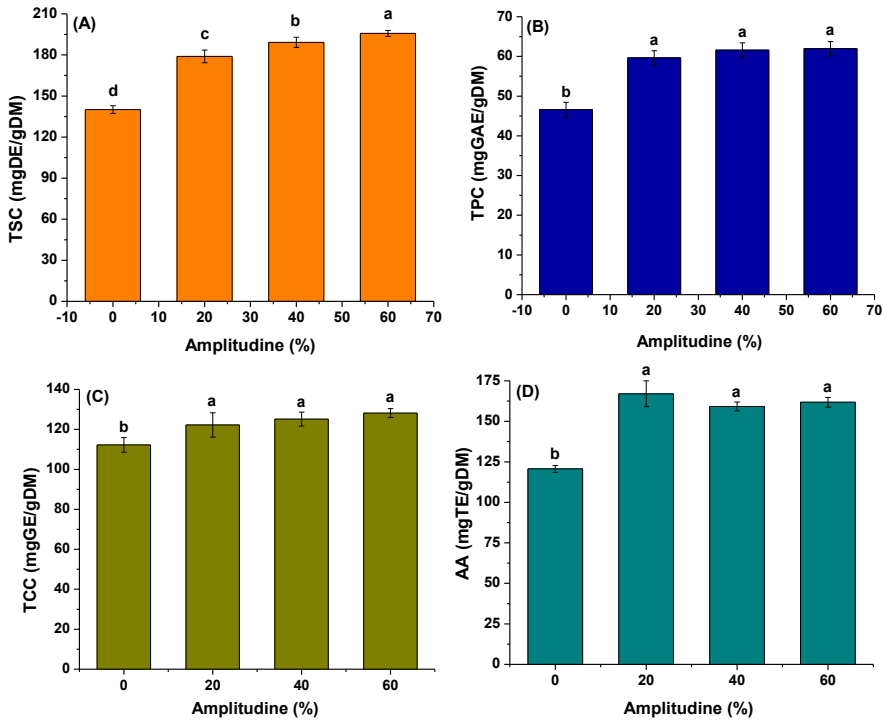


Figure 10.5. Influence of sonication amplitude on the total content of bioactive compounds: A) TSC; B) TPC; C) TCC and D) AA

From **Figure 10.5** it is observed that a higher amplitude of ultrasonic waves contributes to the formation of a larger amount of cavitation bubbles and thus, the collapse is created. The yield and extraction of saponins, polyphenols and carbohydrates from neem leaves increased with the increase in ultrasonic amplitude during extraction, but it was observed that a too high increase in ultrasound power does not lead to a significant increase in the total content of extracted phytocompounds.

10.2.5. Influence of the plant to solvent ratio

Another important parameter of ultrasound-assisted extractions is the plant to solvent ratio. Especially in industrial processes, it is essential to maximize the extraction yield and reduce the consumption of extraction solvent which makes the study of the influence of this ratio on the extraction process extreme-ly important.

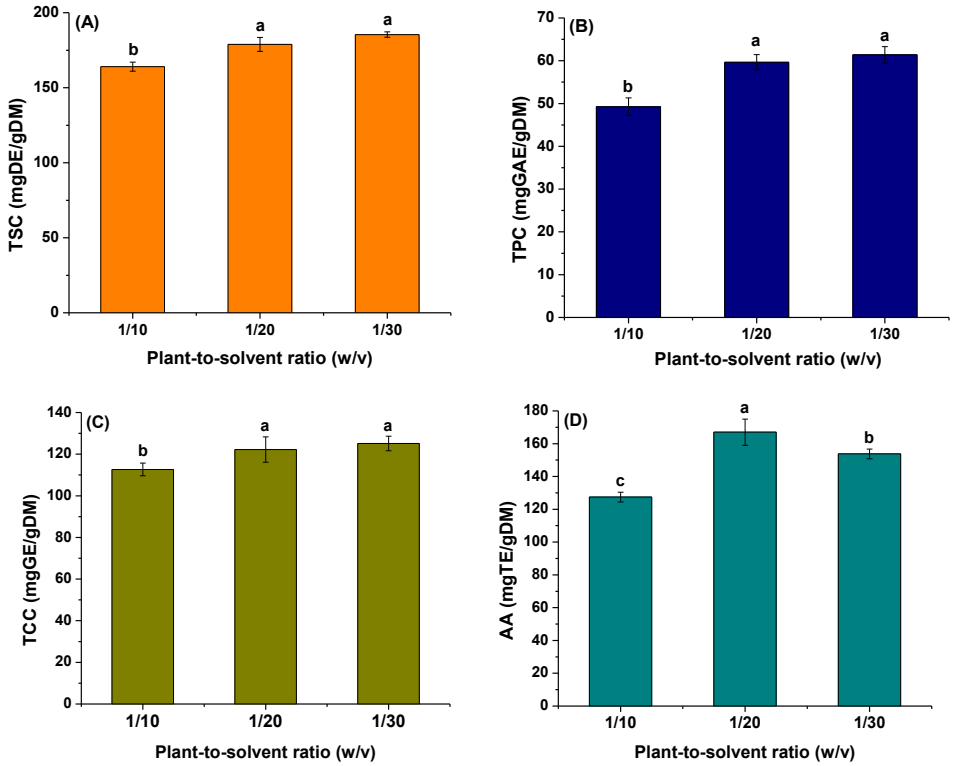


Figure 10.6. Influence of plant to solvent ratio on the total content of bioactive compounds: A) TSC; B) TPC; C) TCC and D) AA

Analyzing the graphs in **Figure 10.6.** it can be seen that at a plant to solvent ratio of 1/10, the lowest values of all the analyzed parameters were obtained because the plant material is much too concentrated and the ultrasound is not sufficiently well adsorbed by the organic matrix

10.2.6. Influence of ethanol concentration

Due to its low toxicity, ethanol has been widely used for the extraction of biologically active compounds from various plants. In ultrasound-assisted extraction, ethanol is commonly used at different concentrations in water to improve the extraction effi-ciency. The high dielectric property of water has made it a good co-solvent for ultra-sound-assisted extraction of phytochemicals. **Figure 10.7** illustrate the effect of ethanol concentration on the extracted amounts of saponins and polyphenols, as well as on the antioxidant capacity.

As can be seen, the amounts of bioactive compounds at an EtOH concentration of 0 % were 125.6 mg DE/g DM (saponins), 15.8 mg GAE/g DM (polyphenols), and anti-oxidant activity 85.4 mg TE/g DM. The subsequent increase in EtOH concentration to 25 % resulted in a slight increase for the other parameters, with the maximum values being recorded at a concentration of 50 % (182.2 mg DE/g DM (saponins), 59.6 mg GAE/g DM (polyphenols) and antioxidant activity 167 mg TE/g DM.). As the ethanol concentration exceeded 75%, a significant decrease was observed, which makes the optimal ethanol concentration in the extraction solvent of neem active principles to be 50 %

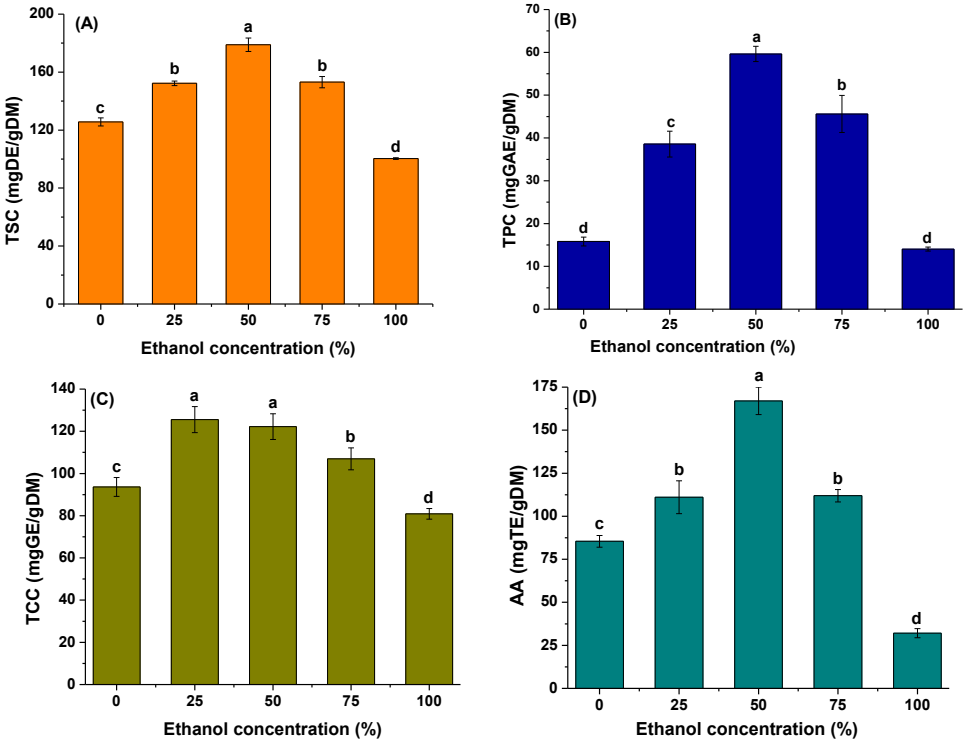


Figure 10.7. Influence of solvent concentration on the total content of bioactive compounds: A) TSC; B) TPC; C) TCC and D) AA: A)TSC, B)TPC, C)TCC și D)AA

10.2.7. Influence of extraction temperature

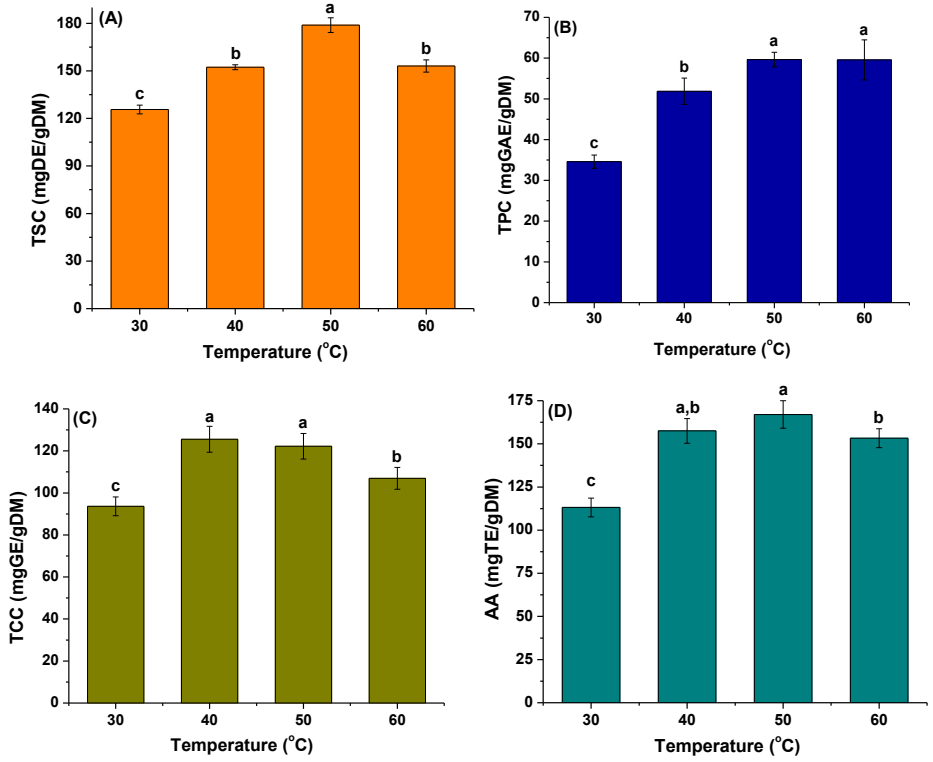


Figure 10.8. Influence of temperature on the total content of bioactive compounds: A) TSC; B) TPC; C) TCC and D) AA

Temperature has a significant impact on the extraction of saponins, especially in terms of preserving thermolabile compounds. As can be seen from the graphs, the increase in the total saponin content is quite low, and in order to obtain maximum extraction yields of all compounds and taking into account the economic factor – increased energy consumption at higher reaction temperatures – it is concluded that the optimal temperature for the ultrasound-assisted extraction process is 50°C..

10.2.8. Principal Component Analysis

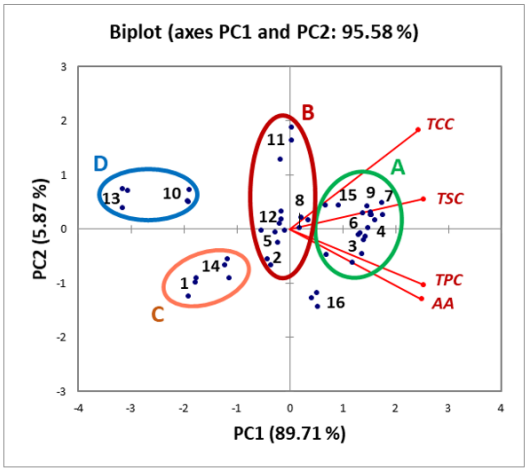


Figure 10.9. PCA of phytocompounds of neem extracts and their extraction conditions

A PCA biplot was conducted to evaluate the potential relationships between the extraction techniques (conventional and UAE), bioactive compounds content (TPC, TSC, and TCC), and antioxidant activity of extracts from neem. (**Figure 10.9**).

PC1 showed strong positive loadings for all measured variables—TPC, TSC, TCC, and AA. Notably, AA shows a strong positive correlation with all bioactive compounds: TPC (r = 0.925), and a positive correlation with TSC (r = 0.852) and TCC (r = 0.806).

11. GENERAL CONCLUSIONS AND RESEARCH PERSPECTIVES

Each chapter was dedicated to individual studies and represent the main objectives of this thesis:

1. Determination of an efficient method for the extraction of saponins from ivy and the influence of the most important parameters of the extraction process; in vitro evaluation of the biocompatibility of *Hedera helix* leaf extracts and their influence on the DNA content of L929 fibroblasts;

Taking into account the experimental data obtained from these studies, the following conclusions were drawn:

- Ultrasound-assisted extraction was evaluated as a simpler and more efficient alternative to the conventional extraction method;

- The highest total amount of saponins, hederacoside C and α-hederin were obtained when a temperature of 50°C was used, for 60 min of extraction, at an ultrasound amplitude of 40%, a plant to solvent ratio of 1:20 (w/v) and an ethanol concentration of 80%;

2. Establishing the best method for phytocompounds extraction from ivy leaves, by comparing the conventional extraction method with microwave-assisted extraction; Evaluation of extraction time, temperature, plant to solvent ratio and ethanol concentration;

- Following the interpretation of the experimental data, it is highlighted that microwave-assisted extraction led to much higher extraction yields, in a shorter time. Thus, the optimal extraction conditions determined were: temperature of 50°C, a P/S ratio of 1/20 and an ethanol concentration of 80%, when the highest saponin content of 93 mg DE/g DM was obtained, having a maximum antioxidant activity of 103 mg TE/g DM.

3. Evaluation of the most efficient method for extracting ivy principles between the conventional method, ultrasound and microwave-assisted extraction;

- A series of parameters were studied such as: extraction time, ethanol concentration in the extraction solvent, temperature and multiple successive extraction. UAE and MAE proved to be the most attractive alternatives, the results indicating the best parameters: extraction time of 10 min, a temperature of 50°C and 80% ethanol as solvent;

- The highest saponin content was obtained by MAE – 77.64 ± 1.79 mg DE/g DM;

- Regarding carbohydrates and polyphenols, the best results were obtained by UAE (TCC = 224.17 ± 8.53 mg GE/g DM and TPC = 30.02 ± 0.75 mg GAE/g DM);

- The PCA method was used to evaluate potential correlations between TSC, TCC, TPC and AA and extraction methods of bioactive compounds from *Hedera helix* leaves. A positive correlation was found between antioxidant activity and TSC (r= 0.867), between AA and TCC (r=0.928) and between AA and TPC (r=0.922);

4. Development of extraction technologies and their combinations; determination of the most efficient method for the extraction of bioactive compounds from ivy leaves, UAE, MAE, UMAE being investigated;

- Synergistic effects were observed when these technologies were combined, leading to increased yields of total bioactive compounds and antioxidant activity of the extracts. Specifically, the hybrid approach – UMAE was identified as an innovative strategy for optimizing extraction performance.

- The results highlight that emerging extraction technologies, especially when combined, can produce *Hedera helix* leaf extracts with improved functional properties, making them suitable for future applications as pharmacological agents and dietary supplements;

5. Extraction of bioactive compounds from neem leaves;

- Experimental data showed that ultrasound-assisted extraction presents major advantages over conventional extraction, obtaining incontestably more valuable results;

- The study of the influence of different working parameters showed that the best reaction conditions for obtaining maximum amounts of phytocompounds were: extraction time 10 min, temperature of 50°C, ultrasonic amplitude of 20%, continuous sonication, plant to solvent ratio 1/20 (w/v), solvent 50% EtOH in water;

- Regarding these reaction conditions, the highest saponin content of 195.5 mg DE/g DM, 60.5 mg GAE/g DM for polyphenols and 173.3 mg TE/ g DM in the case of antioxidant activity was obtained.

The future objectives/research directions refer to:

- The experimental study can be continued especially in the direction of concentrating ivy and neem extracts in order to increase the content of bioactive components used in food supplements for the prevention of various diseases.
- Optimization of the extraction process of important compounds from neem leaves and establishment of separation methods from the obtained extracts.
- Extraction of phytocompounds from neem leaves by using microwave-assisted extraction and hybrid extraction method (microwave combined with ultrasound).

13. DISSEMINATION OF RESULTS

❖ Articole publicate în reviste ISI

1. A. I. Gavrila, R. Tatia, A.M. Seciu-Grama, I. Tarcomnicu, **C.L. Negrea**, I. Calinescu, C.M. Zalaru, L. Moldovan, A.D. Raiciu, I. Popa*, *Ultrasound Assisted Extraction of Saponins from Hedera helix L. and an In Vitro Biocompatibility Evaluation of the Extracts*, Pharmaceuticals, 15(10), nr. 1197 (13 pag), **2022**, eISSN 1424-8247, WOS:000873732500001 (ISI, **Q1, IF = 4.8**).
2. A. I. Gavrila, C.M. Zalaru, R. Tatia, A.M. Seciu-Grama, **C.L. Negrea,** I. Calinescu, P. Chipurici, A. Trifan*, I. Popa, *Green Extraction Techniques of Phytochemicals from Hedera helix L. and In Vitro Characterization of the Extracts*, Plants, 12(9), nr. 3908 (17 pag), **2023**, ISSN: 2223-7747, WOS:001114597100001 (ISI, **Q1, IF = 4.1**).
3. **C. L. Negrea** , I. Popa , I. Calinescu*, C. M. Zalaru , A. I. Gavrila, *A hybrid ultrasound – microwave system for phytocompounds extraction from Hedera helix L*., UPB Sci. Bull., Series B, **2025**, ISSN 1454 – 2331, **acceptat spre publicare**. (ISI, **Q4, IF = 0.3**)

❖ Conferințe internaționale

1. A. I. Gavrila, I. Popa, C.L. Negrea, I. Calinescu, A. Trifan, E. Feizula, *Hybrid green extraction of phytoextracts from Hedera Helix* L, International Chemical Engineering and Material Symposium (SICHEM), 17–18 noiembrie **2022**, București, România (**Prezentare orală**).
2. A. I. Gavrila., I. Popa, C. M. Zălaru, I. Calinescu, **C. L. Negrea**, *Comparison of different techniques for the extraction of bioactive compounds with immunostimulatory activity from ivy leaves*, 22nd Romanian International Conference on Chemistry and Chemical Engineering (RICCCE 22), 7–9 septembrie **2022**, Sinaia, România (**Poster**).
3. A. I. Gavrila, I. Popa, **C.L. Negrea,** I. Calinescu, A. Trifan, E. Feizula, *Hybrid green extraction of phytoextracts from Hedera Helix* L, International Chemical Engineering and Material Symposium (SICHEM), 11–12 aprilie, **2024**, București, România (**Poster**).

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3. Gavrila, A.I., et al., *Ultrasound assisted extraction of saponins from Hedera helix L. and an in vitro biocompatibility evaluation of the extracts.* Pharmaceuticals, 2022. **15**(10): p. 1-12https://doi.org/10.3390/ph15101197.
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