PhD THESIS

Exploration of the physico-chemical profile and the activity of individual compounds of honey produced from *Fallopia japonica* plant with the aim of effectively exploiting its bioactive and beekeeping potential

(SUMMARY OF PhD THESIS)

PhD student **Alexandra-Antonia Cucu**

Scientific coordinator **Prof. PhD. Daniel Severus Dezmirean**

INTRODUCTION

Plants have always played an important role in nourishment and health due to their nutritional and therapeutic benefits, therefore, they are considered to be nutraceuticals (Atazadegan et al., 2021). The last decades have witnessed a constant scientific interest in medicinal plants, natural products or apitherapy, which provide essential components with nutritional value and positive impact on human physical and mental health (Santini & Novellino, 2014).

Fallopia japonica (F. japonica), part of the *Polygonaceae* family, also known as *Reynoutria japonica* or *Polygonum cuspidatum* is an invasive plant native to Asia and North America, widely recognized for its pharmacological attributes (Dong et al., 2014). This last aspect is underlined by studies that have characterized the chemical composition of different anatomical parts of the *F. japonica* plant, revealing a wide range of different bioactive compounds (especially resveratrol), known for their pharmacological effect (Bensa et al., 2020; Glavnik & Vovk, 2020; Lachowicz & Oszmianski, 2019; Nawrot-Hadzik et al., 2018). Moreover, the plant is a rich source of nectar for pollinating insects, such as the bee (Cucu et al. 2021).

However, current knowledge of the honey produced from *F. japonica* plant is particularly limited. Therefore, the present research contributes with valuable information to an area of study that has received limited attention up to this point, laying the foundations for future investigations related to research on the characterization of this distinct type of honey that remains relatively unknown to many beekeepers due to its late flowering of the plant and its invasive tendencies.

The use of this plant for positive purposes, such as beekeeping or alternative medicine, represents an innovative way to reduce its negative impact and allows approaching new perspectives on the sustainable management of invaded areas by creating value-added products.

The present research has a number of 150 pages and is structured on two distinct sections, respectively "Literature review", which highlights the knowledge, information and limitations related to the researched field and the "Personal Contribution" section, where the results of the research carried out are presented.

Thus, the first section is structured in two chapters, namely: "**Chapter 1.** Honey-general notions" and "**Chapter 2.** *Fallopia japonica* plant description, characteristics and beekeeping potential" and the second section of the thesis includes six chapters: "**Chapter 3.** Objectives of the research"; "**Chapter 4.** Particularities of the experimental areas"; "**Chapter 5.** Materials and methods utilized in research"; "**Chapter 6.** Results and discussions"; "**Chapter 7.** Conclusions and recommendations" and & "**Chapter 8**. Innovative contributions of the thesis".

1. Honey – general notions

This chapter presents information related to the complex and unique composition of honey, as well as information related to its therapeutic properties, authenticity, processing and adulteration.

2. *Fallopia japonica (F. japonica)* plant – description, characteristics and beekeeping potential

This chapter presents a brief description of the *F. japonica plant*, its chemical constituents and its pharmacological properties. At the same time, this chapter considers the beekeeping potential of the plant by characterizing the honey produced from it and comparing it with honeys from the same botanical family.

3. Objectives of the research

The main purpose of this thesis aims to **identify the physico-chemical properties and therapeutic effects of the honey produced from *F. japonica* plant, in order to capitalize on its biologically active and melliferous potential.**

In this regard, the research directions have the following **objectives**: "3.1 Determination of the physico-chemical profile of the honey obtained from *F. japonica* plant"; "3.2 Evaluation of macro-, micro- and trace-elements from *F. japonica* plant and their translocation in the related honey"; "3.3 Identification and quantification of the biologically active compounds (phenolic compounds) present in both *F. japonica* plant and honey"; "3.4. Determination of the antioxidant capacity of both *F. japonica* plant and honey"; "3.5. Determination of antibacterial and antibiofilm activity of both *F. japonica* plant and honey".

4. Particularities of experimental areas

The experimental areas for this study were the North-Wester and the Wester part of Romania, namely three different areas: (1) Merişor area, Maramureş County (47°39′25.2″N 23°24′06.7″E) at the hydrographic confluence of the Lăpuş and Someş rivers, Baia Mare depression; (2) Valea Vinului area, Satu Mare county (47°43′46.9″ N 23°10′26.3″E) in the Someş river meadow; (3) Bocsig area, Arad county (46°25′50.4″N 21°57′47.3″E) in the Crişul Alb river meadow.

5. Materials utilized in research

In this chapter, are presented data regarding the plant and honey samples used in this research, respectively the chemical compounds used, the analysis methods and the equipment used to achieve the proposed objectives.

All the experiments of this thesis were carried out at the Center for Advanced and Extension Research in Apiculture (APHIS-DIA) of the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, under the guidance of Prof. Dr. Daniel Severus Dezmirean and the research team.

6. Results and discussions

6.1. Results concerning the physico-chemical profile of *F. japonica* honey

6.1.1. The mellisopalynological evaluation

Melisopalyological analysis was performed for each region separately. Following the palynological analysis, it became evident that the three regions are distinguished by the different types of pollen found alongside the *Fallopia* pollen. None of the pollen types identified constituted a majority (> 45%) of the analyzed samples.

6.1.2. The sensorial evaluation

All samples showed a clean appearance without impurities, the consistency varied from fluid-viscous to finely crystallized, caramel-brown color with some reddish-brown shades, a general coniferous, floral or vegetal aroma, a medium smell and taste sweet, with a metallic and refreshing persistence of fresh mint.

6.1.3. The physico-chemical evaluation

The findings regarding the physico-chemical parameters analyzed in the 9 samples of *F. japonica* honey from the three different areas of Romania (Merișor, Valea Vinului and Bocsig) are presented in Figure 1.

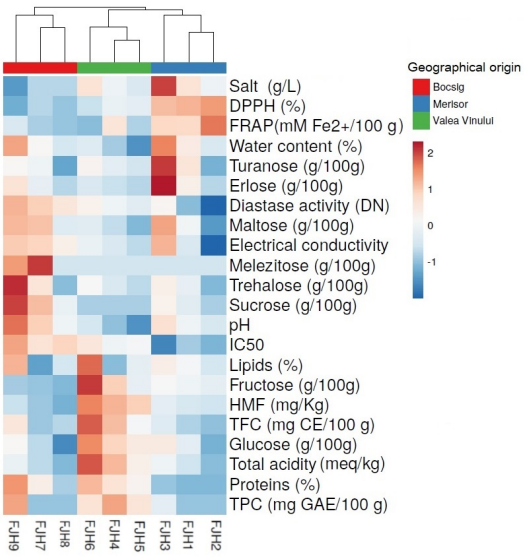

Figure 1. Heatmap and cluster analysis visualization of physicochemical analysis, phenolics and antioxidant activities in FJH samples. Columns indicate the FJH samples according to their collection sites, whereas rows represent the evaluated bioactive compounds, and antioxidant activities. Based on the detected bioactivities, the cells are highlighted accordingly, where blue denotes a significant negative association and red denotes a significant positive association, (Source: original).

The first cluster highlights the clustering of samples collected from the Bocsig area, which showed high levels of melezitose, trehalose, sucrose and pH, especially in the case of samples FJH7 and FJH9. Significant inhibitory levels (IC_{50}) were recorded in all samples. On the contrary, samples from the Bocsig area showed relatively lower levels of glucose and turanose. In the 2nd group, samples from the Valea Vinului area

showed similarities and high levels of fructose, glucose, HMF and total acidity, with respect to samples FJH4 and FJH6. In the final cluster, the samples from the Merişor area presented relatively low diastastic activity and electrical conductivity, especially with regard to sample FJH2.

6.2. Results concerning the determination of macro-, micro- and trace-elements from *F. japonica* plant and its related honey

Among the macro-elements in plant tissues, Ca had the highest concentration, followed by K and Mg. The Valea Vinului area had the highest concentration of Mg and Ca in the stem parts, while Ca was not detected in the roots and rhizomes. Regarding micro-elements, Cu presented the highest content in the Merişor area, for roots, and the lowest concentration was observed in rhizomes and stems (Valea Vinului and Bocsig). Fe had significant differences among plant tissues in all three zones. Mn had higher values in the strain from Valea Vinului. A high value was also detected for Se in stems. The results of the research showed variations in the content of trace-elements (heavy metals) in the tissues of the *F. japonica* plant, but also in the experimental areas. Valea Vinului generally presented the highest concentration of heavy metals.

For honey, both the highest (Ca) and the lowest (Mg) level of macro-elements were obtained in the samples collected from the same experimental area (Bocsig). The descending order of micro-elements in *F. japonica* honey is as follows: Fe >Mn >Cu >Se. The values presented for all heavy metals do not exceed 1 mg/kg; therefore, they are in line with the thresholds set by the EU. The highest proportion of harmful elements found in honey was observed for Ni in the samples from the Merişor area, while Pb was not detected in any analyzed honey sample.

6.2.3. The traceability of macro-, micro- and trace-elements from *F. japonica* plant-honey interaction (Translocation Factor - TF)

The results obtained for TF indicate a strong translocation potential in the case of Mg for all three investigated areas and for Se and K in the Merişor area, where the data exceed the TF reference value (TF>1). In contrast, the values of the other elements were below the reference value (TF<1) indicating that their transfer from the aerial parts of the *F. japonica* plant into honey follows a downward trend: Mg>Se>K>Ca>Ni>Mn>Cr>Fe>Cu >Cd>Pb.

6.3. Results regarding the TPC, TFC and antioxidant capacity of both *F. japonica* plant and honey

The results in Figure 2 revealed a significant antioxidant activity, with an inhibition percentage between 93.66% (IC50-5.31) (the root of plants collected from the Merişor area) and 16.09% (IC_{50}-30.70) (the stems of the plants collected from the Bocsig area). Total phenolic content (TPC) along with total flavonoid content (TFC) and antioxidant potential of the nine *F. japonica* honey samples can be viewed in Table 1.

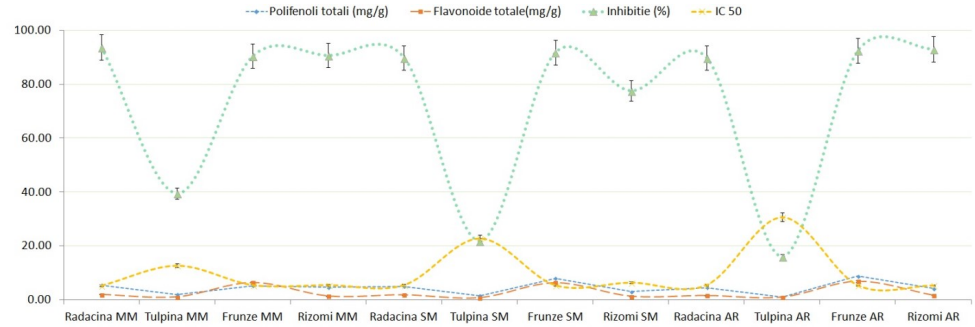

Figure 2. The content in total phenolic compounds, the inhibitory concentration (IC_{50}) and the inhibition percentage of the extracts obtained from different anatomical parts of the plants collected from the experimental areas. Rădăcină MM – roots Maramureș; Tulpină – Stems; Frunze- leaves; Rizomi – rhizomes. MM – Maramureș; SM – Satu Mare; AR-Arad, (Source: original).

Table 1. Polyphenol content and antioxidant capacity of F. japonica honey from the three experimental areas

Parameter	FJH1	FJH2	FJH3	Merișor area	FJH4	FJH5	FJH6	Valea Vinului area	FJH7	FJH8	FJH9	Bocsig area
TPC (mg GAE/100 g)	90.12± 0.10^{d}	90.23± 0.95^{d}	99.79± 1.01^{c}	93.38± 5.55A	120.08 ± 0.62^{a}	110.24± 0.46^{b}	110.71± 0.85^{bc}	113.67± 25.55A	89.87± 0.95^{d}	89.92± 0.79^{d}	119.88± 0.82^{a}	99.89±17.31A
TFC (mg CE/100 g)	19.71± 1.23	18.13± 0.22^{fe}	23.13± 0.20^{d}	20.32± 2.55A	33.75± 0.73b	26.25± 0.40^{c}	39.38± 0.49^{a}	33.12± 6.58A	18.75± 0.38^{ef}	20.17± 0.47^{e}	29.78± 0.98^{c}	22.90±6.00A
Antioxidant activity												
DPPH (%)	54.00± 2.12^{ab}	55.87± 0.36^{a}	53.40± 2.36^{b}	54.42± 1.28A	44.09± 0.26^{c}	43.16± 0.32^{c}	40.09± 0.42^{d}	42.44± 2.09B	39.31± 0.20^{d}	37.69± 0.80^{e}	35.41± 0.15^{f}	37.46±1.95C
IC_{50}	9.9.44 ±0.35^{d}	8.88± 0.10^{e}	7.83± 0.18^{f}	8.71± 0.81B	11.30± 0.09^{c}	11.49± 0.11^{c}	12.54± 0.13^{b}	12.53± 0.72A	12.74± 0.07^{b}	13.27± 0.28^{ab}	14.15± 0.06^{a}	13.38±0.71A
FRAP(mM Fe2+/100 g miere/honey)	1.88± 0.07^{b}	2.32± 0.10^{a}	1.91± 0.08^{b}	2.03± 0.24A	1.78± 0.06^{c}	1.09± 0.09^{e}	0.97± 0.10^{f}	1.28± 0.43B	1.07± 0.05^{e}	1.00± 0.02^{f}	1.28± 0.09^{d}	1.11±0.14B

Data are presented as mean ± standard deviation (n=3). Different lowercase letters in the same row represent significant differences between the accumulation of phenolic compounds and the antioxidant capacity of the samples according to the collection regions. Different capital letters denote significant differences by experimental areas.

6.4. Results concerning the identification and quantification of individual phenolic compounds found in both *F. japonica* plant and honey

A total of 20 phenolic compounds, of which 8 phenolic acids (protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid and rosmarinic acid), 10 flavonoids (catechin, epicatechin, rutin, naringin, quercitrin, isoquercitrin, quercetin, kaempferol, naringenin and apigenin), 1 phenolic aldehyde (vanillin) and 1 stilbene (resveratrol), were identified in the plant tissues of *F. japonica*.

In the case of honey, 15 phenolic compounds were identified, 9 phenolic acids (gallic acid, p-hydroxybenzoic acid, vanillic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid and rosmarinic acid), 5 flavonoids (quercitrin, quercetin, naringenin, kaempferol and galangin) and 1 stilbene (resveratrol).

6.5. Results concerning the antimicrobial and antibiofilm activities of both *F. japonica* plant and honey

The antibacterial mechanisms of different *F. japonica* plant tissues and honey samples were tested against Gram-positive bacteria (*S. aureus, B. cereus, E. faecalis*) and Gram-negative bacteria (*E. coli, P. aeruginosa , S. enteritidis*), as well as yeast (*C. albicans*) and are shown in Figures 3-4.

A clear discrimination was observed between the tissues of the plant and the collection region (Figure 3). Samples from the Bocsig area showed significant accumulations of bioactive compounds but moderate inhibitory activities (*S. aureus, S. enteritidis* and *P. aeruginosa*). Samples from Arad showed significant inhibitory potential against *E. coli, S. aureus* and *S. enteridis* (leaves), and stem extracts showed moderate inhibition against *S. aureus, E. coli, E. faecalis* and *P. aeruginosa* (Merişor and Valea Vinului area).

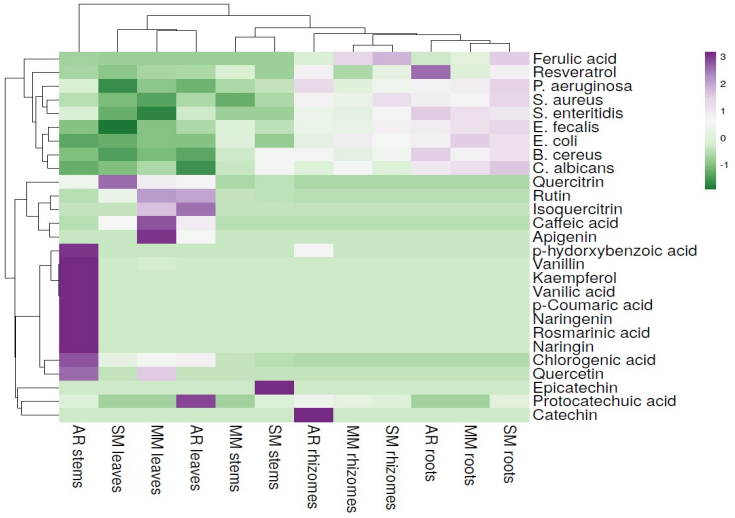

Figure 3. Heatmap visualization of the FJ plant extracts identified phenolic compounds and inhibitory activities against several microorganisms. Rows are represented by the individual phenolic compounds and the bacterial strains, whereas the columns indicate the evaluated plant tissues according to their area of collection. The cells are highlighted according to the influence of both phenolic components and microorganisms, where an intense purple hue denotes a significant positive association and an intense green hue denotes a significant negative association, (Source: original).

Regarding the honey samples (Figure 4), one sample from the Bocsig area (FJH7) and two from the Merişor area (respectively FJH1–2) proved to have distinct accumulations in phenolic compounds compared to the other studied honey samples. The sample collected from the Bocsig area (FJH7) revealed increased levels of gallic acid and p-hydroxybenzoic acid, as seen by the positive values highlighted according to the importance score. Interestingly, although phenolic acids were accumulated in rather low amounts in the mentioned sample, it showed significant inhibitory activity against all tested bacterial strains.

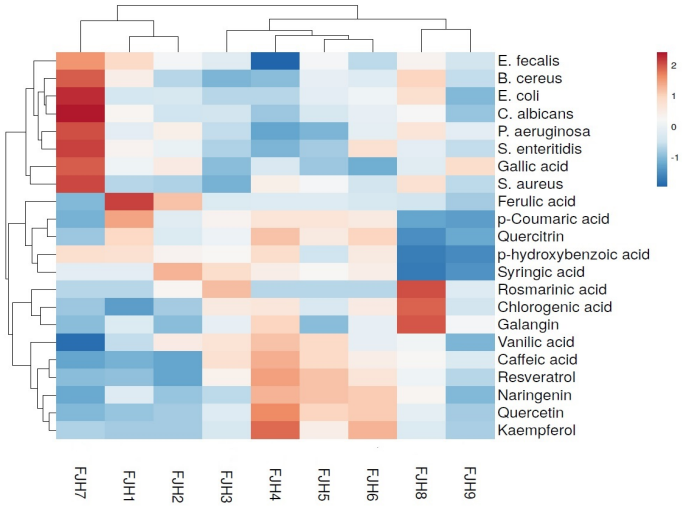

Figure 4. Heatmap visualization of the FJ honey extracts identified phenolic compounds and inhibitory activities against the evaluated bacterial strains. Rows are represented by the individual phenolic compounds and the microorganisms, whereas the columns indicate the evaluated honey samples according to their area of collection. The cells are highlighted according to the influence of both phenolic components and microorganisms, where an intense red hue denotes a significant positive association and an intense blue hue denotes a significant negative association, (Source: original).

The samples from the Merişor area (FJH1-2) showed strong inhibitory capacities, especially regarding *S. aureus, E. faecalis, P. aeruginosa* and *S. enteridis.* Sample 3 from the Merişor area (FJH3) was collectively positioned in the same sub-cluster as the samples from Valea Vinului (FJH4–6). These samples showed similarities in the accumulation of certain phenolic compounds (respectively caffeic acid, chlorogenic acid, syringic acid, vanillic acid and resveratrol). In addition, samples from Valea Vinului accumulated a considerable amount of flavonoids (such as kaempferol, naringenin, quercitin and quercitrin). Surprisingly, despite the fact that sample 1 from the Valea Vinului area (FJH4) showed considerable accumulation of relevant phenolic constituents, it showed significantly reduced antimicrobial activity, especially against *E. faecalis*. The next sub-cluster grouped the other samples from the Bocsig area (FJH8-9) which, as in the case of the first sample (FJH7), showed increased antimicrobial activity against the tested bacterial strains.

6.6. Results concerning the antibiofilm activity of both *F. japonica* plant and honey

The relationships between biofilm eradication after treatment with plant tissues and honey extracts can be seen in Figure 5 A and B. Plant tissue extracts of F. *japonica* showed discrepancies in their biofilm eradication capabilities, influenced by the amount of phenolic compounds accumulated in (Figure 5 A).

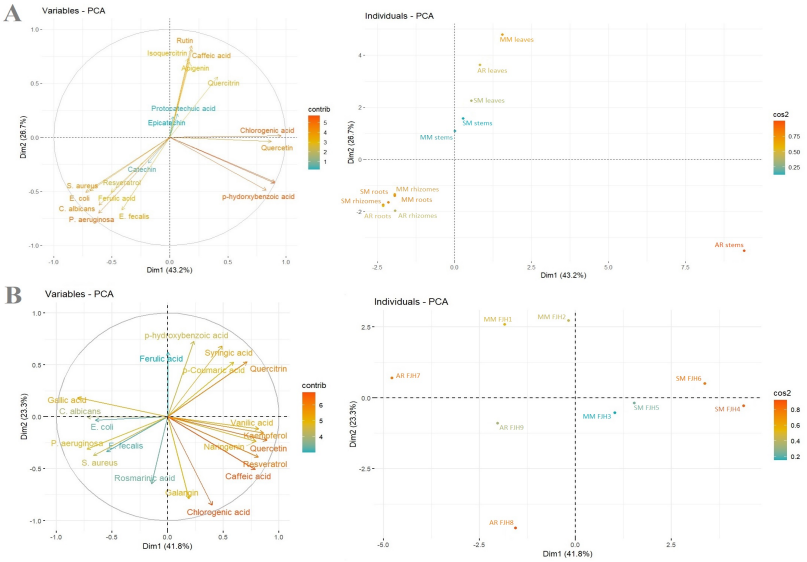

Figure 5. PCA visualizations of the evaluated plant tissue (A) and honey extracts (B) and the relationship between the biofilm eradication (MIC x 4) capability and the identified individual phenolic compounds. The first two dimensions of the plant extracts explained 69.9% of the total variance. Regarding the honey samples, the first dimensions explained 65.1% of the overall variance. MM - Maramureș; SM - Satu Mare; AR – Arad, (Source: original).

Upper quadrants 1 and 2 highlighted the clustering pattern of leaf extracts and stem extracts from Merișor and Valea Vinului areas. Regarding the tissues of the leaves from the Merisor area, biofilm eradication was influenced by the accumulation of phenolic acids (caffeic acid) and flavonoids (apigenin, isoquercitrin, quercitrin and rutin) in these tissues. For the stem samples from the Merișor and Valea Vinului areas, the biofilm activity was determined by the presence of epicatechin and protocatechuic acid. Quadrant 3 highlights stem tissues from the Bocsig area where biofilm eradication was found to be moderate. Lower quadrant 4 highlights that the accumulation of catechin, ferulic acid and resveratrol in rhizomes and roots significantly influenced the biofilm inhibitory activities of the extracts of these tissues.

Regarding the honey samples (Figure 5 B), the honey samples from the Bocsig area showed a significant biofilm eradication potential in all bacterial strains evaluated. These samples accumulated significant levels of gallic acid and rosmarinic acid. The samples from the Merișor area accumulated high levels of ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, syringic acid and quercitrin. The samples from Valea Vinului, which accumulated important levels of flavonoids and stilbenes (resveratrol), with a significant inhibitory potential.

7. Conclusions and recommendations

7.1. The study provides a comprehensive examination of the botanical and sensory profiles applied to *F. japonica* honey, serving as vital tools to discern the authenticity of this specific honey variety.

7.2. The physicochemical parameters of *F. japonica* monofloral honey showed distinctive characteristics of this type of honey, including a high content of sugars, especially fructose and glucose, a higher natural diastase activity and an acidic pH. All analyzed samples were in accordance with the standard values imposed for fresh honey.

7.3. The results confirmed the abundance of crucial macro-nutrients in the *F. japonica* plant samples but also in the honey. The honey obtained from the *F. japonica* plant produced in North-Wester and Wester parts of Romania does not exceed the limits of food contaminants, being suitable for consumption.

7.4. In this research, 20 phenolic compounds from the *F. japonica* plant and 15 compounds from the adjacent honey were identified, using the HPLC-PDA method, resveratrol being identified as a marker for *F. japonica* honey.

7.5. The total content of polyphenols and flavonoids showed a high antioxidant activity, this study demonstrated that the antioxidant potential of the *F. japonica* plant transferred to the related honey.

7.6. Both plant and honey extracts demonstrated the strongest antibacterial activity, but also antibiofilm activity against *E. coli, S. aureus*. Regarding the experimental areas, the samples from Arad and Satu Mare counties (Bocsig and Valea Vinului) showed the highest effectiveness in antimicrobial inhibition and biofilm eradication, followed by Maramureș county (Merișor area).

7.8. Recommendations

It is recommended to analyze a wider spectrum of *F. japonica* honey samples and from other areas of the country to have a comprehensive picture of the characteristics of this type of honey. This would enable us to create a detailed understanding of the biologically active properties transferred from the plant to the honey, thereby elucidating the therapeutic potential of *F. japonica* honey.

8. Innovative contributions of the thesis

The present research represents a pioneering study in the analysis of the physico-chemical and therapeutic characteristics of *F. japonica* honey in an attempt to bring valuable insights into a unique variety of honey that has received limited attention so far and remains relatively unknown to many beekeepers. Moreover, this research could represent a sustainable and integrated approach to counteract the harmful methods of eradicating the plant and to strengthen the efforts of beekeepers in the certification, promotion and commercialization of Romanian monofloral honey resulting from the *F. japonica* plant.

Selective bibliography:

1. Atazadegan, M. A., Bagherniya, M., Askari, G., Tasbandi, A., & Sahebkar, A. (2021). The effects of medicinal plants and bioactive natural compounds on homocysteine. Molecules, 26(11). https://doi.org/10.3390/molecules26113081.
2. Bensa, M., Glavnik, V., & Vovk, I. (2020). Leaves of invasive plants—japanese, bohemian and giant knotweed—the promising new source of flavan-3-ols and proanthocyanidins. Plants, 9(1). https://doi.org/10.3390/plants9010118.
3. Bobis, O., Dezmirean, D. S., Bonta, V., Moise, A., Pasca, C., Domokos, T. E., & Urcan, A. C. (2019). Japanese Knotweed (Fallopia Japonica): Landscape Invasive Plant Versus High Quality Honey Source. Scientific Papers-Series D-Animal Science, 62(1), 231–235.
4. Bogdanov, S. (2009). Harmonised Methods of the International IHC. Bee Product Science, 5, 1–62.
5. Cucu, A.-A.; Baci, G.-M.; Moise, A.R.; Dezsi, Ş.; Marc, B.D.; Stângaciu, Ş.; Dezmirean, D. S. (2021). Towards a Better Understanding of Nutritional and Therapeutic Effects of Honey and Their Applications in Apitherapy. Appl. Sci., 11, 4190. https://doi.org/https://doi.org/10.3390/app11094190.
6. Cucu, A. A., Baci, G. M., Dezsi, Ş., Nap, M. E., Beteg, F. I., Bonta, V., Bobiş, O., Caprio, E., & Dezmirean, D. S. (2021). New approaches on Japanese knotweed (*Fallopia japonica*) bioactive compounds and their potential of pharmacological and beekeeping activities: Challenges and future directions. Plants, 10(12). https://doi.org/10.3390/plants10122621.
7. Cucu, A. A., Paşca, C., Cucu, A. B., Moise, A. R., Bobiş, O., Dezsi, Ş., Blaga Petrean, A., & Dezmirean, D. S. (2024). Evaluation of the Main Macro-, Micro- and Trace Elements Found in Fallopia japonica Plants and Their Traceability in Its Honey: A Case Study from the Northwestern and Western Part of Romania. Plants, 13(3). https://doi.org/10.3390/plants13030428.
8. Dong, X., Fu, J., Yin, X., Li, X., Wang, B., Cao, S., Zhang, H., Zhao, Y., & Ni, J. (2014). Pharmacological and other Bioactivities of the Genus Polygonum - A Review. Tropical Journal of Pharmaceutical Research, 13, 1749–1759.
9. Glavnik, V., & Vovk, I. (2020). Extraction of anthraquinones from japanese knotweed rhizomes and their analyses by high performance thin-layer chromatography and mass spectrometry. Plants, 9(12), 1–16. https://doi.org/10.3390/plants9121753.
10. Lachowicz, S., & Oszmianski, J. (2019). Profile of bioactive compounds in the morphological parts of wild *Fallopia japonica* (houtt) and *Fallopia sachalinensis* and their antioxidative activity. Molecules, 24(7). https://doi.org/10.3390/molecules24071436.
11. Nawrot-Hadzik, I., Granica, S., Domaradzki, K., Matkowski, A., Systems, S. T., State, P. C., Quality, C., State, P. C., & Medical, W. (2018). Isolation and Determination of Phenolic Glycosides and Anthra- quinones from Rhizomes of Various Reynoutria Species. *Planta Med*. 84(15): 1118-1126.doi: 10.1055/a-0605-385784, 1118–1126.
12. Pătruică, S., Alexa, E., Obiștioiu, D., Cocan, I., Radulov, I., Berbecea, A., Lazăr, R. N., Simiz, E., Vicar, N. M., Hulea, A., & Moraru, D. (2022). Chemical Composition, Antioxidant and Antimicrobial Activity of Some Types of Honey from Banat Region, Romania. Molecules, 27(13). https://doi.org/10.3390/molecules27134179.