Theses of Doctoral (PhD) Dissertation

Comprehensive evaluation of plant extracts generated effects using multiple animal models for fish feed development

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1. Introduction and the aim of research

The use of extracts from plants in medicine, for purposes of healing, has been a part of human history, transcending all geographical boundaries and cultures. Be it the ancient Egyptians with their herbal formulas or the modern practice of phytotherapy, the plant uses over time as medicinal agents reflect a quest for natural health. All the ancient civilizations—Egyptians, Chinese, Indians, and the Islamic world—all agreed with the therapeutic value of plants and used them in their practices of healing. This rich heritage has laid the foundation for the development of phytotherapy that includes traditional knowledge along with modern scientific research to harness the medicinal potency effectively either from whole plants or from plant parts.

What really stands different with phytotherapy from orthodox pharmacology is the fact that it deals with the use of plants wholly, and not just isolating a single active ingredient. This, therefore, aims not only to give treatment to certain health problems but also to try to promote the good health of a person, thus strictly observing the holistic principle in medicine. All of this has aroused a new interest in phytotherapy since the limits of synthetic drugs have been overcome in an overwhelming way—mainly at the level of important secondary effects or antibiotic resistances—determining the need to look for other possibilities therapeutically, more natural and holistic, rather than forceful.

Among such multidisciplinary scholarly activities is the study of plant extracts, which, in turn, joins botany, chemistry, pharmacology, and clinical sciences into one logical link. Development of such analytical techniques, including NMR spectroscopy, GC-MS, HPLC, etc., becomes possible for the identification and quantitation of various bioactive compounds in plants, and therefore the exploration of plants for their medicinal property begins. This line makes clear that such efforts have been directed at finding many and various phytochemicals with possible health benefits, including but certainly not limited to antioxidant, anticancer, and antimicrobial activities.

Amongst these secondary metabolites, polyphenols and flavonoids are the ones of therapeutic application, since they hold great potential for anti-inflammatory, antioxidant, and neuroprotective activities. These compounds not only play key roles in the prevention and management of chronic diseases but also in cell metabolism, including signaling during the induction of proliferation and apoptosis.

Thus, with the health benefits related to the polyphenols and flavonoids, yet they form a tippy point to take care of maintaining a diet that has a balance of many plant-based foods. The current study is an effort to look for the medicinal activity of some traditionally known plants with health-providing properties, i.e., olive (*Olea europea*), almond (*Prunus amygdalus*), and blackberry (*Morus nigra*).

The olive tree can produce products like olive oil and leaves, which contain antioxidants and inflammations against the heart. Almonds are highly valued for both nutrition and traditional medicine, they exert activities beneficial to health, including lipid-lowering and anti-stress effects. Blackberry, for its part, due to the high content of phenolic compounds, seems to be related to antihypertensive, anti-diabetic, and anti-inflammatory properties. The following are the research objectives that describe and summarize advanced analytical methods and model organisms that elicited the nutritional effects, cellular mechanisms, and induction by plant extract. This will thus be multi-inter-disciplinary research targeting the intensified understanding of the health benefitting impacts of bioactive plant-derived compounds on cellular events and processes.

The study intends to assess the possible antimicrobial activity of these extracts in view of their microbiological effect on nutrition and metabolism, making their contribution for developing a nutrition and animal feed system aimed at health. Thus, this study characterizes a coherent effort towards bridging the gap of traditional herbal medicine and modern scientific inquiry for complete unlocking of the therapeutic potential from the extracts of plant species. This, through the conduct of basic and applied research, can strengthen our knowledge in relation to health-promoting phytonutrients and, therefore, develop innovative natural health solutions that are able to contribute to the global healthcare reality.

2. Materiel and methods

2.1. Preparation of gemmotherapy extracts (GTEs) for quantitative and qualitative analyses

The paper aimed to develop the Gemmotherapy Extracts (GTEs) comprehensive methods involving collection, preparation, and analysis of the plant materials to assure that the produced extracts are indeed of high quality. The materials used within the scope of this research included those of young olive shoots, sweet almond buds andblack mulberry buds from the regions of Calabria in Italy and from Așchileu Mare, Cluj, Romania. All these were submitted for strict quality control to the SC PlantExtrakt SRL QC Laboratories; voucher specimens have been deposited for reference and further analysis.

The preparation of GTEs was done immediately post-harvest with a preservative mixture of 96% ethanol and glycerol at a ratio of 1:1. The plant material was mixed with it at 1:0.5 plant:solvent ratio, which is important for the maintenance of the integrity and quality of the vegetal material. This would involve the moisture analysis of the samples that would guide in adjusting the quantity of the solvent to achieve an optimum dry plant: solvent ratio of 1:20 so that the extract is not damaged and remains stable.

Maceration of the vegetal material in the mixture of ethanol-glycerol with the corresponding amount of solvent was added for preservation of the samples. After that, it was expected that the extraction was complete by stirring the plant-solvent mixture at intervals of 20 days. The mixture was then subjected to processing to separate solid and liquid parts; pressing of the solid plant material was then performed for further enhancement of the extraction yield. Certainly, such a method of preparation would have helped in the retention of these natural qualities and therapeutic potentials of these plant materials.

For the quantitative determination of these selected extracts, the Shimadzu Nexera I LC-MS-8045 system, located at Kyoto, Japan, was employed. Its parts included Quaternary Advanced UHPLC Pump and an Autosampler. These include an Electrospray Ionization (ESI) probe that can work with solutions containing electrospray solvents; down to the quadrupole mass spectrometer rod components, which determine accurate detection of the trace levels of Chromatographic separation was carried out on a reversed-phase column with size 150 mm × 4.6 mm × 3 μm and 100 Å pore size from Phenomenex in Torrance, CA, USA. The temperature of the column was consistently maintained at 40 °C throughout the analysis.

The applied mobile phase is the gradient composition of methanol with ultrapure water. The purchased methanol and the ultrapure water used in the mobile phase were bought from Merck, Darmstadt. Ultra-pure water was prepared using the Simplicity Ultra-Pure Water Purification System from Merck Millipore, located in Billerica, MA, USA. Formic acid of HPLC grade was used as an organic modifier in the mobile phase from the same supplier. The applied flow rate for the analysis was 0.5 mL/min, and the sum of the total time taken for one complete analytical run, including the post-run, stands at 35 minutes.

In the study, the analysis of protein and carbohydrate contents was meticulously conducted using established analytical methods, ensuring a thorough and accurate evaluation of the samples' nutritional composition. The protein content was determined through the Kjeldahl method, a highly regarded technique for its precision in measuring the nitrogen content, which is then used to estimate protein levels. This method involves digestion, distillation, and titration processes, making it a cornerstone in protein analysis.

For carbohydrate quantification, Carbohydrates were determined by the phenol– sulfuric acid method, the most sensitive and reliable for the qualitative test for monosaccharides, disaccharides, oligosaccharides, and polysaccharides. The method is based on the formation of a colored complex of phenol and sulfuric acid with carbohydrates, which allows the measurement of the total content of carbohydrates in a sample.

The concentration of the reducing sugars in the solution was also determined through the Luff–Schoorl method. The method involves the reaction of reducing sugars with a copper reagent under alkaline conditions. Hence, the sugar directly gives rise to a colored product, which is in direct proportion to the concentration of reducing sugar and specific in this aspect.

This study focuses as well on assessing the antimicrobial properties of various GTEs against a selected group of bacteria and fungi sourced from the National Collection of Agricultural and Industrial Microorganisms (NCAIM). It includes eight bacterial strains*:* *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* subsp. enterica, *Proteus vulgaris* (Gram-negative); *Bacillus cereus*, Staphylococcus aureus, and Enterococcus faecalis (Gram-positive); and five mycotoxigenic fungi: *Aspergillus flavus, A. niger, A. ochraceus*, *Penicillium citrinum*, *P. expansum*, along with the yeast strain *Saccharomyces cerevisiae*. The bacterial strains were cultured on Nutrient agar at 37°C for 24 hours, while the fungi and yeast were grown on a complex medium at 28°C for 72 hours, with all media supplied by VWR International L.L.C., in Debrecen, Hungary.

The study investigated the antimicrobial effects of GTEs using the agar-well diffusion method. Ethanol in the extracts was removed via evaporation under controlled conditions to preserve bioactive compounds, with the ethanol then replaced by purified water. Samples were stored at 4 degrees Celsius until use. GTE concentrations ranged from 0% to 100% (v/v), with 100% being 50 mg/mL and lower concentrations proportionally diluted. Additionally, certain concentrations were combined in a 1:1 ratio to assess synergistic effects. Microbial suspensions with a standard optical density were applied to nutrient media, followed by the introduction of extracts into a central well. Plates were incubated, and the inhibition zones measured to evaluate antimicrobial activity. The methodology applied varied slightly between bacterial and fungal strains, with different incubation temperatures and durations.

2.2. Transitional models

The research conducted extensive experiments on *Drosophila melanogaster* the *wm4h* (white mottled 4) strain and carp (*Cyprinus carpio*) larvae to explore the nutritive or toxic effects of GTEs on growth, viability, and metabolic health. In the *Drosophila* segment, the *wm4h* strain from the Bloomington Stock Center was used, with the fruit flies being subjected to three distinct dietary regimes: a zero nutrient medium (0N) that lacked essential nutrients, a normal medium (NM), and a high-sugar medium (HS). These mediums were prepared with precise compositions to foster different growth conditions, and some vials received various concentrations of GTEs to assess their impact on larval development and adult viability.

For the carp larvae studies, the experiments began following the successful artificial propagation of common carp, with fertilized eggs placed in Zuger glasses connected to a sophisticated recirculation system incorporating both mechanical and biological filtration to simulate a controlled aquatic environment. The larvae underwent initial rearing in a specialized unit before being introduced to a modular aquarium setup, designed to closely monitor growth conditions such as temperature, oxygen levels, and dietary intake, including GTE-infused feed for selected groups.

In both studies, a significant focus was placed on monitoring and assessing the larvae's viability through various stages of development. For Drosophila, this involved the collection of early-stage embryos and their transfer to vials containing the different media types, each possibly supplemented with GTEs. The viability of these flies was meticulously tracked from embryo to adulthood, under controlled temperature and humidity, to determine the effect of dietary conditions and GTE concentration on their development. The larvae's ATP content was also measured as an indicator of metabolic health, using a detailed process of homogenization and centrifugation to obtain accurate readings of energy levels within the larvae, providing insights into the physiological impact of the different diets.

Carp larvae were similarly observed for growth parameters, with their ATP content assessed to gauge metabolic activity and overall health. The creation of GTE-infused feed involved a novel preparation method, ensuring the larvae received a nutritionally enhanced diet aimed at improving their developmental outcomes. Environmental conditions within the aquarium system were rigorously controlled, with parameters such as temperature and oxygen concentration consistently maintained to ensure the reliability of the experimental results.

The research aimed to thoroughly investigate the potential benefits or impacts of GTEs on the development, health, and viability of both Drosophila melanogaster and carp larvae. Through careful design and execution of the experiments, including the replication of conditions across multiple trials, the study sought to provide a comprehensive understanding of how GTEs might influence the growth and metabolic health of these organisms, contributing valuable knowledge to the fields of developmental biology and aquaculture nutrition.

In a detailed experimental study, carp larvae were segregated into two groups: a control group that was fed solely on brine shrimp (Artemia salina) and several test groups that were provided with a diet enriched with Gemmotherapy Extracts (GTEs). The purpose of the study was to observe the effects of GTEs on the development, health, and survival rates of carp larvae. Over the course of the experiment, key growth indicators, such as the length of the larvae and their ATP (adenosine triphosphate) content, were meticulously monitored to gauge the biological impact of the GTE-infused diet compared to the standard diet of brine shrimp.

To design a specific diet for the carp larvae infused with GTEs, a unique preparation process was employed. This involved integrating Fibersol-2, a water-soluble dietary fiber known for its health benefits, with a measured amount of the GTE. The precise ratio used was 0.2 mL of GTE for every gram of Fibersol-2, ensuring a consistent and uniform mixture. After blending, the mix was dried in an incubator set at 35 °C for 2-3 days, then ground to a fine consistency to ensure uniformity in feed particle size. The final product was stored in airtight containers at room temperature, shielded from light to preserve its nutritional quality until it was administered to the larvae.

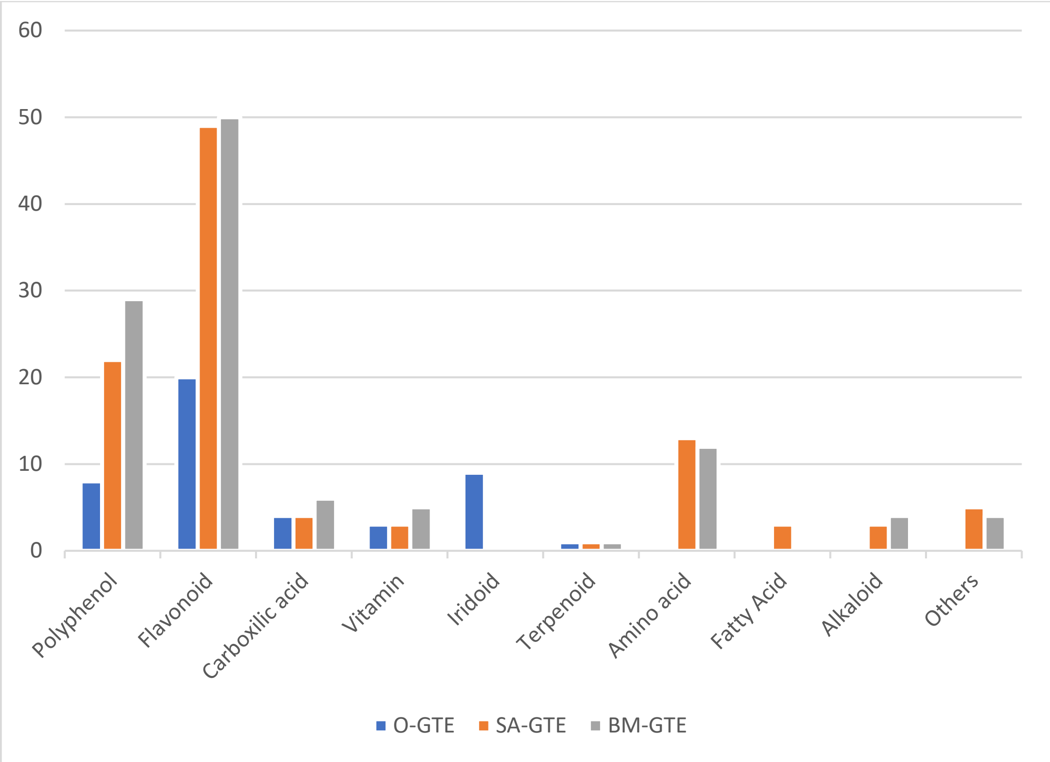
The assessment of the larvae specific ATP content, an indicator of metabolic activity and overall health, was conducted through a systematic process. Selected larvae from each aquarium were homogenized after being rinsed in Phosphate-Buffered Saline (PBS) to remove any external contaminants. The homogenized larvae were then centrifuged to separate the extract, which was analyzed in an ATP test tube using a luminometer. The readings obtained in Relative Light Units (RLU) were converted into picograms of ATP through a specific formula, allowing for precise quantification of the larvae's metabolic energy.

This comprehensive approach not only allowed for the detailed observation of the effects of GTE supplementation on larval growth and metabolic health but also highlighted the potential for using dietary interventions to enhance the development and viability of fish larvae. The inclusion of GTEs in the larvae's diet was explored as a means to potentially improve health outcomes and survival rates, providing valuable insights for aquaculture practices. The methodology, involving careful preparation of the GTE-infused feed and rigorous assessment of larval growth parameters and ATP content, underscores the importance of nutrition in aquacultural development and sets a precedent for future studies on dietary supplements in larval rearing.

3. Results

3.1. Phytonutrient Profiles of Gemmotherapy Extracts (GTEs)

In this comprehensive study, the phytonutrient content of GTEs was examined through the application of Ultra-High-Performance Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (UHPLC–ESI-MS). Utilizing this sophisticated method for qualitative analysis, we were able to detect a wide range of chemical compounds present in different GTEs.



The major phytonutrient compound classes identified in O (olive), SA (almond) and BM (black mulberry)-GTEs.

The study provides an in-depth analysis of the phytonutrient profiles of GTEs, focusing on olive, sweet almond and black mulberry GTEs. Utilizing the UHPLC–ESI-MS, a wide range of bioactive compounds were identified within these extracts.

For olive GTE, 45 distinct phytonutrients were identified, spanning categories like flavonoids, polyphenols, lignans, carboxylic acids, iridoids, terpenoids, and vitamins. These compounds include well-known antioxidants such as quercetin, luteolin, and hydroxytyrosol, showcasing the extract's potential for offering health benefits like anti-inflammatory and antioxidative effects, alongside possibly supporting metabolic and cardiovascular health.

The sweet almond GTE analysis revealed an even broader spectrum of phytonutrients, with 103 identified compounds. This extensive range includes various flavonoids, polyphenols, carboxylic acids, amino acids, alkaloids, carbohydrates, fatty acids, terpenoids, and vitamins. The presence of compounds like quercetin-3-O-rutinoside-7-O-glucoside, kaempferol derivatives, and coumaroylquinic acids further accentuates the extract's potential for health benefits, similar to those suggested by the olive GTE analysis.

The comprehensive phytonutrient profiling of these GTEs highlights the complex and rich composition of bioactive compounds within. This extensive characterization enhances our understanding of the potential health benefits offered by GTEs, underlining their antioxidant properties and suggesting a broad spectrum of possible therapeutic effects. This foundational analysis paves the way for future research into the specific health benefits and applications of these extracts, potentially leading to the development of novel health supplements and natural health solutions.

3.1.1. Olive Gemmotherapy extract Phytonutrient Profile

The investigation into the phytonutrient composition of Gemmotherapy Extracts (GTEs), particularly focusing on olive GTE, used Ultra-High-Performance Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (UHPLC–ESI-MS) for an advanced qualitative analysis. This meticulous process allowed for the identification of a significant variety of chemical constituents present in the extracts, highlighting the complex nature of these natural products. In the olive GTE, specifically derived from Olea europaea, a total of 45 distinct phytochemicals were identified, demonstrating the extract's rich biochemical composition. The identified components span a wide range of phytonutrient categories, including flavonoids, polyphenols, lignans, iridoids, and vitamins, with compounds such as quercetin, luteolin, hydroxytyrosol, and oleuropein being particularly notable for their potent antioxidant properties.

This detailed phytonutrient profiling underscores the potential health benefits of olive GTE, suggesting its role in anti-inflammatory and antioxidative actions, as well as its possible contributions to metabolic and cardiovascular health. The diversity and richness of phytonutrients in olive GTE highlight its therapeutic potential, laying the groundwork for further exploration into its health benefits.

3.1.2. Sweet Almond-Specific GTE Phytonutrient Profile

Extending the phytonutrient analysis to sweet almond GTE revealed an even more extensive array of bioactive compounds. The qualitative analysis through UHPLC–ESI-MS identified a total of 103 constituents within the sweet almond GTE, indicating a broader spectrum of phytonutrients compared to the olive extract. This array includes a variety of flavonoids, polyphenols, carboxylic acids, amino acids, and other essential nutrients that contribute to the almond extract's health-promoting potential.

The detailed table of phytonutrients from the sweet almond GTE showcases an impressive range of compounds, such as quercetin derivatives, kaempferol glycosides, and numerous other flavonoids known for their antioxidant capabilities. This comprehensive nutrient profile suggests the sweet almond GTE's capacity for offering a multitude of health benefits, including anti-inflammatory effects, antioxidant protection, and support for metabolic processes.

The analysis of both olive and sweet almond GTEs through advanced chromatographic techniques has provided valuable insights into their complex phytonutrient compositions. The identification of such a wide variety of bioactive compounds in these extracts underscores their potential as sources of natural health-promoting agents. These findings not only contribute to our understanding of the chemical basis for the therapeutic effects of GTEs but also open up new avenues for future research into their specific applications in health and medicine. This research forms a critical foundation for the development of novel natural health solutions, emphasizing the importance of phytonutrients in the promotion of well-being and disease prevention.

Sweet almond (SA) seeds are highlighted for their nutritional content, offering a rich blend of about 50% lipids, 25% proteins, 20% carbohydrates, along with a variety of phytonutrients known for their beneficial effects, particularly in combating cardio-metabolic diseases. UPLC–ESI-MS analysis of SA-GTE underscored its rich phytonutrient composition, including a notable presence of polyphenols, amino acids, carboxylic acids, and fatty acids. Among these, flavonoids stand out with 49 identified compounds, including flavanols such as quercetin, kaempferol, myricetin, along with other flavonoid categories like flavones and flavanones, featuring compounds like triacetin, naringenin, and eriodyctiol. Additionally, the extract contains significant non-flavonoid polyphenols, including hydroxycinnamic acids like chlorogenic, caffeic, coumaric, and ferulic acids.

3.1.3. Black mulberry GTE Phytonutrient Profile

The black mulberry (BM) GTE analysis further reveals an even more complex phytochemical profile, with the identification of 111 distinct chemical constituents. This diversity reflects the rich, natural compound blend in black mulberry, underscoring its substantial potential for health benefits. This broad spectrum of phytonutrients suggests the extract's promising applications in herbal medicine and nutrition, highlighting its role in supporting overall health and potentially offering therapeutic benefits in various contexts.

The Black Mulberry Gemmotherapy Extract (BM-GTE) analysis revealed a rich diversity of bioactive compounds, spanning various categories known for their health-enhancing properties. The phytonutrient profile of BM-GTE encompasses both non-flavonoid and flavonoid polyphenols, alongside amino acids, carboxylic acids, vitamins, and alkaloids. Notably, the flavonoid category is well-represented with 50 compounds, including flavanols like quercetin, kaempferol, myricetin, along with flavones such as luteolin and apigenin, and flavanones like naringenin. Non-flavonoid polyphenols in the extract feature hydroxybenzoic and hydroxycinnamic acids, including notable compounds like chlorogenic acid and ferulic acid, as well as stilbenes such as resveratrol.

Further quantitative analysis of GTEs focused on a selection of polyphenols for their renowned antioxidant and anti-inflammatory properties. This detailed examination involved both flavonoid and non-flavonoid compounds, specifically highlighting caffeic and chlorogenic acids due to their significant health benefits and strong antioxidant capabilities. An additional set of ten flavonoid compounds, including apigenin, kaempferol, and quercetin, was identified for quantification, recognizing their varied biological activities that contribute to neuroprotection, cardiovascular health, and potential cancer prevention.

The extensive range of polyphenolic compounds identified underscores the potential of GTEs as rich natural sources of anti-inflammatory and antioxidant agents. The highlighted flavonoids demonstrate a broad array of positive health impacts, making them subjects of interest in research on antioxidant potential and the modulation of inflammatory pathways. The quantitative analysis provided in this study, especially for specific polyphenols in the olive GTE (O-GTE), offers valuable insights into the concentrations of these bioactive compounds, aiding in the understanding of the therapeutic benefits and efficacy of these extracts.

This study comprehensively elucidates the intricate phytochemical composition of Gemmotherapy Extracts (GTEs) and underscores the significance of their bioactive components in augmenting the potential health benefits of the extracts. The findings are poised to guide future research and applications in herbal medicine, nutraceuticals, and dietary supplements, particularly emphasizing the demand for natural substances that promote health.

Analysis across various GTEs disclosed unique polyphenol profiles, with flavonoids like luteolin-7-O-glucoside notably predominant in some extracts, surpassing other polyphenols in concentration. This hierarchy was followed by rutoside, hyperoside, chrysin, quercetin, apigenin, luteolin, vitexin, and naringenin. Non-flavonoid polyphenols such as chlorogenic acid were identified, while caffeic acid was absent in the olive GTE (O-GTE), as validated through UPLC–ESI-MS chromatogram analysis.

In the sweet almond GTE (SA-GTE), quantitative analysis showcased a predominance of flavonoids like rutoside and hyperoside, which constituted about 90% of the assessed polyphenols, with non-flavonoids including chlorogenic and caffeic acid detected in lesser quantities. Other flavonoids present at lower levels were chrysin, kaempferol, anrigenin, and apigenin, while luteolin and its derivatives were not detected in SA-GTE in both types of analyses.

The black mulberry GTE (BM-GTE) quantitative analysis suggested chlorogenic acid and rutoside as the most abundant polyphenols, with others like hyperoside, apigenin, and chrysin in lesser quantities. Polyphenols such as luteolin-7-O-glucoside, naringenin, and luteolin were found in minimal amounts.

Each GTE displayed a distinctive polyphenol profile, though some commonalities were observed across different extracts. Moreover, the selected polyphenols in these GTEs, including olive, sweet almond, and black mulberry, demonstrated metabolically mediated antidiabetic and anti-inflammatory effects, highlighting their potential therapeutic applications in managing diabetes and inflammation.

The analysis further examined the Total Polyphenol Content (TPC) and Total Flavonoid Content (TFC) across the GTEs, revealing that the SA-GTE exhibited significantly higher polyphenol content compared to BM-GTE and O-GTE. The TPC analysis highlighted that SA-GTE's polyphenol content was roughly twice as high as that in BM-GTE and O-GTE, emphasizing SA-GTE's potential as a potent source of phytonutrients conducive to health improvement. Polyphenols are crucial for combating oxidative stress linked to numerous chronic diseases, indicating SA-GTE's efficacy in promoting well-being and disease prevention.

Additionally, the TFC analysis showed SA-GTE to have a considerably higher flavonoid concentration compared to other extracts, with its flavonoid content notably higher than O-GTE and BM-GTE. The proportions of flavonoids relative to total polyphenols in each extract highlighted the significant presence of these compounds in O-GTE and SA-GTE, suggesting their exceptional potential as sources of beneficial flavonoids. Conversely, BM-GTE's composition suggested a balanced mix of flavonoid and non-flavonoid polyphenols.

This comprehensive analysis underlines the rich polyphenolic content and diverse bioactive compound profiles of GTEs, underscoring their significant health-promoting potential and encouraging further exploration into their specific benefits and applications in health-related fields.

3.2. Analysis of the Anti-microbial effect of GTEs

The study meticulously examined the antimicrobial capabilities of Gemmotherapy Extracts (GTEs) using the agar diffusion method, revealing varying degrees of antimicrobial activity across different GTEs. The olive (Olea europaea) GTE showed effectiveness against five microbial strains, making it the most potent among the extracts tested. It demonstrated significant activity particularly against Gram-negative bacteria within a concentration range of 50-100%. The almond (Prunus amygdalus) GTE followed, showing efficacy against four strains, while the black mulberry (Morus nigra) GTE was effective against two species.

Notably, none of the GTEs inhibited the growth of Escherichia coli, Salmonella enterica, Pseudomonas aeruginosa, and certain yeast and mold species. This highlights the specificity of the antimicrobial effects of GTEs and suggests the need for targeted application based on microbial susceptibility.

The minimum inhibitory concentration (MIC) analysis provided deeper insight into the antimicrobial strength of each GTE, with all tested extracts displaying inhibitory effects at varying concentrations for all microorganisms tested. Olive GTE was particularly effective with lower MIC values for several bacteria, indicating strong antimicrobial properties. Almond GTE and black mulberry GTE also showed promising MIC values, although generally higher than those of olive GTE.

The minimum bactericidal concentration (MBC) test further identified the concentration at which GTEs could kill bacteria, with olive GTE showing a broad spectrum of bactericidal activity. Interestingly, almond GTE was the only extract to demonstrate any effect against Pseudomonas aeruginosa, yet it showed no activity against yeast in the MBC test.

Experiments with mixed GTEs revealed that combinations could either maintain or reduce the antimicrobial efficacy compared to individual extracts. Some mixtures exhibited synergistic effects, potentially enhancing the antimicrobial activity beyond that observed with single extracts alone.

This comprehensive analysis underscores the potential of GTEs as sources of natural antimicrobial agents. Their varied effectiveness against different microbial strains highlights their potential applications in herbal medicine, dietary supplements, and as natural preservatives in food and cosmetic products. Further research into the mechanisms of action and the optimization of extract combinations could enhance their therapeutic efficacy and broaden their application in combating microbial infections.

3.3. Transitional models

The study embarked on an intricate exploration of the nutritional effects of Gemmotherapy Extracts (GTEs) on Drosophila melanogaster, utilizing diets devoid of nutrients (0N), supplemented with normal media (NM), and high in sugar (HS) to discern the impacts of these extracts on the survival, growth, and development of fruit fly larvae and pupae. Grounded in the substantial body of research emphasizing the pivotal role of diet in the lifecycle of Drosophila melanogaster, where even the absence of a single essential amino acid could drastically reduce lifespan, this study meticulously observed the transition of larvae to the third instar stage and their progression to the pupal stage. Such developmental benchmarks were instrumental in assessing the nutritional potency of the various diets infused with distinct GTEs.

In the context of a zero-nutrient diet supplemented with GTEs, the study unveiled that while Olive GTE (O-GTE) failed to promote larval or pupal development, both Sweet Almond GTE (SA-GTE) and Black Mulberry GTE (BM-GTE) exhibited a range of efficacies in bolstering larval and adult viability. Notably, SA-GTE enhanced survival at lower concentrations of about 2-4%, whereas BM-GTE significantly improved survival rates by up to 26%, showcasing the nuanced differences in the nutritional impacts of various GTEs on fruit fly development and survival.

This nuanced exploration into the differential effects of GTEs not only provides insights into the nutritional benefits and constraints associated with these plant extracts but also underscores the complexity of dietary needs in model organisms like Drosophila melanogaster. Such findings bear implications for the broader application of these extracts in health research, dietary supplementation, and the development of nutraceutical products.

Further investigations under normal and high-sugar dietary conditions shed light on the complex interplay between GTE supplementation and developmental outcomes in fruit flies. Specifically, the research highlighted that while O-GTE could enhance viability under normal dietary conditions, the high-sugar diet presented a challenging environment where GTEs' effects varied, with some extracts causing developmental delays without a corresponding improvement in viability. These observations suggest a delicate balance between the concentration of GTEs, the dietary environment, and the developmental and survival outcomes of Drosophila melanogaster.

Expanding the scope of the study to encompass the nutritional potential of GTEs on carp (Cyprinus carpio) larvae offered additional insights. This comparative analysis aimed at evaluating the ATP production and growth effects of substituting standard brine shrimp feed with GTEs. While the initial developmental stages exhibited no significant differences between dietary groups, the critical feeding phase post-hatching revealed that ATP production varied with the dietary source, underscoring the influence of nutritional composition on energy metabolism and growth potential in carp larvae. Notably, O-GTE emerged as a particularly promising nutritional source, demonstrating the highest initial ATP levels. However, as the development progressed, the observed ATP content across different GTEs suggested varying degrees of nutritional efficacy, with brine shrimp-fed larvae ultimately exhibiting the highest survival rate by day 9 post-hatching.

Collectively, this comprehensive study delineates the specific and concentration-dependent viability enhancements conferred by SA- and BM-GTEs, while also pointing to the potential contributions of other plant extract components to observed nutritive effects. The variability in the effectiveness of different GTEs across dietary conditions and developmental stages in

Drosophila melanogaster and carp larvae illuminates the intricate dynamics between diet, nutritional content, and the health and development of model organisms. These findings not only highlight the potential of GTEs in providing essential macronutrients and other nutritional components but also stress the importance of delving deeper into the unique chemical compositions of various plant extracts and their specific impacts on organismal growth and development.

4. New contributions to academic knowledge

1. Distinct Phytonutrient Profiles: The study identified unique compositions for each GTE: O-GTE is characterized by high concentrations of luteolin-7-O-glucoside, SA-GTE by hyperoside, and BM-GTE by chlorogenic acid. This differentiation allows for the use of these compounds as quantitative markers in identifying each extract.

2. Antioxidant Capacity Analysis: Comparative antioxidant assessments using DPPH and FRAP methods indicated that O-GTE possesses the highest antioxidant capacity, followed by SA-GTE, and BM-GTE, with O-GTE demonstrating approximately twice the antioxidant efficiency compared to SA-GTE and three times that of BM-GTE.

3. Total Polyphenol and Flavonoid Content: The Total Polyphenol Content (TPC) and Total Flavonoid Content (TFC) analysis revealed significant quantities, with SA-GTE showing a polyphenol content twice as high as O-GTE and BM-GTE, and a flavonoid content 2.5 times greater than O-GTE and 3.6 times that of BM-GTE.

4. Nutritional Impact on Development: BM-GTE significantly supported larval and pupal viability under a zero-nutrient (0N) diet, showing up to 26% viability at the highest concentration tested, demonstrating the importance of specific polyphenols and flavonoids in biological systems.

5. Role in Caloric Restriction-Mimetic Mechanisms: Despite varied antioxidant capabilities, all GTEs contain polyphenols like resveratrol and quercetin, which are known for their caloric restriction-mimetic (CRM) effects. However, it remains uncertain whether GTEs directly induce CR-specific cellular mechanisms.

6. Diverse Physiological Influences: The study revealed that GTEs influence a range of physiological processes, such as antidiabetic, anti-inflammatory, and neuroprotective effects, through their unique polyphenol and flavonoid profiles. Notably, the high

rutoside content in BM-GTE suggests its potential in counteracting the adverse effects of high-sugar diets.

7. Dual-Species Experimental Model: Using both Drosophila melanogaster and Cyprinus carpio larvae, the study established a robust model system that enhances the reliability of findings and allows for a comprehensive comparative analysis of the nutritional impacts of GTEs across different species. This dual-species approach confirms the broad applicability and relevance of GTEs in nutrition science.

5. Practical use of the results

- The presence of specific polyphenols suggests their usefulness in treating inflammatory and metabolic disorders, highlighting overlapping therapeutic potentials among the extracts.
- The diverse polyphenolic content in each GTE underscores their potential as natural remedies, contributing to their therapeutic efficacy and offering opportunities for developing natural health products and alternative therapies.
- The use of a consistent experimental framework across Drosophila melanogaster and Cyprinus carpio larvae, establishing a robust dual-species model system for assessing the extracts' specific nutritive properties and biological impacts.
- The lack of negative effects emphasizes the value of these plant extracts as positive supplements across different dietary scenarios, especially where increased viability is sought.
- The study shows each GTE's unique composition and shared potential in treating inflammatory and metabolic disorders.
- This detailed characterization of GTEs sets the stage for further research and the development of GTE-based treatments and supplements, opening avenues for new natural health solutions.

6. List of publications

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Registry number: DEENK/165/2024.PL
Subject: PhD Publication List

Candidate: Amina Alaya
Doctoral School: Doctoral School of Animal Husbandry
MTMT ID: 10065282

List of publications related to the dissertation

Foreign language scientific articles in Hungarian journals (1)

1. **Alaya, A.**, Máthé, A. B., Frecska, E., Máthé, E.: The olive (Olea europaea) and the almond (Prunus amygdalus) related phytonutrients, and the associated health-promoting biological effects, a review.
   Agrártud. közl. 2021 (1), 11-24, 2021. ISSN: 1587-1282.
   DOI: http://dx.doi.org/10.34101/actaagrar/1/8558

Foreign language scientific articles in international journals (3)

2. Héjja, M., Mihok, E., **Alaya, A.**, Jolji, M., György, É., Mészáros, N., Turcuş, V., Oláh, N. K., Máthé, E.: Specific Antimicrobial Activities Revealed by Comparative Evaluation of Selected Gemmotherapy Extracts.
   Antibiotics-Basel. 13 (2), 1-31, 2024. EISSN: 2079-6382.
   DOI: http://dx.doi.org/10.3390/antibiotics13020181
   IF: 4.8 (2022)

3. **Alaya, A.**, Mihok, E., Pecsenye, B., Jolji, M., Kertész, A., Bársony, P., Vigh, S., Cziáky, Z., Máthé, A. B., Burtescu, R. F., Oláh, N. K., Neamtu, A. A., Turcuş, V., Máthé, E.: Phytoconstituent Profiles Associated with Relevant Antioxidant Potential and Variable Nutritive Effects of the Olive, Sweet Almond, and Black Mulberry Gemmotherapy Extracts.
   Antioxidants. 12 (9), 1-36, 2023. EISSN: 2076-3921.
   DOI: http://dx.doi.org/10.3390/antiox12091717
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4. Neamtu, A. A., Maghiar, T. A., **Alaya, A.**, Oláh, N. K., Turcuş, V., Pelea, D., Totolici, B. D., Neamtu, C., Maghiar, A. M., Máthé, E.: A Comprehensive View on the Quercetin Impact on Colorectal Cancer.
   Molecules. 27 (6), 1-24, 2022. EISSN: 1420-3049.
   DOI: http://dx.doi.org/10.3390/molecules27061873
   IF: 4.6

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List of other publications

Foreign language scientific articles in international journals (2)

5. Jolji, M., Pecsenye, B., Mposula, Z., **Alaya, A.**, Kiss, T., Máthé, E.: Development and comparative analysis of protein-polyphenol-fibre bars as nutritional supplements suitable for healthy senior consumers.
   Acta Univ. Sapientiae, Alim. 16 (1), 103-125, 2023. ISSN: 1844-7449.
   DOI: http://dx.doi.org/10.2478/ausal-2023-0008

6. **Alaya, A.**, Oláh, N. K., Pripon Furtuna, F. R., Burtescu, R. F., Chise, E., Hepcal Cuc, I. M., Hanganu, D., Bota, V., Ivănescu, L. C., Ungureanu, O., Arsene, G. G., Turcuş, V., Máthé, E.: Regulating mechanism of blood pressure by the hawthorn and olive phyto- and gemmo-therapic extracts.
   Stud. Univ. "Vasile Goldiş" Arad, Ser. Ştiinţ. Econ. 32 (1), 37-43, 2022. ISSN: 1584-2339.

Foreign language abstracts (2)

7. Héjja, M., Mihok, E., **Alaya, A.**, Oláh, N. K., György, É., Máthé, E.: Analytical and microbiological examination of gemmotherapy extracts = Gemmoterápiás extraktumok analitikai és mikrobiológiai vizsgálata.
   In: XXIX. Nemzetközi Vegyészkonferencia = 29th International Conference on Chemistry. Ed.: Majdik Kornélia, Erdélyi Magyar Műszaki Tudományos Társaság, Kolozsvár, 1, 2023, (ISSN 2734-7109)

8. **Alaya, A.**, Máthé, E.: Comprehensive evaluation of gemmotherapy extracts generated effects using animal models.
   In: 19th Wellmann International Scientific Conference : Book of abstract. Ed.: Kiss Orsolya, University of Szeged Faculty of Agriculture, Hódmezővásárhely, 16, 2022. ISBN: 9789633068601

Total IF of journals (all publications): 16,4
Total IF of journals (publications related to the dissertation): 16,4

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

24 April, 2024