THESES OF DOCTORAL (PHD) DISSERTATION

INVESTIGATION OF THE BIOACTIVE EFFECTS OF PLANT EXTRACTS IN A TRANSLATIONAL MODEL AND IN FISH LARVAE NUTRITION

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1. INTRODUCTION AND AIM OF THE DISSERTATION

The active compounds of bud preparations may have therapeutic potential, contributing to the prevention and treatment of certain diseases. Their mechanisms of action are worth investigating, as understanding them may provide insights into the prevention and treatment of pathological conditions. The macro- and micronutrients contained in bud preparations influence cellular metabolism and stress responses. Due to their low calorie and fat content, they are suitable for both human and animal use and are rich in phytonutrients that may enhance the efficiency of cellular processes. Considering the occurrence of plant-derived bioactive compounds (PDBC), they are often classified as micronutrients (CICERO and COLLETTI, 2016). Increasing experimental evidence supports their beneficial effects on human health, although their cellular (GULDIKEN et al., 2018; RESCIGNO et al., 2018) and antimicrobial (BANDERIA et al., 2018; JODAA HOLM et al., 2016) mechanisms are less well understood. In this context, any research aimed at exploring the physiological effects of PDBCs is timely and forward-looking, as it forms a prerequisite for health-oriented nutrition and quality animal feeding.

PDBCs can be studied individually in a concentration-dependent manner or as complex extracts, where complementary physiological effects and synergies can be expected, often in a stochastic rather than deterministic fashion. Their effects on consumers are frequently characterized by dose-dependent hormesis (CALABRESE et al., 2012). However, in contrast to antimicrobial effects, relatively little information is available on the bioactive compounds present in the studied plant species and their physiological effects on *Drosophila melanogaster* w^{m4h}. To gain a deeper understanding of the compounds occurring in these plants and the mechanisms of their generated effects, I combined *Drosophila* and carp models. The nutritional conditions characteristic of the life stages of *Drosophila melanogaster* w^{m4h} are relatively well established (PIPER et al., 2014; BASS et al., 2007), similarly to those of *Cyprinus carpio* (TAKEUCHI et al., 2002). For this reason, I selected these two model species, as such a linked system ensures the interdisciplinary and multidisciplinary interpretation of causal relationships between nutrition, gene expression regulation, and development. This represents a novel translational approach, based on the evolutionary conservation of cellular mechanisms.

OBJECTIVES

1. Phytochemical Objectives

- Preparation of gemmotherapy extracts (GTEs) from bilberry (*Vaccinium myrtillus*), blackberry (*Rubus fruticosus*), and blackcurrant (*Ribes nigrum*).
- Determination of the plant-derived bioactive compound profiles of the extracts using the UHPLC-ESI-MS method, along with the quantitative measurement of total polyphenol and flavonoid contents.
- Comparison of the obtained phytochemical profiles and a literature review of the known biological effects of the identified components.
- Formulation of predictive evaluations regarding the potential physiological effects of the extracts, which may define further research directions.

2. Antimicrobial Activity Tests

- Investigation of the antimicrobial effects of GTEs against microorganisms that may occur in feed and food, potentially causing diseases in animals and humans.
- Assessment of microbial growth inhibition using the agar diffusion method, followed by the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC).
- Evaluation of the antimicrobial activity of GTEs based on the obtained results, in relation to the microbiota characteristic of consumers.

3. Nutritional Effects and Metabolic Evaluation

- Transcriptome analysis of *Drosophila melanogaster* populations reared on normal and high-carbohydrate diets, with particular emphasis on hemolymph hormones and cytoskeletal gene expression.
- Examination of the nutritional effects of GTEs in a *Drosophila melanogaster*-based nutritional model using three different dietary compositions.
- Investigation of the effects of GTEs in feeding carp larvae, including the measurement of viability parameters and quantitative analysis of ATP production.

4. Model Systems and Translational Applications

- Development of a translational model system integrating results obtained from *Drosophila melanogaster* and *Cyprinus carpio* to better understand the mechanisms of action of GTEs and to provide a scientific basis for the development of fish larval feeds.

2. MATERIALS AND METHODS

2.1. Preparation and extraction of plant extracts

In this study, I investigated three different plant buds: blieberry (*Vaccinium myrtillus* L. – Vm), black currant (*Ribes nigrum* L. – Rn), and blackberry (*Rubus fruticosus* L. – Rf). The plant species were harvested from their natural habitat in Romania. The extracts were prepared from freshly collected plant bud samples, which were preserved in a 1:1 mixture of 96% (v/v) ethanol and glycerin, with a plant-to-solvent ratio of 1:2 (EDQM, 2023).

2.2. Composition analysis of plant GTEs by UHPLC-ESI-MS

For the identification of the extracts, I used an ultra-high-performance liquid chromatography (UHPLC) system, the Dionex Ultimate 3000RS. The quantitative analysis of selected polyphenols was performed using a high-end Shimadzu Nexera I LC/MS-8045 UHPLC system.

2.3. Prediction of the physiological effects of the bud extracts

In the assessment of the physiological effects, after performing component analysis, I searched the literature for each component present in the GTE to identify any reported health benefits. Based on this literature review, I categorized the various physiological effects and expressed them as a percentage relative to each GTE.

2.4. *In Vitro* antioxidant properties of the bud extracts

The total polyphenol content and total antioxidant capacity (FRAP), as well as the antioxidant capacity based on DPPH radical scavenging, were assessed at the Department of Food Science, Sapientia Hungarian University of Transylvania, in Miercurea Ciuc (Csíkszereda).

2.5. Investigation of the antimicrobial effect of GTE-k

The various reference bacterial and microscopic fungal strains I used in this study were obtained from the National Collection of Agricultural and Industrial Microorganisms (NCAIM). I evaluated the antimicrobial activity of the GTEs by testing eight bacterial strains and six microscopic fungal species.

I cultivated the bacterial strains on Nutrient agar and incubated them at 37 °C for 24 hours, while I grew the molds and yeasts on complex medium at 28 °C for 72 hours.

I determined the antimicrobial effect of the plant GTEs using the agar diffusion method. I measured the diameters of the inhibition zones (including the well, measured in millimeters) using a digital caliper (BAUER et al., 1996).

I also used the microdilution method in liquid media. I performed the antimicrobial microdilution test in 96-well microtiter plates filled with broth. I serially diluted the GTE samples from the stock solution in the microplate wells to obtain 100 µl of mixed solution in each well, resulting in a concentration range between 10% and 100%.

I determined the resistance of the antimicrobial agents and demonstrated their bacteriostatic effectiveness using the minimum inhibitory concentration (MIC) test, while I assessed the direct lethal effect of the antimicrobial agent using the minimum bactericidal concentration (MBC) test.

2.6. Investigation of the translational model system

2.6.1. *Drosophila melanogaster*: cultivation, viability, and transcriptomic analysis

In the pharmacological studies of GTE, I employed the *Drosophila melanogaster* model system, obtained from the Bloomington Stock Center. For the viability assessment of *D. melanogaster*, fertilized eggs were collected from the crossbreeding of male and female individuals. The majority of the eggs corresponded to 0-2 hour-old embryos. Subsequently, the embryos were placed into vials containing appropriate NM and HS medium, which were supplemented with different concentrations of GTE. After the first 24 hours, I monitored the progression of the embryo-larva stages, observing the migration/completion phase, followed by the transition from larva to pupa and the development of the adult imago. The entire hatching process was monitored for 30 days.

For the gene expression analysis of the entire *Drosophila melanogaster* genome, I collected 120-hour-old third-stage larvae. The comprehensive transcriptomic analysis of the *D. melanogaster* larvae, which included RNA isolation and sequencing, was conducted by Tamirna, a Vienna-based company, under a contract agreement.

2.6.2. Production and viability assessment of early feeding common carp (*Cyprinus carpio*)

I conducted my experiments on early feeding common carp (*Cyprinus carpio*) were conducted in the Fish Laboratory of the Institute of Animal Science, Biotechnology and Nature Conservation, University of Debrecen. I conducted my experiments on early feeding common carp larvae (*Cyprinus carpio*) at the Institute of Animal Science, University of Debrecen.

Following artificial propagation, I placed the fertilized eggs in recirculating Zuger jars. I then transferred the viable but not yet feeding larvae to a separate recirculation system, where I observed that within 48 hours they began to breathe air and inflated their swim bladders. After this, I moved the larvae into modular aquarium units, which served as the experimental sites. Throughout the experiment, I fed the fry *ad libitum*. I applied four different experimental treatments, each in triplicate: the control groups were fed brine shrimp (*Artemia salina*), while the other groups received diets supplemented with GTE-specific ingredients. To bind the plant GTE, I used Fibersol-2, a maltodextrin-based, water-soluble dietary fiber. At designated time points, I measured the size and ATP content of the early feeding carp. At the end of the experiment, I counted the number of surviving individuals and calculated the survival rate.

2.7. Statistical analysis

All experiments were performed in triplicate, and I present the data as means ± standard deviation (SD). I conducted statistical analyses using IBM SPSS Statistics 26. I evaluated antimicrobial activity using one-way ANOVA and Tukey's HSD test to assess significant differences ($p < 0.05$) between various concentrations and extracts. I also analyzed the viability data of *Drosophila melanogaster* by one-way ANOVA and tested the homogeneity of variances using Levene's test. Since $p > 0.05$ in all cases, I performed group comparisons using Tukey's HSD test. For ATP measurements in carp fry, due to the time requirements of the method, I was able to analyze only 10 individuals per sample; I took this limitation into account during data interpretation.

3. RESULTS

3.1. Phytonutrient profiling of black currant, blackberry, and bilberry bud extracts

In my research, I analyzed the phytochemical composition of three types of alcohol-based bud extracts using the UHPLC-ESI-MS method. From the blackcurrant (*Ribes nigrum*) gemmotherapy extract (Rn-GTE), I identified 139 phytochemical constituents; from the blackberry (*Rubus fruticosus*) gemmotherapy extract (Rf-GTE), 95 constituents; and from the bilberry (*Vaccinium myrtillus*) gemmotherapy extract (Vm-GTE), 85 constituents (Figure 1). These compounds fall into the following bioactive categories: polyphenols, flavonoids, iridoids, alkaloids, amino acids, carboxylic acids, esters, terpenes, vitamins, and other derivatives. Among the GTEs analyzed, Rn-GTE proved to be the richest in terms of quantity, containing 84 different flavonoids and 23 polyphenols. In contrast, the composition of Rf-GTE was less diverse, with 36 flavonoids and 24 polyphenols identified. In Vm-GTE, the number of flavonoid components was outstanding (47), whereas the proportion of polyphenols was lower (8) compared to both Rf-GTE and Rn-GTE.

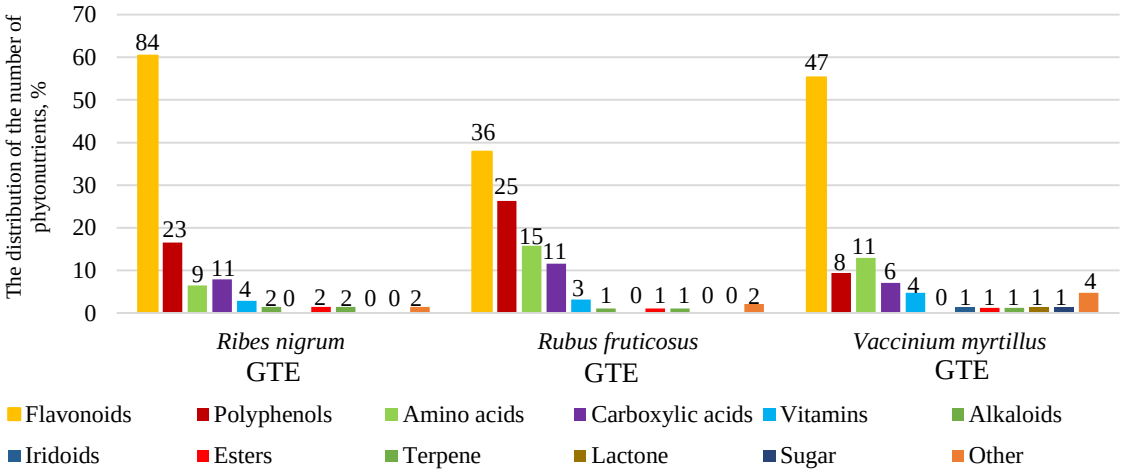

Figure 1. **Distribution of phytonutrients in GTEs**

3.1.1. Identification of the bioactive compound composition in GTEs

During UHPLC-ESI-MS analysis, I was able to identify for the first time in blackcurrant bud extract (Rn-GTE) several flavonoids and polyphenols recognized as bioactive in the literature, such as ampelopsin, dihydroxy-dimethoxy-isoflavan, myricetin-O-xyloside, naringenin-6,8-di-C-glucoside, and pentahydroxyflavone. Notably, 73% of the identified compounds had not previously been reported in the genus *Ribes*, significantly expanding the known bioactive spectrum of this species. In the blackberry bud extract (Rf-GTE), I also identified for the first time pinocembrin, pentahydroxyflavone, ducheside A, kaempferol-3-O-

rutinoside (nicotiflorin), and naringenin-6,8-di-C-glucoside. Approximately 60% of the components are new to the genus *Rubus*; I detected 18 polyphenols, 22 flavonoids, and 17 additional new compounds. In the bilberry extract (Vm-GTE), 40% of the identified compounds had not previously been reported in the literature, and three characteristic phytochemicals (cinnamtannin B1, cinnamtannin D1, and quercetin-3-O-galactoside) had only been identified in other species until now. From the buds, I identified 17 flavonoids, 6 polyphenols, and 11 other new compounds, which are now described for the first time in the genus *Vaccinium*.

My findings confirm that the bioactive compound content significantly depends on the species, altitude, habitat type, and growing conditions (SKROVANKOVA et al., 2015; LASLO and KÖBÖLKUTI, 2017).

3.1.2. Literature review on the physiological effects of identified phytonutrients

Among the phytonutrients identified in the qualitative analysis, I will describe the most significant ones, which are most prevalent based on their physiological effects. The slices of the pie charts represent phytonutrients with a specific physiological effect, and based on these proportions, one can infer the potential physiological effects of the corresponding GTE. In Rn-GTE, the proportion of anti-inflammatory phytonutrients is approximately 34%, while antioxidants account for 32%. The main physiological effects of these compounds include the inhibition of oxidative processes in the organism (Figure 2), which can be exerted both directly and/or indirectly. Antidiabetic components constitute 25%, whereas phytonutrients supporting tumor prevention represent 24% of the Rn-GTE.

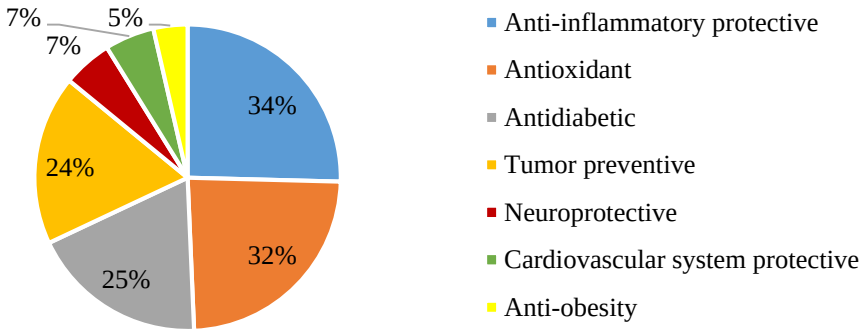

Figure 2. **Distributions of physiological effects of Rn-GTE components**

The blackberry-specific Rf-GTE also presents an interesting phytonutrient profile (Figure 3): anti-inflammatory compounds predominate, accounting for 26.7%. Phytonutrients with antioxidant physiological effects represent 20%, while those with antidiabetic properties account for 15%.

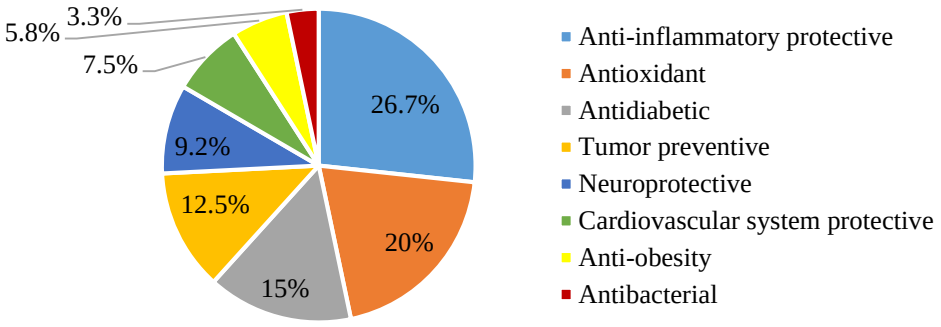

Figure 3. **Distributions of physiological effects of Rf-GTE components**

The most significant phytonutrients in Vm-GTE are antioxidants, which represent approximately 34% of the total composition (Figure 4). Anti-inflammatory phytonutrients account for 28% of the extract, while 22% of the active compounds in Vm-GTE possess antidiabetic properties.

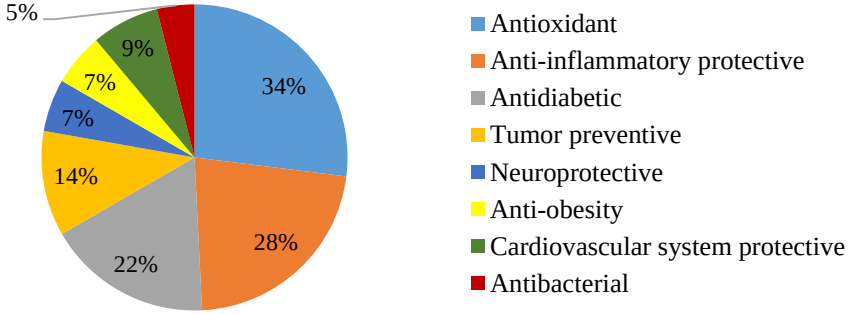

Figure 4. **Distributions of physiological effects of Vm-GTE components**

3.1.3. Determination of total polyphenol and flavonoid content of the examined GTEs

In my analyses, I determined the total polyphenol content of the GTEs using the TPC method, and the results were expressed as gallic acid equivalents (GAE). Among the three GTEs, Vm-GTE had the highest total polyphenol content (Table 1), measuring 300.35 ± 3.5 mg GAE/100 ml. Furthermore, Rf-GTE exhibited a lower antioxidant potential (237.56 ± 5.57 mg GAE/100 ml) compared to Vm-GTE. The lowest total polyphenol content was measured in Rn-GTE, with a value of 83.8 ± 1.2 mg GAE/100 ml.

Using the FRAP method, I determined the antioxidant capacity based on iron-reducing ability in the examined GTEs. Rn-GTE exhibited extremely low antioxidant potential compared to Rf-GTE and Vm-GTE. Among the latter two extracts, Vm-GTE had the highest antioxidant capacity (162.61 ± 3.13 mg ASA/100 ml), while the value for Rf-GTE was lower (133.73 ± 0.92 mg ASA/100 ml). The analysis also revealed that the GTEs contain significant amounts of flavonoids, which also possess strong antioxidant properties.

Table 1. **Evaluation of the characteristic antioxidant capacity of GTEs**

GTE sample	Total polyphenol TPC (mg GAE/ 100 ml)	Total flavonoids - FRAP (mg ASA/100 ml)	DPPH antioxidant activity, %
Ribes nigrum	83,8 ± 1,2	40,11 ± 4,36	80,29 ± 0,81
Rubus fruticosus	237,56 ± 5,57	133,73 ± 0,92	88,83 ± 0,47
Vaccinium myrtillus	300,35 ± 3,5	162,61 ± 3,13	87,91 ± 0,24

Results are the mean ± SD.

The antioxidant activity of the GTEs was determined using the DPPH free radical scavenging assay. The obtained antioxidant activity values were within a similar range to the total polyphenol and flavonoid contents of the analyzed GTEs, with only minor differences between them (Table 1). This means that Rf-GTE exhibited the highest antioxidant activity, followed by Vm-GTE, while Rn-GTE had the lowest antioxidant content. Based on the results, both the TPC and FRAP, as well as the DPPH antioxidant capacity measurements, showed that Rf-GTE and Vm-GTE values were outstanding.

3.1.4. Quantitative analysis of selected polyphenols in the studied GTEs

During the analytical investigations, the presence of several polyphenols was observed, both from the phenolic acid class and flavonoids. Generally, the main identified compounds were chlorogenic acid, caffeic acid, as well as quercetin and its derivatives, such as hyperoside and rutin. Naturally, each GTE has its own characteristics, which define its specific composition. For the quantitative determination of the GTEs (Table 2), five non-flavonoid polyphenols (caffeic acid, chlorogenic acid, ferulic acid, gallic acid, and salicylic acid) and ten flavonoids (apigenin, catechin, chrysin, hyperoside, kaempferol, luteolin, luteolin-7-O-glucoside, naringenin, quercetin, and rutin) were identified.

Table 2. **Selected polyphenol content of GTEs**

Studied components	***Ribes nigrum* GTE**	***Rubus fruticosus* GTE**	***Vaccinium myrtillus* GTE**
Phenolic acids			
Caffeic acid	1,693 ± 0,0101	-	1,693 ± 0,0188
Chlorogenic acid	0,227 ± 0,0057	0,157 ± 0,0057	7,552 ± 0,0217
Ferulic acid	0,109 ± 0,0086	-	-
Gallic acid	0,049 ± 0,0008	0,049 ± 0,0010	-
Salicylic acid	0,071 ± 0,0017	0,895 ± 0,0202	0,066 ± 0,0009
Flavonoids			
Apigenin	0,043 ± 0,0011	0,330 ± 0,0108	-
catechin	0,028 ± 0,0009	-	0,044 ± 0,0018
Chrysin	0,114 ± 0,0027	0,101 ± 0,0022	0,117 ± 0,0085
Hyperosid	0,547 ± 0,0187	0,172 ± 0,0089	0,392 ± 0,0102
Kaempferol	-	-	0,033 ± 0,0009
Luteolin	-	0,013 ± 0,0008	-
Luteolin-7-*O*-glucoside	0,074 ± 0,0021	0,078 ± 0,0012	-
Naringenin	-	0,043 ± 0,0009	0,036 ± 0,0005
Quercetin	0,210 ± 0,0100	-	0,989 ± 0,0118
Rutoside	1,662 ± 0,0198	0,278 ± 0,0047	0,105 ± 0,0028

The concentrations are expressed in mg/ml, mean ± RSD.

Among the three GTEs analyzed, the most significant component among the identified phenolic acids was chlorogenic acid (7.552 ± 0.0217 mg/ml), which was present in the highest concentration in Vm-GTE. Both Vm-GTE and Rn-GTE contained a high amount of caffeic acid (1.693 ± 0.0101 mg/ml). Among the flavonoids, the concentrations of quercetin (0.989 ± 0.0118 mg/ml) and hyperoside were particularly high in Vm-GTE. In Rf-GTE, the most prominent phenolic acid was salicylic acid (0.895 ± 0.0202 mg/ml), while among the flavonoids, apigenin (0.330 ± 0.0108 mg/ml) and rutin (0.278 ± 0.0047 mg/ml) were present in notable amounts. In Rn-GTE, rutin (1.662 ± 0.0198 mg/ml) was the most abundant flavonoid, followed by hyperoside (0.547 ± 0.0187 mg/ml). Among the non-flavonoid compounds, in addition to caffeic acid (1.693 ± 0.0101 mg/ml), chlorogenic acid was also present, and ferulic acid was detected as the only unique compound, although at a lower concentration.

3.2. Antimicrobial activity of the bud extracts

3.2.1. Results from the agar diffusion method

The size of the inhibition zones formed due to the bud extracts varied depending on the microorganisms tested. Among the Gram-positive bacteria, *L. monocytogenes* showed the highest sensitivity to the Rf-GTE, followed by the Rn-GTE, while no inhibition effect was observed with the Vm-GTE (Table 3). The effectiveness of the blackberry bud extract is indicated by the fact that it had an inhibitory effect on *L. monocytogenes* even at a 20% concentration. In the case of *S. aureus*, inhibition zones formed in the concentration range of 100-40% with the Rf-GTE. A dose-dependent hormesis effect was also observed with *S. aureus*, where the inhibitory effect at 50-70% concentrations was significantly greater than at 80-100% concentrations. *B. cereus* and *E. faecalis* were only inhibited by the Vm-GTE, with measurable inhibition zones formed at higher concentrations. Among the Gram-negative bacteria tested, inhibition was detectable only in the case of *P. vulgaris* with both Vm-GTE and Rf-GTE (Table 3). Vm-GTE inhibited growth at a 30% concentration, whereas inhibition zones were only observed with the Rf-GTE at 80% concentration.

Table 3. **Antimicrobial activities of the investigated GTEs (*n* = 3).**

Microorganisms studied	Conc. (%)	*Ribes nigrum* GTE	*Rubus fruticosus* GTE	*Vaccinium myrtillus* GTE
Gram-positive bacteria				
B. cereus	100	nd	nd	10,45 ± 0,55 ^{c,d}
	90	nd	nd	10,70 ± 0,85 ^{c,d,e,f}
	80	nd	nd	10,56 ± 0,65 ^{c,d,e}
	70	nd	nd	9,91 ± 0,57 ^{a,b,c}
	60	nd	nd	nd
	50	nd	nd	nd
	40	nd	nd	nd

	30	nd	nd	nd
S. aureus	100	nd	12,2 ± 0,51 ^{c,d}	nd
	90	nd	10,47 ± 0,40 ^{a}	nd
	80	nd	10,79 ± 1,06 ^{a,b,c}	nd
	70	nd	13,95 ± 0,63 ^{e,f}	nd
	60	nd	13,29 ± 0,65 ^{d,e}	nd
	50	nd	13,22 ± 0,58 ^{d,e}	nd
	40	nd	9,81 ± 0,56 ^{a}	nd
	30	nd	nd	nd
E. faecalis	100	nd	nd	10,91 ± 0,46 ^{d,e,f}
	90	nd	nd	10,49 ± 0,63 ^{c,d}
	80	nd	nd	9,97 ± 0,18 ^{a,b,c,d}
	70	nd	nd	10,00 ± 0,20 ^{a,b,c,d}
	60	nd	nd	9,84 ± 0,26 ^{a,b,c}
	50	nd	nd	10,14 ± 0,42 ^{b,c,d}
	40	nd	nd	nd
	30	nd	nd	nd
	20	nd	nd	nd
L. monocytogenes	100	10,81 ± 0,74 ^{b}	19,20 ± 0,87 ^{i}	nd
	90	10,77 ± 0,41 ^{b}	18,71 ± 0,60 ^{h,i}	nd
	80	nd	18,59 ± 0,41 ^{h,i}	nd
	70	nd	17,84 ± 0,71 ^{g,h,i}	nd
	60	nd	17,37 ± 2,31 ^{g,h}	nd
	50	nd	17,33 ± 0,46 ^{g,h}	nd
	40	nd	16,93 ± 0,68 ^{g}	nd
	30	nd	15,4 ± 0,73 ^{f}	nd
	20	nd	13,4 ± 0,53 ^{d,e}	nd
	10	nd	nd	nd
Gram-negative bacteria				
P. vulgaris	100	nd	12,77 ± 0,64 ^{d,e}	12,76 ± 0,80 ^{h,i}
	90	nd	11,18 ± 0,45 ^{a,b,c}	13,55 ± 0,75 ^{i,j}
	80	nd	10,99 ± 0,30 ^{a,b,c}	15,04 ± 1,03 ^{k}
	70	nd	nd	14,24 ± 0,86 ^{j,k}
	60	nd	nd	11,91 ± 0,48 ^{g,h}
	50	nd	nd	10,59 ± 0,38 ^{c,d,e}
	40	nd	nd	11,64 ± 0,57 ^{f,g}
	30	nd	nd	10,15 ± 0,45 ^{b,c,d}
	20	nd	nd	nd
P. aeruginosa	100	nd	nd	nd
E. coli	100	nd	nd	nd
S. enterica	100	nd	nd	nd
Yeast				
S. cerevisiae	100	9,63 ± 0,35 ^{a}	10,39 ± 0,43 ^{a}	10,84 ± 0,37 ^{c,d,e,f}
	90	10,61 ± 0,96 ^{a,b}	11,97 ± 0,64 ^{b,c,d}	11,68 ± 0,9 ^{f,g}
	80	10,90 ± 0,20 ^{b}	10,43 ± 0,42 ^{a}	11,52 ± 0,56 ^{e,f,g}
	70	9,78 ± 0,38 ^{a}	10,56 ± 0,55 ^{a,b}	9,90 ± 0,48^{a,b,c}
	60	nd	nd	nd
	50	nd	nd	nd
Mould fungi				
A. niger	100	nd	nd	nd
A. flavus	100	nd	10,4 ± 0,27	nd
	90	nd	10,13 ± 0,25	nd
	80	nd	9,76 ± 0,54	nd
	70	nd	9,42 ± 0,25	nd
	60	nd	nd	nd
A. ochraceus	100	nd	10,47 ± 0,7	nd
	90	nd	10,07 ± 0,26	nd
	80	nd	9,96 ± 0,22	nd

	70	nd	9,56 ± 0,19	nd
	60	nd	nd	nd
P. citrinum	100	nd	14,02 ± 0,64	9,34 ± 0,25
	90	nd	13,22 ± 0,32	9,09 ± 0,31
	80	nd	12,81 ± 0,36	8,83 ± 0,35
	70	nd	11,91 ± 0,24	9,00 ± 0,18
	60	nd	nd	nd
P. expansum	100	nd	9,09 ± 0,07	nd
	90	nd	8,87 ± 0,19	nd
	80	nd	8,90 ± 0,22	nd
	70	nd	8,83 ± 0,14	nd
	60	nd	nd	nd

Note: nd - not detectable. The results were expressed as the mean mm ± SD. Inhibition zones, including the diameter of the hole, which is 8 mm. Values with different letters (a–n) within a column are statistically different at $p < 0.05$, based on Tukey's test.

In the case of *Saccharomyces cerevisiae*, all three GTEs exhibited antimicrobial effects in the concentration range of 100-70%. In the examination of microscopic molds, *Aspergillus niger* was resistant to the tested bud extracts. In contrast, inhibition zones formed against *A. flavus*, *A. ochraceus*, *P. expansum*, and *P. citrinum* when treated with the blackberry bud extract. The cranberry bud extract inhibited *P. citrinum* molds at concentrations ranging from 100-70%. The black currant bud extract did not show inhibition against the tested molds.

Table 4. **Minimum antimicrobial inhibitory concentration (%) of GTEs tested by the agar diffusion method**

Microorganisms studied	*Ribes nigrum* GTE	*Rubus fruticosus* GTE	*Vaccinium myrtillus* GTE
Gram-positive bacteria			
B. cereus	nd	nd	70
S. aureus	nd	40	nd
E. faecalis	nd	nd	50
L. monocytogenes	90	20	nd
Gram-negative bacteria			
P. vulgaris	nd	80	30
P. aeruginosa	nd	nd	nd
E. coli	nd	nd	nd
S. enterica	nd	nd	nd
Yeast			
S. cerevisiae	70	70	70
Mould fungi			
A. niger	nd	nd	nd
A. flavus	nd	70	70
A. ochraceus	nd	70	nd
P. citrinum	nd	70	nd
P. expansum	nd	70	nd

Note: nd – not detectable

The bud extracts tested exhibited varying antimicrobial activity against the microorganisms examined. The blackberry bud extract demonstrated antimicrobial effects against three bacterial species and five microscopic fungi. The cranberry extract inhibited the growth of three bacterial strains and two microscopic fungi, while the black currant extract inhibited the growth of *Listeria monocytogenes* and *Saccharomyces cerevisiae* (Table 4).

3.2.2. MIC testing of bud extracts using a cultivation-based method

Based on the results of the MIC testing, most of the bud extracts exhibited inhibitory effects on all the tested microorganisms at varying concentrations (Table 5). In the case of Vm-GTE, even a 20% extract concentration showed inhibitory effects on *Bacillus cereus*, while for *Staphylococcus aureus*, *Enterococcus faecalis*, and *Listeria monocytogenes*, the MIC value reached 30%. Among the Gram-negative bacteria, *Proteus vulgaris* was the most sensitive, as inhibition was observed at a concentration of 20%. *Salmonella enterica* was found to be slightly less sensitive, with the MIC value corresponding to a 40% concentration.

Table 5. **The tested bud extracts have a minimum inhibitory concentration and a minimum bactericidal concentration**

Microorganisms studied	MIC			MBC		
	***Ribes nigrum* GTE**	***Rubus fruticosus* GTE**	***Vaccinium myrtillus* GTE**	***Ribes nigrum* GTE**	***Rubus fruticosus* GTE**	***Vaccinium myrtillus* GTE**
Gram-positive bacteria						
B. cereus	40	20	20	-	-	-
S. aureus	70	60	30	-	60	80
E. faecalis	-	60	30	-	70	30
L. monocytogenes	70	40	30	-	60	60
Gram-negative bacteria						
P. vulgaris	20	10	20	-	-	-
P. aeruginosa	50	40	50	-	-	70
E. coli	70	50	50	-	-	-
S. enterica	100	60	40	-	-	80
Yeast						
S. cerevisiae	80	60	60	-	100	100

The most resistant microorganisms were *Pseudomonas aeruginosa*, *Escherichia coli*, and *Saccharomyces cerevisiae*. Only the Vm-GTE had an inhibitory effect at concentrations above 50% against these two Gram-negative bacteria, while yeast proved to be the most resistant, requiring a concentration of 60% for growth inhibition.

The Rf-GTE exhibited a significant growth-inhibitory effect against the Gram-negative bacterium *P. vulgaris* at a 10% extract concentration and against the Gram-positive bacterium *B. cereus* at a 20% extract concentration. For *L. monocytogenes*, a 40% concentration was required to achieve the MIC effect. *S. aureus* and *E. faecalis* proved to be more resistant, as a 60% extract concentration was necessary to suppress their growth. Higher concentrations, ranging from 40% to 60%, were required to inhibit other Gram-negative bacteria. The MIC for *S. cerevisiae* yeast was found to correspond to a 60% concentration of blackberry bud extract.

For Rn-GTE, we obtained varying results for the tested microorganisms (Table 5). For example, *P. vulgaris* was inhibited by the 20% extract, but *E. faecalis* was not affected by the extract.

The *S. aureus* and *L. monocytogenes* Gram-positive bacteria exhibited higher resistance to the black currant bud extract, while the *B. cereus* bacterium was more sensitive, with an MIC value of 40%. Among the Gram-negative bacteria, *S. enterica* required a 100% concentration for inhibition. The yeast species tested showed greater resistance to the black currant bud extract, with the minimum inhibitory concentration (MIC) being 80%.

It was concluded that the studied GTEs (bud extracts) possess bacteriostatic activity against bacteria that can cause significant human infections, including *S. aureus*, *L. monocytogenes*, *E. coli*, and *S. enterica*, as indicated by the determined MIC values.

3.2.3. MBC testing results of the bud extracts

In the MBC test, the Vm-GTE proved to be the most effective extract, showing significant efficacy against six bacteria (Table 5). Both Rf-GTE and Vm-GTE exhibited bactericidal activity against the same Gram-positive bacteria, with the exception of *B. cereus*. The lowest minimum bactericidal concentration (MBC) was achieved with a 30% concentration of Vm-GTE against *E. faecalis*. This was followed by 60% concentration for *L. monocytogenes* and 80% concentration for *S. aureus*.

Among the Gram-negative bacteria, Vm-GTE showed a 70% MBC against *P. aeruginosa* and 80% MBC against *S. enterica*. The Rf-GTE had a bactericidal effect specifically against Gram-positive bacteria. Among the obtained MBC values, more favorable results were observed against *L. monocytogenes* and *S. aureus* with the 60% Rf-GTE. A 70% concentration of Rf-GTE proved to be much weaker against *E. faecalis* compared to Vm-GTE. Against Gram-negative bacteria, Rf-GTE showed no inhibition. Both Rf-GTE and Vm-GTE were highly effective (100% concentration) against *S. cerevisiae*. In contrast, Rn-GTE did not show bactericidal effects against the examined microorganisms.

3.3. Investigation of the nutritional effect of bud extracts on the development of *Drosophila melanogaster*

3.3.1. Effect of normal and high carbohydrate diets on the development of *Drosophila melanogaster* w^{m4h}

To examine how the two types of diet (Normal Diet - NM and High-Sugar Diet - HS) affect the development of *Drosophila melanogaster* in the w^{m4h} genotype, I placed 0-2 hour-old early embryos of the w^{m4h} genotype on the mentioned media and monitored their development daily at 25°C in normoxic conditions. This is a comparative study, where, considering that the individuals have the same genotype, age, and different nutrient availability, any observed

differences in developmental duration can be attributed to the diet. In this experiment, I observed that on the HS medium, the larvae of the w^{m4h} genotype took three extra days to reach the larval stage, compared to those on the NM diet (Figure 5). The duration of pupation and the emergence of adults (i.e., imago hatching) was the same (4 days each).

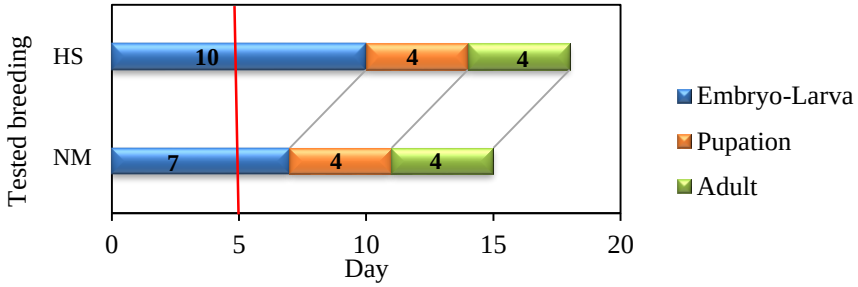

Figure 5. **The length of the vinegar fly's life cycle depends on the carbohydrate content of the nutrient**

The results of this comparative and in vivo experiment suggest that the high-sugar (HS) medium extends the developmental duration by approximately 3 days. However, this delay specifically affects the larval stage, while the duration of the pupal stage remains unchanged. A similar observation was made by Musselman et al. (2011) in *Drosophila melanogaster* with a normal genotype. Therefore, the extension of the larval stage can be associated with hyperglycemia, insulin resistance, inflammation, and obesity.

3.3.2. Transcriptome results of *Drosophila* w^{m4h} larvae under NM and HS diets without GTE treatment

In the complete transcriptome analysis of the *Drosophila melanogaster* w^{m4h} genotype, I identified 15,113 genes. The goal of the experiment was to compare the gene expression patterns of *Drosophila melanogaster* larvae fed with normal (NM) and high-sugar (HS) diets and identify those genes whose expression changes significantly due to the diet. The genes with significant transcriptional differences were grouped by their functions, creating 19 functional gene clusters. The assignment of each gene to a particular cluster was done by reviewing the gene information in the Flybase database. Flybase contains all the scientific results related to *Drosophila* genes, meaning that information about genomic positions, gene-transcript-protein structures, functions, tissue-organ and life cycle-specific expression patterns, as well as interactome analyses of the genes, are available. This makes it possible to study all essential information in the published bibliographic sources, as the full interactome of *Drosophila* is known. During my research, I focused on two functional gene clusters: the hemolymph/hormone (Table 6) and cytoskeleton (Table 7) functional genes. When selecting

relevant genes, I used a critical expression value of 5000 units. If the expression of a gene exceeded this value in the NM or HS experimental sample, it was placed into the appropriate cluster group based on its known or presumed function.

HEMOLYMPA gene cluster

In the following Table 6, the genes associated with hemolymph function are listed. These genes were identified in the transcriptomic analysis of the third larval stage (120 hours) under the normal (NM) and high-sugar (HS) diet conditions. A total of 25 genes were categorized into the hemolymph/hormones cluster, of which 5 genes will be discussed in more detail below.

Under the HS diet, the expression of *Fer1HCH* (–37%) and *Fer2LCH* (–38%), two genes with a key role in iron homeostasis, was significantly reduced compared to the control (NM) culture. This indicates an impairment of ferritin complex function and disturbances in cellular iron storage, suggesting an increased risk of oxidative stress in developing larvae.

Table 6. **Some relevant representatives of the hemolymph function gene cluster of vinegar muslica**

Environment / Genes	NM diet	HS diet
Fer1HCH Ferritin 1 heavy chain homolog	62,868 **Cellular iron ion homeostasis**	23,153 ↓
Fer2LCH Ferritin 2 light chain homolog	66,770 **Cellular iron ion homeostasis**	25,596 ↓
Lsp1α Larval serum protein 1α	297,054 **Energy and amino acid supply during**	316,442 ↑
Lsp1β Larval serum protein 1β	395,386 **Energy and amino acid supply during**	728,257 ↑
Lsp2 Larval serum protein 2	508,909 **Energy and amino acid supply during**	570,206 ↑

Note: NM = normal carbohydrate medium; HS = medium with high carbohydrate content;
Legend: red ↑ = increased gene expression; blue ↓ = decreased gene expression.

In contrast, the expression of the hemolymph-associated *Lsp* gene family (*Lsp1α*: +107%, *Lsp1β*: +184%, *Lsp2*: +112%) increased under the HS diet. The most pronounced change was observed for *Lsp1β* (nearly a twofold increase compared to NM), highlighting its potential role in carbohydrate-induced metabolic adaptation. Meanwhile, *Lsp2* expression remained stable, indicating that the developmental stage of larvae was not markedly affected by the different diets.

Furthermore, the comparable expression levels of *Lsp1α* and *Lsp2* under HS and NM diets suggest that larvae in both groups were at a similar developmental stage. In contrast, the strong upregulation of *Lsp1β* under HS conditions points to its possible key role in

carbohydrate-driven metabolic changes and implies that the HS diet may induce broader dysregulation of hemolymph-related genes.

CYTOSKELEYON – actin and microtubules

After culturing third-instar *Drosophila melanogaster* larvae on NM and HS media, I examined gene expression changes, this time focusing on genes with major cytoskeletal functions. The genes listed in Table 7 encode proteins that influence the function of actin microfilaments and microtubules. Based on whole-genome expression analysis and the criterion of strong transcriptional changes, 126 genes were classified into the cytoskeletal cluster, of which the functions of 10 genes were considered in more detail.

The HS diet induced significant alterations in cytoskeletal gene expression. Several actin- and tubulin-related genes were downregulated (*Act42A*: –75%, *Act5C*: –60%, *Act87E*: –24%, *αTub84B*: –19%, *βTub60D*: –65%), while others showed increased expression (*Act57B*: +181%, *βTub56D*: +16%, *Mlc2*: +19%, *Mlp60A*: +242%, *Mp20*: +13%). These results suggest that high carbohydrate intake reduces the stability of actin filaments while excessively activating muscle-related cytoskeletal genes. This imbalance may impair cell dynamics, muscle function and neuromuscular integrity. Transcriptome-based comparisons confirm that the HS diet influences developmental programs in a complex way, extending beyond classical hyperglycemia-related metabolic defects and opening new perspectives for the study of diabetes-related complications and potential antidiabetic effects.

Table 7. **Some relevant representatives of the gene cluster with cytoskeletal function in vinegar muslica**

Environment / Genes	NM diet	HS diet
Act42A	28,692	7,075 ⬇
Act57B	167,899	471,939 ⬆
Act5C	180,010	72,459 ⬇
Act87E	20,286	15,450 ⬇
αTub84B	26,584	21,616 ⬇
βTub56D	27,143	31,494 ⬆
βTub60D	3,107	1,073 ⬇
Mlc2	127,046	150,575 ⬆
Mlp60A Muscle LIM protein 60A	45,691	156,228 ⬆
Mp20 Muscle protein 20	53,785	60,692 ⬆

Note: NM = normal carbohydrate medium; HS = medium with high carbohydrate content;
Legend: red ⬆ = increased gene expression; blue ⬇ = decreased gene expression.

3.3.3. Examination of the effects of bud extracts on the development of *Drosophila*

The physiological effects of the w^{m4h} ecetmuslica were studied under in vivo conditions. In Table 8, the results of the experiments with NM and HS-based breeding mediums (at 25 °C) are shown in relation to varying concentrations of Rn-GTE. During these investigations, we monitored the pupation timing (in developmental days) and the eclosion time of the imagos (adults) on both the NM and HS media. These milestones were chosen because they reflect the viability of the individuals. The data collected from these experiments provide insights into how varying concentrations of Rn-GTE influence the development of the larvae and the overall life cycle, as well as the potential nutritional impacts of the extracts. The results are expected to offer a better understanding of how Rn-GTE interacts with the metabolic and genetic processes of the *Drosophila melanogaster* under different dietary conditions.

Table 8. **Effect of Rf-GTE on *Drosophila melanogaster* w^{m4h} development**

***Ribes nigrum* GTE**	**Puppeting**			**Metamorphosis**		
	Individual development days	**Unit number**	**%**	**Individual development days**	**Unit number**	**%**
NM/Control	4-8	24,8 ± 2,4	50	9-13	23,9 ± 3,4	48
NM/0,5 ml	5-15	27,8 ± 12,2	56 ↑	9-16	18,0 ± 10,8	36 ↓
NM/1 ml	5-9	23,5 ± 7,5	47 ↑	9-14	23,6 ± 6,7	47 ↑
NM/1,5 ml	6-7	26,6 ± 4,5	53 ↑	9-17	25,6 ± 6,1	51 ↑
NM/2 ml	6-10	28,8 ± 7,7	58 ↑	9-13	19,0 ± 9,7	38 ↓
NM/3 ml	5-9	18 ± 7,2	36 ↓	9-14	17,8 ± 6,0	36 ↓
HS/Control	8-11	21,6 ± 7,0	43	11-16	15,5 ± 10,3	31
HS/0,5 ml	8-13	23,4 ± 5,1	47 ↑	12-18	22,3 ± 5,0	45 ↑
HS /1 ml	10-19	26,3 ± 7,9	53 ↑	14-20	25, 9 ± 9,8	52 ↑
HS /1,5 ml	9-13	23,4 ± 3,4	47 ↑	13-18	20, 7 ± 7,6	41 ↑
HS /2 ml	10-17	18,3 ± 13,7	37 ↓	14-21	16,4 ± 12,1	33 ↑
HS /3 ml	14-22	2,1 ± 1,8	4 ↓	18-24	4,7 ± 2,0	9 ↓

Explanation: NM = normal carbohydrate medium; HS = high carbohydrate medium; **nt:** not tested.
Legend: green ↑= increase; red ↓= decrease in individual number.

The concentrations of Rn-GTE have a significant impact on the larval stages of *Drosophila melanogaster* and may play an important role in regulating populations. Higher concentrations generally have a negative effect on larval development, while lower concentrations were associated with increased growth. Furthermore, different concentrations trigger distinct effects on the developmental cycles of the fruit flies, indicating a concentration-dependent effect of Rn-GTE.

For instance, on the NM medium, the 1.5 ml concentration showed the best results for pupation and metamorphosis, although the developmental time was longer in the latter case. In contrast, on the HS medium, 1 ml of Rn-GTE proved most effective for pupation and the eclosion of imagos, although both processes exhibited an increased developmental time. It is also noteworthy that the 3 ml concentration of Rn-GTE on the HS medium prevented the w^{m4h} fruit flies from reaching the early pupal stage, indicating a growth-inhibitory effect of this concentration. Based on these findings, it can be concluded that Rn-GTE likely has a biphasic effect on the viability of the fruit fly.

Additionally, the results suggest that Rf-GTE concentrations significantly influence the pupation process and the viability of the emerging imagos (adults), as shown in Table 9. Notable results were observed on the NM diet with 2 and 3 ml concentrations, while on the HS diet, 1 ml affected pupation, and 2 ml affected the emerging imagos. These concentrations also exhibited the best developmental times. Rf-GTE positively supports the viability of *Drosophila melanogaster*, indicating that the extract has a beneficial impact on their development, in addition to containing significant anti-inflammatory, antioxidant, and antidiabetic phytochemicals.

Table 9. **Effect of Rf-GTE on *Drosophila melanogaster* w^{m4h} development**

***Rubus fruticosus* GTE**	**Puppeting**			**Metamorphosis**		
	Individual development days	**Unit number**	**%**	**Individual development days**	**Unit number**	**%**
NM/Control	7-12	28,7 ±1,2	57	10-15	23,3 ± 3,3	47
NM /0,5 ml	4-9	27,1 ± 0,5	54 ↓	8-14	23,3 ± 1,8	47
NM /1 ml	5-11	23,8 ± 1,5	48 ↓	8-15	24,1 ± 3,4	48 ↑
NM /2 ml	5-9	33,2 ± 3,8	66 ↑	9-13	32,7 ± 2,8	65 ↑
NM /3 ml	5-9	34,5 ± 3,7	69 ↑	8-13	26,1 ± 3,4	52 ↑
HS/Control	7-11	24,6 ± 1,3	49	10-16	22, 8 ± 2	46
HS /0,5 ml	7-11	27,5 ± 1,5	55 ↑	10-15	26,6 ± 2,6	53 ↑
HS /1 ml	7-12	28,7 ± 1,2	57 ↑	10-15	23,3 ± 3,3	47 ↑
HS /2 ml	7-12	28,1 ± 0,7	56 ↑	11-15	27,1 ± 3,3	54 ↑
HS /3 ml	7-12	25,1 ± 0,2	50 ↑	11-16	23,5 ± 1,0	47 ↑

Explanation: NM = normal carbohydrate medium; HS = high carbohydrate medium; **nt:** not tested.
Legend: green ↑ = increase; red ↓ = decrease in individual number.

Both on NM and HS media, the application of different concentrations of Vm-GTE did not show any significant positive effects on pupation or the viability of the emerging imagos (Table 10). It was observed that the 3 ml concentration had a positive effect on the viability of the imagos on the NM medium, while for pupation, the effect was similar to the control. On the

other hand, on the HS medium, a mild increase in viability was observed for both life stages (pupation and imago eclosion) with the 1 ml concentration.

Based on the *Drosophila melanogaster* viability assays, it can be concluded that the most effective extract was Rf-GTE, followed by Rn-GTE and then Vm-GTE. The pupation of *Drosophila melanogaster* was significantly influenced by Rf-GTE on both NM and HS media. Similarly, the viability of the emerging imagos was increased by certain concentrations of Rf- and Rn-GTE, while no such effect was observed for Vm-GTE in either of the media with different carbohydrate content.

Table 10. **Effect of Vm-GTE on *Drosophila melanogaster* w^{m4h} development**

***Vaccinium myrtillus* GTE**	**Puppeting**			**Metamorphosis**		
	Individual development days	**Unit number**	**%**	**Individual development days**	**Unit number**	**%**
NM/Control	4-10	28,1 ± 1,5	56	8-13	19,8 ± 3,0	40
NM /0,5 ml	nt	nt	nt	nt	nt	nt
NM /1 ml	4-8	24,9 ± 2,9	50 ↓	8-12	21,9 ± 3,0	44 ↑
NM /2 ml	4-11	27,1 ± 1,2	54 ↓	8-13	22,1 ± 1,7	44 ↑
NM/3 ml	5-10	27,8 ± 1,8	56	9-13	24,3 ± 2,6	49 ↑
HS/Control	7-15	22,9 ± 1,7	46	11-19	23,4 ± 2,1	47
HS /0,5 ml	nt	nt	nt	nt	nt	nt
HS /1 ml	6-16	25,1 ± 2,9	50 ↑	11-18	24,2 ± 1,9	48 ↑
HS /2 ml	7-14	18,9 ± 2,3	38 ↓	12-19	20,9 ± 2,4	42 ↓
HS /3 ml	8-16	19,5 ± 2,9	39 ↓	11-20	16,0 ± 4,2	32 ↓

Explanation: NM = normal carbohydrate medium; HS = high carbohydrate medium; **nt:** not tested.
Legend: green ↑ = increase; red ↓ = decrease in individual number.

The results of the study support that the phytonutrients present in Rf-GTE have an impact on the treatment of the diabetic symptoms we induced. Analytical and literature data confirm that, under the high-sugar (HS) diet, *Drosophila melanogaster* supplemented with Rf-GTE are able to exert strong antidiabetic physiological effects on the developmental process of the fruit flies. Furthermore, the extract is characterized by a high antioxidant content, which likely contributes to achieving the observed viability parameters during the development of *Drosophila melanogaster*.

3.4. Examination of the nutritive effect of bud extracts in the feeding of juvenile carp with supplementary diet

The aim of the study was to compare the feeding efficiency of brine shrimp on diets supplemented with GTEs. During the experiment, a parallel could be established between the amount of ATP produced and the evaluation of larval body size growth, starting from day three.

The data analysis revealed that during the stages involving fertilized eggs and non-feeding larvae, the body size doubled under conditions of high ATP levels. Three days post-hatching, during the feeding larval stage, significant differences in ATP yields were observed between the groups. Since ATP measurements had to be performed exactly one hour after feeding to ensure the comparability of samples and the reproducibility of the experiment (considering the stability of ATP and the ATP level fluctuations influenced by diurnal feeding cycles), it was possible to analyze only 10 individuals per sample due to the time required for sampling and measurement. In the future, the proximity of the fish laboratory and the ATP measurement site, as well as the involvement of additional personnel in the sampling process, could greatly facilitate multiple replications of the method and its broader application. While the body sizes of the larvae were comparable, there were considerable variations in ATP production (Table 11). These results suggest that ATP production could be more efficient with greater food variety, potentially influencing the larvae's growth and development. Therefore, it is crucial to consider the type and diversity of food to optimize the development of the larvae.

Table 11. **Nutritional effects of GTEs on carp larvae**

Larvae Development in days	**Measured values**	**Supplementary feed**			
		***Ribes nigrum* GTE**	***Rubus fruticosus* GTE**	***Vaccinium myrtillus* GTE**	***Artemia salina***
Eggs (Day 0)	**Body size (mm)**	1,95 ±0,05			
	ATP (pg/10 individuals)	77,29			
Non-Feeding Larvae (Day 1)	**Body size (mm)**	5,84 ±0,10			
	ATP (pg/10 individuals)	51,71			
Feeding Larvae (Day 3)	**Body size (mm)**	7,21 ±0,09			
	ATP (pg/10 individuals)	128,02	168,32	247,16	156,92
Feeding Larvae (Day 5)	**Body size (mm)**	7,21 ±0,09			
	ATP (pg/10 individuals)	292,03	37,52	285,19	99,37
Feeding Larvae (Day 7)	**Body size (mm)**	7,77 ±0,16			8,74 ±0,09
	ATP (pg/10 individuals)	244,12	189,11	173,14	240,83

Note: *Artemia salina* (brine shrimp) control group. Measured parameters: body size (mm), ATP content (pg).

The above results indicate that by the third day post-hatching, the Vm-GTE demonstrates excellent ATP-producing ability (58%), similar to the larvae fed with control brine shrimp (Table 11). However, on the fifth day, ATP levels increased significantly only in the larvae fed with Rn-GTE (194%) and Vm-GTE (187%), while the control group showed a critical decrease in ATP content compared to the others, and the body size of the larvae remained almost unchanged. By the seventh day, only the larvae fed with Rn-GTE exhibited a slight increase in ATP levels, which was comparable to the control group.

Summarizing the results, it can be concluded that GTE positively influences the increase of ATP concentration during larval development, directly demonstrating the nutritional quality of GTEs. However, the larvae's sensitivity at every life cycle stage was confirmed. Since ATP

is the primary source of cellular energy and essential for normal cellular function, the study revealed that replacing standard brine shrimp feed with different GTEs increased ATP levels in the larvae. This suggests that GTEs have nutritional properties for newly hatched fish larvae, reinforcing the fact that nutrients in fish feed are efficiently utilized in cellular energy production. Based on the findings, it can be concluded that these GTEs could serve as a potential alternative food source during the early developmental stages of carp. This discovery could be important for aquaculture, as it may help optimize the composition and quantity of fish feed to improve the health and growth of carp.

During the experiment, it was observed that by the ninth day, only the larvae fed exclusively with brine shrimp survived, highlighting the critical role of diet type in larval survival. Furthermore, the method applied in this study for measuring ATP content represents a novel approach that has not yet been documented in the literature, offering the opportunity to monitor cellular energy levels under various feeding regimes. This approach may contribute to the scientific development of nutrition strategies in aquaculture.

3.5. The Importance of translational model systems in evaluating GTEs

By integrating the results obtained in *Drosophila melanogaster* and *Cyprinus carpio*, a novel translational model system was established to study the nutritive and potentially antidiabetic effects of GTEs. The basic research findings in *Drosophila* confirmed the concentration- and diet-dependent effects of GTEs, which were partly verified in carp larvae as well. This model provides an opportunity to transfer knowledge from basic research into practical applications in aquaculture.

3.5.1. Advantages and limitations of the translational model system

The rapid life cycle of *Drosophila melanogaster* allowed the investigation of concentration- and diet-dependent effects of GTEs, while experiments with common carp (*Cyprinus carpio*) larvae provided insights into their practical feeding potential. The results confirmed that GTEs support energy production and viability in the short term, but cannot substitute for a complex nutrient composition in the long term. The two models complement each other: *Drosophila* proved useful for studying mechanisms and dose-dependencies, whereas carp larvae were suitable for assessing nutritional applicability. ATP levels were identified as a sensitive marker of early effects, but long-term evaluation requires monitoring growth and survival parameters.

3.6. Practical implications

GTEs may represent promising natural feed additives in aquaculture, particularly in early developmental stages where they can support energy metabolism and enhance immunity. However, their incorporation poses technological challenges (stability, dosage, palatability), requiring further developments such as microencapsulation and stability testing.

4. NEW SCIENTIFIC RESULTS

1. The present research stands out for its comparative approach and provides a detailed analysis of the compositional profiles of the examined bud extracts, as follows:

- In the *Ribes nigrum* (Rn-GTE) bud extract, 139 bioactive components were identified, of which 104 compounds had not previously been reported in bud extracts. Among these, 72 were flavonoids, 19 polyphenols, 6 carboxylic acids, 2 alkaloids, 2 esters, and 3 other metabolites.
- In the *Rubus fruticosus* (Rf-GTE) bud-shoot gemmotherapeutic extract, among 95 identified bioactive components, 57 compounds had not previously been reported in bud extracts. These included 22 flavonoids, 18 polyphenols, 6 amino acids, 4 carboxylic acids, 2 vitamins, 1 ester, and 4 other metabolites.
- In the *Vaccinium myrtillus* (Vm-GTE) bud-shoot extract, 85 bioactive components were identified, of which 34 compounds had not previously been reported in bud extracts. These included 16 flavonoids, 6 polyphenols, 1 terpenoid, 1 iridoid, 1 amino acid, 2 carboxylic acids, 1 lactone, 1 sugar, and 4 other metabolites.

2. The component composition of the GTEs showed a positive effect against microorganisms, which opens the possibility for the future application of gemmotherapeutic extracts not yet investigated.

- During antimicrobial studies, under broth culture conditions, the minimum bactericidal concentrations (MBC) for Rf-GTE were as follows: 60% (30 mg/ml) – *L. monocytogenes*, *S. aureus*; 70% (35 mg/ml) – *E. faecalis*; 100% (50 mg/ml) – *S. cerevisiae*.
- For Vm-GTE, the MBC values were: 30% (15 mg/ml) – *E. faecalis*; 60% (30 mg/ml) – *L. monocytogenes*; 70% (35 mg/ml) – *P. aeruginosa*; 80% (40 mg/ml) – *S. aureus*, *S. enterica*; 100% (50 mg/ml) – *S. cerevisiae.*

3. Within the framework of the translational model system, I performed comparative studies on *Drosophila melanogaster* and *Cyprinus carpio* larvae to evaluate the biological effects of GTEs.

- In the fruit fly model, *Rubus fruticosus* (Rf-GTE) extracts showed significant effects on pupation and adult emergence: under NM diet pupation was 2 ml (66%) and 3 ml (69%), under HS diet 1 ml (57%) and 2 ml (56%); the highest rate of emerging adults at 2 ml

was NM (65%) and HS (54%). *Ribes nigrum* (Rn-GTE) extracts resulted in a biphasic response, while *Vaccinium myrtillus* (Vm-GTE) extracts did not show significant positive effects. None of the extracts proved toxic.

- In carp larvae, all three GTEs showed nutritive potential in the early developmental stage. As a new methodological development, I performed the direct determination of ATP content in feeding common carp larvae, which provided direct evidence for the effect of GTE on metabolic activity and nutrient utilization.

5. PRACTICAL APPLICABILITY OF THE RESULTS

1. Biological and Nutritional-Physiological Effects

- The GTEs exerted a significant influence on larval and pupal viability, which showed a close relationship with the nutritional conditions (normal vs. high carbohydrate level).
- Under normal carbohydrate conditions, Rf-GTE and Rn-GTE resulted in different degrees of viability enhancement, indicating the role of compositional diversity among phytonutrients.
- In a high-carbohydrate environment, the viability-promoting effect of the GTEs was even more pronounced, suggesting a potential supportive role under metabolic stress conditions.
- The high polyphenol and flavonoid content of Vm-GTE was associated with reduced viability of larvae and pupae, while it increased the ATP level in carp larvae, indicating metabolic activation.

2. Antimicrobial and Health-Promoting Potential

- Vm-GTE exhibited inhibitory effects against several microbial species, while Rf-GTE showed the strongest antimicrobial activity.
- These results suggest that GTEs may serve as natural alternatives for the prevention or complementary treatment of infections; however, further studies are required before their practical implementation.

3. Methodological and Technological Innovation

- A new, custom-developed ATP measurement method was developed, enabling the accurate and direct determination of ATP content in feeding common carp larvae.
- This method represents a milestone in aquaculture, as it:
    - provides objective, quantitative assessment of the metabolic activity of organisms,
    - allows rapid and biochemically grounded evaluation of the effectiveness of different feed supplements, and
    - directly contributes to the development of optimal starter feeds and the improvement of fish health.

- The method can be applied in both research and practical aquaculture programs, potentially establishing a new standard in the biochemical evaluation of nutrient utilization.

4. Translational research model and application possibilities

- The comparative use of *Drosophila melanogaster* and *Cyprinus carpio* developmental models provided a translational framework linking basic research findings to practical aquaculture applications.
- Based on the results, targeted modification of the composition and concentration of GTEs may improve the efficiency of fish rearing and support the development of more sustainable nutritional strategies.

The research provides a scientifically grounded starting point for the safe and effective application of GTEs in aquaculture.

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7. PUBLICATIONS ON THE TOPIC OF THE THESIS

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List of publications related to the dissertation

Foreign language scientific articles in Hungarian journals (1)

1. **Mihok, E.**, György, É., Máthé, E.: The Carpathian lingonberry, raspberry and blackberry fruit extracts feature variable antimicrobial efficiency.
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11. András, C. D., Salamon, B., György, É., **Mihok, E.**, Szép, A.: Essential oil extraction from herbs and their use in the food industry.
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