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Research Article

Effect of dietary supplementation with *Gontscharovia popovii* on growth performance, whole body composition, and hematological parameters in *Litopenaeus vannamei*

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Abstract

The study analyzed the effect of *Gontscharovia popovii* supplement on growth performance, whole body composition, and hematological parameters of *Litopenaeus vannamei*. The shrimp were fed with 0.5%, 1%, and 2% *G. popovii* powder for 45 days. The essential oil of *G. popovii* was analyzed and found to contain a total of 49 compounds. The main compounds identified in the oil were carvacrol, γ-terpinene, and (E)-caryophyllene. The highest activities of phenol oxidase and superoxide dismutase were observed in the control group (5.64±0.14 µg/mL) and 2% concentration (56.24±0.5 µg/mL), respectively. The hematological parameters indicated that the highest values of triglyceride (108±0.88 mg/dl), cholesterol (103.3±2 mg/dl), IgM (268.5±4 mg/dl), and hyaline (5.5±0.22 10^6 cell/mL) were observed in the 2% concentration. The highest amounts of hemocyte (1.22±0.01 10^7 cell/mL) and large-granular cells (2.3±0.05 10^6 cell/mL) were observed in the 1% concentration, while the highest amount of semi-granular cells (4.9±0.15 10^6 cell/mL) was observed in the 0.5% concentration. The results of digestive enzyme activities showed that the highest activities of amylase (1265.2±22.1 n/kg), lipase (57.1±2.7 n/kg), and protease (245.2±6.12 n/kg) were observed in the 2% concentration, respectively. The results of growth performance indices showed that the highest amounts of weight gain percentage (417.58±3.55 %), weight gain rate (12.5±0.03 g), and specific growth ratio (1.73±0.01 %/days) were observed in the 2% concentration, while the highest amount of food conversion ratio (1.46±0.02) was observed in the control group. Furthermore, the results of whole body composition showed that the highest level of crude protein (18.05±0.1 %), crude lipid (1.08±0.04 %), and moisture (4.7±0.15 %) were observed in the 2% concentration, while the highest level of ash (76.8±0.4 %) was observed in the control group. In general, the findings indicate a beneficial impact of dietary supplementation with *G. popovii* at 2% to improve blood biochemical and immune indices, digestive enzyme activities, and growth performance in *L. vannamei*.

Keywords: *Gontscharovia popovii*, Hematological Parameters, Growth Performance, Enzyme activities, *Litopenaeus vannamei*

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Introduction

In recent times, the decline in aquatic resources has prompted the expansion of the aquaculture industry in many countries. The increasing demand for protein resources has led to a focus on studying and introducing new species, such as crustaceans and mollusks, into aquaculture systems (Mirzaei *et al*., 2021; Jahanbakhshi *et al*., 2022). Penaeid shrimps, with a consumption rate of 4.5 tons according to FAO statistics, are highly sought after in the market. Since 2003, the whiteleg shrimp (*Litopenaeus vannamei*) has gained popularity over other farmed shrimp species due to its cost-effectiveness and adaptability to environmental changes. *L. vannamei* is native to the Pacific Ocean's west coast in Latin America, ranging from Peru in the south to Mexico in the north. It has been successfully cultivated on a commercial scale in Asia since the late 1990s, with a growth rate of up to 3 g per week and a maximum weight of 20 g in intensive culture systems. However, the growth of this industry also brings potential risks, including environmental concerns and health hazards for humans and animals (Pazir, 2016; Khanjani *et al*., 2020). Compared to other species, *L. vannamei* exhibits higher disease resistance. The larval stage poses challenges during the rearing process, as rapid growth is accompanied by a high mortality rate (Pazir *et al*., 2012; Jahanbakhshi *et al*., 2022). Therefore, the ability to produce larvae with high survival rates and quality is crucial for the development of shrimp farming in vulnerable areas. Consequently, improving food quality becomes a critical factor in promoting the growth and survival of shrimp larvae (Abdirad *et al*., 2022; Salehpour *et al*., 2022).

Bacterial, viral, and fungal disease factors and the lack of stable immune systems in shrimps have caused many problems in this industry. The use of some drugs such as antibiotics can promote the aquaculture industry, but their use is not recommended because they remain in the aquatic tissue and cause drug resistance (Pazir, 2016). The use of medicinal plants as immune stimulants is a suitable alternative to antibiotics, vaccines, and synthetic compounds. The annual consumption of medicinal plants has made significant progress in European and developing countries due to the increase in the resistance of infectious disease-causing agents to chemical drugs (Asadi *et al*., 2018). The use of antibiotics and chemical drugs increases the resistance of pathogens, accumulation in the body of farmed fish, and pollution in the environment during a period (Zare *et al*., 2014; Mirbakhsh *et al*., 2021). Shrimp diseases are caused by a complex relationship between shrimp, pathogens, and the environment. Shrimp health is also affected by a wide range of environmental stressors, including pH, salinity, temperature, and dissolved oxygen (Forouzani *et al*., 2021; Houshmand *et al*., 2022).

The plant *Gontscharovia popovii*, which belongs to the Lamiaceae family, has recently been identified as a native species of Iran, specifically found in the Fars and Hormozgan regions.

Traditionally, *G. popovii* has been used as a decoction to address various ailments such as fever, cold, bone pain, flatulence, and heartache (Soltanipoor and Asadpoor, 2006). The utilization of plant products in aquatic feed strategies has resulted in increased production volume. Plant extracts and spices possess appetizing properties and can enhance growth performance by stimulating digestion in the host organism (Jain *et al*., 2008). Extensive research has been conducted on the use of medicinal herbs in shrimp nutrition, with studies conducted by Babanezhad Abkenar *et al*. (2019), Tazikeh *et al*. (2020), Chirawithayaboon *et al*. (2020), Moghadam *et al*. (2022), Abidin *et al*. (2022), and Rahmani and Pratama (2023). Some studies have observed improvements in immune parameters, such as increased lysozyme activity, phagocytosis activity, and elevated levels of plasma globulin and albumin, in certain species after intraperitoneal injection or ingestion of plant extracts. Additionally, significant increases in erythrocytes, lymphocytes, monocytes, hemoglobin, and hematocrit levels have been reported in fish treated with medicinal plants (Jahromi *et al*., 2021; Abidin *et al*., 2022). Considering the beneficial properties of *G. popovii*, this study aims to investigate the effects of *Gontscharovia popovii* as a feed supplement on the growth performance, whole body composition, and hematological parameters of *Litopenaus vannamei* shrimps.

Material and methods

In this study, the essential oil of the *Gontscharovia popovii* plant was extracted using the Clevenger method. Gas chromatography and mass spectrometry (GC/MS) were employed to identify the constituent components present in the essential oil. The amount and type of compounds were determined using the inhibition coefficient and Kovats index. The extracted oil was then utilized to assess the phenolic compounds and antioxidant properties. To evaluate the effects on the growth, health, and chemical composition of Vannamei shrimp, varying percentages of plant powder were added to their food in the laboratory of the Shrimp Research Center. To minimize stress and losses, the purchased shrimp post-larvae were fed with vitamin C. The study consisted of four concentrations, including one control group and three experimental concentrations, each with three replications. A total of 180 shrimp post larvae, with an average weight of 2.00±1.00 g, were placed in glass aquariums and fed with food supplements containing plant powder at levels of 0.5%, 1%, and 2% after 3 days. Feeding was conducted four times a day for a duration of 45 days, after which the samples were weighed. At the end of the experiment period, growth indices (such as average weight gain, weight gain percentage, specific growth factor, and food conversion ratio), blood parameters, and body chemical composition were evaluated (Hoseinifar *et al*., 2013; Labrador *et al*., 2016).

Laboratory measurements

Extraction of essential oil

To prepare the dried plant samples for essential oil extraction, an electric mill was used to powder the samples. 50 grams of each powdered sample were then placed into a balloon connected to the Clevenger apparatus after weighing. Next, 500 mL of distilled water was added to the mixture. The extraction of essential oil was carried out for a duration of 3 hours, starting from the point when the liquid inside the balloon began to boil. After extraction, the obtained essential oil was dehydrated using sodium sulfate. The essential oils were collected in special small vials, and the weight percentage of the essential oil was calculated. To ensure preservation, the collected essential oils were stored in dedicated containers at a temperature of 5^{o}C in a refrigerator prior to analysis. This process of extracting essential oils was repeated four times for each concentration (Sefidkon and Jamzad, 2006).

GC/MS mass spectrometer to identify essential oil compounds

The identification of compounds in the essential oil was carried out using a Hewlett-Packard (6890 series II) GC/MS device. The device was equipped with a DB-5 column, which had a length of 30 meters, an inner diameter of 0.25 mm, and a thickness of 0.25 mm. The initial temperature programming involved setting the temperature at 60^{o}C for 3 minutes, while the final temperature was set at 240^{o}C for 5 minutes. The injection chamber was maintained at a temperature of 240^{o}C, and the detector chamber was set at 250^{o}C. Helium gas was used as the carrier gas, and the temperature of the injection chamber was set to 10^{o}C higher than the final temperature of the column. The essential oil sample was injected using the split method with a ratio of 1/50 (Sefidkon and Jamzad, 2006).

Extraction to determine phenolic and antioxidant compounds

To begin the process, 7.5 grams of dry plant material was weighed. Next, 200 milliliters of petroleum benzene was added to the plants for degreasing purposes. Following that, 100 milliliters of 90% methanol were added to each plant sample. After a 48-hour period, the resulting methanolic extract was filtered using Whatman paper (Whatman Ltd., England). The obtained extract was then subjected to dewatering for 30 minutes using a rotary evaporator to purify it and remove any remaining solvents. The volume of the extract was reduced, and the resulting extract was freeze-dried for 24 hours at a temperature of -47 ^{o}C and a pressure of 25-30 atmospheres. The weight of the extract was accurately measured using a digital scale, considering different concentrations and replications, in order to calculate the performance percentage of the extract. The obtained extract was stored in a refrigerator for further analysis of phenolic compounds and antioxidant properties. The determination of phenolic compounds was conducted using the Folin Ciocaltio method (Singleton and Rossi, 1965). To do this, a 2.5 mL solution (diluted 10 times) of folinic acid

and 2 mL of calcium carbonate (7.5%) were added to 50 microliters of each sample (in triplicate). The absorbance of the sample was measured at a wavelength of 765 nm using a spectrophotometer (Perkin-Elmer UV/Vis double beam lambda 1, USA). A range of concentrations of methanolic gallic acid solution was used to create a standard curve. The amount of phenolic substances obtained was then calculated in terms of milligrams of gallic acid per gram of dry matter (dw mg GAE/g) (Sonboli *et al*., 2006).

The antioxidant property of the plant was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method, as described by Brand-Williams *et al*. (1995). Four different concentrations of methanol extract were prepared to achieve a total volume of 4 mL and a DPPH amount of 100 micromoles in the solution. After a reaction time of 30 minutes, the absorbance was measured at a wavelength of 517 nm using a spectrophotometer. To compare the antioxidant properties of the plant extracts with synthetic antioxidants, two indicators, Trolox and Quercetin, were used. Methanol was used as a blank. All solutions were prepared daily, and each experiment was repeated four times. The percentage inhibition value was calculated using the following formula:

Percentage inhibition (%I) = [(A blank -A sample) / A blank] × 100

Where, A blank is the amount of DPPH absorption and A sample is the amount of DPPH absorption and extract sample. These values were calculated using the Sigma Plot software program. The antioxidant property of the samples was expressed as IC50, which represents the concentration of the plant sample required to cause a 50% recovery of DPPH.

Whole body composition

For the assessment of total body composition, four shrimp were randomLy selected from each tank. The measurements of protein, fat, moisture, and ash were conducted following standard methods outlined by AOAC (1990). Crude protein was determined through nitrogen content analysis using the Kjeldahl method with a factor of 6.25. Crude fat was measured using the Soxhlet method, which involved petroleum ether extraction (Folch *et al*., 1957). Moisture content was determined by drying the samples in an oven at 105°C until a constant weight was achieved. Ash content was measured through combustion at 550°C for 24 hours using a Carbolite apparatus from Germany (Adineh *et al*., 2020).

Growth performance of shrimp

The growth performance of *Litopenaeus vannamei* shrimps was examined following the procedures outlined in the study conducted by Khan *et al*. (2004). Various growth indices were calculated to assess the shrimps' growth, including average weight gain (AWG), weight gain percentage (WGP), specific growth ratio

(SGR), and food conversion ratio (FCR). These indices were determined using the following formulas:

Body Weight Gain (gr) = (Final weight (gr) - Initial weight (gr))

$$\text{Fed conversion ratio (FCR)} = \frac{\text{Dry Weight of feed fed (g)}}{\text{Fish Weight gained}}$$

$$\text{Percentage Weight gain PWG (\%)} = \frac{\text{Final mean body weight}}{\text{Initial mean body weight}} \times 100$$

$$\text{SGR} = \frac{\text{L}_\text{n}\text{W}_2 - \text{L}_\text{n}\text{W}_1}{\text{Time (days of experiment)}} \times 100$$

Where, W_1 is the initial weight gained, W_2 is the final weight gained, and Ln is the natural logarithm.

Hematological Parameters

To collect hemolymph from the second swimming leg of *Litopenaus vannamei* shrimps, a 21-gauge hypodermic needle and a 2 mL syringe were used. Each syringe was pre-filled with 0.3 mL of anticoagulant solution containing 10 mM Tris–HCl, 250 mM sucrose, and 100 mM sodium citrate at pH 7.6. The anticoagulant was added in an equal volume ratio to the hemolymph. A volume of 50 mL of the anticoagulated hemolymph was mixed with an equal volume of neutral buffered formalin (10%) and fixed for 30 minutes to measure the total hemocyte count (THC) and differential hemocyte count (DHC). The remaining anticoagulated hemolymph was centrifuged at 300×g for 10 minutes at 4°C to separate the hemocytes from the plasma. The total hemocytes were counted using a hemocytometer (Marienfeld, Germany) and a light microscope with a magnification of 100×. Additionally, fixed hemolymph was smeared on a slide and stained with Giemsa solution (10%) for 10 minutes. The differential hemocytes, including Hyaline, Semi-granular, and Granular cells, were characterized, and 100 cells from each smear were counted under a light microscope (Samadi *et al*., 2016).

Digestive enzyme activities

The impact of *G. popovii* on digestive enzymes, specifically amylase, protease, and lipase activities in the digestive tract, was assessed using the methodology described by Gamboa-Delgado *et al*. (2003). To prepare enzyme extracts, shrimps were subjected to a 24-hour fasting period after 45 days and then euthanized using sterile scissors. The digestive tracts were carefully removed, washed with sterile distilled water, weighed, and homogenized using a cooled phosphate buffer (0.65%). The resulting supernatant obtained through centrifugation (3000 rpm for 20 minutes at 4°C) was utilized for enzyme assays. Amylase activity was determined by measuring the amount of maltose liberated over a 10-minute period at 30°C using an α-Amylase kit (DIALAB, Austria). Protease activity was quantified

by assessing the quantity of tyrosine released within 15 min under the specified assay conditions using an API-ZYM kit (Bio-Merieux, France). Additionally, lipase activity was determined by measuring the amount of 0.025 N NaOH required to neutralize the fatty acids released during an 18-hour incubation period (at pH 6.9 and a temperature of 30°C) using a lipase kit (Bionik, Iran). The activity of digestive enzymes was expressed as enzyme units per gram of tissue, following the methodology outlined by Akbary *et al*. (2017).

Data analysis

The normality of the data was determined by the Kolmogorov-Smirnov test. Significant differences among *G. popovii* dietary levels were performed by one-way analysis of variance (One-way ANOVA). Duncan's test was used at a significant level of 0.05 to compare the means. Statistical analyzes were performed by SPSS 21 and Excel 2013 software.

Results

Essential oil composition

Table 1 presents the compositions of the essential oil extracted from the aerial parts of *Gontscharovia popovii*. A total of 49 compounds were identified in the essential oil of *G. popovii*, with the main compounds being carvacrol (69.64 %), γ-terpinene (7.04%), and (E)-caryophyllene (4.69%), respectively.

Table 1: Essential oil compositions of Gontscharovia popovii.

Row	Compound	RI	%
1	α-Thujene	924	1.132
2	α-Pinene	931	0.664
3	Camphene	946	0.173
4	Sabinene	970	0.056
5	β-Pinene	974	0.250
6	6-methyl-5-Hepten-2-one	983	0.020
7	Myrcene	988	0.884
8	3-Octanol	992	0.057
9	α-Phellandrene	1004	0.280
10	δ-3-Carene	1009	0.082
11	α-Terpinene	1015	1.690
12	p-Cymene	1022	2.643
13	Limonene	1025	0.137
14	β-Phellandrene	1026	0.366
15	1,8-Cineole	1029	0.026
16	(Z)- β-Ocimene	1034	0.100
17	Benzene acetaldehyde	1041	0.009
18	(E)- β-Ocimene	1044	0.295
19	γ-Terpinene	1057	7.041
20	cis-Sabinene hydrate	1064	0.494
21	Terpinolene	1086	0.119
22	Linalool	1100	3.761
23	Borneol	1163	1.117
24	Terpinene-4-ol	1174	0.526
25	α-Terpineol	1188	0.055
26	trans-Dihydro carvone	1204	0.110
27	Nerol	1233	0.125
28	Carvacrol methyl ether	1241	0.549
29	Geraniol	1253	0.314
30	Geranial	1267	0.195
31	Thymol	1290	0.790
32	Carvacrol	1299	69.644
33	Eugenol	1356	0.049
34	Carvacrol acetate	1371	0.089
35	*n*-Tetradecane	1389	0.052
36	(Z)-Jasmone	1396	0.209
37	α-Gurjunene	1406	0.110
38	(E)-Caryophyllene	1418	4.698
39	β-Gurjunene	1435	0.019
40	α-Humulene	1450	0.244
41	Germacrene D	1477	0.026
42	β-Selinene	1482	0.036
43	α-Selinene	1491	0.069
44	Bicyclogermacrene	1492	0.042
45	γ-Cadinene	1510	0.034
46	β-Bisabolene	1516	0.069
47	δ-Cadinene	1520	0.092
48	Spathulenol	1572	0.035
49	Caryophyllene oxide	1578	0.341
	Total		**99.910**

Antioxidant status

Based on the obtained results, it was found that the control group exhibited the highest level of Phenol oxidase, measuring 5.64±0.14 µg/mL. There was a significant difference observed between the 0.5% concentration and both the control group, as well as the 1% and 2% concentrations (p<0.05). Furthermore, the highest level of superoxide dismutase was observed in the 2% concentration, measuring 56.24±0.5 µg/mL. Significant differences were observed between the levels of superoxide dismutase in the 0.5% and 1% concentrations compared to the 2% concentration and the control group (Table 2; p<0.05).

Table 2: Comparison (Mean±SE) of phenol oxidase and superoxide dismutase levels in *Litopenaeus vannamei* fed with different levels of *Gontscharovia popovii.*

Antioxidant	Control	0.5%	1%	2%
Phenol oxidase (µg/mL)	5.64 ± 0.14^{a}	4.14 ± 0.04^{c}	4.52 ± 0.1^{b}	4.67 ± 0.11^{b}
Superoxide dismutase (µg/mL)	44.12 ± 0.5^{c}	53.12 ± 0.6^{b}	54.1 ± 0.8^{b}	56.24 ± 0.5^{a}

Mean values with different superscripts are significantly different. The significance level is defined as p<0.05.

Blood and immune indices

Table 3 presents the measured blood biochemical and immune indices of *Litopenaeus vannamei* shrimps fed with different levels of *G. popovii* powder. The results indicate significant differences in triglyceride levels between the control group and the 0.5% concentration, as well as the 1% and 2% concentrations (p<0.05). The highest triglyceride level was observed in the 2% concentration (108±0.88 mg/dl). There were significant differences in cholesterol levels between the control group and the 1% and 2% concentrations, as well as between the 0.5% and 2% concentrations (p<0.05). The highest cholesterol amount was observed in the 2% concentration (103.3±2 mg/dl). Significant differences were found in IgM levels between the control group and the 2% concentration, as well as the 0.5% and 1% concentrations (p<0.05). The highest IgM amount was observed in the 2% concentration (268.5±4 mg/dl). Total IgM levels showed significant differences between the control group and the 0.5%, 1%, and 2% concentrations (p<0.05). The highest total IgM amount was observed in the 2% concentration (69.3±2.3 mg/dl). Hemocyte levels exhibited significant differences among all concentrations (p<0.05). The highest hemocyte amount was observed in the 1% concentration (1.22±0.01 107 cell/mL). There were significant differences in hyaline levels between the control group and the 1% and 2% concentrations, as well as between the 0.5% and 1% concentrations (p<0.05). The highest hyaline amount was observed in the 2% concentration (5.5±0.22 106 cell/mL). Semi-granular cell levels showed significant differences among all concentrations (p<0.05). The highest semi-granular cell amount was observed in the 0.5% concentration (4.9±0.15 106 cell/mL). Significant differences were found in large-granular cell levels between the control group and

the 0.5% concentration, as well as the 1% and 2% concentrations (p<0.05). The highest large-granular cell amount was observed in the 1% concentration (2.3±0.05 106 cell/mL).

Table 3: Blood biochemical and immune indices (Mean±SE) of *Litopenaeus vannamei* fed with different levels of *Gontscharovia popovii*.

Blood and immune indices	Control	0.5%	1%	2%
Triglyceride (mg/dl)	104.6 ± 2.5 [a]	95.5 ± 2.5 [b]	104.2 ± 1.8 [a]	108 ± 0.88 [a]
Cholesterol (mg/dl)	86.9 ± 1.16 [c]	90.5 ± 1.5 [bc]	94.13 ± 1.46 [b]	103.3 ± 2.0 [a]
IGM (mg/dl)	232.2 ± 3.0 [c]	254.2 ± 3.2 [b]	253.3 ± 1.77 [b]	268.5 ± 4.0 [a]
Total IGM (mg/dl)	57.3 ± 1.3 [b]	66.5 ± 1.0 [a]	68.2 ± 1.8 [a]	69.3 ± 2.3 [a]
Hemocyte (10^7 cell/mL)	1.02 ± 0.01 [d]	1.13 ± 0.02 [c]	1.22 ± 0.01 [a]	1.18 ± 0.0 [b]
Hyaline (10^6 cell/mL)	5.1 ± 0.2 [b]	4.6 ± 0.25 [c]	5.3 ± 0.12 [ab]	5.5 ± 0.22 [a]
Semi-granular cell (10^6 cell/mL)	3.0 ± 0.20 [d]	4.9 ± 0.15 [a]	4.6 ± 0.1 [b]	4.2 ± 0.12 [c]
Large-granular cell (10^6 cell/mL)	1.8 ± 0.02 [c]	1.8 ± 0.03 [c]	2.3 ± 0.05 [a]	2.1 ± 0.02 [b]

Mean values with different superscripts are significantly different. The significance level is defined as p<0.05.

Digestive enzyme activities

Table 4 shows the measured digestive enzyme activities of *Litopenaeus vannamei* fed with different levels of *G. popovii* powder. The results indicate a significant difference (p<0.05) in the levels of amylase and lipase across all concentrations. Additionally, there is a significant difference (p<0.05) in the protease levels between the 1% and 2% concentrations compared to both the control group and the 0.5% concentration. The highest amounts of amylase (1265.2±22.1 n/kg), lipase (57.1±2.7 n/kg), and protease (245.2±6.12 n/kg) were observed in the 2% concentration.

Table 4: Digestive enzyme activities of *Litopenaeus vannamei* fed with different levels of *Gontscharovia popovii*.

Digestive enzyme activities	Control	0.5%	1%	2%
Amylase (n/kg)	645.25 ± 15.05 [d]	954.15 ± 12.05 [c]	1170.5 ± 19.3 [b]	1265.2 ± 22.1 [a]
Lipase (n/kg)	35.12 ± 2.18 [d]	45.21 ± 1.91 [c]	52.04 ± 1.51 [b]	57.1 ± 2.7 [a]
Protease (n/kg)	201.2 ± 5.2 [b]	205.67 ± 9.29 [b]	235.05 ± 3.1 [a]	245.2 ± 6.12 [a]

Mean values with different superscripts are significantly different. The significance level is defined as p<0.05.

Growth performance

Table 5 shows the measured growth coefficients of *Litopenaeus vannamei* shrimps fed with different levels of *G. popovii* powder. The results indicate a significant difference (p<0.05) in the weight gain percentage and weight gain rate levels between the control group and the 0.5% concentration compared to the 1% and 2% concentrations. Additionally, there is a significant difference (p<0.05) in the specific growth rates (SGR) and feed conversion ratio (FCR) levels across all concentrations. The highest amounts of weight gain percentage (417.58±3.55%), weight gain rate

(12.5±0.03 g), and SGR (1.73±0.01 %/days) were observed in the 2% concentration. Furthermore, the control group exhibited the highest FCR (1.46±0.02).

Table 5: Growth coefficients of *Litopenaeus vannamei* fed with different levels of *Gontscharovia popovii*.

Growth coefficients	Control	0.5%	1%	2%
Weight gain percentage (%)	417.58 ± 3.55^{c}	430.85 ± 5.77^{c}	466.73 ± 4.47^{b}	499.12 ± 6.87^{a}
Weight gain rate (g)	11.31 ± 0.02^{c}	11.33 ± 0.01^{c}	12.22 ± 0.06^{b}	12.5 ± 0.03^{a}
SGR	1.58 ± 0.00^{d}	1.61 ± 0.01^{c}	1.67 ± 0.03^{b}	1.73 ± 0.01^{a}
FCR	1.46 ± 0.02^{a}	1.39 ± 0.03^{b}	1.31 ± 0.01^{c}	1.19 ± 0.01^{d}

Mean values with different superscripts are significantly different. The significance level is defined as $p<0.05$.

Whole body composition

Table 6 shows the whole body compositions of *Litopenaeus vannamei* fed with different levels of *G. popovii* powder. The results indicate a significant difference ($p<0.05$) in the crude protein and lipid percentages between the control group, 0.5% concentration, 1% concentration, and 2% concentration. However, there is no significant difference ($p>0.05$) in the moisture percentages among the experimental concentrations. Additionally, there is a significant difference ($p<0.05$) in the ash percentages between the control group, 0.5% concentration, and 2% concentration. The highest percentages of crude protein (18.05±0.1%), crude lipid (1.08±0.04%), and moisture (4.7±0.15%) were observed in the 2% concentration. Furthermore, the highest percentage of ash (76.8±0.4%) was observed in the control group.

Table 6: Whole body composition of *Litopenaeus vannamei* fed with different levels of *Gontscharovia popovii*.

Whole body composition	Control	0.5%	1%	2%
Crude Protein (%)	17.58 ± 0.07^{b}	17.64 ± 0.05^{b}	17.7 ± 0.05^{b}	18.05 ± 0.1^{a}
Crude Lipid (%)	0.93 ± 0.03^{b}	0.94 ± 0.02^{b}	1.0 ± 0.04^{b}	1.08 ± 0.04^{a}
Moisture (%)	76.8 ± 0.40^{a}	76.72 ± 0.39^{a}	76.52 ± 0.40^{a}	76.33 ± 0.41^{a}
Ash (%)	4.34 ± 0.10^{b}	4.4 ± 0.07^{b}	4.55 ± 0.13^{ab}	4.7 ± 0.15^{a}

Mean values with different superscripts in row are significantly different. The significance level is defined as $p<0.05$.

Discussion

During the study, a total of 49 compounds were identified in the essential oil of *G. popovii*. The main compounds found were carvacrol (69.64%), γ-terpinene (7.04%), and (E)-caryophyllene (4.69%). Sefidkon and Jamzad (2006) investigated the chemical composition of the essential oil of *G. popovii* plant in Fars and Hormozgan provinces which 35 compounds were identified in the essential oil of this plant. The most important components of the plant essential oil were carvacrol, gamma-terpinene, linalool, para cymene, and beta-caryophyllene. Sonboli *et al*. (2006) reported that 31 compounds were identified in the *G. popovii* essential oil which carvacrol (71.9%), linalool (5.5%), para cymene (4.5%) and gamma-

terpinene (4.4%) had the highest percentage in the composition. Zareiyan *et al*. (2018) reported that the main components of the *G. popovii* essential oil include carvacrol (76.7%), gamma-terpinene (4.25%), para cymene (3.8%), and E-caryophyllene (2.4%).

According to the obtained results, the control group exhibited the highest amount of phenol oxidase (5.64±0.14 µg/mL). Additionally, the concentration of 2% showed the highest amount of superoxide dismutase (56.24±0.5 µg/mL). Zareiyan *et al*. (2018) reported the IC50 value of *Satureja rechingeri* essential oil as 395.77 µg/mL. Alizadeh and Shaabani (2012) reported the antioxidant content of *Satureja rechingeri* at different phenological stages ranging from 46.2 to 50.2 mg/liter. The results also revealed that *Gontscharovia popovii*, *Satureja rechingeri*, and *Allium eriophyllum* contained phenolic compounds with contents of 38.44, 36.23, and 38.44 mg of gallic acid equivalent per gram of dry weight, respectively. Zareiyan *et al*. (2018) reported a total phenol amount of approximately 20.01 mg of gallic acid per gram of dry weight in *Gontscharovia popovii*. According to Alizadeh and Shaabani (2012), the total phenolic content of *Satureja rechingeri* ranged from 35.5 to 37.5 mg of gallic acid per gram of dry weight at different phenological stages. The type of plant, extract concentration, their interactions, and the type of plant have a significant impact on immune responses in shrimp, such as phenol oxidase, total protein, and superoxide dismutase. In this study, the level of superoxide dismutase increased significantly with the increase in *Gontscharovia popovii* levels. Kasornchandra *et al*. (2005) reported that incorporating garlic plant powder in shrimp diet stimulates hemocyte cell production, enhances phagocytotic activity, generates superoxide anions, and increases phenol oxidase activity. Khodadadi *et al*. (2013) noted that the immune stimulation and increased resistance in fish fed with different forms of garlic depend on the presence of compounds like allicin, vitamin A, and vitamin C in the plant.

The measurement of blood parameters is widely recognized as an important tool for assessing the physiology and health status of aquatic animals (Akinrotimi *et al*., 2010). In the case of *Litopenaeus vannamei* fed with different levels of *G. popovii* powder, significant differences were observed in the measured blood biochemical and immune indices levels ($p<0.05$). Hemocytes, which play a crucial role in the immune responses of the host, encompass various functions such as pathogen identification, phagocytosis, melanin production, and cell toxicity. These cells are categorized into hyaline cells (HC), semi-granular cells (SGC), and granular cells (LGC) based on nucleus-to-cytoplasm ratio, granule count, color, and size. Hemocytes are continuously replaced by new ones in the hemolymph, and the number of hemocytes in crustacean species varies depending on individual characteristics and environmental conditions (van de Braak, 2002). Studies have shown that the inclusion of mangroves in the diet of

black tiger shrimps increases total hemocyte count and immunity due to the presence of flavonoids, glycosides, and steroids (Avenido and Serrano, 2012). Similarly, biologically active substances such as gallic acid, lutein phenoloid, and luteolin 7-O glucoside found in mangroves exhibit antioxidant activity. Other substances like anthraquinone in rhubarb (Liu, 2002), allicin in garlic (Nya and Austin, 2009), and carotenoid in astaxanthin (Farhangi *et al*., 2013) have been found to increase total hemocyte count in shrimp and leukocytes in fish. The use of edible garlic powder in the diet of Beluga has been reported to improve food conversion ratio, weight gain, and specific growth indices, as well as significantly affect blood parameters (Nobahar *et al*., 2013). Additionally, the density of blood hemocyte cells in concentration shrimps fed with diets containing garlic plant powder was found to increase significantly compared to the control group (Pazir, 2016). This difference may be attributed to the stimulation of the non-specific immune system by the compounds present in garlic plant powder. Garlic extract has also been shown to enhance resistance to salinity and pH stress in *L. vannamei* due to the presence of allicin, vitamin A, and vitamin C (Gholaghaie *et al*., 2016). Furthermore, the effect of rosemary essential oil on Beluga hematological parameters has been investigated, revealing significant improvements in the immune system and physiological conditions of fish fed with certain levels of rosemary essential oil. Additionally, rosemary essential oil has been found to reduce cholesterol and triglyceride levels (Ebrahimi *et al*., 2020). Changes in blood plasma protein, albumin, and globulin levels reflect the reactive nature of the biochemical system in response to internal or external environmental conditions. An increase in blood plasma protein, albumin, and globulin is associated with a stronger innate immune response, which enhances the functioning of organs involved in protein production, such as the liver (Lee *et al*., 2012; Akrami *et al*., 2015).

The inclusion of medicinal plants in the diet has been found to enhance the digestion process and food conversion rate. This is attributed to various factors such as the increase in bile acids, stimulation of hepatopancreas, and elevation of digestive enzymes (Yasemi *et al*., 2017). Plants, plant extracts, and spices possess appetizing properties and contribute to improved digestion by enhancing food absorption and digestion in animals, thereby promoting growth (Jain *et al*., 2008). The results showed a significant difference between the Amylase, Lipase, and Protease levels in experiment concentrations with each other (p<0.05). Shahraki *et al*. (2021) reported that the inclusion of ginger extract in the diet of western white leg shrimp resulted in increased activity of amylase, lipase, and protease digestive enzymes. Medicinal plants and their extracts have been shown to enhance animal performance and growth through their antibacterial effects on the microbial flora of the digestive system, stimulation of digestive system enzymes, and protein

synthesis (Lee *et al*., 2012; Akrami *et al*., 2015; Adeshina *et al*., 2019).

The obtained results showed that the use of different levels of *G. popovii* powder in the diet of farmed shrimps had significant effects on their growth indices. The increase in the occurrence of antibiotic resistance in aquaculture has led to the increasing use of plant-based growth and immunity stimulants. Ahmad *et al*. (2011) reported that the use of cinnamon powder in the diet of tilapia fish caused a significant increase in growth indicators including average final weight, body weight gain, weight gain percentage, specific growth rate, and the food conversion rate also decreased. Foroughi *et al*. (2016) reported that the use of chicory powder in the diet of western white-leg shrimp post-larvae improved the growth indices. Asadi *et al*. (2018) reported that the used cumin powder in the diet caused a significant increase in growth indices of shrimp, including average final weight, body weight, specific growth rate and reduction in food conversion ratio. Babanezhad Abkenar *et al*. (2019) also stated that different levels of mangrove leaf powder increased the growth and survival rates of Juvenile shrimps of *L. vannamei*.

The chemical composition of aquatic animals' bodies is influenced by various factors, including age, gender, environmental conditions, season, type and quality of food, density, and water quality. The highest percentages of Crude Protein, Crude Lipid, and Moisture were observed at a concentration of 2%. The primary difference in body composition is attributed to the animal's nutrition type and daily feeding amount (Turchini *et al*., 2009). Ebrahim Dorche *et al*. (2013) found that different levels of garlic essential oil had a positive impact on the growth indices, nutrition, and carcass chemical composition of juvenile Beluga. Carcass protein percentage is influenced by the digestibility of the diet and increased energy utilization, leading to improved growth indices. Protein is the most significant biochemical component in shrimp muscle. Fats serve as the primary source of energy, with twice the energy content of carbohydrates and protein. Environmental variables, such as temperature, can affect the amount of fat and protein in the body. There is a proven relationship between moisture and fat content, where an increase in fat leads to a decrease in moisture, and decomposed fats are replaced by an equal volume of water. Harmful microbes in the digestive tract can have negative effects, including increased breakdown of protein and amino acids and deamination activity. Consumption of medicinal plants can reduce the microbial population in the digestive system, resulting in slower digestion of protein and amino acids and increased absorption by the body (Lee *et al*., 2012). Gholaghaie *et al*. (2016) reported that a diet containing raw garlic powder increased the protein content in western white leg shrimp, but did not significantly affect the amount of fat, ash, and moisture. Asadi *et al*. (2018) found that a diet containing cumin powder caused a significant increase in fat and protein, and a decrease in moisture in shrimp carcass composition. According

to Ahmad *et al*. (2011), the inclusion of cinnamon powder in the diet did not have a significant impact on the composition of tilapia fish carcass.

In conclusion, the analysis of *G. popovii*'s essential oil revealed the presence of 49 compounds, with carvacrol, γ-terpinene, and (E)-caryophyllene identified as the main components. The results demonstrated that the 2% concentration exhibited the highest weight gain percentage, weight gain rate, SGR, crude protein, crude lipid, and moisture percentage. Conversely, the control group showed the highest ash percentage. Additionally, the findings indicated that *G. popovii* supplementation at 2% had a significant and positive impact on the blood biochemical and immune indices, digestive enzyme activities, and growth performance. The findings of this study indicate that *G. popovii* has the potential to serve as a beneficial dietary supplement for *Litopenaeus vannamei.*

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