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Research Article

Detection and separation of fatty acids and proteins in monogenean flatworm *Polystoma integerrimum*

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Abstract
Metabolite methodology in parasites assists in gaining a better comprehension of their roles in infection, adaptation, pathogenesis, taxonomy, diagnosis, and host-parasite interactions. The present study aimed to isolate and detect fatty acids and proteins in tissue extract of the whole flatworm Polystoma integerrimum that settle in the urinary bladder of the frog Bufo viridis, using Reversed-phase high-performance liquid chromatography (RP-HPLC) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) techniques, respectively.The results showed that the number of fatty acids in flatworm extract was ten. Six of them were three unsaturated fatty acids, which included oleic (C18:1n-9), linoleic (C18:2n-6), and linolenic (C18:3n-3) acids, and three other saturated fatty acids, which included myristic (C14:0), palmitic (C16:0) and stearic acids (C18:0). The palmitic (49.022%) and stearic (35.499%) fatty acids were the highest among the ten total fatty acids. In addition, the SDS-PAGE technique showed that the number of protein bands in the flatworm extract was six, where the second (76859 D), fourth (23356 D), and fifth (13966 D) bands of the extract matched the first (bovine serum albumin), fourth (pepsin), and fifth (alpha-lactalbumin) bands of the standard proteins, respectively. The other three bands, the first, third, and sixth, were not matched by any of the standard protein bands. The present study could be a starting point for studying more metabolites and increasing the present-day knowledge of their types, mechanism of action, and their role in the biological and taxonomical fields to fill the gap in data about this flatworm.

Keywords: Flatworm, Metabolites, RP-HPLC, Separation techniques, SDS-PAGE

INTRODUCTION

The biochemistry of parasites is very important for understanding the relationships between parasites and their hosts at the pathogenic, habitational, adaptive, and immunological levels, as well as classifying and diagnosing these parasites on a genomic basis (Rehman and Abidi, 2023). Parasitic helminths are classified into nematodes and flatworms, which include cestodes and trematodes (Mathison *et al*., 2023). Flatworms can parasitize mammals (Al-Tikrity *et al*., 2014; Zhang *et al*., 2023), poultry (Al-Marsomy and Al-Hamadaani, 2016), fish (Al-Niaeemi *et al*., 2020), and amphibians such as frogs (Sales *et al*., 2023), turtles (Lignon *et al*., 2023), and salamanders (Leeming *et al*., 2023). Monogeneans are parasitic flatworms that frequently transmit to just one host (Martínez-González *et al*., 2022). Polystoma integerrimum is a dimorphic monogenetic trematode; the adult form lives in the urinary bladder of amphibian hosts, while the neotenic form develops quickly in the gill chamber of the tadpole (Chaabane *et al*., 2019).

Previously, frog farming did not take up a large economic space in the interest of countries, but with the increasing interest in consuming white and healthy meat, it is anticipated that frogs will serve as an alternative source of protein, particularly in some Asian countries and Brazil (Moreira *et al*., 2013). Frogs are regarded as a delicious and expensive food in Europe, the United States, and Australia (Grano, 2020). Therefore, more attention must be given to frog farms and controlling parasitic diseases that affect them to maintain their economic and nutritional returns (Auliya *et al*., 2023).

The anatomy, physiology, and morphology of parasitic helminths have been extensively studied in literature; however, helminthic metabolomics techniques are being applied more frequently to gain a deeper compre-

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hensive level of infections, life cycles, and host-parasite interactions (Wangchuk *et al*., 2023). The field of "omics" technology in parasites is relatively new (Montaño *et al*., 2021).

Lipid metabolism plays a crucial role in parasitism and the survival of organisms (Sunshine and Iruela-Arispe, 2017). Lipids, including phospholipids, glycolipids, glycerides, sterols, and fatty acids, which may be saturated or unsaturated, play a crucial role in cell signaling, are constituents of cellular membranes and contribute to their stability and permeability, and serve as a major source of energy and precursors to synthesize large, important molecules in organisms (Gyamfi *et al*., 2019). Moreover, some polyunsaturated fatty acids (PUFAs) have different medicinal properties and may have an influential role in many diseases that affect animals (Satyanarayana and Chakrapani, 2021).

The interest in lipidomes is mainly due to the development of new techniques that deal with the separation and diagnosis of these compounds, as many studies have been conducted on lipids in fungi (Martínez-Ramírez *et al*., 2023), yeasts (Liu *et al*., 2021), plants (Guevara-Zambrano *et al*., 2023), and animal cells (Palma *et al*., 2023) using modern techniques. As for the studies conducted on parasites, Wang *et al*. (2020) studied the amount of lipid in the nematode Ascaris suum, and they found that significant variations in the abundance and composition of lipids with essential roles in cellular functions and processes exist among organ systems at different growth stages. They identified more than 500 kinds of lipids that return to 18 classes belonging to three lipid categories.

Polyunsaturated fatty acid (PUFA) such as arachidonic acid (ARA, C20:4n-6), cannot be synthesized by the microfilariae of the nematode Brugia malayi, but it can obtain the essential fatty acid, linoleic acid, from plasma to synthesize this acid (Liu *et al*., 1990). Palmitic (C16:0) and stearic (C18:0) fatty acids are the most abundant among the saturated fatty acids (SFAs), while unsaturated oleic acid (C18:1) is the most abundant in the tachyzoites phase of Toxoplasma gondii (Besteiro *et al*., 2008).

Proteins are key compounds in the parasites; they participate in vital metabolic pathways by synthesizing enzymes, some hormones, and structural components in membranes, as well as their contribution to immune responses (Robinson and Cwiklinski, 2021). Also, parasite proteins are one of the ultimate byproducts of the genome, and therefore, the genes of a parasite give specific characteristics of that parasite. Using molecular data, Haemonchus contortus in sheep was first detected in Iraq using accession numbers LC552170 and LC552171, which depend on the ITS-2 spacer and 28S gene sequences (Hade *et al*., 2022).

By using the electrophoresis technique, the two worms, Paragordius tricuspidatus and Spinochordodes tellinii, contain 689 and 575 protein bands, respectively, and only 36.2% of the bands were shared with these two worms (Biron *et al*., 2005). As for the taxonomy of nematodes, a taxonomic system for nematodes was developed based on their protein content; 1000 families of parasitic and free-living nematodes were diagnosed, and parasitic nematodes in animals included 53 families (Wang *et al*., 2009).

Although in the previous years, metabolomics has grown significantly in biotechnological and biomedical fields (McVeigh, 2020), research in this field has only been done on a small number of parasites. Currently, there is not a single study that discusses the metabolites in tissue extracts of Polystoma integerrimum; therefore, the aim of this study was formulated as a conclusion through which fatty acids will be detected by using High-performance liquid chromatography (HPLC), as well as studying the protein content using the Polyacrylamide gel electrophoresis (PAGE) technique in the tissue extract of this worm.

MATERIALS AND METHODS

Collection of frogs

Fifty frogs, Bufo viridis, were collected in May 2023 from the Alghabat, Besan, and Rashidiya regions of the city of Mosul and transferred to the Research Laboratory in the Biology Department, College of Science, University of Mosul, and dissected. The urinary bladder of the frogs was isolated in Petri dishes containing normal saline after washing them with distilled water. The urinary bladder was torn to confirm its infection with the monogenic flatworm *P. integerrimum.*

Preparation of monogenic flatworm *Polystoma integerrimum* extract

The method of Al-Niaeemi *et al*. (2019) was adopted to prepare the worm extracts. The worms were washed after being isolated from the urinary bladder with normal saline solution, then with 50 mmol Tris-HCl buffer pH 7.2 and sucrose 0.25 M to remove suspended materials.

One gram of the flatworm's whole body, P. integerrimum, was crushed in 5 ml of Tris-HCl buffer using MSE -Homogenizer for 3 minutes under cold conditions to maintain the viability of the extract. The crushing was completed using ultrasonic devices (Romany, PG-1545) at 16,000 Hz for 30 seconds using an ice bath, and this process was repeated four times with a pause of five minutes after each time in order to maintain a low temperature of the extract. Then, separating the supernatant from the precipitate using a cooled ultracentrifuge (PEP1406 Hettich) at a speed of 10,000 rpm for 15 minutes and at a temperature of 4°C to sediment cell debris and membranes, then discard the precipitate and use the supernatant to detect the types of fatty

acids and proteins.

Detection and separation of fatty acids

The fatty acids in the monogenic P. integerrimum extract were analyzed using the reversed phase-high performance liquid chromatography (RP-HPLC) technique (Shimadzu LC-2010A, Japan).

Extraction of fatty acids from the worm extract

The worm extract that was previously prepared was dried by a rotary evaporator RE 300 (Stuart Company, United Kingdom) under vacuum at 50°C; then 100 mg of the dry extract was weighed and dissolved in 10 ml of NaOH 7.5 N in methanol diluted with distilled water in a ratio of 1.5:1; then the mixture was heated at 105° C for 90 minutes and left to cool; then 12 ml of distilled water were added to the mixture, and the acidity was adjusted at pH 2 using sulfuric acid 20%; then the fatty acids were extracted using 30 ml of diethyl ether and using a separating funnel to ensure that all the fats were withdrawn and separated from the acid and water. The final solution was dried by the rotary evaporator at 50°C. This separation process was carried out according to the method of Hajji *et al*. (2022) with modifications.

Fatty acid analysis

Analysis of fatty acids using RP-HPLC included the preparation of esters of fatty acids. According to the previously prepared extract, 1 ml of methanol-acetyl chloride 25:1 v/v was added, which was prepared immediately and remains usable for a period not exceeding a week according to Stoffel *et al*. (1959) with modification.

The standard fatty acids and worm extract fatty acids were analyzed in the laboratories of Ibn Sina Company, Ministry of Industry and Minerals, Baghdad, Iraq, under the following conditions, according to Elliott *et al*. (1989). A C18 reversed-phase column (250 mm × 4.6 mm) was used; the mobile phase was acetonitrile-water 60:40 v/v, flow rate 0.4 ml/sec, and wavelength 254 nm.

Detection and separation of proteins

The total protein in the worm extract (9.34 mg/g wet weight) was estimated by the Bradford method and analyzed using the sodium dodecyl sulfate and polyacrylamide gel electrophoresis (SDS-PAGE) technique (Cleaver Scientific, United Kingdom). SDS-PAGE was conducted according to the method of Laemmli (1970) on 12% PAG-reducing SDS as follows:

Preparation of separation gel

The components of 10 ml of separation gel included 4 ml of stock solution (30% acrylamide/bis-acrylamide in a ratio 29:1), 2.5 ml of 0.375 M of Tris-HCl (pH 8.8), 0.1 ml of 10% sodium dodecyl sulfate (SDS), and 3.4 ml of distilled water (DW). The gel mixture was mixed gently, and then 0.05 ml of 10% ammonium persulfate (APS) and 0.005 ml of tetramethylethylenediamine (TEMED) were added and mixed. The gel was poured into the casting frame (10 cm x 8 cm x 1 mm) with about 1 cm of space left at the top for stacking gel, and then it was allowed to polymerize at room temperature for about 30–60 minutes.

Preparation of stacking gel

The components of 3 ml of stacking gel included 0.75 ml of stock solution (30% acrylamide/bis-acrylamide in a ratio 29:1), 1 ml of 0.125 M of Tris-HCl (pH 6.8), 0.05 ml of 10% SDS, and 1.2 ml of DW. The gel mixture was mixed gently, and then 0.03 ml of 10% APS and 0.005 ml of TEMED were added and mixed. The gel was poured to the top of the separation gel, and the combs were inserted into the stacking gel immediately after pouring to create wells for sample loading. The gel was then allowed to polymerize at room temperature until it solidified.

Sample preparation

The protein sample (1.8 µg/µL) was diluted with an equal volume of Laemmli's buffer, which consists of 2% SDS, 5% beta-mercaptoethanol (BME), 62.5 mM Tris (pH 6.8), 10% glycerol, and 0.01% bromophenol blue dye. Heat the mixture at 95°C for five minutes.

Sample loading and running conditions

Once both gels were polymerized, combs were carefully removed, and the wells were rinsed with deionized water and then with running buffer containing 0.1% SDS, 25 mM Tris base, and 192 mM glycine. 30 µL of sample preparation (27 µg protein) was loaded into wells along with standard proteins (14.4–67 kDa), by which it will be possible to know the molecular weight of the unknown proteins (worm extract proteins). Electrophoresis was carried out at a voltage of 120 V with a current of 30 mA until the dye front (bromophenol blue) reached 1 cm from the end of the plate. Proteins were fixed in gel with 50% trichloroacetic acid (TCA) overnight, then stained with a 0.1% Coomassie brilliant blue solution freshly made in 50% TCA for 1 hour at 37°C to visualize protein bands.

Statistical analysis

The GraphPad Prism program, version 7, was used to conduct statistical analysis of the present research data at ($P \leq 0.01$). The Pearson correlation coefficient (r) was estimated between relative migration distance (Rf) values and the logarithm of molecular weights of standard proteins. A simple linear regression was conducted to find the regression equation to calculate the unknown proteins' molecular weights.

RESULTS AND DISCUSSION

Since the present study is the first global study providing information about the isolation and detection of some metabolites, such as proteins and fatty acids, in the flatworm parasite P. integerrimum, therefore, the content of these metabolites and their biological role in general parasitic organisms is focused on the helminths that are most closely related to the worm under study. The variations in fatty acids and protein have been reported by the researchers in other parasites, such as trematodes, nematodes, digenean cercariae, and others (Lin and Siddique, 2024; Colomb and McSorley, 2025; Wang *et al*., 2025).

Fig. 1 shows that the number of fatty acids in the present P. integerrimum was ten, and six of them were identified based on a comparison of their retention time with those of the standard fatty acids, as shown in Fig. 2 and Table 1, which were also analyzed using RP-HPLC. The six fatty acids included three unsaturated fatty acids, including linolenic acid (LNA, C18:3n-3), linoleic acid (LA, C18:2n-6), and oleic acid (OA, C18:1n -9), in addition to three saturated fatty acids, including myristic acid (MA, C14:0), palmitic acid (PA, C16:0), and stearic acid (SA, C18:0) (Table 2).

In the present study, the palmitic and stearic acids had the highest concentration among the total fatty acids in worm P. integerrimum, 49.022% and 35.499%, respectively. Moreover, the results showed four peaks belonging to myristic, oleic, linoleic, and linolenic acids in the flatworm extract, and their percentages were 0.278%, 1.989%, 0.196%, and 10.871%, respectively. Besteiro *et al*. (2008) mentioned that the palmitic and stearic acids are the most abundant in the extract of the tachyzoites of Toxoplasma gondii. Mondal and Dey (2015) also reported the concentration of palmitic acid in trematode Isoparorchis hypselobagri being the highest of all saturated fatty acids; however, in the present finding regarding linolenic acid on P. integerrimum, oleic was the highest among all unsaturated fatty acids. In terms of the number of fatty acids separated and the highest fatty acid concentration, Wangchuk *et al*. (2019) identified fourteen fatty acids in the tapeworm Dipylidium caninum (isolated from dogs), and stearic acid was the major one. Eight lipids are reported in the nematode Trichinella papuae (Mangmee *et al*., 2020).

The appearance of the aforementioned two fatty acids in high percentages in the present study can be attributed to the fact that palmitic acid is the main product of the fatty acid synthesis pathway; also, other fatty acids can be synthesized by adding two carbon atoms to palmitic acid. Palmitic and stearic acids are considered precursors for two monounsaturated fatty acids, palmitoleic and oleic acids (Pratt and Cornely, 2024).

The present study showed that the flatworm extract P. integerrimum contained ten fatty acids (FAs) with different chain lengths, divided into saturated ones, and the other one was unsaturated. These results were close to the result of Mondal and Dey (2015), who found the total lipid was 3.81 % of the wet weight of the body tissue of a trematode, Isoparorchis hypselobagri inhabiting the swim bladder of a freshwater catfish Wallago attu, and the lipid contained saturated, monoenes, dienes and polyenes of unsaturated fatty acids, as well as the present result was close to the result of Hoskin and Bier (1983) , who found that the fourth stage larva

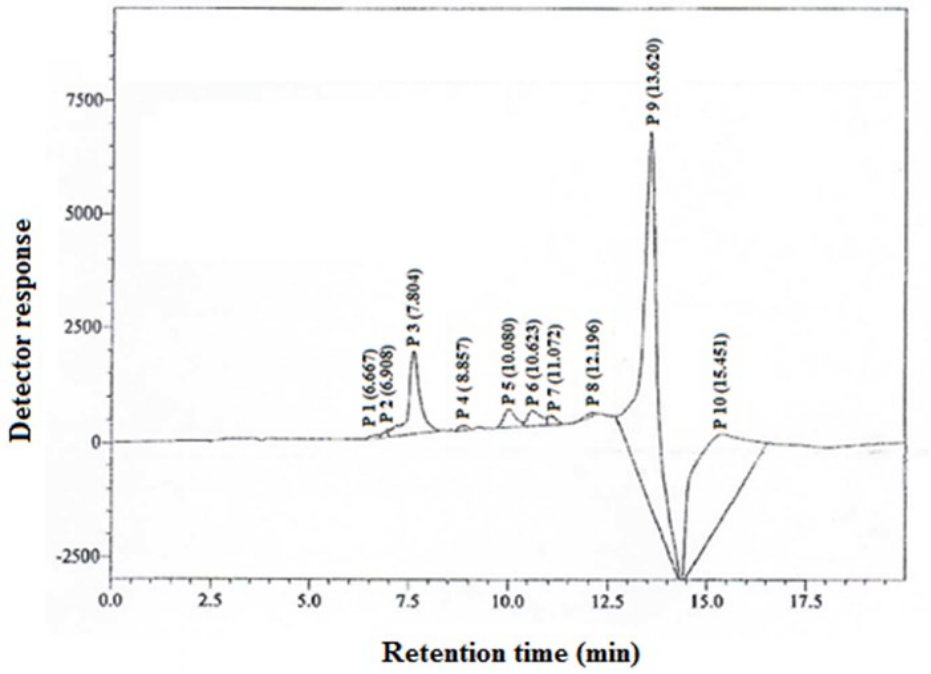

Fig. 1. Separation of fatty acids from worm extract *Polystoma integerrimum* using RP-HPLC

Table 1. Retention time of standard fatty acids

Name of fatty acids	Number of carbons	Number of double bonds	Symbol	Retention time (min)
Stearic acid	18	0	SA, C18:0	15.374
Palmitic acid	16	0	PA, C16:0	13.563
Myristic acid	14	0	MA, C14:0	12.112
Oleic acid	18	1	OA, C18:1n-9	10.021
Linoleic acid	18	2	LA, C18:2n-6	8.879
Linolenic acid	18	3	LNA, C18:3n-3	7.624

Table 2. Percentile of fatty acids of flatworm extract *Polystoma integerrimum* and their retention time compared with standard fatty acids

Extract Fatty Acids (EFA)	Retention Time of EFA(min)	Standard Fatty Acids (SFA)	Retention time of SFA (min)	Percentage of EFA (%)
Peak 1	6.667	---	---	0.081
Peak 2	6.908	---	---	0.098
Peak 3	7.804	Linolenic acid (C18:3n-3)	7.624	10,871
Peak 4	8.857	Linoleic acid C18:2n-6)	8.879	0.196
Peak 5	10.080	Oleic acid (C18:1n-9)	10.021	1.989
Peak 6	10.623	---	---	1.302
Peak 7	11.072	---	---	0.632
Peak 8	12.196	Myristic acid (C14:0)	12.112	0.278
Peak 9	13.620	Palmitic acid C16:0)	13.563	49.022
Peak10	15.451	Stearic acid (C18:0)	15.374	35.499

of the nematode Sulcascaris sp. parasitizes in oyster Argopecten gibbus, an intermediate host, contained seven fatty acids myristic, myristoleic, palmitic, palmitoleic, stearic, oleic and linoleic acids.

Metabolism of lipids and protein is of crucial importance for parasitism, and it changes dramatically as the parasite transits through the various stages of its life cycle (Ramakrishnan *et al*., 2013). Becker *et al*. (2017) shed light on possible adaptations of fatty acid composition during the transition of the nematode Dictyocaulus viviparus from free-living to parasitic stages in bovine lungs. This suggests that D. viviparus parasitic stages absorb FAs from their surroundings (host). The present study also aligns with the results of Al-Mowla (2010) when she studied fatty acids in six models of larvae and adults of parasitic nematodes in frogs and fish. The difference in the number of fatty acids in the present study and the studies mentioned above may be attributed to the host's nature and type of food sources involved in synthesizing the fatty acids to meet the host's

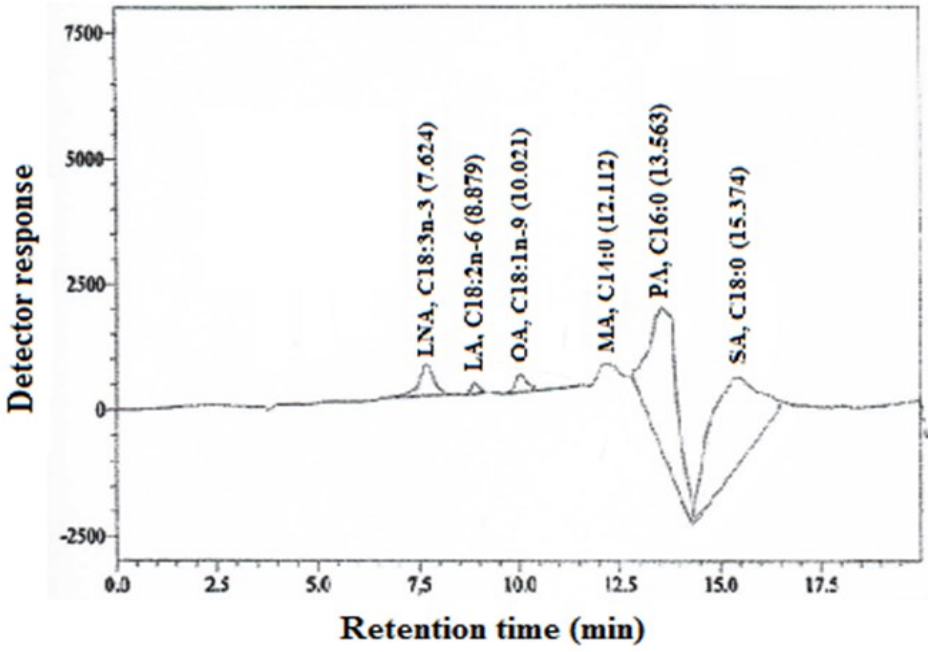

Fig. 2. *Standard fatty acid separation using RP-HPLC*

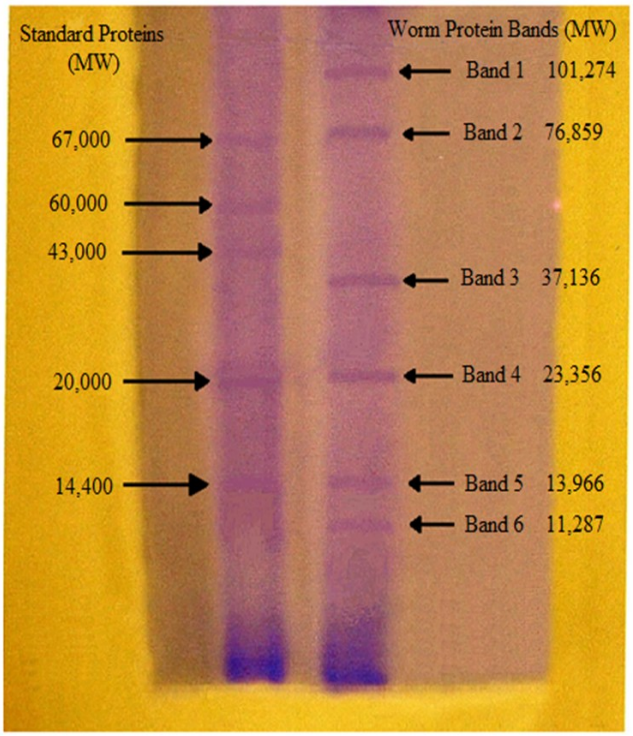

Fig. 3. Protein bands of flatworm extract *Polystoma integerrimum* and standard proteins separated by SDS-PAGE

needs (Besteiro *et al*., 2008).

With regard to how metabolites affect and interact with the association between parasite and its host, Babaran *et al*. (2021) analyzed fatty acids in trematode Plagiorchis sp. and its hosts, freshwater snails Stagnicola elodes, which were fed with three different diets (diatoms, green algae, and cyanobacteria), and the results explored parasite-containing snail tissue contained more polyunsaturated fatty acids compared to parasite-free snail tissue. These results suggest that the hosts, and possibly their parasites, could be nutritional upgraders of polyunsaturated fatty acids (PUFAs). Lipid modifications in the intermediate hosts, molluscs Littorina littorea and Mytilus edulis, were mainly caused by their parasite trematode Himasthla elongate (Fokina *et al*., 2018).

The presence of the two fatty acids, linolenic and linoleic, in the flatworm in this study is attributed to either obtaining these two fatty acids from their hosts, and this refers to the adaptation of the parasite to its host (Liu *et al*., 1990), or to the possibility of these worms being able to synthesize these two fatty acids, and this was confirmed by Morimoto *et al*. (2005). Also, the above are considered essential in the synthesis of long-chain unsaturated fatty acids (LC-PUFAs) such as arachidonic acid (AA; C20:4n-6) (Pratt and Cornely, 2024).

Separation and detection of proteins

The SDS-PAGE technique was conducted to separate the proteins contained in worm extract P. integerrimum as shown in Fig. 3 and Table 3, and these protein bands were detected according to five standard proteins, as shown in Table 4 using a simple linear regression equation $\hat{y} = 1.43761X + 3.70955$. Also, the Pearson correlation coefficient (r) was estimated between relative migration distance (Rf) and the logarithm of molecular weight (Log MW) of standard proteins, and the (r) value was 0.9899. Fig. 4.

The electrophoresis results in Fig. 3 revealed that the number of protein bands that appeared in P. integerrimum extract was six. It was noted that there was a relative similarity between the second band in the worm extract and the standard protein, BSA, with a MW of 67,000 daltons, while the fourth band was similar to the PPS with a MW of 20,000 daltons. Also, the fifth band of the extract was similar to the band of the α-LA with a MW of 14,400 daltons. The third band of the extract was relatively less than the molecular weight of OVA with a MW of 43,000 daltons. In addition, Fig. 3 shows two bands in the extract, one represented by the first band, which was at a lower level than the band of the standard protein, BSA, and another band represented by the sixth band in the extract, which was higher than

Table 3. Molecular weight of protein bands of flatworm extract *Polystoma integerrimum*

Band number of flatworm extract	Relative migration distance (Rf)	Molecular weight (Dalton)	Log molecular weight
1	0.9015	101274	5.0055
2	0.8181	76859	4.8857
3	0.5984	37136	4.5698
4	0.4583	23356	4.3684
5	0.3030	13966	4.1451
6	0.2386	11287	4.0526

Table 4. Molecular weight of standard proteins

Standard proteins	Relative migration distance (Rf)	Molecular weight (Dalton)	Log molecular weight (Log MW)
Alpha-lactalbumin (α-LA)	0.2992	14400	4.1583
Pepsin (PPS)	0.4431	20000	4.3010
Ovalbumin (OVA)	0.6363	43000	4.6334
Catalase (CTS)	0.7045	60000	4.7781
Bovine serum albumin (BSA)	0.8030	67000	4.8260

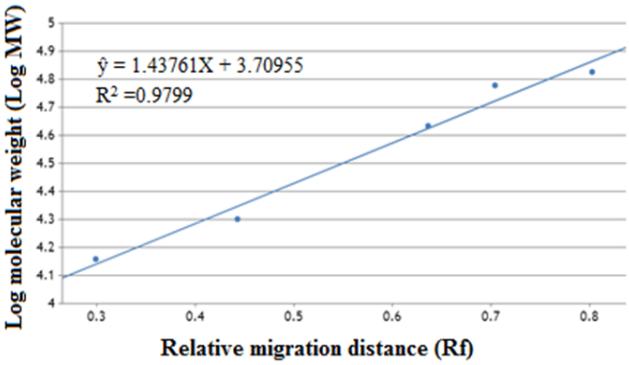

Fig. 4. *Scatter plot and equation of simple linear regression of standard proteins to estimate the unknown molecular weight of worm extract Polystoma integerrimum*

the band of the standard protein, α-LA.

The results of this study align with the study of Al-Daoody (2006) in terms of the especial band's presence in five digenean cercariae models. This may be attributed to the fact that this protein band is specific to the penetration process in cercariae that penetrate the appropriate host directly or by migrating through tissues and organs in the case of juvenile worms, such as the migration of juvenile hepatic worms from the intestine to the liver and then penetrating the liver capsule and then parenchyma and settling in the bile ducts. As for the three special protein bands in the present worm P. integerrimum, they may have a role in sexual maturity, especially with regard to the gill stages in the gills of tadpoles, which mature within three weeks and can lay eggs during this period, or they are resulting from the host's immune response, where a change occurs in the parasite's proteins as a response to the host's immune system (Gazzinelli-Guimaraes and Nutman, 2018), or perhaps some of these three bands have an important role in distinguishing between the stages of monogenan flatworm *P. integerrimum* that live in the gills of tadpoles from those that live in the urinary bladder in adult frogs.

The number of bands in the extract *P. integerrimum* was similar to the number of protein bands in the nematode worm Rhabdias bufonis (Al-Mowla, 2010), where similarity was observed between the second band in the present study and the first band in Rhabdias bufonis, which in turn resembles the standard protein BSA with a MW of 67,000 daltons. Also, the second in the P. integerrimum was similar to the second band in the third stage larva of the nematode Contracaecum sp. (Al-Naftachi, 2006), and this similarity in the protein bands may indicate the existence of genetic closeness between the different parasitic worms.

On the other hand, the present findings of Tak *et al*. (2015) found only four bands of protein in an extract of the intestinal helminth *Haemonchus contortus*. Zawistowska-Deniziak *et al*. (2021) reported eight protein bands for microfilariae and twelve bands for the adult stage of the parasitic nematode Dirofilaria repens. In Schistosoma haematobium and S. mansoni, 127 metabolites were found, with the metabolic pathways related to lipids and proteins accounting for 11.8% (Midzi *et al*., 2023).

Metabolomes of parasites play a key role in host immunity (Perera and Ndao, 2021). Depending on a combination of mass spectrometry, immunoblot, and two-dimensional electrophoresis, ten distinct immunogenic proteins were identified in sheep infected with the parasite trematode Fasciola hepatica serum. These findings could contribute to understanding the host-parasite dynamics in fasciolosis (Becerro-Recio *et al*., 2021).

Proteomics and genomics are metabolomics branches that play an important role in diagnosing parasites. Somatic proteome analysis of parasites may be employed in diagnosis to differentiate closely related species. Bennett and Robinson (2021) mentioned that differentiating between Fasciola gigantica and Fasciola hepatica solely on the basis of morphology can be challenging, but PCR methods and gene markers are needed for their identification.

Finally, metabolomics is a relatively emerging field of study that uses advanced technologies, through different modern separation, purification, and detection techniques, to identify suitable helminthic biomarkers as diagnostic tools and gain a deeper comprehension to design biochemical pathways that could shed light on the drug treatment, host-parasite relationship, differentiation of helminth populations, helminthic infection, and parasite life cycle processes (Ríos-Valencia *et al*., 2023).

The present study sheds some light on some metabolites of flatworm P. integerrimum, and there is an urgent need for further research in this field to improve the present understanding of metabolite-associated signaling pathways, their modifications and synthesis, as well as the role of this flatworm in its host and its genomic convergence with other species. It is also necessary to discover novel tools that can be used to control monogenic diseases, thus providing new and more background data about this flatworm.

Conclusion

The present study concluded that the tissue extract of the flatworm parasite Polystoma integerrimum contained fatty acids and proteins, which were studied for the first time represented by ten fatty acids and six protein bands that may have a vital role in the worm's metabolic pathways. This study could serve as a starting point towards investigating other biometabolites. Future studies can clarify the crucial roles of these metabolites in the physiological, pathological, immunological, taxonomical, and diagnostic aspects of this worm

and employ these concepts in a direction that serves human life in all health, nutritional, agricultural, and economic fields.

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Conflict of interest

The authors declare that they have no conflict of interest.

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