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Differential Gastrointestinal Effects Of Pongamia Pinnata Extracts In Drosophila Melanogaster Larvae: Influence Of Compound Polarity On Gut Ph And Morphology

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Abstract
Gastrointestinal (GI) drug screening is a valuable approach for evaluating the safety and potential applications of bioactive compounds. This study utilized Drosophila melanogaster third instar larvae as an in vivo model to examine the effects of Pongamia pinnata extracts on gut pH regulation. Petroleum ether, water, and acetone extracts were administered orally, with bromophenol blue serving as the pH indicator control. All three extracts induced measurable disturbances in the larval midgut pH, with the petroleum ether fraction producing the most pronounced alterations.
Phytochemical screening and GC–MS analysis revealed the presence of glycosides, flavonoids, tannins, and terpenoids—compounds known for their therapeutic and insecticidal properties. The results indicate that P. pinnata phytoconstituents can modulate gut physiology in insects, highlighting their potential dual role in gut health promotion and pest management. Furthermore, the study reinforces the relevance of D. melanogaster as a cost-effective, OECD-aligned model for preliminary screening of plant-derived bioactive agents.
Keywords: *Pongamia pinnata, Drosophila melanogaster, Polarity, Gastrointestinal tract, pH profile disturbance.*

INTRODUCTION

Drosophila melanogaster, commonly known as the fruit fly, is a well-established model organism in toxicological research due to its short life cycle, high reproductive rate, and conserved metabolic and detoxification pathways (Pandey & Nichols, 2011). Its physiological parallels with higher organisms make it an ideal system for evaluating the toxicity of novel compounds, including plant-derived bioactives and pesticides (Rand, 2010). Moreover, its ease of maintenance and suitability for high-throughput assays facilitate cost-efficient preliminary screenings. Although *D. melanogaster* is not part of the official OECD test species list for pesticide toxicity, its application follows OECD guidelines for Good Laboratory Practice (GLP) and aligns with the 3Rs principle (Replacement, Reduction, and Refinement), making it invaluable for mechanistic toxicology and early hazard detection (OECD, 1998).

In recent developments, *Drosophila* has emerged as a robust model for eco-environmental toxicology, with studies highlighting its sensitivity to pollutants such as micro- and nanoplastics—demonstrating physical damage, oxidative stress, reproductive effects, and gut damage in a size-dependent manner (Wang *et al.*, 2024). It has also proven useful for evaluating the toxicity of heavy metals—specifically lead and copper—across different genotypes, reinforcing its versatility in comparative toxicology (Ioan *et al.*, 2024).

Pongamia pinnata (L.) Pierre, a leguminous tree highly regarded in traditional medicine, is recognized for its pharmacological attributes. It contains flavonoids, glycosides, tannins, and terpenoids, and its seeds are rich in furanoflavonoids such as karanjin and pongamol—compounds known for their insecticidal, antimicrobial, and anti-inflammatory properties (Kumar *et al.*, 2010; Shoba & Thomas, 2001). Contemporary studies further substantiate its insecticidal applications: one report demonstrated high mortality of *Scirtothrips dorsalis* when exposed to seed extracts, with methanolic extract showing 100% mortality at 10% concentration after 96 hours (Chetia *et al.*, 2024) Another investigation revealed dose-dependent antifeedant activity against *Papilio demoleus* larvae, with a 2% seed oil emulsion achieving up to 84.65% feeding inhibition after 24 hours (Lingakari *et al.*, 2024)

In the present study, we leveraged *D. melanogaster* larvae as a model to investigate the gastrointestinal effects of *P. pinnata* extracts by assessing midgut pH disturbances. Petroleum ether, water, and acetone extracts were evaluated, using bromophenol blue as a positive control. Phytochemical screening and GC–MS profiling confirmed the presence of glycosides, flavonoids, tannins, and terpenoids. The observed alterations in gut pH suggest that *P. pinnata* phytoconstituents can modulate digestive physiology—highlighting dual relevance in both gut health research and biopesticide development.

MATERIALS AND METHODS

Plant Material Collection and Preparation

Fresh leaves of *Pongamia pinnata* were collected in bulk during February 2024 from RVS College of Arts and Science, Sulur, Coimbatore, Tamil Nadu, India. The plant was taxonomically identified using standard floras, particularly *Flora of the Presidency of Madras* (Gamble & Fischer, 1915–1936; reprinted by the Botanical Survey of India, Calcutta), and authenticated by a qualified botanist. A voucher specimen was deposited in the institutional herbarium for future reference. The collected leaves were washed thoroughly under running tap water to remove adhering dust and debris, followed by shade-drying at ambient temperature to prevent degradation of heat-sensitive phytoconstituents. The dried leaves were then ground into a fine powder using a mixer grinder and stored in airtight containers under dry conditions until further use for phytochemical extraction.

Extraction of Phytoconstituents

The dried leaf powder of *Pongamia pinnata* was subjected to successive solvent extraction using petroleum ether, acetone, and distilled water to obtain a broad range of phytoconstituents with varying polarities. For each solvent, 50 g of powdered leaf material was extracted using a Soxhlet apparatus for 6–8 hours until the solvent became colorless, indicating complete extraction. The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at temperatures not exceeding 40 °C to preserve thermolabile compounds. Filters were dried in a hot air oven at 60 °C for 2 hours, transferred to sterile containers, and immersed in PBS for 1 hour at room temperature before a final filtration step to obtain a clear extract (Kumar *et al.*, 2011). All extracts were stored at 4 °C until further use for phytochemical screening and bioassays.

Phytochemical Screening

The petroleum ether, acetone, and aqueous extracts of *Pongamia pinnata* leaves were subjected to qualitative phytochemical screening to detect the presence of major secondary metabolites, following standard procedures described by Rao *et al.* (2016).

Test for Saponins

One millilitre of the extract was diluted with distilled water to a total volume of 20 mL in a graduated cylinder and shaken vigorously for 15 minutes. The formation of a stable foam layer approximately 1 cm in height indicated the presence of saponins.

Test for Phenols

A small quantity of extract was mixed with 1 mL of distilled water in a test tube, followed by the addition of 1–2 drops of ferric chloride ($FeCl_3$) solution. The appearance of blue, green, red, or purple coloration was considered a positive indication for phenols.

Test for Glycosides

A small quantity of extract was combined with 1 mL of distilled water, and a few drops of aqueous sodium hydroxide (NaOH) solution were added. The development of a yellow coloration suggested the presence of glycosides

Test for Flavonoids

One to five drops of concentrated hydrochloric acid (HCl) were added to a small amount of extract. Immediate development of a red coloration indicated the presence of flavonoids.

Test for Alkaloids

Two millilitres of extract were mixed with 0.2 mL of dilute hydrochloric acid, followed by the addition of 1 mL of Mayer's reagent. The formation of a yellowish precipitate confirmed the presence of alkaloids.

Test for Tannins

Five millilitres of extract were treated with 2 mL of 5 % $FeCl_3$ solution. The appearance of a greenish-black precipitate was indicative of tannins.

Test for Terpenoids

In a test tube containing 2 mL of chloroform, 0.5 mL of extract was added, followed by 3 mL of concentrated sulphuric acid (H_2SO_4) to form a separate layer. A reddish-brown coloration at the interface confirmed the presence of terpenoids.

Gas Chromatography–Mass Spectrometry (GC–MS) analysis

Gas Chromatography–Mass Spectrometry (GC–MS) analysis of *Pongamia pinnata* leaf extracts was carried out using an Agilent Technologies 7890B GC system coupled with a 5977A Mass Selective Detector (MSD) and equipped with a HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. An aliquot of 1 µL of the extract was injected in split mode (1:20) at an injector temperature of 250 °C. The oven temperature was initially set at 60 °C (held for 2 min), then increased at 10 °C/min to 300 °C and held for 10 min. The MS was operated in electron impact (EI) mode at 70 eV, with a scan range of m/z 50–600 and an interface temperature of 280 °C. The mass spectra of the detected peaks were compared with those in the National Institute of Standards and Technology (NIST) library to identify the compounds based on their molecular weight, probable structure, and spectral similarity. The relative abundance of each identified compound was determined from peak area normalization (Adams, 2007).

Maintenance and Larval Collection of Drosophila melanogaster for In Vivo Gut pH Analysis

Drosophila melanogaster (Wild – type) were procured from the University of Calicut and maintained in the Cytogenetics Laboratory, BHU, on an agar–maize powder–sugar food medium following the method of Clara de Castro *et al.* (2013). Cultures were kept at room temperature (21–23 °C) under a 12 h light:dark photoperiod. After 7 days of incubation, adult flies were transferred to an agar–sugar medium prepared according to the protocol of Upendra Nongthomba (Indian Institute of Science, Bengaluru) to facilitate larval collection. Third-instar larvae were harvested and used for *in vivo* experiments investigating pH-mediated alterations in gut physiology of *D. melanogaster*.

In Vivo Gut pH Assay in Drosophila melanogaster

To assess the gut pH, 25 mg of yeast granules were dissolved in 500 µL of distilled water and mixed with different concentrations of three extracts of *Pongamia pinnata* to form a thick paste. This mixture was added to fresh food vials, and 4–5 third-instar larvae were introduced. Larvae were allowed to feed for 20 minutes. Post-feeding, each larva was placed on a glass slide in a drop of PBS and observed under a light microscope. Dissection was performed by gently pulling apart the cuticle using two dissecting needles, followed by careful straightening of the digestive tract for microscopic examination. For the control group, 25 mg of yeast granules were dissolved in 500 µL of distilled water along with 2 µL of 2% bromophenol blue pH indicator solution to form a paste. The indicator-fed larvae served as pH controls (Lakhotia & Ranganath, 2021).

RESULTS

Extraction, Phytochemical Profiling, and GC-MS Analysis of Pongamia pinnata

The extraction of *Pongamia pinnata* was performed using three solvents of varying polarity—water, acetone, and petroleum ether—to evaluate their efficiency in isolating bioactive compounds (Table 1). Solvent polarity significantly influenced the extraction yield and chemical composition. Water, being highly polar, produced the highest yield due to its capacity to dissolve polar phytoconstituents. Acetone, with intermediate polarity, extracted a balanced mix of polar and non-polar compounds, while petroleum ether, a non-polar solvent, effectively isolated hydrophobic constituents such as oils and lipids.

Preliminary phytochemical screening revealed distinct profiles for each extract. The aqueous extract contained glycosides, flavonoids, and terpenoids, but lacked saponins, phenols, alkaloids, and tannins. The acetone extract tested positive for flavonoids, tannins, and terpenoids, but was negative for saponins, phenols, glycosides, and alkaloids. The petroleum ether extract showed the presence of glycosides, flavonoids, tannins, and terpenoids, while saponins, phenols, and alkaloids were absent.

GC–MS analysis further identified the major phytoconstituents in each extract. The aqueous extract showed prominent peaks for Lupeol, Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl, and Lup-20(29)-en-3-one. The acetone extract contained Phytol, Benzoic acid 4-ethoxy-, ethyl ester, and Dibutyl phthalate, while the petroleum ether extract exhibited high peak areas for Pentacosane, Heneicosane, and

Di-n-octyl phthalate. (Table. 2,3&4) (Fig. 1,2 & 3).

In Vivo Analysis of pH-Mediated Disturbance of Gut pH in Drosophila melanogaster Larvae

Third-instar larvae of *Drosophila melanogaster* were fed for 20 minutes on a diet containing a pH indicator solution (2% phenol red and bromophenol blue), which produced distinct color changes in the digestive tract, indicating pH variations along the gut; microscopic observation confirmed that bromophenol blue shifted from yellow in acidic regions to blue in alkaline regions. To assess the effect of *Pongamia pinnata* leaf extracts on gut pH, larvae were exposed to different concentrations of aqueous, acetone, and petroleum ether extracts prepared in phosphate-buffered saline (PBS). All tested concentrations of the aqueous extract altered the GI pH profile, while higher concentrations of the acetone extract (0.7:0.3, 0.6:0.4, and 0.5:0.5 extract: PBS, v/v) caused pH disturbances and lower concentrations (0.4:0.6 and 0.3:0.7) induced both visible pH shifts and morphological changes in the GI tract. In contrast, all concentrations of the petroleum ether extract consistently disrupted the GI pH and caused morphological alterations, indicating that *P. pinnata* extracts exert extract-specific and concentration-dependent effects on larval gut physiology, with petroleum ether extract showing the strongest and most consistent impact. (Table 5).

Table1: Preliminary phytochemical screening of *Pongamia pinnata* leaves

Phyto chemicals	Water	Acetone	Petroleum ether
Saponins	-	-	-
Phenols	-	-	-
Glycosides	+	-	+
Flavonoids	+	+	+
Alkaloids	-	-	_
Tannins	_	+	+
Terpenoids	+	+	+

(+) – Presence; (-) – Absence

Fig 1. GCMS of aqueous extract *of Pongamia pinnata* leaves

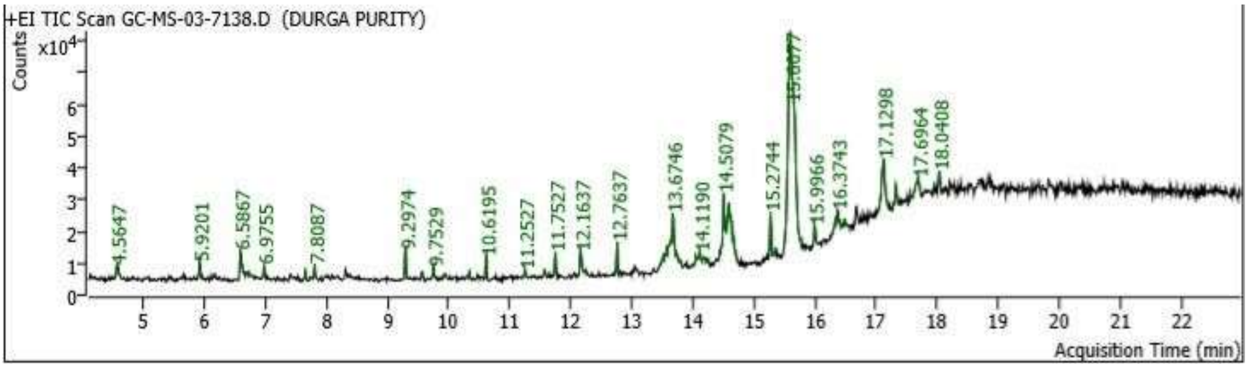

Fig 2. GCMS of acetone extract *of Pongamia pinnata* leaves

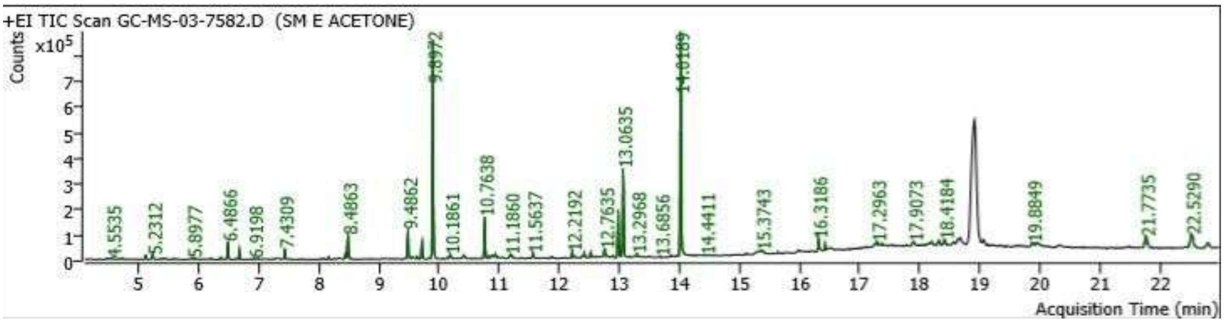

Fig 3. GCMS of petroleum ether extract *of Pongamia pinnata* leaves

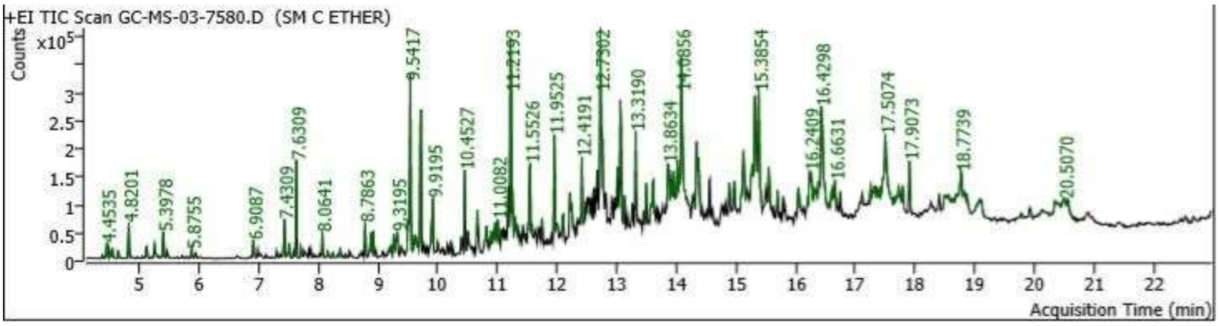

Table 2: Phytochemical constituents of aqueous extract of *Pongamia pinnata* leaves

S. No	RT	Compound Name	Formula	Area
1	15.607	Lupeol	C30H50O	505588
2	13.6746	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15hexadecamethyl	C16H50O7Si8	124637
3	14.5968	Lup-20(29)-en-3-one	C30H48O	114022
4	14.1190	Folic Acid	C19H19N7O6	36743
5	16.3743	Vitamin E	C29H50O2	36503
6	17.6964	3-Methoxymethoxy-3,7,16,20-tetramethyl-heneicosa1,7,11,15,19-pentaene	C27H46O2	31387
7	6.5867	Phenol, 4-ethyl-	C8H10O	27738
8	15.2744	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15hexadecamethyl-	C16H50O7Si8	23442
9	12.1637	Isopropyl myristate	C17H34O2	21751
10	9.2974	Cycloheptasiloxane, tetradecamethyl-	C14H42O7Si7	14175

Table 3: Phytochemical constituents of acetone extract of *Pongamia pinnata* leaves

S. No	RT	Compound Name	Formula	Area
1	9.8972	Benzoic acid, 4-ethoxy-, ethyl ester	C11H14O3	990060
2	14.0189	Phytol	C20H40O	1263325
3	12.9857	Diphenyl sulfone	C12H10O2S	228469
4	10.7638	Diphenylamine	C12H11N	228942
5	13.0635	Dibutyl phthalate	C16H22O4	469311
6	9.7195	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	87503
7	6.4866	Benzenemethanol, alpha-ethyl-	C9H12O	90017
8	7.4309	Benzene, 1,3-bis(1,1-dimethylethyl)-	C14H22	49315
9	6.6754	Benzoic acid, ethyl ester	C9H10O2	37779

Table: 4: Phytochemical constituents of petroleum ether extract of *Pongamia pinnata* leaves

S. No	RT	Compound Name	Formula	Area
1	17.5074	Pentacosane	$C_{25}H_{52}$	1014002
2	14.0856	Heneicosane	$C_{21}H_{44}$	898913
3	16.4298	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	841467
4	11.2193	Heptadecane	$C_{17}H_{36}$	733034
5	15.3188	Pentacosane	$C_{25}H_{52}$	629143
6	20.5070	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	564700
7	12.7302	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	506866
8	18.7739	Hentriacontane	$C_{31}H_{64}$	483916
9	15.3854	1-Cyclohexyldimethylsilyloxy-3, 5-dimethylbenzene	$C_{16}H_{26}OSi$	402013
10	15.1299	Heptadecane, 9-octyl-	$C_{25}H_{52}$	242774

In Vivo Analysis of pH-Mediated Disturbance of Gut pH in Drosophila melanogaster Larvae

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Table 5: pH profile of Different Extract of *Pongamia pinnata* on GI Tract of D*rosophila melanogaster*

S. No	Water	Disturbed/ Not Disturbed	Acetone	Disturbed/ Not Disturbed	Petroleum ether	Disturbed/ Not Disturbed
1	70%	Disturbed	70%	Disturbed	70%	Disturbed with morphological changes
2	60%	Disturbed	60%	Disturbed	60%	Disturbed with morphological changes
3	50%	Disturbed	50%	Disturbed	50%	Disturbed with morphological changes
4	40%	Disturbed	40%	Disturbed with morphological changes	40%	Disturbed with morphological changes
5	30%	Disturbed	30%	Disturbed with morphological changes	30%	Disturbed with morphological changes

DISCUSSION

Pongamia pinnata is well recognized in Ayurveda and Unani medicine for its anti-hyperglycaemic, anti-inflammatory, and antibacterial properties, and its seed oil is valued as a renewable biodiesel source (Qadir *et al.*, 2023). In this study, *P. pinnata* leaf extracts were evaluated for gastrointestinal (GI) safety using Drosophila melanogaster third-instar larvae—a cost-effective, genetically conserved (~60% similarity to humans) in vivo model with physiological parallels to the mammalian digestive tract (Apidianakis & Rahme, 2011). Results revealed distinct, extract-specific effects on gut pH and morphology. Aqueous extracts altered GI pH profiles at all tested concentrations, indicating that polar phytoconstituents can modulate digestive homeostasis. Acetone extracts displayed a biphasic effect—higher concentrations caused pH disturbances, whereas lower concentrations induced both pH shifts and visible morphological changes, suggesting concentration-dependent toxicity thresholds. Petroleum ether extracts consistently caused pronounced pH alterations and structural disruptions at all concentrations, likely due to their highly lipophilic constituents penetrating gut epithelium more readily and potentially disturbing epithelial integrity or ion balance.

These findings align with recent advances in insect gut research. For example, micro- and nanoplastic exposures in Drosophila have been shown to disrupt gut microbiota composition, induce dysbiosis, and compromise midgut integrity (Li *et al.*, 2023,). Similarly, emerging work demonstrates that gut physiology—including detoxification capacity—is finely modulated by compound polarity and host–microbe interactions. In the present case, the consistent morphological alterations caused by petroleum ether extracts may reflect lipophilic compound-mediated changes in membrane permeability, microbiota balance, or epithelial stability. Phytochemical and GC–MS analyses confirmed the presence of glycosides, flavonoids, tannins, and terpenoids—bioactives known for gut-modulating functions. Flavonoids exert antioxidant and microbiota-balancing effects (González-Gallego *et al.*, 2010), tannins display antimicrobial and astringent actions (Chung *et al.*, 1998), and terpenoids/glycosides contribute to mucosal protection and motility regulation (Ríos & Recio, 2005). Given the pivotal role of gut pH in drug solubility, ionization, and absorption (Vertzoni *et al.*, 2019), disruptions induced by lipophilic extracts could have direct implications for pharmacokinetics and safety. Overall, this work underscores the value of Drosophila for early-stage, ethically sustainable herbal safety assessments and highlights the need for future studies to isolate active constituents, probe mechanisms in mammalian models, and validate safety profiles before clinical translation (Markstein *et al.*, 2014).

CONCLUSION

This study demonstrates that *Pongamia pinnata* extracts exert differential effects on the gastrointestinal physiology of *Drosophila melanogaster* larvae, with petroleum ether extracts consistently causing marked pH alterations and gut morphological damage, acetone extracts producing concentration-dependent effects, and aqueous extracts altering pH without significant structural disruption. These outcomes highlight the influence of compound polarity on gut epithelial stability and digestive homeostasis, aligning with emerging insights into gut–compound and host–microbe interactions. The findings underscore the utility of *Drosophila* as a rapid, low-cost, and ethically sustainable model for preliminary safety screening of herbal products. Further research involving mammalian models, detailed mechanistic studies, and compound isolation is essential to confirm safety profiles and guide the therapeutic application of *P. pinnata*.

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Declarations

- *Conflict of Interest Disclosure*

The authors, [Marimuthu Dhanalakshmi, Periyasamy Thirunavukkarasu, Bhoopathy Sajitha, Udayan Elangovan, Rajagopal Renugadevi, Subramani Muthamilselvan Kanagathara, Ravi Kalaiyarasan] declare that: They have no conflicts of interest to disclose.

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- *Authors' Contribution*

Both authors contributed equally to this manuscript.

- *Data Availability Statement*

This manuscript, titled [Differential Gastrointestinal Effects of *Pongamia pinnata* Extracts in *Drosophila melanogaster* Larvae: Influence of Compound Polarity on Gut pH and Morphology], is an original work and has not been published elsewhere.

- *Ethical Approval Statement*

This study was exempt from ethical approval as it did not involve human subjects.

- *Informed Consent Statement*

This study did not involve any individual participants, and therefore, informed consent was not applicable.

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