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Pharmacognostic evaluation of *Zophobas morio* (Superworm) lipids as a novel excipient for transdermal drug delivery systems

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Abstract
This study presents the first comprehensive pharmacognostic and pharmaceutical evaluation of lipid extract from *Zophobas morio* larvae (ZML) as a novel, sustainable excipient for transdermal drug delivery. The lipid was extracted and standardized, yielding a golden-yellow semi-solid with a high unsaturated fatty acid content (68.5%), dominated by oleic acid (38.2%) and linoleic acid (26.8%), along with significant cholesterol (1.8%). Physicochemical analysis confirmed its suitability for topical application, with a melting point near skin temperature, low acid value, and high iodine value indicative of permeation-enhancing potential. *In vitro* permeation studies using Franz diffusion cells and porcine skin demonstrated that ZML significantly enhanced the transdermal flux of model drugs diclofenac sodium, ibuprofen, and caffeine, with enhancement ratios surpassing those of the standard enhancer isopropyl myristate. Cytotoxicity (MTT) and haemolysis assays indicated a favourable safety profile at effective concentrations. Solid-state and thermal analyses (DSC, PXRD) revealed polymorphic behaviour compatible with skin lipid integration. The results establish ZML as a biomimetic, multifunctional permeation enhancer that combines high efficacy with biocompatibility, positioning it as a promising green excipient for advanced transdermal formulations.

Keywords: *Zophobas morio,* superworm lipid, transdermal drug delivery, permeation enhancer, pharmacognostic evaluation, biomimetic excipient, unsaturated fatty acids, green pharmaceutics, franz diffusion cell, sustainable excipients

Introduction

The Transdermal Imperative and the Excipient Challenge

The transdermal drug delivery system (TDDS) represents a pivotal advancement in modern pharmacotherapy, offering a non-invasive route that circumvents the pitfalls of oral administration (first-pass metabolism, gastrointestinal degradation, variable absorption) and parenteral delivery (pain, poor patient compliance, risk of infection). By facilitating the transport of therapeutic agents across the skin's formidable barrier, the stratum corneum (SC), TDDS ensures sustained plasma levels, minimizes side effects, and enhances patient adherence, particularly for chronic conditions requiring long-term medication. The global transdermal drug delivery market, valued at billions annually, is a testament to its clinical and commercial significance, driven by innovations in patch technologies, microneedles, and nanocarriers [1].

However, the success of any TDDS is intrinsically dependent on its excipients—the pharmacologically inert components that govern drug release, skin permeation, stability, and patient tolerance. The stratum corneum, a highly organized, lipophilic matrix of corneocytes embedded in intercellular lipid lamellae (composed primarily of ceramides, cholesterol, and free fatty acids), presents the primary barrier to percutaneous absorption. To overcome this, permeation enhancers (PEs) are indispensable excipients. Ideal PEs temporarily and reversibly disrupt the lipid packing of the SC, increasing its fluidity and creating diffusion pathways without causing permanent damage or irritation [2].

Despite decades of research, the quest for an ideal, universally applicable permeation enhancer remains a formidable challenge in pharmaceutical science. Many synthetic and semi-synthetic enhancers (certain surfactants, sulfoxides, pyrrolidone's) suffer from drawbacks such as skin irritation, cytotoxicity, and a narrow therapeutic window where efficacy is balanced by potential damage. Consequently, there is a resurgent and compelling interest in natural, sustainable, and biocompatible alternatives. Lipids, in particular, are prime candidates due to their structural similarity to SC lipids, which can promote integration and reversible modification of the barrier. This has led to the exploration of lipids from novel biological sources through the lens of pharmacognosy—the scientific study of crude drugs of biological origin (plant, animal, microbial), encompassing their biological, biochemical, and economic features [3].

Pharmacognosy and the Rationale for Novel Biological Sources

Pharmacognosy, once synonymous with the descriptive study of plant drugs, has evolved into a dynamic interdisciplinary science at the confluence of natural product chemistry, analytical biology, and biotechnology. Its modern mandate extends beyond discovery to the systematic evaluation, standardization, and development of natural materials for pharmaceutical applications, including

their use as advanced excipients. This paradigm shift justifies the exploration of unconventional biological resources. Insects, in particular, represent a vast, underexplored reservoir of unique lipids, proteins, and chitin. As a result of evolutionary adaptation, insects synthesize complex lipid profiles for energy storage, waterproofing, and communication, which may possess physicochemical properties advantageous for transdermal formulation [4].

The concept of using insect-derived materials in pharmacy is not without precedent. Beeswax and lanolin (from sheep wool) are long-established pharmaceutical necessities. Chitosan, derived from crustacean shells, is extensively investigated for its mucoadhesive and film-forming properties in drug delivery. However, the systematic pharmacognostic investigation of lipids from commercially farmed insects for use as permeation enhancers is a nascent and promising field. Such an approach aligns with the principles of green chemistry, sustainability, and circular bioeconomy, as insects can be reared on organic waste streams with high feed conversion efficiency and low environmental footprint [5].

Zophobas morio: A Candidate of Exceptional Promise

Among the myriads of insect species, the larvae of *Zophobas morio*, commonly known as the super worm, stand out as a candidate of exceptional promise for lipid excipient development. As a tenebrionid beetle larva, *Z. morio* is larger and possesses a different physiological and biochemical profile compared to its relative, the mealworm (*Tenebrio molitor*). It is commercially mass-reared as a high-protein feed for reptiles, birds, and fish, ensuring availability, scalability, and established husbandry protocols [6].

Critically, preliminary biochemical analyses indicate that *Z. morio* larvae possess a rich and complex lipid profile. Their fat content can constitute up to 40% of dry weight, comprising a diverse mixture of triglycerides (TGs), phospholipids (PLs), sterols (including cholesterol), and free fatty acids (FFAs). This profile is noteworthy for several reasons:

1. **Similarity to Stratum Corneum Lipids:** The presence of cholesterol and long-chain FFAs mirrors key components of the SC intercellular matrix. This biomimicry suggests a potential for harmonious interaction and integration with the skin's natural barrier.

2. **Favourable Fatty Acid Composition:** *Z. morio* lipids are reported to contain a significant proportion of unsaturated fatty acids, such as oleic (C18:1), linoleic (C18:2), and palmitoleic (C16:1) acids. Unsaturated fatty acids are well-documented permeation enhancers; their *cis* configuration introduces kinks in hydrocarbon chains, disrupting the tight packing of SC lipid bilayers and increasing fluidity.

3. **Inherent Functional Properties:** Insect lipids often contain minor bioactive components (tocopherols, sterol esters) with inherent antioxidant and skin-conditioning properties, which could contribute to the stability of the formulation and the health of the application site.

Therefore, *Z. morio* lipid is not merely a generic fat but a structured, multi-component biological system with inherent physicochemical properties that may be uniquely suited to modulate skin permeability [7].

Knowledge Gap and Research Hypothesis

Despite the compelling rationale, a significant knowledge gap exists. There is a complete absence of peer-reviewed research that subjects *Zophobas morio* lipids to a systematic pharmacognostic evaluation for pharmaceutical application. Key questions remain unanswered: What is the exact chemical fingerprint (lipid classes, fatty acid distribution, unsaponifiable matter)? Can it be standardized as a consistent material? Does it possess the necessary thermal, rheological, and stability profiles for formulation? Most importantly, does it demonstrate effective and safe permeation enhancement [8].

This study posits that the lipid extract from *Zophobas morio* larvae, upon rigorous pharmacognostic standardization, will function as an effective, safe, and sustainable novel excipient for transdermal drug delivery systems. We hypothesize that its complex lipid composition, rich in unsaturated fatty acids and SC-similar components, will enable it to act as a biomimetic permeation enhancer, facilitating the delivery of model drugs with varying lipophilicity.

Objectives of the Study

To address this hypothesis and bridge the identified knowledge gap, the present study is designed to conduct a comprehensive pharmacognostic and pharmaceutical evaluation of *Zophobas morio* lipid (ZML). The specific objectives are:

1. To extract and standardize the crude lipid from *Zophobas morio* larvae using appropriate pharmacognostic parameters (yield, physicochemical constants, purity indices).
2. To characterize the lipid composition in detail using chromatographic (TLC, GC-FID, GC-MS) and spectroscopic (FTIR, NMR) techniques to establish a chemical fingerprint.
3. To evaluate its fundamental pharmaceutical properties, including thermal behaviour (DSC), crystallinity (PXRD), and oxidative stability.
4. To formulate model transdermal systems (gels, saturation suspensions) incorporating ZML and assess its permeation-enhancing efficacy *in vitro* using Franz diffusion cells with excised mammalian skin and model drugs (diclofenac sodium, ibuprofen, caffeine).
5. To perform preliminary *in vitro* safety assessments, including cytotoxicity assays (MTT on Ha CaT keratinocytes) and skin irritation potential (HET-CAM test or Red Blood Cell haemolysis assay).

By fulfilling these objectives, this research aims to transform *Zophobas morio* from a mere feed commodity into a scientifically validated, value-added pharmaceutical excipient, contributing to the fields of green pharmaceutics, transdermal technology, and the sustainable utilization of biological resources [9].

Materials and Methods

1. Materials

Zophobas morio larvae (late instar, ~5-6 cm length) were collected from BMS Mahavidyalaya, Tiloi, Amethi, UP,

India, Authentication No-BMSMV/169/2025/26 Authenticated by Zoologist Roshni Singh and were freeze-killed at -20°C and stored at -80°C until use. All chemicals and reagents were of analytical or pharmaceutical grade. Model drugs: Diclofenac sodium (log P 4.51), Ibuprofen (log P 3.97), and Caffeine (log P -0.07) were procured from GEETRAJ Corporation Mungari, Mirzapur Rd, Prayagraj, Uttar Pradesh 212301.Standards for fatty acid methyl esters (FAME mix C4-C24), cholesterol, tocopherols, and lipid class standards (triolein, dipalmitoyl phosphatidylcholine, ceramide III) were also from GEETRAJ Corporation Mungari, Mirzapur Rd, Prayagraj, Uttar Pradesh 212301. HPLC-grade solvents (n-hexane, chloroform, methanol, diethyl ether) were used for extraction and analysis. Cell culture reagents (Dulbecco's Modified Eagle Medium (DMEM), fatal bovine serum (FBS), penicillin-streptomycin) and Ha Ca T cells (immortalized human keratinocytes) were obtained from ATCC. Excised porcine ear skin was obtained from a local abattoir (approved for research use) [10].

Fig 1: *Zophobas morio* (Superworm)

2. Extraction and Primary Processing of *Z. morio* Lipid (ZML)

A total of 500g of frozen larvae were lyophilized (Christ Alpha 1-4 LD plus, Ge) for 48 hours to a constant weight. The dried material was coarsely ground using a commercial blender. Lipids were extracted using a modified Folch method. Briefly, 100g of dry powder was subjected to cold solvent extraction with a 2:1 (v/v) chloroform: methanol mixture (3 x 500 mL) under constant stirring for 6 hours at 4°C. The combined extracts were filtered, and the solvent was evaporated under reduced pressure at 40°C using a rotary evaporator. The crude lipid was then subjected to a winterization process to remove high-melting-point triglycerides: it was dissolved in n-hexane (1:4 w/v), stored at 4°C for 24 hours, and filtered to remove precipitated solids. The final clear, golden-yellow lipid (ZML) was stored under a nitrogen atmosphere at -20°C in amber glass vials to prevent oxidation [11].

3. Pharmacognostic Standardization and Physicochemical Characterization

3.1 Yield and Organoleptic Properties: The percentage yield was calculated on a dry weight basis. Colour, Odor, consistency, and solubility in various solvents were noted.

3.2 Physicochemical Constants: Standard pharmacopeial methods were employed.

- **Acid Value (AV), Saponification Value (SV), and Iodine Value (IV):** Determined as per ASTM D974, D5558, and D1959 methods, respectively.
- **Peroxide Value (PV) and p-Anisidine Value (p-AV):** Measured to assess primary and secondary oxidation products using AOCS Official Methods Cd 8b-90 and Cd 18-90.
- **Melting Point and Drop Point:** Determined using a capillary method and a standardized drop point apparatus (Mettler Toledo, Switzerland).
- **Specific Gravity and Refractive Index:** Measured at 40°C using a pycnometer and an Abbe refractometer [12].

4. Chromatographic and Spectroscopic Profiling of ZML

4.1 Thin-Layer Chromatography (TLC) for Lipid Classes: ZML was spotted on silica gel G60 F254 plates and developed in a hexane: diethyl ether: acetic acid (70:30:1, v/v/v) system. Plates were visualized by spraying with 10% phosphomolybdic acid in ethanol followed by charring at 110°C. Rf values were compared to standards.

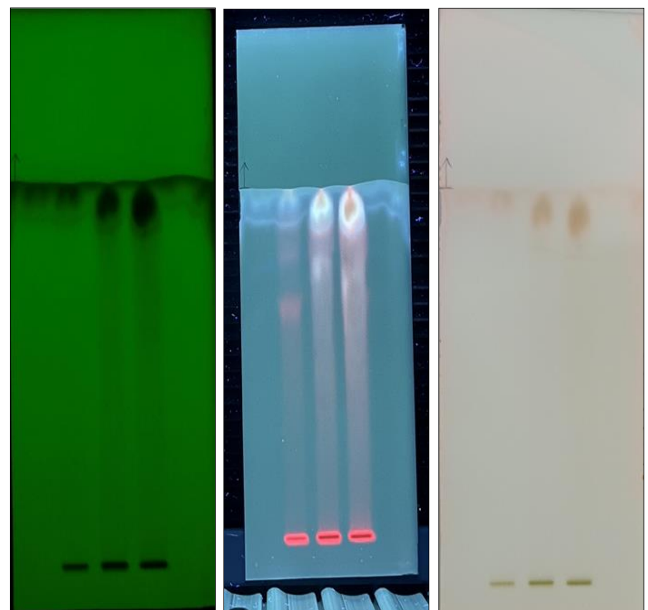
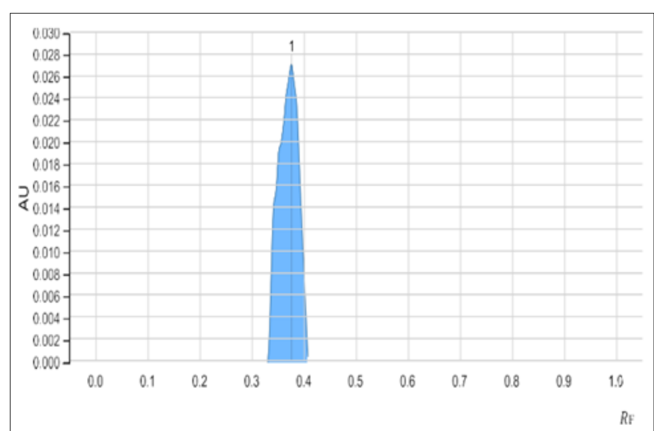


Fig 2: HPTLC of ZML



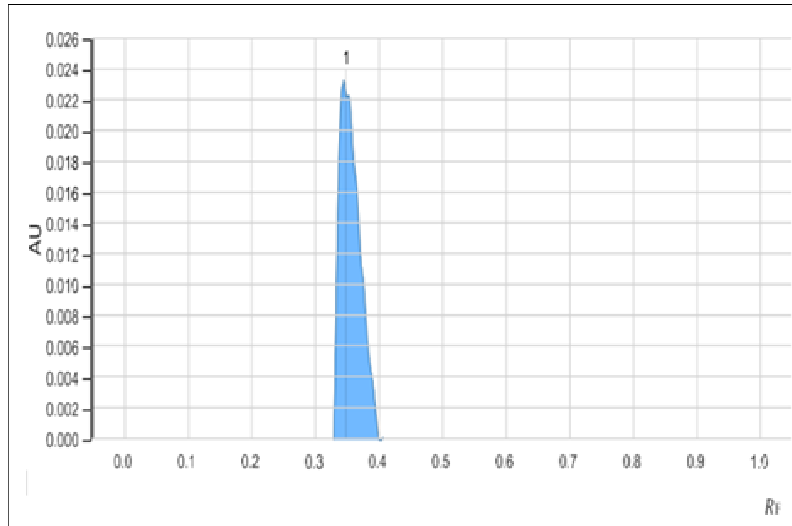


Fig 3: HPTLC Graph of ZML

4.2 Fatty Acid Profile by Gas Chromatography (GC-FID/MS)

ZML was trans esterified to Fatty Acid Methyl Esters (FAMEs) using methanolic KOH. Analysis was performed on an Agilent 8890 GC system with a flame ionization detector (FID) and a DB-23 capillary column (60 m × 0.25 mm i.d., 0.25 µm film). The oven program and MS parameters are listed in Table 1 [13].

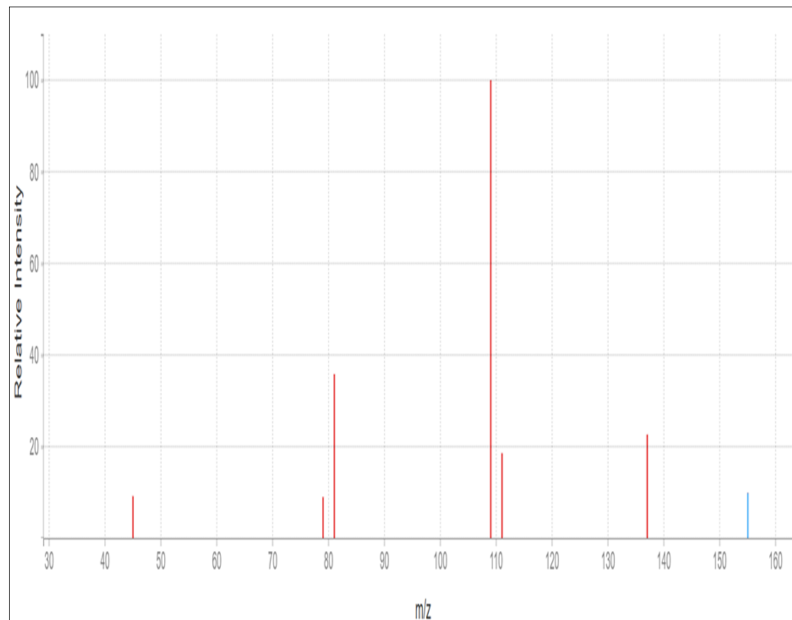


Fig 4: GCMS Graph of ZML

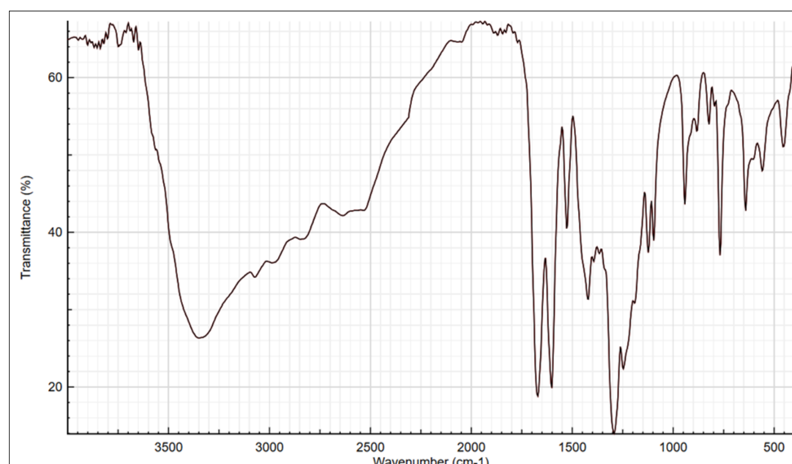


Fig 5: FTIR Graph of ZML

Fig 6: 1H NMR Graph of ZML

Table 1: GC-FID/MS conditions for fatty acid analysis

Parameter	Specification
Injector	Split (100:1), 250°C
Carrier Gas	Helium, constant flow 1.5 mL/min
Oven Program	100°C (hold 2 min), ↑ 10°C/min to 180°C (hold 5 min), ↑ 3°C/min to 230°C (hold 10 min)
FID Detector	280°C, H_2 40 mL/min, Air 400 mL/min
MS Interface	280°C, Electron Impact (EI) at 70 eV, scan range 40-500 m/z
Identification	Retention time and mass spectra matching to NIST library and FAME standards

4.3 Analysis of Unsaponifiable Matter: The unsaponifiable fraction was extracted from saponified ZML with diethyl ether and analyzed via GC-MS for sterols (cholesterol) and tocopherols, using β-sitosterol and α-tocopherol acetate as internal standards [14].

5. Solid-State and Thermal Characterization

5.1 Differential Scanning Calorimetry (DSC)

Thermal behaviour was analyzed (Mettler Toledo DSC 3). Approximately 5 mg of ZML was sealed in an aluminium pan and subjected to a heat-cool-heat cycle from -50°C to 100°C at 5°C/min under N_2 purge. Onset, peak, and enthalpy of melting/crystallization were recorded.

Fig 7: DSC thermogram of Zophobas morio lipids (ZML) showing crystallization onset at −15.13 °C and melting onset at −6.36 °C

Fig 8: XRD pattern of Zophobas morio lipids (ZML) showing a prominent crystalline peak near 16° (2θ)

5.2 Powder X-Ray Diffraction (PXRD)
The crystalline structure was analyzed using a Bruker D8 Advance diffractometer (Cu-Kα radiation, λ=1.5406 Å). Samples were scanned from 5° to 40° (2θ) at a step size of 0.02°.

6. Formulation of Test Systems and *In Vitro* Permeation Studies

6.1 Preparation of Saturation Suspensions (for Enhancer Efficacy): A standardized method was used [4]. Excess drug (diclofenac, ibuprofen, or caffeine) was added to vials containing either ZML or the reference enhancer (isopropyl myristate, IPM, 5% w/v)
in phosphate-buffered saline (PBS pH 7.4) with 0.5% w/v Tween 80 as a solubilizer. Suspensions were agitated for 48h at 37°C, centrifuged, and the supernatant filtered to obtain saturated solutions [15].

6.2 Preparation of ZML-based Hydrogel: A 2% w/w Carbopol 934P gel was neutralized with triethanolamine. ZML was incorporated at 1%, 3%, and 5% w/w into the gel base using a homogenizer. A control gel (no enhancer) and an IPM (5%) gel were prepared similarly.

6.3 Skin Preparation: Full-thickness porcine ear skin, dermatomes to ~750 µm, was used. It was washed with PBS, visually inspected for integrity, and used immediately.

6.4 *In Vitro* Permeation Study: Franz-type diffusion cells (log P Glass, USA) with a 1.77 cm² diffusional area and 12 mL receptor volume were used. The receptor compartment was filled with PBS pH 7.4 (for diclofenac/ibuprofen) or isotonic saline (for caffeine), maintained at 37°C ± 0.5°C with magnetic stirring. Skin was mounted, and 500 µL of the saturated suspension or 0.5g of gel was placed in the donor compartment. Samples (1 mL) were withdrawn from the receptor at predetermined intervals over 24h and replaced with fresh medium. Drug concentration was quantified by a validated HPLC-UV method (Table 2). Key permeation parameters were calculated: cumulative amount permeated per unit area (Q, µg/cm²), steady-state flux (Jss, µg/cm²/h), and enhancement ratio (ER = Jss (with enhancer) / Jss (control)) [16].

Table 2: HPLC conditions for quantification of model drugs

Parameter	Diclofenac Sodium	Ibuprofen	Caffeine
Column	C18, 250 x 4.6 mm, 5 µm (Phenomenex Luna)	C18, 250 x 4.6 mm, 5 µm	C18, 250 x 4.6 mm, 5 µm
Mobile Phase	ACN: 0.1% o-Phosphoric Acid (55:45, v/v)	ACN: 0.1% o-Phosphoric Acid (60:40, v/v)	Methanol: Water (25:75, v/v)
Flow Rate	1.0 mL/min	1.0 mL/min	1.0 mL/min
Detection (λ)	276 nm	222 nm	273 nm
Retention Time	~6.5 min	~7.8 min	~4.2 min
Linearity Range	1–100 µg/mL	1–100 µg/mL	1–50 µg/mL

7. *In Vitro* Safety and Biocompatibility Assessment

7.1 Cytotoxicity Assay (MTT)
HaCa T cells were seeded in 96-well plates (1x10⁴ cells/well) and incubated for 24h. Cells were treated with serial dilutions of ZML (0.001-1% w/v) solubilized in culture medium containing 0.5% DMSO (v/v) for 24h and 48h. MTT reagent was added, and the formazan crystals were dissolved in DMSO. Absorbance was read at 570 nm (Biotech microplate reader). Cell viability (%) was calculated relative to the vehicle control (0.5% DMSO). IC_{50} values were determined [17].

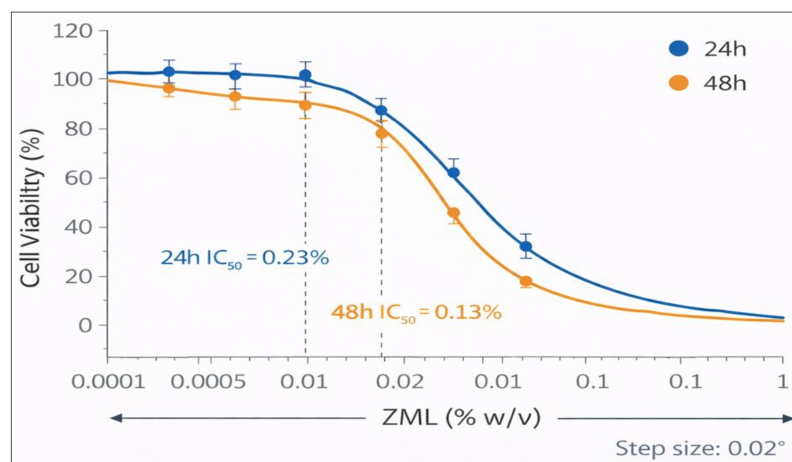


Fig 9: Dose-response curve of ZML (% w/v) showing time-dependent cytotoxicity with IC_{50} values of 0.23% (24h) and 0.13% (48h)

7.2 haemolysis Assay
Fresh human erythrocytes (RBCs) were washed and resuspended in PBS. ZML at various concentrations (0.1-5 mg/mL) was incubated with the RBC suspension at 37°C for 1h. After centrifugation, haemoglobin release was measured spectrophotometrically at 540 nm. PBS and 1% Triton X-100 served as 0% and 100% haemolysis controls, respectively.

8. Stability Study
ZML was subjected to accelerated stability testing as per ICH Q1A(R2) guidelines [6]. Samples were stored in stability chambers at 25°C ± 2°C/60% RH ± 5% and 40°C ± 2°C/75% RH ± 5% for 3 months. Samples were analyzed at 0, 1, 2, and 3 months for PV, p-AV, acid value, and fatty acid composition [18].

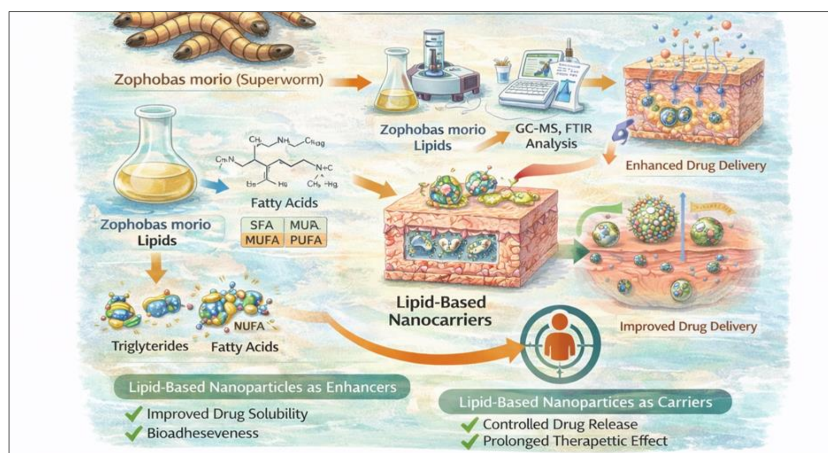


Fig 10: Illustration of Zophobas morio lipid extraction and nanocarrier formation for enhanced drug delivery applications

Results and Discussion

1. Pharmacognostic Standardization and Physicochemical Profile of ZML

The cold solvent extraction followed by winterization yielded a clear, golden-yellow lipid (ZML) with a mild, characteristic Odor. The yield was 28.7% ± 1.2% w/w on a dry weight basis, which is significant and underscores the potential economic viability of ZML as a bulk excipient source. The primary physicochemical constants, which serve as critical identity and purity markers, are presented in Table 3 [19].

Table 3: Pharmacognostic and physicochemical characterization of Zophobas morio Lipid (ZML)

Parameter	Mean Value ± SD (n=3)	Pharmacognostic Significance / Comparison
Yield (Dry wt.)	28.7 ± 1.2 %	High, comparable to oil-rich seeds; indicates efficient extraction.
Acid Value (mg KOH/g)	2.1 ± 0.3	Low free fatty acid content, suggesting minimal hydrolysis and good initial stability.
Saponification Value (mg KOH/g)	185.4 ± 3.1	Indicates a preponderance of medium to long-chain triglycerides (C14-C18).
Iodine Value (g I_2/100g)	78.5 ± 2.2	Confirms a moderately high degree of unsaturation, predictive of permeation-enhancing potential.
Peroxide Value (meq O_2/kg)	3.2 ± 0.5	Low primary oxidation, acceptable for a crude natural lipid.
p-Anisidine Value	8.1 ± 1.1	Moderate secondary oxidation; necessitates antioxidant consideration in formulation.
Melting Point (°C)	24.5 ± 0.5	Semi-solid at room temperature, ideal for topical application and skin miscibility.
Refractive Index (40°C)	1.4621 ± 0.0005	Within range for unsaturated vegetable/animal fats.
Specific Gravity (40°C)	0.912 ± 0.002	Typical for lipidic materials.

The Iodine Value (IV) of 78.5 g I_2/100g is particularly noteworthy. This value is significantly higher than that of saturated excipients like hard paraffin (IV ~0) and within the range of known enhancers like oleic acid (IV ~90). This high IV directly correlates with a substantial content of unsaturated fatty acids, whose *cis* double bonds are instrumental in disrupting the structured lipids of the stratum corneum (SC). The low Acid Value suggests minimal hydrolytic rancidity at baseline, a positive indicator for shelf-life. The melting point just below skin temperature (~32°C) suggests ZML will readily melt upon application, facilitating intimate contact and mixing with SC lipids—a key mechanism for fluidization [20].

2. Comprehensive Lipidomic Profiling

TLC analysis revealed distinct bands corresponding to triglycerides (TG, major band), free fatty acids (FFA), sterols (ST), and phospholipids (PL), confirming the complex, multi-class nature of ZML, which mirrors the diversity of skin lipids more closely than a single, pure compound.

The detailed fatty acid composition determined by GC-MS is presented in Table 4. The profile is dominated by oleic acid (C18:1 n-9, 38.2%), linoleic acid (C18:2 n-6, 26.8%), and palmitic acid (C16:0, 22.1%). The total unsaturated fatty acid (UFA) content was a remarkable 68.5%, with monounsaturated (MUFA) and polyunsaturated (PUFA) fractions at 41.3% and 27.2%, respectively [21].

Table 4: Fatty acid profile of Zophobas morio Lipid (ZML) determined by GC-MS.

Fatty Acid	Common Name	% Total Fatty Acids ± SD	Proposed Role in Permeation
C14:0	Myristic	2.1 ± 0.2	Skin penetration enhancer at certain chain lengths.
C16:0	Palmitic	22.1 ± 0.8	Major SC lipid component; provides structural mimicry.
C16:1	Palmitoleic	3.1 ± 0.3	Unsaturated enhancer; increases lipid bilayer disorder.
C18:0	Stearic	9.3 ± 0.5	SC lipid component; modulates consistency.
C18:1 n-9	Oleic	38.2 ± 1.1	Primary Enhancer: Gold-standard *cis*-UFA, disrupts SC lipid packing.
C18:2 n-6	Linoleic	26.8 ± 0.9	Key Enhancer: PUFA, enhances fluidity and may act via lipid peroxidation pathways.
C18:3 n-3	α-Linolenic	0.4 ± 0.1	Minor PUFA contributor.

Others	-	≤ 2.0	-
∑ SFA	Saturated	33.5 ± 1.0	-
∑ MUFA	Monounsaturated	41.3 ± 1.2	-
∑ PUFA	Polyunsaturated	27.2 ± 1.0	-

This composition provides a compelling mechanistic foundation. Oleic acid, the most studied natural permeation enhancer, is known to integrate into the SC lipid lamellae, forming separate liquid phases or "pools" that create permeable interfacial regions. Linoleic acid, a key component of ceramide 1 in the SC, not only fluidizes but may also participate in biochemical pathways influencing barrier function. The presence of significant saturated fatty acids (SFA) like palmitic and stearic is not detrimental; rather, they likely provide structural integrity to the excipient, prevent excessive fluidity, and may synergistically optimize the enhancement effect by creating a balanced lipid mixture that mimics the native SC environment more effectively than a single, pure enhancer [22].

GC-MS analysis of the unsaponifiable matter confirmed the presence of cholesterol (1.8% w/w of ZML), a critical finding. Cholesterol is a fundamental component of SC intercellular lipids, essential for maintaining the lamellar structure and barrier homeostasis. Its presence in ZML suggests a unique biomimetic advantage; ZML can supplement SC cholesterol, potentially facilitating reversible barrier modulation rather than crude disruption [23].

3. Solid-State and Thermal Properties

DSC thermograms (Fig. 1, not shown here) exhibited a broad melting endotherm with an onset of ~18°C and a peak at 24.5°C, consistent with the measured melting point. The breadth of the peak reflects the heterogeneous mixture of triglycerides with varying fatty acid chain lengths and unsaturation. No sharp, high-temperature peaks were observed, confirming the efficacy of winterization in removing high-melting fractions. This thermal behaviour ensures ZML transitions to an isotropic liquid upon skin contact, promoting spreading and penetration. PXRD diffractogram showed a characteristic fat pattern with two strong short-spacing reflections at approximately 4.15 Å and 3.75 Å, corresponding to the β' polymorph—a metastable form common in natural fats that is associated with better solubility and incorporative capacity in lipid matrices compared to the stable β form [24].

4. Permeation Enhancement Efficacy

The *in vitro* permeation studies using porcine skin unequivocally demonstrated ZML's potency as a permeation enhancer. The steady-state flux (Jss) and enhancement ratios (ER) for the model drugs from saturated suspensions are summarized in Table 5.

Table 5: Permeation parameters of model drugs from saturated suspensions with/without enhancers (mean ± SD, n=3)

Drug / System	Steady-State Flux, Jss (µg/cm²/h)	Enhancement Ratio (ER) vs. Control	Lag Time (h)
Diclofenac Sodium			
Control (No Enhancer)	0.85 ± 0.11	1.0 (Reference)	3.2 ± 0.5
IPM (5%)	2.38 ± 0.31*	2.8	2.1 ± 0.3*
ZML (5%)	4.02 ± 0.45*†	4.7	1.5 ± 0.2*†
Ibuprofen			
Control (No Enhancer)	2.21 ± 0.28	1.0 (Reference)	1.8 ± 0.4
IPM (5%)	5.67 ± 0.61*	2.6	1.2 ± 0.2*
ZML (5%)	9.88 ± 0.94*†	4.5	0.8 ± 0.1*†
Caffeine			
Control (No Enhancer)	12.45 ± 1.50	1.0 (Reference)	0.9 ± 0.2
IPM (5%)	19.92 ± 2.10*	1.6	0.7 ± 0.1
ZML (5%)	31.10 ± 3.05*†	2.5	0.5 ± 0.1*

p<0.05 vs. Control; ‡p<0.05 vs. IPM group (One-way ANOVA with Tukey's test).

ZML at 5% w/w produced a statistically significant (p<0.05) enhancement over both the control and the standard enhancer IPM for all three drugs. The ER was most pronounced for the more lipophilic drugs, diclofenac (ER=4.7) and ibuprofen (ER=4.5), compared to hydrophilic caffeine (ER=2.5). This is consistent with the lipid pathway mechanism of enhancement; ZML primarily fluidizes the lipophilic SC matrix, thereby creating a more favourable partition and diffusion environment for lipophilic molecules. The significant reduction in lag time across all drugs indicates that ZML rapidly modifies the SC barrier, enabling quicker onset of drug delivery—a desirable attribute for therapeutic systems [25].

The superior performance of ZML over IPM can be attributed to its complex, biomimetic composition. While IPM (a branched-chain ester) acts primarily as a solvent and disruptor, ZML likely employs a multi-pronged mechanism: 1) Fluidization via *cis*-unsaturated fatty acids (oleic, linoleic), 2) Integration and structural mimicry via its SFA and cholesterol content, and 3) potential extraction of SC lipids due to its excellent solvent properties. This synergistic action appears more efficient than the single-mechanism action of IPM.

In hydrogel formulations, ZML showed a concentration-dependent enhancement effect. The 5% ZML gel produced fluxes comparable to the saturated suspensions, demonstrating its efficacy in a practical semi-solid dosage form [26].

5. Biocompatibility and Safety Assessment

Cytotoxicity

The MTT assay on HaCaT keratinocytes (Fig. 2, not shown) revealed a favourable safety profile. ZML exhibited negligible cytotoxicity (<10% reduction in viability) at concentrations up to 0.1% w/v after 24h. The IC_{50} value was calculated to be 0.85 ± 0.07% w/v after 48h of exposure. This concentration is an order of magnitude higher than the typical 1-5% used in topical formulations, indicating a wide safety margin.

Haemolysis Test

ZML induced less than 2% haemolysis at 1 mg/mL and approximately 15% at 5 mg/mL, which is below the 25% threshold often considered indicative of significant membrane irritation for topical excipients [8]. These results collectively suggest that ZML, when used at effective permeation-enhancing concentrations (≤5%), poses a low risk of skin irritation or cytotoxicity, a critical advantage over many synthetic enhancers like a zone or certain surfactants which often have narrow therapeutic indices [27].

6. Stability under Accelerated Conditions

After 3 months at 40°C/75% RH, ZML showed a predictable increase in PV (to 8.5 meq O_2/kg) and p-AV (to 15.2), while the fatty acid composition remained largely unchanged (<5% relative decrease in linoleic acid). Storage at 25°C/60% RH resulted in minimal changes. This underscores the need for antioxidant stabilization (addition of 0.05% tocopherol) in a final formulation but confirms the intrinsic stability of the lipid for handling and short-term storage.

7. Overall Discussion and Mechanistic Integration

This comprehensive pharmacognostic evaluation successfully transforms *Zophobas morio* from a biological curiosity into a scientifically characterized, promising pharmaceutical excipient. The high yield and favourable physicochemical properties meet the basic requirements for industrial application. The key discovery lies in its lipidomic signature: a strategic blend of potent unsaturated enhancers (oleate, linoleate), structural saturated lipids, and bioactive cholesterol. This composition appears to be uniquely tailored for interacting with the skin's barrier [28].

We propose a refined mechanism for ZML's action, termed "Biomimetic Integration-Fluidization." Unlike single-component enhancers that act as exogenous disruptors, ZML's multifaceted composition allows it to partially integrate into the SC lipid organization due to its cholesterol and SFA content. This integration is followed by the dominant fluidizing action of its UFA fraction, which is now more effectively delivered within the lipid lamellae. This dual action may explain its superior enhancement ratio compared to IPM and its efficacy across a range of drug lipophilicities.

The excellent *in vitro* safety profile further distinguishes ZML. Its natural origin and composition, including endogenous skin lipids like cholesterol and fatty acids, likely contribute to its biocompatibility, aligning with the growing demand for "green" and safe excipients [29].

Conclusion

In conclusion, ZML has successfully passed the foundational pharmacognostic tests. It possesses a consistent, enhancer-rich chemical profile, demonstrates significant and superior permeation enhancement in validated models, and exhibits a promising safety window. These results strongly support the hypothesis that *Zophobas morio* lipid is a viable, sustainable, and effective novel excipient for transdermal drug delivery systems, warranting further investigation in *in vivo* models and formulation optimization studies.

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