Research Article

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# The impact of drying and extraction methods on total lipid, fatty acid profile, and cytotoxicity of *Tenebrio molitor* larvae

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**Abstract:** The rising cultivation of edible insects at an industrial level highlights the importance of employing appropriate post-harvest methods for processing safe and fine-quality insect-based products. The study investigated the impact of different drying and extraction methods on the quality of *Tenebrio molitor* larvae powder. Two drying treatments, microwave and oven drying, were evaluated followed by total lipid extraction using various methods. Soxhlet extraction with an *n*-hexane–EtOH (3:1) mixture was effective, yielding 37.85% (microwave) and 38.85% (oven). The highest fatty acid (FA) content was 9-octadecenoic acid, methyl ester (*E*)-(**C18:1**). The Schlechtriem and Bligh and Dyer (BD) methods resulted in higher total lipid yields, with BD yielding 56.66% in oven-dried samples. FA profiles were consistent, with monounsaturated FAs predominant. Oven-dried samples had higher saturated FAs. Phenolic content was greater in oven-dried samples, with the *n*-hexane–EtOH extraction showing the highest total phenolic content (0.09 ± 0.02 mg GAE/100 mg). BD extract from oven-dried samples was the most cytotoxic, significantly reducing cell survival against A549 cells ($IC_{50}$ = 458.6 µg/mL) and inducing apoptosis at 250 µg/mL. This study underscores the importance of post-harvest methods in producing high-quality insect-based products, revealing that oven drying and specific solvent extractions can enhance total lipid yield, phenolic content, and cytotoxic effects on cancer cells.

**Keywords:** cytotoxicity, drying methods, fatty acid, Soxhlet, yellow mealworm

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## 1 Introduction

Entomophagy, or eating insects as food, dates back approximately 7,000 years [1]. Recently, there has been a significant shift toward cultivating insects to produce protein and oil-based products, explicitly focusing on fatty acids (FAs) obtained through various extraction methods. This has become a central area of interest for research, development, and commercialization efforts [2,3].

Among the most studied, black soldier fly larvae (*Hermetia illucens*) are recognized for their high-fat content, which positions them as a valuable source of oil rich in lauric acid, which has applications in both food and cosmetics industries [4]. Additionally, crickets (*Acheta domesticus*) have been explored for their oil, which is rich in unsaturated fats and has potential as a functional food ingredient [5]. Mealworms (*Tenebrio molitor*) are another well-researched species, valued not only for their protein but also for their oil, which contains a balanced profile of essential FAs such as oleic acid, which is valued for its anti-inflammatory, anticancer, and wound-healing properties, making it an important component in topical formulations and pharmaceutical ointments. Linoleic acid is crucial for maintaining skin barrier function and is extensively used in skincare products and treatments for inflammatory skin conditions. Linoleic acid was also reported for its antitumor effect. Additionally, palmitic acid plays a significant role in pharmaceutical synthesis, particularly in modifying drug solubility and absorption [6–11]. Insect lipids have undergone extensive research, not only for their potential as valuable food additives and other high-value products but also as sources for biofuel production [12,13].

Lipid extraction from insects for commercial oil production poses several challenges despite the various extraction processes described in the literature. The effectiveness of lipid extraction is influenced by the polarity of the solvent or its combination used [14]. Typically, the utilization of solvent blends comprising both non-polar and polar solvents results in enhanced lipid extraction [15]. One example is the Bligh and Dyer (BD) method, incorporating a blend of

non-polar (chloroform), polar (EtOH), and water. This method has found widespread application in lipid extraction from diverse biological samples [16].

Recently, sonication technology has become widely used for extracting bioactive compounds in the pharmaceutical sectors [17,18]. Ultrasonic extraction, when compared to traditional liquid solvent methods, presents numerous benefits, including shorter extraction durations, decreased solvent usage, heightened extraction efficiency, and superior extract quality. This technique is economically viable and environmentally sustainable for extracting bioactive constituents from sample matrices [19]. A comparative analysis of various technological and physicochemical processes for oil extraction has been conducted on mealworm larvae. The efficient extraction of oil from cellular biomass is crucial in assessing its suitability and yield [20]. Given the absence of a standardized extraction process for FAs, this research seeks to examine the effects of different lipid extraction techniques on insect lipid research. Specifically, we compare lipid extractions from mealworm biomass that was either oven-dried or microwave-dried. We employed Soxhlet extraction to recover lipids using single solvents or mixtures. Additionally, we utilized two extraction methods previously successful in insect lipid extraction from biomass: (1) the monophasic ternary system of chloroform:methanol:water, a commonly used method [21], and (2) a combination of cyclohexane, propan-2-ol, and water as suggested by Schlechtriem et al. [22]. The innovation and originality of this study lie in evaluating various drying and extraction methods to highlight the differences in total lipid yield and FA profiles obtained from mealworms.

# 2 Materials and methods

## 2.1 Insect culture

The breeding of *T. molitor* larvae was conducted following previously established protocols at King Saud University. Larvae were cultured in a controlled environment maintained at 25 ± 2°C and 35% humidity level. They were nourished with a diet consisting of wheat bran and provided with cabbage for hydration after hatching. After 30 days of feeding, larvae were separated from the wheat bran and excrement. Following a fasting period of 24 h, the insects were subjected to freezing at −80°C using a Hera freezer (Thermo Scientific, USA) before the drying process.

## 2.2 Drying procedures

*T. molitor* frozen larvae were collected and then subjected to further drying utilizing two distinct methodologies: microwave and oven drying. Before drying, the larvae were subjected to boiling (100°C) for 3 min. For the oven drying method, 150 g of larvae were placed onto a baking plate measuring 20 cm × 25 cm × 3 cm and subjected to oven drying at 60°C for 24 h, as outlined by Siemianowska et al. [23]. In contrast, for microwave drying, 150 g of frozen larvae were spread on a plate measuring 20 cm × 25 cm × 3 cm, positioned at the centre of a conventional microwave (Haam, model P10043AP-G2, China), and dried for 5 min (Power level 10, 1,000 W). Upon completion of the drying processes, the mealworms were collected and finely ground into particles using an electric blender (Stardust, Japan) before analysis. Each group was subjected to three replicates in the experiment.

## 2.3 Extraction methods

### 2.3.1 Soxhlet extraction

In a Soxhlet extractor (Glassco Scientific, Korea), 4 g of microwave- and oven-dried powder were used in the extraction process. The extraction method was performed at 85°C for 6 h. Two solvent systems were used for crude fat recovery: 200 mL of *n*-hexane alone and a mixture of 150 mL of *n*-hexane with 50 mL of ethanol ($n$ = 3). The extracts obtained were filtered using a 0.25 hydrophobic syringe filter (Millipore, USA) and vacuum-dried using a rotary evaporator (Heidolph, Germany). The resulting oily extract was quantified and expressed as crude fat content (% dried weight).

### 2.3.2 Schlechtriem method

The samples (4 g) of microwave and oven-dried powder were put into a flask and mixed with 120 mL of propane-2-ol and cyclohexane (1:1.25, v/v; $C_3H_8O/C_6H_{12}$) following [22] method with minor modifications. The tubes were homogenized (IKA, Germany) for 1 min and ultrasonicated (Wise Clean, Korea) at 60°C for 30 min. Subsequently, 50 mL of water was added. After vortexing, the phases were centrifuged at 4,000 rpm for 5 min, and the organic phases were pooled together and vacuum dried at 45°C.

#### 2.3.3 BD method

A modification of the BD [21] extraction method was used. Initially, the sample (4 g) was mixed with chloroform ($CHCl_3$) and methanol (75:37.5, v/v; $CHCl_3$/MeOH), homogenized for 2 min, and then treated in an Ultrasonic Cleaner for 10 min at 25°C. Then, 37.5 mL of $CHCl_3$ was added to 50 mL of water; this mixture was homogenized for another 2 min and sonicated for another 10 min. The resulting suspension was filtered, and the liquid was collected into a separatory funnel for phase separation. Finally, the lipid extract was vacuum-dried at 45°C.

## 2.4 Gas chromatography–mass spectrometry (GC–MS)

#### 2.4.1 FA methyl ester preparation

A 200 mg of mealworm extract was mixed with 1 mL of *n*-hexane. Subsequently, 0.2 mL of methanolic sodium hydroxide (1 M) was added, and the mixture was agitated in a water bath at 50°C for 20 s. After cooling, 0.2 mL of methanolic hydrochloric acid (1 M) was introduced, followed by vortexing for 10 s and reheating for an additional 10 s at 50°C. Approximately 500 µL of the upper phase was carefully transferred to a separate tube. The extraction was repeated with 250 µL of *n*-hexane, and the combined upper phases were collected. The resulting FA methyl esters were dissolved in 500 µL of *n*-hexane and stored in glass vials for subsequent analysis.

#### 2.4.2 GC–MS analysis

Following the methodology outlined by Abutaha and Al-Mekhlafi [24], the analyses were performed using an Agilent Technologies 7890B GC–MS system (Santa Clara, CA, USA) with a DB-5 MS capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness). Helium was utilized as the carrier gas at a flow rate of 1 mL/min, with an inlet temperature of 250°C and a split ratio of 50. The oven temperature was programmed from 50 to 250°C over a total run time of 73 min, with an injection volume of 0.9 µL via autosampler. The mass spectrometer operated with a scan range of 40–500 g/mol, a scan speed of 1.56, a 2-min solvent delay, and a source temperature of 230°C. Product identification was conducted using the NIST Mass Spectrometry database software.

## 2.5 Determination of total phenolics (TP) in the extracts

The TP concentration in the sample was evaluated using the Folin–Ciocalteu (FC) method with gallic acid as a standard. A 5 µL extract was mixed with 20 µL of FC reagent and 80 µL of $Na_2CO_3$ (7.5%). After incubation (60 min) at 25°C, the absorbance (765 nm) was measured using a plate reader (ChroMate, USA). Results were quantified as milligrams of gallic acid equivalent (GAE) per 100 mg of extract, determined based on the reference gallic acid calibration with the equation $y = 0.0042x + 0.0489$ and $R^2 = 0.974$. Each measurement was conducted in triplicate [25].

## 2.6 Radical scavenging assay

The scavenging potential of the extracts against DPPH˙ was assessed according to Al-Zharani and Abutaha [25]. Briefly, 10 µL of the extract (50 mg/mL), diluted in dimethyl sulfoxide (DMSO), was mixed with 190 µL/well of DPPH˙ solution (0.008% w/v in methanol) and left to react for 30 min at 25°C. A blank sample was prepared by adding DPPH˙ solution to 10 µL of DMSO. Then, the absorbance (515 nm) was measured, and the scavenging % was calculated. The DPPH radical scavenging ability was measured as a percentage of inhibition, determined using the equation:

$$\text{DPPH scavenging activity}(\%) = (A_0 - A_1)/(A_0) \times 100,$$

where $A_0$ represents the absorbance of the control and $A_1$ represents the absorbance of the sample.

## 2.7 *In vitro* cytotoxic potential

#### 2.7.1 Culturing of cell line

Lung adenocarcinoma cell lines (A549) were cultured in Dulbecco's minimum essential medium, supplemented with 10% fetal bovine serum and penicillin/streptomycin. These cells were placed in a T25 cell culture flask and maintained in a humidified chamber with 5% $CO_2$ at 37°C.

#### 2.7.2 Determination of cell viability

For the (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) (MTT) assay, A549 cells (50,000 cells/mL) were seeded in 1,000 µL of DMEM media per well in a

**Table 1:** Relative abundance of fatty acids in lipids and non-fatty acid compounds extracted from *T. molitor* larvae dried by microwave method

| Compound | Soxhlet (hexane) | Soxhlet (*n*-hexane–EtOH) | Schlechtriem | BD | *F* value |
|---|---|---|---|---|---|
| Tetradecanoic acid, methyl ester (**C14:0**) | 2.27 ± 0.03a | 2.33 ± 0.09a | 1.38 ± 0.06b | 1.315 ± 0.00b | 101.74 |
| 9-Hexadecenoic acid, methyl ester, (*Z*)- (**C16:1**) | 1.72 ± 0.04a | 1.74 ± 0.12a | 1.05 ± 0.05b | 1.22 ± 0.14b | 11.891 |
| Hexadecanoic acid, methyl ester (**C16:0**) | 16.25 ± 0.14a | 16.52 ± 0.05a | 14.23 ± 0.08b | 12.17 ± 0.01c | 548.36 |
| 9,12-Octadecadienoic acid (*Z*,*Z*)-, methyl ester (**C18:2**) | 28.52 ± 0.00a | 31.69 ± 0.05a | 30.33 ± 1.53a | 29.02 ± 0.44a | 3.18 |
| 9-Octadecenoic acid, methyl ester, (*E*)- (**C18:1**) | 39.22 ± 0.10c | 38.36 ± 0.11c | 44.22 ± 0.60b | 47.58 ± 0.29a | 163.15 |
| Methyl stearate (**C18:0**) | 6.14 ± 0.02b | 7.60 ± 0.18a | 5.50 ± 0.14b | 5.37 ± 0.31b | 27.90 |
| 3′,8,8′-Trimethoxy-3-piperidyl-2,2′-*B* inaphthalene-1,1′,4,4′-tetrone | 0.55 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | — |
| Cis-13-Eicosenoic acid (**C20:1**) | 0.85 ± 0.00a | 0 ± 0.00b | 0 ± 0.00b | 0 ± 0.00b | 123.86 |
| Cholest-5-En-3-Ol (3α)- | 2.65 ± 0.08a | 1.77 ± 0.01a | 2.02 ± 0.43a | 0 ± 0.00b | 27.18 |
| Methyl 7-ethyl-10-hydroxy-11-hydroxy(18o)-3,11-dimethyl-2,6-tridecadienoate | 0.81 ± 0.08a | 0 ± 0.00b | 0 ± 0.00b | 0 ± 0.00b | 106.67 |
| 1,2-Benzenedicarboxylic acid | 0 ± 0.00c | 0 ± 0.00c | 0.56 ± 0.01b | 0.76 ± 0.08a | 105.95 |
| 1-Heptatriacotanol | 0 ± 0.00a | 0 ± 0.00a | 0.44 ± 0.44a | 0 ± 0.00a | 1.00 |
| 4*H*-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-Methoxy- | 0 ± 0.00a | 0 ± 0.00a | 0.29 ± 0.29a | 0 ± 0.00a | 1.04 |
| 9,12-Octadecadienoic acid (*Z*,*Z*)- | 0± | 0± | 0± | 0± | — |
| Stigmast-5-en-3-Ol, (3α)- | 0 ± 0.00a | 0 ± 0.00a | 0 ± 0.00a | 0.43 ± 0.23a | 3.45 |
| Stigmast-5-en-3-Ol, (3α,24S)- | 0 ± 0.00a | 0 ± 0.00a | 0 ± 0.00a | 2.54 ± 0.20a | 162.31 |
| Stigmasta-5,22-dien-3-Ol, acetate, (3α)- | 0± | 0± | 0± | 0± | — |
| SFA (% TFA) | 24.66 | 26.44 | 21.10 | 18.85 | — |
| MUFA (% TFA) | 42.83 | 40.09 | 45.26 | 48.8 | — |
| PUFA (% TFA) | 29.70 | 32.26 | 31.36 | 30.01 | — |
| PUFA/SFA | 1.15 | 1.19 | 1.43 | 1.53 | — |

The data are presented as means ± SD, with a sample size of $n = 3$. Letters accompanying the means signify statistically significant differences ($P < 0.05$) among the extraction solvents. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

24-well plate. The cells were then treated with increasing concentrations from 100 to 900 µg/mL. To evaluate cell viability, the medium was replaced with an MTT working solution (5 mg/mL) and incubated for 2 h. Afterwards, DMSO (500 µL) was added to each well. The absorbance at 570 nm was determined, and the findings were expressed as a percentage compared to the control group (0.1% DMSO). The $IC_{50}$ concentration, representing 50% inhibition of cancer cells, was determined using the non-linear regression method [25].

### 2.7.3 Analysis of alterations in cell and nuclear morphology

Morphological changes induced by the different extraction methods in A549 cancer cell lines were observed and photographed using an EVOS microscope (USA). To study nuclear morphology, the treated and control cells were fixed in ice-cold ethanol, washed with PBS, stained with 4′,6-diamidino-2-phenylindole (DAPI) (1 mg/mL), and left for 5 min at 25°C. The cells were then observed for nuclear morphology changes using a fluorescence microscope (EVOS, USA).

### 2.7.4 Dual staining assay for apoptosis detection

Following previously reported protocols [25], the dual dye (AO–EB) method was employed to assess apoptotic changes induced by extracts in liver cancer cells. Cells were seeded and treated the next day with the extract (250 µg/mL) for 24 h. Subsequently, the cells were stained with AO–EB for 2 min. Microscopic images were then captured using a fluorescence microscope.

## 2.8 Statistical analysis

All statistical analyses were performed with the SPSS software package. The collected data were subjected to a one-way

analysis of variance. The Tukey's (honestly significant difference) multiple tests determined significant differences between means at $P \leq 0.05$.

# 3 Result

## 3.1 Total lipid recovery performance

The study investigated the total lipid yields obtained from *T. molitor* larvae using different drying and extraction methods (Tables 1 and 2). The Schlechtriem method revealed that microwave-dried samples had a lipid yield of 36.8 ± 0.3%, while oven-dried samples yielded 32.65 ± 0.2%. BD method showed that microwave-dried samples had a lipid yield of 28.1 ± 1.1%, and oven-dried samples had a slightly higher yield of 29.7 ± 1.3%. The *n*-hexane method yielded 36.45 ± 0.84% lipids from microwave-dried samples and 31.7 ± 0.3% from oven-dried samples. The *n*-hexane:EtOH (3:1) method provided the highest lipid yields, with microwave-dried samples at 37.85 ± 0.2% and oven-dried samples at 38.85 ± 0.3%; comparing these methods, the oven-drying combined with the *n*-hexane:EtOH (3:1) extraction achieved the highest lipid yield, indicating this method's efficiency in lipid recovery from *T. molitor* larvae.

## 3.2 Comparative FA profile of four extraction methods

The efficiency of FA extraction was notably influenced by the drying and extraction methods, with statistical significance observed ($P < 0.05$, Table 1). Our comparative analysis revealed significant FA variations across four extraction methodologies. These variances in the percentage of FAs and their profiles (Table 1) are influenced by

**Table 2:** Relative abundance of fatty acids in lipids and non-fatty acid compounds extracted from *T. molitor* larvae dried by oven method

| Compound | Soxhlet (hexane) | Soxhlet (*n*-hexane–EtOH) | Schlechtriem | BD | *F* value |
|---|---|---|---|---|---|
| Tetradecanoic acid, methyl ester (**C14:0**) | 1.98 ± 0.00a | 1.47 ± 0.13b | 1.00 ± 0.00c | 1.17 ± 0.01 bc | 36.30 |
| 9-Hexadecenoic acid, methyl ester, (*Z*)- (**C16:1**) | 1.37 ± 0.04b | 2.23 ± 0.13a | 1.12 ± 0.01b | 1.34 ± 0.11b | 30.56 |
| Hexadecanoic acid, methyl ester (**C16:0**) | 13.49 ± 0.20b | 16.52 ± 0.15a | 12.38 ± 0.07c | 12.89 ± 0.03bc | 198.74 |
| 9,12-Octadecadienoic acid (*Z,Z*)-, methyl ester (**C18:2**) | 22.72 ± 0.08c | 31.21 ± 0.12a | 23.59 ± 0.02b | 22.50 ± 0.13c | 1855.50 |
| 9-Octadecenoic acid, methyl ester, (*E*)- (**C18:1**) | 54.72 ± 0.02c | 42.49 ± 0.10d | 55.35 ± 0.01b | 56.66 ± 0.03a | 16335.04 |
| Methyl stearate (**C18:0**) | 3.52 ± 0.22b | 5.03 ± 0.04a | 3.97 ± 0.01b | 3.79 ± 0.03b | 35.56 |
| 3′,8,8′-Trimethoxy-3-piperidyl-2,2′-*B* inaphthalene-1,1′,4,4′-tetrone | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | — |
| Cis-13-eicosenoic acid(**C20:1**) | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | — |
| Cholest-5-En-3-Ol (3α)- | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | — |
| Methyl 7-ethyl-10-hydroxy-11-hydroxy(18o)-3,11-dimethyl-2,6-tridecadienoate | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | — |
| 1,2-Benzenedicarboxylic acid | 0.61 ± 0.00a | 0 ± 0.00b | 0 ± 0.00b | 0 ± 0.00b | 11163.00 |
| 1-Heptatriacotanol | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | — |
| 4*H*-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy- | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | — |
| 9,12-Octadecadienoic acid (*Z,Z*)- | 0 ± 0.00b | 0 ± 0.00b | 1.05 ± 0.03a | 0 ± 0.00b | 1083.00 |
| Stigmast-5-en-3-Ol, (3α)- | 0 ± 0.00c | 1.06 ± 0.00b | 0 ± 0.00c | 1.65 ± 0.08a | 381.69 |
| Stigmast-5-en-3-Ol, (3α,24S)- | 1.60 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | 0 ± 0.00 | — |
| Stigmasta-5,22-dien-3-Ol, acetate, (3α)- | 0 ± 0.00b | 0 ± 0.00b | 1.46 ± 0.09a | 0 ± 0.00b | 250.29 |
| SFA (% TFA) | 18.99 | 23.01 | 17.44 | 17.84 | — |
| MUFA (% TFA) | 56.09 | 44.72 | 56.47 | 58 | — |
| PUFA (% TFA) | 23.28 | 31.53 | 24.19 | 22.87 | — |
| PUFA/SFA | 1.19 | 1.35 | 1.35 | 1.26 | — |

The data are presented as means ± SD, with a sample size of $n = 3$. Letters accompanying the means signify statistically significant differences ($P < 0.05$) among the extraction solvents. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

the chosen extraction and drying methods. The major FAs identified in the extracts were hexadecanoic acid, methyl ester (**C16:0**) (palmitic acid), 9,12-octadecadienoic acid (*Z*,*Z*)-, methyl ester (**C18:2**) (linoleic acid), and 9-octadecenoic acid, methyl ester, (*E*)- (**C18:1**) (oleic acid). The highest FA content across all extracts was 9-octadecenoic acid, methyl ester, (*E*)- (**C18:1**). The Schlechtriem method yielded 55.35%, while the BD method yielded 56.66% in oven-dried samples.

Both drying methods yielded high monounsaturated fatty acid (MUFA) content, with the oven-dried method consistently exhibiting the highest MUFA levels. The findings clearly show that the BD, Schlechtriem, and Soxhlet extraction using *n*-hexane resulted in the highest yields of MUFA. Interestingly, MUFA content was notably higher in the oven drying method across all extraction methods, including BD (58.0%), Schlechtriem (56.4%), and Soxhlet extraction with *n*-hexane (56.0%). Whereas MUFA content was predominantly lower in the microwave drying method when using the BD (48.8%), Schlechtriem (45.2), and Soxhlet extraction methods with *n*-hexane (42.2%). Our findings also revealed that polyunsaturated fatty acid (PUFA) content was higher in the microwave drying methods, including *n*-hexane:ethanol (32.2%), Schlechtriem (30.39%), and BD (30.0%) methods. In contrast, the oven drying method with hexane:ethanol (31.5%), Schlechtriem (24.1%), and Soxhlet extraction using *n*-hexane (23.2%) showed lower PUFA content. The ratio PUFA to saturated fatty acids (SFA) varied with the BD method (using microwave drying) scoring the highest ratio (PUFA/SFA = 1.53), followed by the Schlechtriem method (PUFA/SFA = 1.43).

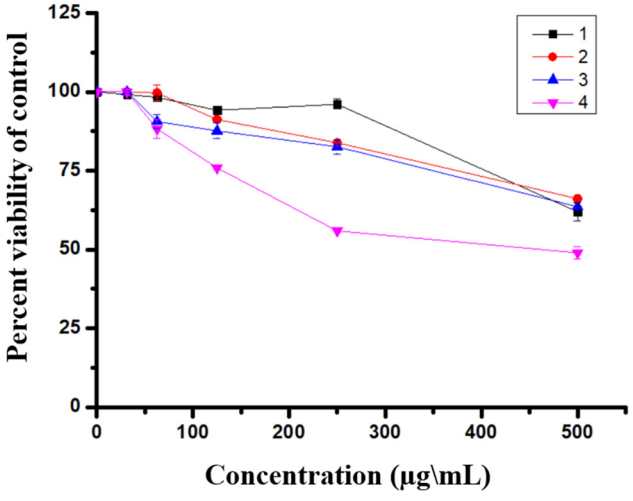


**Figure 1:** The anticancer potential of oven-dried mealworm extract, extracted through four distinct methods: 1: Soxhlet using *n*-hexane, 2: Schlechtriem, 3: Soxhlet using *n*-hexane:ethanol, and 4: Bligh and Dyer. This evaluation was conducted against lung cancer cell lines A549.

## 3.3 TP and antioxidant activity in the extracts

The analysis revealed that the oven-dried method produced higher TP content than the microwave-dried method. However, the *n*-hexane–EtOH extraction showed the highest phenolic content at 0.09 ± 0.02 mg GAE/100 mg, followed by *n*-hexane using the Soxhlet method at 0.075 ± 0.009 mg GAE/100 mg, BD at 0.069 ± 0.01 mg GAE/100 mg, and Schlechtriem at 0.06 ± 0.01 mg GAE/100 mg. Similarly, within the microwave-dried method, BD exhibited the highest TP content at 0.07 ± 0.05 mg GAE/100 mg, followed by *n*-hexane–EtOH at 0.064 ± 0.009 mg GAE/100 mg, Schlechtriem at 0.062 ± 0.05 mg GAE/100 mg, and *n*-hexane using the Soxhlet method at 0.051 ± 0.01 mg GAE/100 mg. Notably, no antioxidant activity was observed in any of the tested extracts.

## 3.4 Mealworm extracts induce cytotoxicity to A549 cells

The cytotoxic effects of all extraction methods from both oven-dried and microwave-dried samples were evaluated using an MTT assay on A549 cancer cells. In the microwave-dried method, no cytotoxicity was observed for any of the extracts tested at 500 µg/mL (the highest concentration), showing 100% viability. However, toxicity was observed in the oven-dried method, particularly at the highest concentration (500 µg/mL). However, the BD (oven-dried method (was the most cytotoxic extract. The results indicated a dose-dependent decrease in cell survival percentage for the BD method, with an $IC_{50}$ value of 458.6 µg/mL (Figure 1).

## 3.5 Mealworm extracts induce changes in liver cancer cells

The BD extraction from the oven-dried samples exhibited morphological changes indicative of cytotoxicity toward A549 cells, as observed using light microscopy (Figure 2). After 24 h treatment with mealworm BD extract, these changes encompassed cell shrinkage and detachment from the culture plate. Fluorescence microscopy with DAPI staining revealed a marked increase in cells exhibiting fragmented nuclei, condensed, and bright blue in the treated samples, in contrast to the evenly blue-stained nuclei observed in the vehicle control group (Figure 2).

### 3.6 Acridine orange/ethidium bromide staining for apoptosis

Morphological changes correlated with BD (oven-dried (-induced apoptosis were studied using fluorescence microscopy and dual staining. Figure 2 illustrates the differential staining between treated A549 cells and control cells. In the control cells, a bright green nucleus indicates viable cells, as acridine orange penetrates intact cell membranes. In contrast, *n*-hexane:ethanol-treated cells exhibit early-stage apoptotic characteristics, marked by granular yellow-green acridine orange nuclear staining or crescent-shaped (Figure 2f).

## 4 Discussion

Edible insects such as mealworms are rich in moisture and nutrients; therefore, they must be dried to preserve quality. Drying larvae is essential for subsequent processing, ensuring

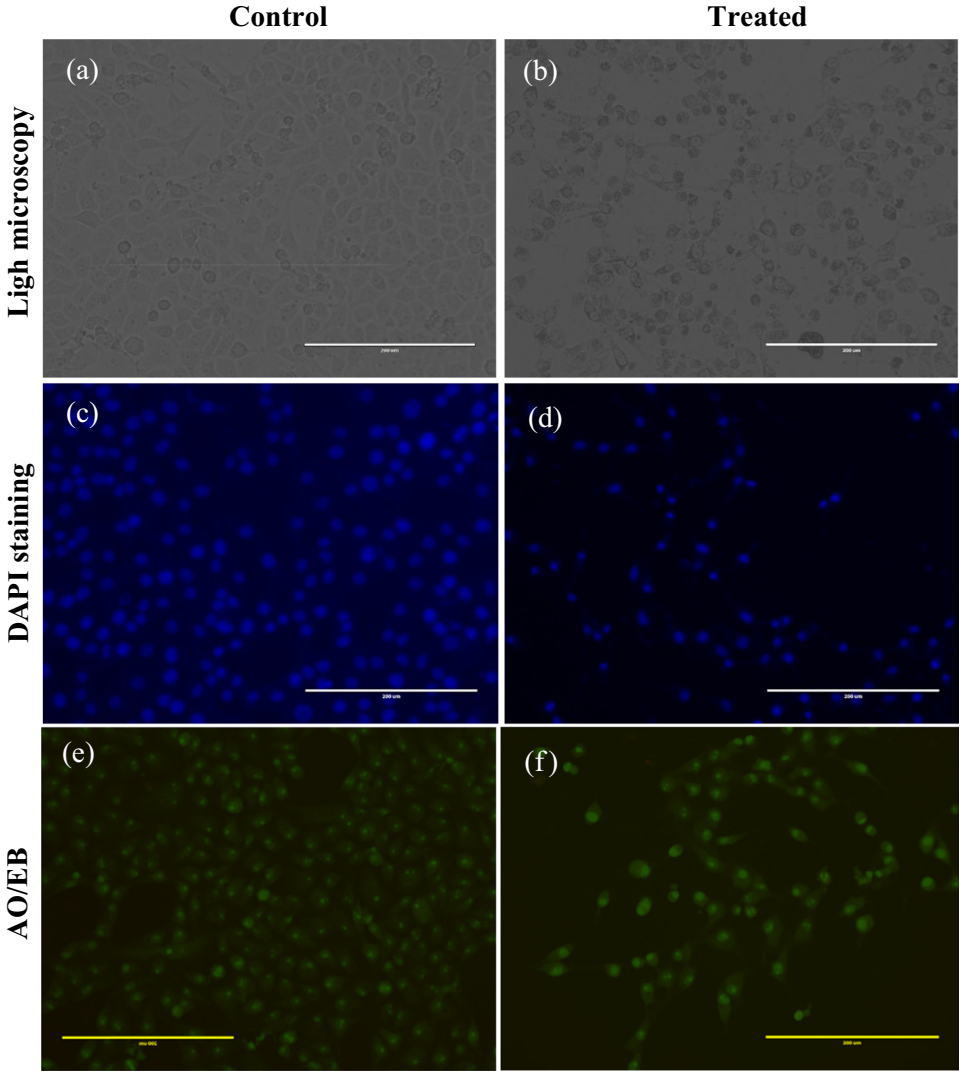


**Figure 2:** Light and fluorescence microscopy of cells stained with DAPI and AO/EB. (a and b) These images show cells observed under light microscopy. (a) The cells are densely packed, displaying typical morphology with clear cell boundaries and nuclei visible within the cytoplasm. (b) Treatment, resulting in a less dense cell population. The cell morphology appears altered, floating, and detached, indicating cell damage or death. (c and d) Fluorescence microscopy (DAPI staining). Apoptosis assay by DAPI staining of A549 nucleus. (c) Control group and (d) treated. White arrows depict chromatin condensation and fragmented nuclei. (e) Negative control group (normal cells): the nucleus is centrally positioned and evenly circular. (f) Experimental group (early apoptotic cells): the nucleus exhibits green fluorescence from acridine orange (AO) staining, forming a crescent or granular pattern on one side of the cell. Cells were treated with *n*-hexane-EtOH extract at a 250 µg/mL concentration for 24 h. The scale bar represents 200 µm.

nutritional quality and safety. Various reports have noted quality alternations in edible insect flours related to different drying methods [26–28]. Thus, this study aimed to assess the different drying processes and extraction methods to observe their impact on yellow mealworm larvae's total lipids and fatty composition. The findings obtained from employing various extraction methods of the mealworm larvae using microwave and oven drying methods exhibited a significant ($P < 0.05$) efficacy in extracting total lipids using a hexane and ethanol mixture (Soxhlet extraction) (Figure 3). As ethanol is a polar solvent, it has a greater affinity for polar lipids and can penetrate the cellular membrane, thereby enhancing the accessibility of neutral lipids to the non-polar solvent (*n*-hexane). Consequently, the yield of lipid was higher when using the combination of *n*-hexane and ethanol than *n*-hexane alone. This finding aligns with a prior study by Gharibzahedi and Altintas [29], indicating that extraction solvents combining polar and non-polar components tend to yield higher lipid quantities. A similar result was also recorded in different mixtures as well, such as ethanol:isopropanol, isopropanol:*n*-hexane, ethanol/*n*-hexane, and where different ratios of solvents caused different extraction efficiencies on lesser mealworms (*Alphitobius diaperinus* L.) larvae lipid extraction. This suggests that only specific polar and non-polar solvent combinations can attain increased lipid yields compared to single-solvent extraction [30].

The Soxhlet extraction technique has been employed to extract numerous types of biological specimens [31,32]; however, this method is time-consuming and can lead to thermal degradation of specific compounds (e.g., ω-3 FAs) [31]. Therefore, we also compared other extraction methods, namely Schlechtriem and BD. The results revealed that both the Schlechtriem and BD extractions resulted in higher total lipid yields in the microwave method than in the oven-dried method. However, the yield was higher in the Schlechtriem extraction method.

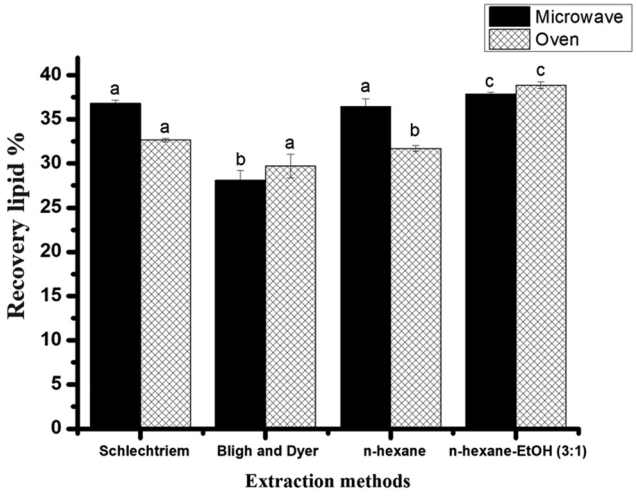


**Figure 3:** Comparison of crude lipid recovery using Bligh and Dyer, Schlechtriem, and Soxhlet extraction methods with both *n*-hexane and *n*-hexane–EtOH (3:1) solvent systems. Error bars denote the standard deviation calculated from three replicates. Different letters signify a significant distinction ($P < 0.05$) between the employed extraction techniques and solvent systems.

According to previous studies, mealworms are rich in unsaturated fatty acids (USFA), predominantly oleic acid and linoleic acid, along with the saturated FA palmitic acid [33,34]. Tables 1 and 2 reveal the SFA, MUFA, and PUFA composition in the mealworms extracted using various drying and extraction methods. Notably, regardless of the drying or extraction method, the composition pattern remained consistent across all samples, with MUFA being the highest, followed by PUFA, and then SFA. However, the different drying techniques influenced the composition of the FAs. Oven-dried samples consistently had significantly higher levels of USFA than those dried using microwave methods, regardless of the extraction technique. Both microwave and oven-dried mealworms showed increased levels of MSFA and reduced levels of PUFA, rendering them more prone to oxidation. The reduced PUFA content in mealworms may be due to higher oxidative degradation, as PUFA are more susceptible to oxidation [35].

The results agree with Lenaerts et al. [34] who reported that blanched and microwave-dried mealworm larvae have the highest proportion of MUFA (41.69 ± 0.09), an intermediate proportion of PUFA (32.57 ± 0.04%), and a lower proportion of SFA (25.75 ± 0.12%). They also noted that combining blanching with microwave drying had minimal impact on the FAs composition compared to microwave drying alone. Likewise, the FAs profile of mealworms blanched and dried in a microwave dryer remained unchanged when a vacuum was applied [34]. Contrary to our findings, Kröncke et al. reported an equal ratio of PUFA (35.08%) and MUFA (38.51%) using the rack oven drying method [36].

The PUFA/SFA being close to 1 is useful for human health [33]. A diet with a PUFA/SFA ratio below 0.45 is connected to increased blood cholesterol levels. The oils extracted in this research showed consistently elevated PUFA/SFA ratios in all samples. Results indicate that the extracted oils from mealworms have favorable and balanced FA profiles, which may benefit human dietary habits. Additionally, PUFA-rich oils are in demand for their possible applications in skincare formulations across the pharmaceutical sectors [37]. Our findings suggest that utilizing the oven dry method and the BD extraction method is advisable, as they yield products with higher UFA content.

Interestingly, only the oven-dried method exhibited cytotoxicity against A549 cells, whereas none of the extracts obtained from microwave drying showed any cytotoxic effects. This variation could be related to the higher phenolic content in the extracts obtained through the oven-dried method. Several studies have reported that oven drying of plant extracts results in higher phenolic contents and antioxidant activity than microwave drying. For instance, a study conducted by Özcan et al. showed that hot air oven drying significantly influenced various factors, including antioxidant capacity, total phenols, carotenoids, flavonoid content, and FAs in orange and lemon peel powders, compared to other methods like microwave and infrared oven drying [38].

# 5 Conclusion

The study demonstrated that both drying and extraction methods significantly impact the lipid yield, FA profile, and bioactivity of *T. molitor* larvae extracts. Among the methods tested, oven drying combined with the *n*-hexane:EtOH (3:1) extraction method proved to be the most effective in recovering lipids, yielding the highest lipid content. The analysis also revealed that the oven-dried samples consistently produced higher MUFA levels across different extraction methods, while the microwave-dried samples exhibited higher PUFA content. Additionally, the TP content was generally higher in the oven-dried extracts, particularly when using the *n*-hexane:EtOH method, although no significant antioxidant activity was detected in the extracts. The cytotoxicity studies indicated that the oven-dried extracts, especially those obtained using the BD method, were more potent in inducing cell death in A549 cancer cells compared to microwave-dried extracts, which showed no cytotoxic effects at the highest concentration tested. Moreover, the BD extract from oven-dried samples induced significant apoptotic changes in liver cancer cells, as evidenced by cell morphology and nuclear fragmentation. These findings highlight the importance of selecting appropriate drying and extraction methods to maximize lipid recovery and modulate the bioactive properties of mealworm larvae, with potential implications for their use in nutraceutical and therapeutic applications.

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**Ethical approval:** The conducted research is not related to either human or animal use.

**Data availability statement:** Data will be made available on request from the corresponding author.

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