# Statistical Evaluation of the Biological Effects of Desert Locust Extract Via Dose-Response Curves in Esophageal Cancer Cells

Mohammed Nazar Hussain Alagele and Ammar Ahmed Sultan
*Department of Biology, College of Education for Pure Science, University of Diyala, 32001 Baqubah, Iraq*
*agele.1993@gmail.com, drammarmohamed@yahoo.com*

Keywords: IC50 KYSE, Bioanalytical, Schistocerca Gregaria, Esophageal Cancer KYSE, MTT, GC Mass.

Abstract: A preliminary statistical and bioanalytical study was conducted based on the cytotoxic potential of Schistocerca gregaria (desert locust) extract on esophageal cancer and normal cell lines. The research provided a preliminary chemical composition of the extract characterized through Gas Chromatography–Mass Spectrometry (GC–MS) and was subjected to bioinformatics to assess the potential pharmacological importance of compounds such as the flavonoids and other identified phenolic compounds, omega-3 and omega-6 fatty acids, and glucosamine which are known to activate oxidative stress and alter pathways of cell cycle regulation. The MTT assay was used to measure the extract's inhibitory effects on cancer cell proliferation (cytototoxicity) and the results were converted to OD (optical density) values in order to assess the percentage of cancer cell proliferation that was inhibited through the equation to determine the inhibition of cancer cell proliferation. [ Percentage change in growth = (Optical density of drilling parameter- Optical density of drilling control )/(Optical density for drilling control) x 100 ]. The results indicated explicit dose-dependent suppression inhibition, which increased inhibition (%) from 43.7 % to 67.3 % at 50 mg/mL and at 1600 mg/mL, respectively. The half-maximal inhibition concentration from the dose-inhibition was calculated from the dose-inhibition curve to be an $IC_{50}$ value of 117.2 µg/mL. Normal HFF cells, on the other hand, exhibited almost no inhibition, which shows high therapeutic selectivity index for the extract. The findings give evidence that suggests Schistocerca gregaria extract has promising anticancer potential as a natural source of highly specific, bioactive compounds leading to the advancement of the extract for further drug discovery and development activities.

## 1 INTRODUCTION

The National Cancer Institute (NCI) now defines cancer as "a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body." This description is broadly consistent with the majority of definitions of the disease [1]. It is also defined as an abnormal and continuous growth of body tissue, resulting from excessive cell proliferation or a failure in the programmed cell death (apoptosis) mechanism, and continues even after the removal of initial stimuli [2]. It is sometimes referred to as a collection of illnesses that are typified by the body's cells growing out of control and having the capacity to spread to other areas. [3]. this definition has recently been updated to include the evolutionary dimension, considering it a disease resulting from cellular evolution through natural selection. The genetic and hereditary alterations that build up within a population of cancer cells and result in a fatal phenotype are included in this revised definition. Natural selection causes cancer, a disease caused by the unchecked growth of altered cells [2].

Esophageal cancer (EC) is one of the deadliest cancers worldwide. Studies have shown that hundreds of thousands of people are diagnosed with and die from esophageal cancer annually. While esophageal squamous cell carcinoma is more prevalent in East Asia and North Africa, adenocarcinoma is becoming more common in the West because of increased obesity, GORD, and Western dietary patterns [4], [5]. Esophageal cancer is divided into:

Esophageal Squamous Cell Carcinoma (ESCC). One of the two primary forms of esophageal cancer, esophageal squamous cell carcinoma (ESCC), develops from the squamous epithelial cells that line the esophagus. This kind is common throughout a lot of Asia and Africa. [6]. It commonly affects smokers, alcohol consumers, those who drink hot beverages, or individuals suffering from malnutrition [7]. Genetic studies mentioned by [8] have shown that the TP53 gene mutation is the most frequent mutation in ESCC, followed by mutations in NOTCH1, PIK3CA, and genes related to chromatin remodeling.

Esophageal Adenocarcinoma (EAC). Barrett's esophagus, a disorder in which the usual stratified squamous epithelium lining the lower esophagus is replaced by simple columnar epithelium with goblet cells, chronic esophagitis, and obesity, is associated with Esophageal Adenocarcinoma (EAC). In Western nations, its prevalence has grown during the previous few decades [9].

Over the past ten years, there has been a growing interest in insects as a substitute source of protein and other minerals. However, insects have been suggested as experimental anti-cancer treatments since they create a variety of chemicals that are lethal to human cells and linked to anti-tumor action [10]. Many species-dependent bioactive compounds that are essential for survival are produced by insects. Because of this, pharmaceutical research has been interested in insects, and numerous studies have demonstrated negative effects on different types of human cancer cells [11]. Research has shown that administering varying doses of extracts from Schistocerca gregaria and Gryllus bimaculatus to colon cancer cells (Caco-2) reduces their viability. [10]. the extract of housefly larvae musca domestica has also demonstrated significant efficacy against breast cancer (MCF-7) cells through the enhancement of potent antioxidant activity [12]. Similarly, the silkworm extract exhibited effectiveness against esophageal cancer by targeting the cell membranes of cancer cells [13]. Moreover, the extract of the rhinoceros beetle induced apoptosis and necrosis in hepatocellular carcinoma cells, in addition to disrupting cancer cell metabolism to promote autophagy [14].

# 2 MATHERIALS AND METHODS

## 2.1 Extract Preparation

After the desert locust Schistocerca gregaria was gathered, specimens were sent to the Natural History Museum for classification in order to verify its taxonomic identity. To get rid of any impurities that might have adhered, the insects were cleaned with distilled water. After that, the wings, legs, and abdominal area were taken out and thrown away.

The insects were then pulverized into a fine powder after being dried. A Soxhlet equipment was used for the extraction, and the solvent used was 95% hexane. To stop the powder from leaking, 100 grams of the insect powder were put in a cone-shaped filter paper thimble and sealed tightly. After that, the thimble was put in the Soxhlet extractor, and 500 mL of solvent was injected for a continuous extraction period of 24 hours. The resulting extract was concentrated using a rotary evaporator to remove the solvent [15].

## 2.2 Identification of Active Compounds

Gas chromatography–mass spectrometry (GC–MS) study of the previously made alcoholic extract of the desert locust *Schistocerca gregaria* was used to identify the bioactive chemicals in the insect (Fig. 1). Gas chromatography (GC) with a flame ionization detector (FID) and gas chromatography–mass spectrometry (GC–MS) for mass detection (MSD) utilizing an Agilent GC–Mass system was used in the Basra Oil Company's labs to analyze the bioactive components. An Agilent 5975 GC–MSD system was used for the GC–MS studies, and helium was used as the carrier gas [16] - [18].

## 2.3 Cytotoxicity Assay

In tissue culture flasks measuring 25 cm³, the esophageal cancer cell line (KYSE) and the normal cell line (HFF) were cultivated using RPMI-1640 media that was enhanced with 10% fetal bovine serum (FBS). For 24 hours, the culture flasks with the growth medium and cell suspension were kept at 37 °C in a humidified environment with 5% $CO_2$. To be sure there was no microbial contamination, the flasks were inspected under an inverted microscope after incubation.

Subcultures were then made, incubated once more for twenty-four hours, and then reexamined under a microscope to make sure the culture was sterile and growing properly. The wasted media was then aspirated after the cells were moved to a biosafety cabinet. Two 10-minute washes with phosphate-buffered saline (PBS) were performed on the cells. After covering the cell monolayer with an adequate volume of Trypsin/Versene enzyme solution, the flasks were incubated for 30 to 60 seconds at 37 °C. Fresh culture medium containing 10% serum was added to halt the enzymatic reaction when the cells separated from the culture surface and transformed from a confluent monolayer to single, scattered cells.

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide] technique was used to carry out the cytotoxicity test. [19], [20]. Using a micropipette, cells were seeded into 96-well tissue culture plates at a density of 10,000 cells/well. The Schistocerca gregaria insect extract was administered for 24 hours at different doses after the establishment of a confluent monolayer was verified. After being exposed, the growth media was taken out, and the cells underwent three PBS washes. Following the addition of 30 µL of MTT solution (2 mg/mL) to each well, the plate was incubated for three hours at 37 °C. To dissolve the formazan crystals, 25 µL of dimethyl sulfoxide (DMSO) was added to each well after incubation, and the plate was then incubated once more for 10 minutes at 37 °C. A microplate ELISA reader was used to measure the optical density (OD) at 492 nm. The following formula was then used to determine the proportion of cytotoxicity:

Cytotoxicity killing % = CO.O mean – txO.Omean / CO.O mean x 100

Where:

- CO.O. stands for optical density of cells without nanomaterial treatment.
- txO. O mean: The optical density of cells exposed to nanoparticles

## 2.4 Statistical Analysis

Using GraphPad Prism 6 software, I computed the mean ± standard error of the mean (SEM) in triplicate experiments using an unpaired t-test with a significance value of $P<0.05$, as reported in [21].

# 3 RESULTS AND DISCUSSION

## 3.1 GC-MS Analysis Results

By computer matching their mass spectra with those in commercial spectrum libraries, such as the Wiley GC/MS Library, the MassFinder 3 Library, and the Baser Library of essential oil components, the specific components of the insect extract were identified. Together, these databases include reference mass spectra and retention index information for over 3,200 real substances. As shown in the Table 1, the results showed that the insect extract contained a few bioactive substances, such as different fatty acids and their derivatives, amino acids produced by protein breakdown, phenolic compounds with antioxidant qualities, and steroids (cholesterol). These results align with those published by [22].

Table 1: The active ingredients in the insect extract are displayed using the GC-Mass method.

| No | Compound Name | | Chemical Formula |
|---|---|---|---|
| 1 | Fatty Acids & Derivatives | Tetradecanoic acid | C14H28O2 |
| | | n-Hexadecanoic acid (Palmitic acid) | C16H32O2 |
| | | 9-Octadecenoic acid, (E)- (Elaidic acid) | C18H34O2 |
| | | Octadecanoic acid (Stearic acid) | C18H36O2 |
| | | 9-Octadecenamide, (Z)- (Oleamide) | C18H35NO |
| | | Pentanoic acid, 3-methyl- | C6H12O2 |
| 2 | Amino Acids & Derivatives (Peptides) | 2-Amino-5-methylbenzoic acid | C8H9NO2 |
| | | [(2-Amino-3-hydroxypropanoyl)amino]acetic acid | C5H10N2O4 |
| | | Cyclo(L-prolyl-L-valine) | C10H16N2O2 |
| | | 2,5-Piperazinedione, 3-methyl-6-(1-methylpropyl)- | C9H16N2O2 |
| | | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- | C7H10N2O2 |
| | | Hexahydro-3-(1-methylpropyl)pyrrolo[1,2-a]pyrazine-1,4-dione | C11H18N2O2 |
| | | Octahydrodipyrrolo[1,2-a:1',2'-d]pyrazine-5,10-dione-, (5aR,10aR) | C10H14N2O2 |
| | | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)- | C14H16N2O2 |
| 3 | Phenolic & Aromatic Compounds | Benzeneacetaldehyde | C8H8O |
| | | 3,4-(Methylenedioxy)toluene | C8H8O2 |
| | | Benzeneacetic acid | C8H8O2 |
| | | Phenol, 4-ethenyl-2,6-dimethoxy- | C10H12O3 |
| 4 | Steroids (an isomer of Cholesterol) | Cholest-5-en-3-ol, (3.alpha.)- | C27H46O |

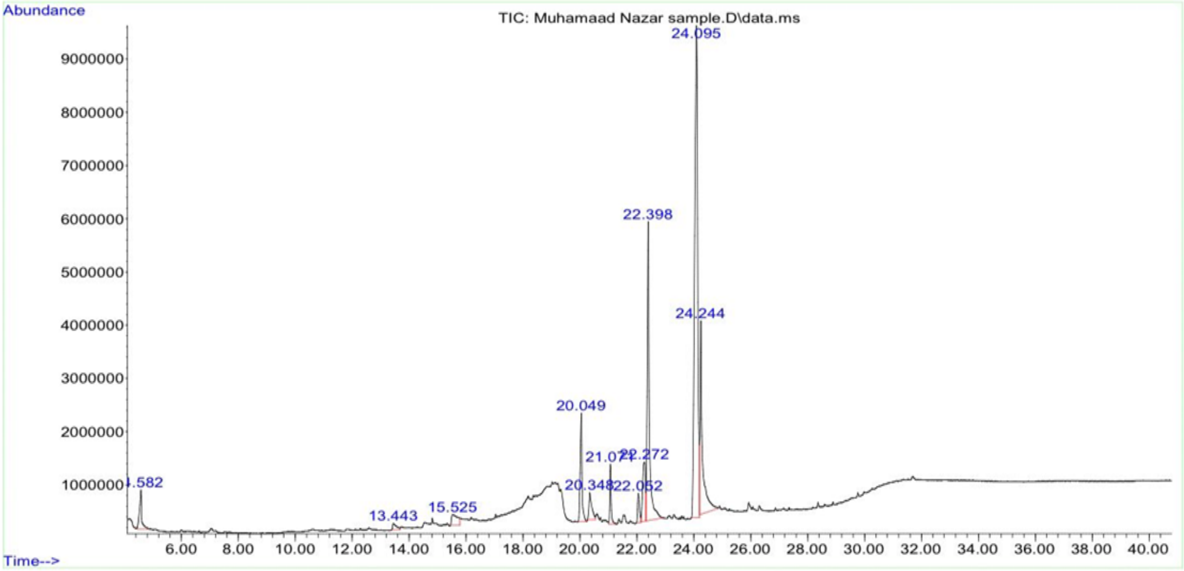

Figure 1: Gas chromatography-mass spectrometry of desert locust extract *Schistocerca gregaria.*

## 3.2 Cytotoxicity Experiments

Schistocerca gregaria insect extract was used at several dosages to assess cytotoxicity in esophageal cancer cells and compare it to the normal HFF cell line. Using an ELISA microplate reader set to 492 nm, the absorbance intensity of the cells was measured to assess the cytotoxic effect. The percentage change in cell growth was then computed using the following:

Percentage change in growth =

$$\frac{\text{Optical density of drilling parameter} - \text{Optical density of drilling } \mathit{control}}{\mathit{Optical\ density\ for\ drilling\ control}}$$

**x.100**

After 24 hours of exposure at 37 °C, the optical density (OD) values that represent cell viability were measured in order to examine and compare the cytotoxic effects of different concentrations of the insect extract on the proliferation of esophageal cancer cells with the normal HFF cell line. Eight distinct concentrations were used in the triplicate experiment: 50, 100, 200, 400, 800, and 1600 µg/mL. The MTT method was used to conduct the cytotoxicity assay, and the mean percentage of growth inhibition and cell viability were used to analyze the findings. Figure 3 illustrates how the concentration of the Schistocerca gregaria extract rose in proportion to the suppression of esophageal cancer cell viability. When treated with the same extract, the HFF normal cell line showed very low inhibition levels, as shown in Figure 4. The proportion of cell viability inhibition for both the normal HFF cells and the esophageal cancer cells at each concentration is shown in Table 2.

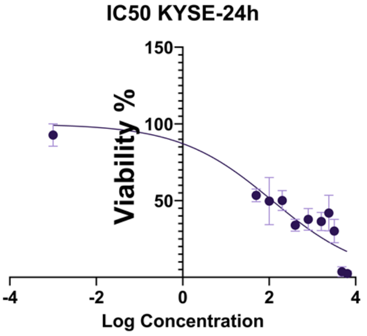

Figure 2: Median lethal dose on esophageal cancer cells.

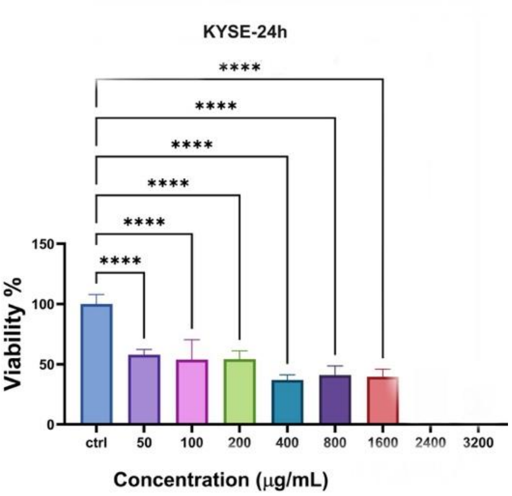

Figure 3: The inhibitory effect of insect extract on the viability of esophegase cancer cells.

Table 2 demonstrates a dose-dependent decrease in cell viability, indicating an inverse relationship between extract concentration and cell survival. Cell viability decreased to 56.3% at a dose of 50 µg/mL, representing the first notable reduction in esophageal cancer cell viability. With increasing concentrations, viability further declined, reaching 32.7% at 1600 µg/mL. In contrast, normal HFF cells maintained high viability (96.3%) even at the highest tested concentration, indicating selective cytotoxicity toward cancer cells. The median lethal dose ($IC_{50}$) for esophageal cancer cells was 117.2 µg/mL, as shown in Figure 2.

These findings suggest that the extract exerts selective cytotoxic effects without significant toxicity to normal cells. This activity may be attributed to the generation of reactive oxygen species (ROS), which can induce oxidative stress in cancer cells. Similar mechanisms have been reported for other insect-derived extracts [12]. In addition, the cytotoxic effect may involve disruption of cancer cell membranes [13] and induction of apoptotic and necrotic pathways, leading to metabolic dysfunction and cell cycle arrest [14]. Collectively, these mechanisms support both the antioxidant and anticancer potential of the Schistocerca gregaria extract.

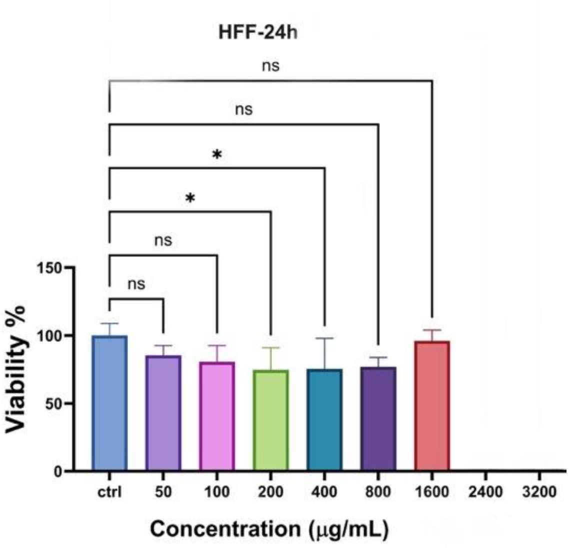

Figure 4: The inhibitory effect of insect extract on the viability of natural HFF strain cells.

Figure 5 illustrates the cytotoxic effects of the insect extract on both esophageal cancer (KYSE) and normal (HFF) cell lines after 24 hours of incubation at 37°C, as assessed by the MTT assay.

Table 2: Percentage reduction in cell viability and Inhibition of both cell lines at different concentrations of the insect extract.

| No. | Concentration (µg/mL) | Percentage of inhibition and cell viability | | | |
|---|---|---|---|---|---|
| | | Esophageal Cancer Cells KYSE | | Normal HFF Cells | |
| | | Viability % | Inhibition % | Viability % | Inhibition % |
| 1 | 50 µg/ml | 56.3 % | 43.7 % | 92.4 % | 7.6 % |
| 2 | 100 µg/ml | 52.8% | 47.2 % | 89.7 % | 10.3 % |
| 3 | 200 µg/ml | 54.6 % | 45.4 % | 84.3 % | 15.7 % |
| 4 | 400 µg/ml | 42.1 % | 57.9 % | 85.1 % | 14.9 % |
| 5 | 800 µg/ml | 40.2 % | 59.8 % | 87.4 % | 12.6 % |
| 6 | 1600 µg/ml | 32.7 % | 67.3 % | 96.3 % | 3.7 % |

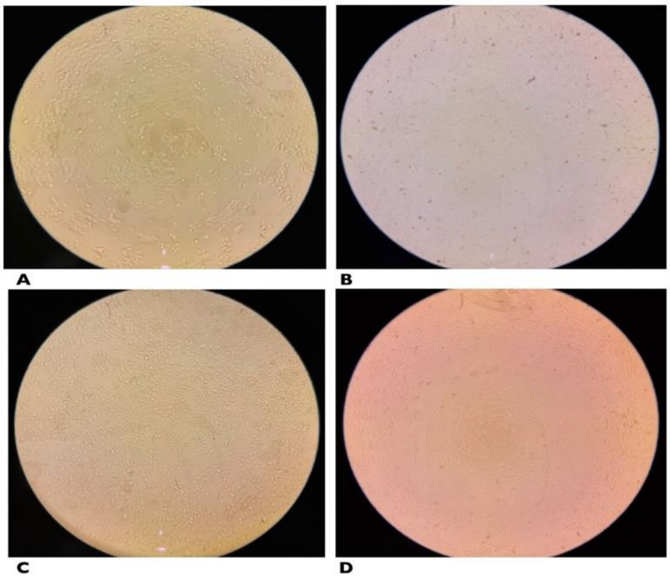

Figure 5: Cytotoxic effect of the insect extract on cell lines assessed by the MTT assay after 24 h of incubation at 37°C: a) Untreated esophageal cancer cells, b) Esophageal cancer cells treated with the insect extract (400 µg/mL), c) Untreated normal HFF cells, d) Normal HFF cells treated with the insect extract (400 µg/mL).

# 3 CONCLUSIONS

This study thoroughly examined the statistical and bio-analytical methods demonstrating the selective and strong cytotoxicity of Schistocerca gregaria extract on esophagus cancer cells, biochemical composition, and quantitative cell assays. The combination of biochemical analysis with bioinformatical methods confirmed the presence of compounds, tied to biological activity, that may influence pathways involving the modulation of oxidative stress as well as the cell cycle. MTT assay results consistently with optical density value and $IC_{50}$ were strong evidence of antiproliferative activity, albeit in a dose-dependent fashion, on cancer cells. On the other hand, HFF cells that were used as a normal control were not sensitive to the extract representing therapeutic selectivity of the extract. Together, evidences show significant biomedical potential of S. gregaria in cancer treatment and warrant the inclusion in anticancer drug discovery, as the S. gregaria extract with cancer cell and normal cell specificity may yield bioactive molecules to be used for novel therapeutic interventions for esophagus cancer.

# REFERENCES

[1] A. Saeed, A. Jabeen, I. Mushtaq, and I. Murtaza, “Cancer: Insight and Strategies to Reduce,” Pathophysiology of Cancer: An Interdisciplinary Approach, vol. 22, p. 49, 2025.

[2] J. S. Brown et al., “Updating the definition of cancer,” Mol. Cancer Res., vol. 21, no. 11, pp. 1142–1147, 2023.

[3] World Health Organization, WHO Framework for Meaningful Engagement of People Living with Noncommunicable Diseases, and Mental Health and Neurological Conditions, Geneva: WHO, 2023.

[4] L. Qi et al., “Global esophageal cancer epidemiology in 2022 and predictions for 2050: A comprehensive analysis and projections based on GLOBOCAN data,” Chin. Med. J., vol. 137, no. 24, pp. 3108–3116, 2024.

[5] AL-Ammari, A. M., Hussain, A. F., & Ismael, T. K., “Prevalence of fungal pathogens among respiratory tract infections,” Ann. Trop. Public Health, vol. 22, no. 8, S227, 2019.

[6] W. Jin et al., “Global, regional, and national burden of esophageal cancer: A systematic analysis of the Global Burden of Disease Study 2021,” Biomark. Res., vol. 13, no. 1, p. 3, 2025.

[7] Y. Wang et al., “Esophageal cancer,” in StatPearls, Treasure Island, FL: StatPearls Publishing, 2024.

[8] D. Zhao et al., “Multi-omics characterization of esophageal squamous cell carcinoma identifies molecular subtypes and therapeutic targets,” JCI Insight, vol. 9, no. 10, e171916, 2024.

[9] W. Jiang et al., “Current status and perspectives of esophageal cancer: A comprehensive review,” Cancer Commun., vol. 45, no. 3, pp. 281–331, 2025.

[10] Dobermann, D. and Scheers, N., “Cytotoxicity of insects (T. molitor, G. bimaculatus, S. gregaria and A. domesticus) in a simulated gastrointestinal digestion/human intestinal cell model,” Food Research International, vol. 143, 110276, 2021.

[11] A. M. Zedan et al., “Oriental hornet (Vespa orientalis) larval extracts induce antiproliferative, antioxidant, anti-inflammatory, and anti-migratory effects on MCF7 cells,” Molecules, vol. 26, no. 11, p. 3303, 2021.

[12] El-Garawani et al., “Enhanced antioxidant and cytotoxic potentials of lipopolysaccharides-injected Musca domestica larvae,” Pharmaceutics, vol. 12, no. 11, p. 1111, 2020.

[13] F. Ramos-Martín, C. Herrera-León, and N. d’Amelio, “Molecular basis of the anticancer, apoptotic and antibacterial activities of Bombyx mori Cecropin A,” Arch. Biochem. Biophys., vol. 715, p. 109095, 2022.

[14] J. E. Lee et al., “Hepatoprotective and anticancer activities of Allomyrina dichotoma larvae,” J. Life Sci., vol. 25, no. 3, pp. 307–316, 2015.

[15] R. A. Wu et al., “Caspase 3-mediated cytotoxicity of mealworm larvae (Tenebrio molitor) oil extract against human hepatocellular carcinoma and colorectal adenocarcinoma,” J. Ethnopharmacol., vol. 250, p. 112438, 2020.

[16] M. W. M. Alzubaidy and M. N. Hussain, “Biosynthetic of green zinc oxide nanoparticles with effect on cancer cell line HeLa,” Revis Bionatura, vol. 8, no. 2, p. 22, 2023.

[17] A. F. Hussain et al., “An annotated bibliography of medical mycology in Iraq: 1962–2021,” Microb. Biosyst., vol. 6, no. 1, pp. 11–31, 2021.

[18] A. A. J. Al-Karawi, N. Y. Al-Bayati, and M. A. A. Shafeeq, “Comparative Analysis of Vegetable Chemical Compounds from Orange Peel Extract and Thuja Plants Using Gas Chromatography Mass Spectrometry Technique,” in Proc. Int. Conf. Appl. Innovation IT, vol. 13, no. 2, pp. 615–622, 2025.

[19] S. Wu and D. K. Shah, “Determination of ADC cytotoxicity in immortalized human cell lines,” in Antibody-Drug Conjugates, New York, NY: Humana, 2020, pp. 329–340.

[20] B. M. A. Latif and M. W. M. Alzubaidy, “Effect of zinc oxide nanoparticles on activity of skin cancer cell line (A-375),” AIP Conf. Proc., vol. 2593, no. 1, p. 050003, 2023.

[21] M. Y. Ahn et al., “Anti-cancer effect of dung beetle glycosaminoglycans on melanoma,” BMC Cancer, vol. 19, 2019.

[22] X. Cheseto et al., “Potential of the desert locust Schistocerca gregaria as an unconventional source of dietary and therapeutic sterols,” PLoS One, vol. 10, no. 5, e0127171, 2015.