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A Novel Antibacterial and Anticancer Property of Iraqi Honey Bee Venom

Nisreen Kaddim Radi*, Russell Issam AL-Daher, Hawraa Jawad Kadhim

Department of Biology, College of Science for Women, Babylon University, Republic of Iraq. *Corresponding Author's Email: nesreenzain.83@gmail.com

Abstract

The antibacterial and anticancer roles of Iraqi bee venom were detected in the current study, and their medical applications were highlighted for future uses. The FTIR analysis of bee venom produced that amide Bands I and II (proteins, peptides) were present. Band I is generally observed at 1658.07 cm^{-1} and is attributable to stretching of the C=O vibrations in the peptide backbone. Band II occurs at approximately 1534.42 cm^{-1}, indicating the existence of peptides (such as melittin). The multiple MICs were done, and it showed good antibacterial activity. It was prominent for Pseudomonas aeruginosa (MIC = 14 mm at 0.1 mg/L) and Staphylococcus aureus (MIC = 18 mm). The anticancer effects were evaluated by MTT cell line cytotoxicity assays. Cancer cell line cytotoxicity of bee venom revealed decreased viability for both cell lines, but it more affected the MCF-7 cancer cells than the normal fibroblast HDFN, indicating selective cytotoxic properties. Note that 400 µg/ml represented the maximum selectivity toward the normal cell line HdFn with Mean ± Std. Deviation (72.33800 ± .6940; 29.20500 ± .4818) for HdFn and MCF-7, respectively. The output was consistent with sustainable goals.

Keywords: Apitoxin, Ebrubicin, GC MASS, MCF-7, MDR, MTT.

Introduction

Bee venom (BV) is a natural substance that shows promise in the fight against clinical illnesses and foodborne pathogens due to its strong antibacterial characteristics. In fact, there's evidence BV can stop various bad bugs—even those resistant to multiple drugs hinting that one day it could stand in for traditional antibiotics. Researchers have previously developed (1). The MIC of BV was between 6.25 and 12.5 mg/L^{-1}, which indicated that the drug is highly potent against multidrug-resistant bacteria. Honeybee Toxin Inhibition Against Pathogen and Non-Pathogen Bacteria: Antibacterial Activities of Anti-Microbial Agents. The newer study found BV was much more effective than conventional antibiotics like the older drugs ampicillin and tetracycline against pathogens such as Klebsiella pneumoniae and Staphylococcus aureus (2). Previous studies have shown that BV has been utilized as an organic preservative in a soft cheese and other food products. Scientists have destroyed dangerous bacteria and yeast, guaranteeing foods can be safely stored. (3, 4). Melittin and apamin, two components of BV, rupture the microbial cell membrane and block bacteria growth. Despite its promising promise as a natural antibacterial agent, further research is necessary to optimize the use of BV in clinical and food safety settings, particularly in terms of dosage and safety profiles (5). Breast cancer is among the most prevalent cancers in women, representing 30% of all recently discovered cases. The American Cancer Society reports that around 2.3 million new breast cancer cases have been identified and 685,000 deaths occurred due to breast cancer in 2020, rendering it the seventh greatest cause of cancer mortality globally. Radiation therapy, especially post-mastectomy, reduces local recurrence risk and enhances survival. However, a decade-long study found loco-regional recurrence and arm lymphedema symptoms. proven, causing severe symptoms. (6) Cancer patients often seek alternative therapies to decrease negative effects of mainstream therapy. Natural materials from plants and animals are used as medicinal treatments for many disorders. Clinical evaluation of toxins that harm other species has been conducted for oncological illnesses (7).

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In cancer radiation, botulinum toxin can decrease tumor development and induce apoptosis in cancer cells, acting as an anesthetic (8). In bee venom, active components include melittin, mast cell degranulating peptide apamin, digestive enzymes (such as phospholipase A2), and amino acids (9). Melittin, the main constituent of bee venom, accounts for 40-60% of its composition and is the primary source of pain (10). Melittin can be injected into membranes through non-selective pore creation and disruption, leading to antibacterial, anticancer, and hemolytic actions. Thus, bee venom requires a suitable delivery vehicle. Numerous studies on bee venom have been undertaken to identify the best carrier for delivering the optimum dose to cancer cells (11). The honeybees in Iraq, especially those located at Babylon, have unique bioactivities compared with previous studies from anywhere else in the world on the production of venom and honey. This uniqueness is determined by the extreme climatic environment of Iraq and the rich floral diversity of the area, positively affecting the accumulation of phenolics and flavonoids into bee products. These time-related effects could be the reason for their high levels of antimicrobial and antioxidant activity, comparable to that in honeys from stressful habitats. From the high incidence of infectious and chronic diseases in Iraq, this substance may be regarded as a natural therapeutic product matching the local health problem (12). This study aims to assess the antibacterial efficacy of local bee venom to inhibit pathogenic bacteria, clinical infection, and therapeutic applications. The aim of this study is to investigate the Minimum Inhibitory Concentration (MIC) on the multidrug-resistant bacteria strains, including *Pseudomonas aeruginosa* and *Staphylococcus aureus*, as well as anticancer activities in honey bee venom, which could be determined for exploiting a novel natural antibacterial agent.

Material and Methods

Samples Collecting

The College of Agriculture at Al Qasim Green University (Babylon Province/Iraq) provides bee venom (BV) gathered from local *Apis mellifera*. Past studies report a continuous improvement in bee venom collection devices (13). Collecting bacterial isolates from clinical samples for the testing of Staphylococcus aureus and P. aeruginosa from urinary tract infection and wound infection patients at AL-Sadiq Teaching Hospital in Babylon, Iraq, gave urine swabs and clinical isolates of Staphylococcus aureus and Pseudomonas aeruginosa during the collection period from 11-3-2024 to 1-12-2024. A nearby hospital provided all samples for this study, which were aseptically collected after ethical permission and biosafety compliance. Traditional microbiological methods for identifying the bacterium included colony morphological Gram staining and VITEK biochemical testing (14).

Bacterial Isolation and Identification

The lab isolated identified *Staphylococcus aureus* and *Pseudomonas aeruginosa* by culturing ambient and clinical samples on selective media. Gram staining, coagulation enzyme tests, and biochemical assays were used to confirm the isolation of *Staphylococcus* species from mannitol salt agar. The colony shape, pigmentation, and an oxidase test were utilized to confirm the isolation of *Pseudomonas aeruginosa* from cetrimide agar. Biochemical studies, such as the Vitek test, confirmed the identification of bacterial isolates (15).

Bee venom (Apitoxin) Preparation and Chemical Characterization

Bee venom (BV) powder (10 mg) was added to 100 ml of a DW solution, and then centrifugation followed by decanting was performed to obtain a concentration of 0.1 mg/ml for antibacterial tests and anti-cancer techniques as a low-concentration form of the toxin. Bee venom chemical composition and general structure Bee venom was characterized in terms of its chemical constituents, as well as general construction, by a number of analytical methods. The functional groups of bee venom particles were analyzed using FTIR. The Perkin Elmer Spectral Two model was employed to obtain analysis, which ranged from 400 cm^{-1} to 4000 cm^{-1}. The UV-Vis spectrometer (Lambda 25, PerkinElmer) was used to measure the absorbance spectra of metal oxide nanoparticles. The elemental composition of the venom was determined employing energy dispersive X-ray spectroscopy (EDX). This model paved the way to assign carbon, oxygen, and nitrogen, which is an essential step for understanding the atomic structure of venom (16).

GC MASS: Extraction and analysis technique was applied in analyzing bee venom using GC-MS. At first, we employed a purpose-built electrical stimulator to induce the wasps to sting through glass so that venom could be collected without harming bees. The poison was freeze-dried (14) as a powder for further study. In preparation for GC-MS analysis, bioactive chemicals were obtained following maceration of frozen bee venom with suitable organic solvents (normally including methanol or chloroform). The GC-MS instrument then volatilized the compound via a heated injection port. For mass/volatility-based component separation, a gas chromatography (GC) column was employed, and chemical detections/identifications based on mass-to-charge ratios were conducted by a mass spectrometer (MS) (17). By comparison of the spectra with the known chemicals, bioactive compounds were identified from bee venom using mass spectral databases in a previous study (18).

Antibiotic Susceptibility Test: The antimicrobial sensitivity profiles of MDR microorganisms were ascertained using the Kirby-Bauer method for disk diffusion (4, 19). Different discs were used in routine antibiograms unique to each species. Resistance to antibiotics is displayed in Table 2, 3.

Cytotoxicity Evaluation (MTT Assay): Cell Cultures and Cell Maintenance This investigation involved the cultivation of breast cancer cells MCF-7 (ATCC: HTB-22) and normal human dermal fibroblast neonatal HDFn (ATCC: PCS-201-010) in RPMI-1640 media supplemented with 10%. Fetal bovine serum supplemented with 1% penicillin-streptomycin antibiotic. The climate required humidity. Upon achieving 80-90% confluency, we transplanted the cells to new plates and refreshed them with RPMI-1640 medium every two to three days, contingent upon their density (20). The methyl thiazole tetrazolium (MTT) assay for anticancer detection. The demonstration entailed the amalgamation of bee venom. We evaluated the cytotoxicity of these compounds on the MCF-7 breast cancer cell line with the MTT proliferation assay. We acquired a human breast cancer cell line from the Biotechnology Research Centre, Department of Biomedical and Molecular Technology, AL-Nahrain University, Iraq. Maintenance of Cell Line Cultures We cultivated MCF-7 human breast cancer cells in Rosewell Park Memorial Institute 1640 (RPMI) (EuroClone) media. We included 10% fetal bovine serum (FBS), 2 mM glutamine, and 50 µg/ml penicillin-streptomycin into the media. A combination of a standard humidified incubator maintained at 37°C with 5% CO_2 was utilized (21, 22). MTT Assay After growing the MCF-7 cell line, 1×10^4 cells/cm^2 were cultivated on flat-bottom 96-well plates for 24 hours prior to research. We performed cytotoxicity assays with MTT on MCF-7 cells subjected to bee venom. We compared the results of Epirubicin HCL 2 mg/ml (a human breast cancer medication, Pfizer, USA) as a positive control (23). whereas the untreated cell line getting serum devoid of DMEM served as a negative control. Cancer cells were cultivated in the preparation medium (control) or with varying concentrations of the material (25, 50, 100, 200, and 400 µg/mL) for 24 hours at 37°C and 5% CO_2. Twenty-four hours later, we administered MTT dye (5 mg/mL, 20 µL/well) to the cells and then incubated them for three hours. Following the removal of a 125 µl aliquot, 50 µl of DMSO was introduced, and the plates were incubated at 37°C for 45 minutes. The negative control consisted of 150 µl of medium combined with 15 µl of MTT stock solution. Absorption was quantified at 570 nm with a microplate reader. The experiment was conducted five times to augment the results. The survival cell ratio was calculated using the subsequent formulas (23).

Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics for Windows (IBM Corp., Armonk, NY) (Version 26.0). The data were first tested for normality of distribution and homogeneity of variances. ANOVAs were used to determine differences between the two substance concentrations in each of the cancerous and non-cancerous cell lines. In cases where differences were significant after ANOVA ($p < 0.05$).

Results

This dataset was used to record the ultraviolet-visible (UV-Vis) absorption spectrum of bee venom that had been collected by the authors to discover and quantify the presence of chromophores and specific compounds. Spectroscopic analysis Venom of known concentration was dissolved in an appropriate solvent and examined by means of spectrophotometry. The final spectrum can be seen in Figure 1 and represents characteristic

absorption maxima at particular wavelengths (279 nm).

A characteristic absorption maximum at 279 nm is noted, which indicates the presence of aromatic acids in proteins of venom.

The UV-Vis spectroscopy study confirmed the aromatic amino acid content of bee venom, including tryptophan and tyrosine, with a strong absorption peak at around 279 nm (Figure 1). This finding is in line with the fact that bee venom contains a high amount of proteins, especially phospholipase A2 and major peptides like melittin. The intensity of the peak indicated the high concentrations of proteins with antibacterial and anti-inflammatory properties. Researchers have extensively used the 280 nm absorbance to evaluate the protein concentration, purity, and stability of bee venom (2). The detected peak provided further evidence that the venom's protein structure is intact, which is essential for its antibacterial film and other practical uses

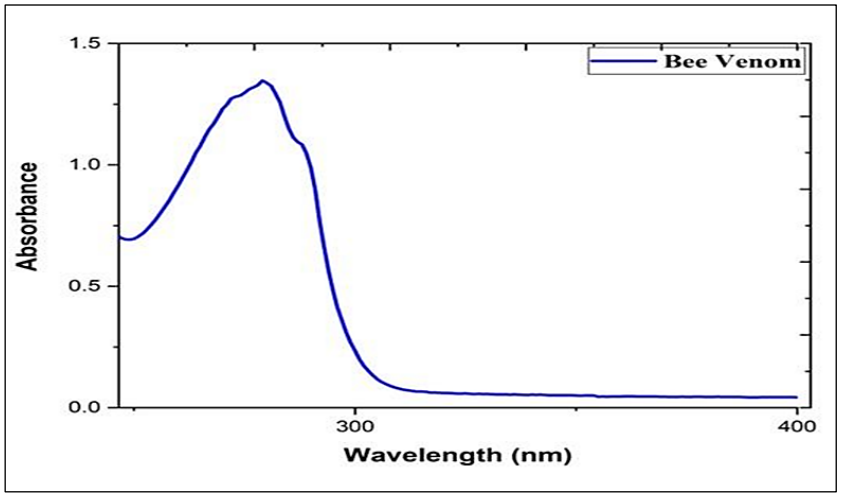


Figure 1: The UV–Vis Absorption Spectrum of the Iraqi Bee Venom

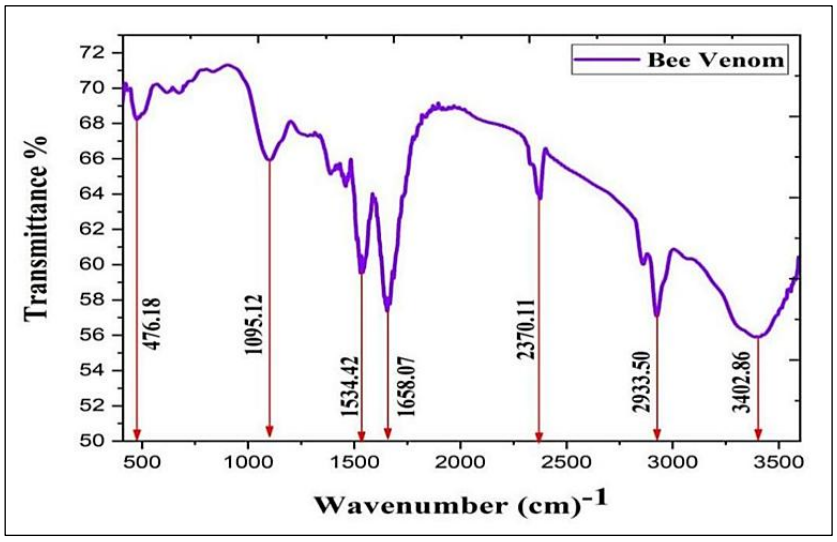


Figure 2: The FTIR Spectrum of Bee Venom

Fourier-Transform Infrared spectroscopy (FTIR)

The FTIR test explained both the molecular structure of bee venom and the identification of functional groups. To observe the vibrational modes of various chemical bonds, we had to perform the test on a dry sample (Figure 2).

This test introduced the absorption peak, which belongs to the functional groups that belong to bee venom. Bands I and II of Amide refer to peptides and proteins as 1658.07 cm^{-3}, which is caused by C=O vibrations that stretch in the peptide backbone. While Amide II results from the vibrations of N-H stretching and C-N stretching that are detected at around 1534.42 cm^{-1}.

Energy-dispersive X-ray Spectroscopy (EDS) Analysis

The chemical analysis of the bee venom sample was performed by energy dispersion X-ray (EDX) analyser. The elemental constitution of the venom contains light elements such as carbon (C), oxygen (O) and nitrogen (N), or sulfur (S) abundant in the identified proteins/peptides based on it molecular structure. Within the main components, among these, according to the EDX spectrum many microelements were found (such as K and Cu). The amounts of these components were estimated and together they matched the clear distinctive elemental fingerprint of BV (Figures 3 and 5).

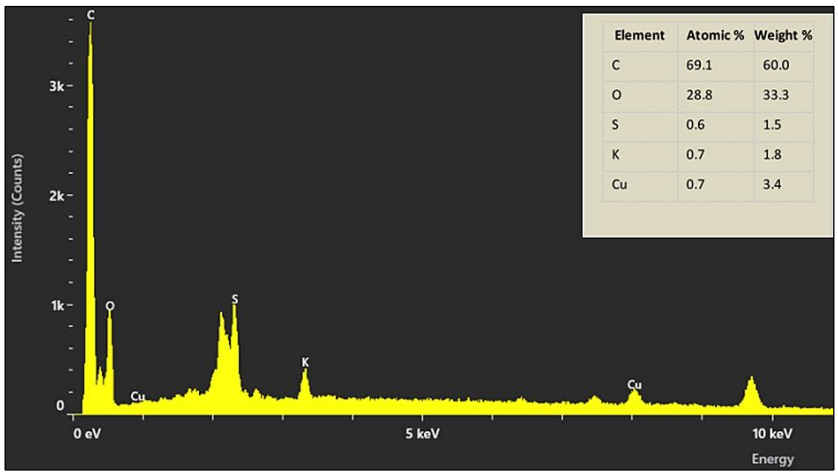


Figure 3: The Elemental Composition of Bee Venom as Determined by EDS Analysis

Cu 3.4% refers to copper (atomic weight), S 1.5% to sulfur, K 1.8% to potassium, C 60% to carbon, and O 33% to oxygen.

Fundamental Bee Venom Component

The EDS test showed the bee venom's elemental composition as denoted in Table 1. Carbon (C) recorded 69% of the atomic weight and 60% of the weight of organic molecules, while oxygen (O) made up 28.33%, connected with amino acid carboxylic acid groups. Sulfur (S) was 0.6% in atomic % and 1.5% in weight %, representing the amino acid cysteine, found in apamin and other peptides.

Table 1: Bee Venom Components Organized by Atom and Weight as Analyzed Using Energy Dispersive X-ray Techniques

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
C	69.1	0.5	60.0	0.5
O	28.8	0.7	33.3	0.8
S	0.6	0.0	1.5	0.1
K	0.7	0.0	1.8	0.1
Cu	0.7	0.1	3.4	0.4

Scanning Electron Microscope (SEM) Examination

Under a scanning electron microscope, bee venom appears as a complex network of linked granules with multilayered and porous crystalline aggregation. These layers may result from physical scraping from the collecting plate (Figure 4).

Figure 4(A) has a magnification power of 1 mm, and Figure 4(B) has 50 µm. The SEM technique used a total of 199,630 counts, with a standard counting rate of 2,551 points per second (cps) and an acceleration voltage of 30 kV. The acquisition of the entire analysis took 84 seconds. The scanning electron microscopy (SEM) examination of the bee venom particles showed various shapes and sizes, including some big, amorphous fragments.

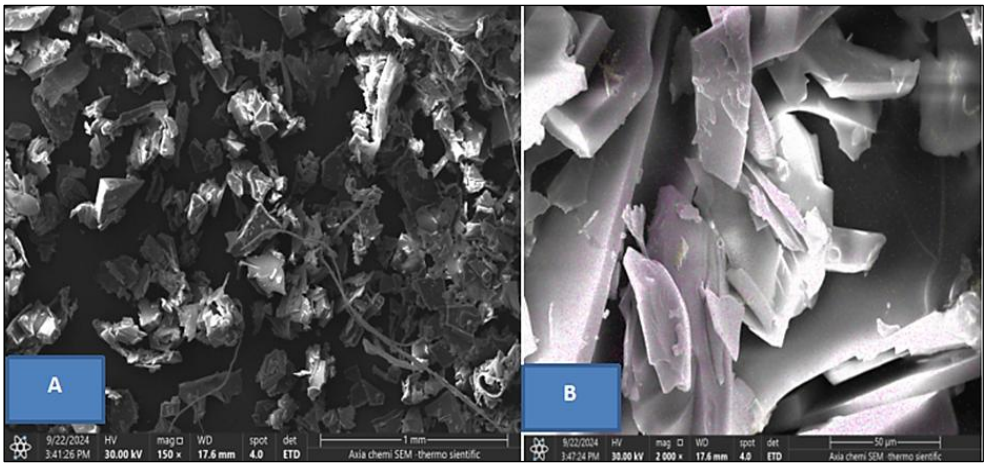


Figure 4: SEM Microscopy of Bee Venom Particles

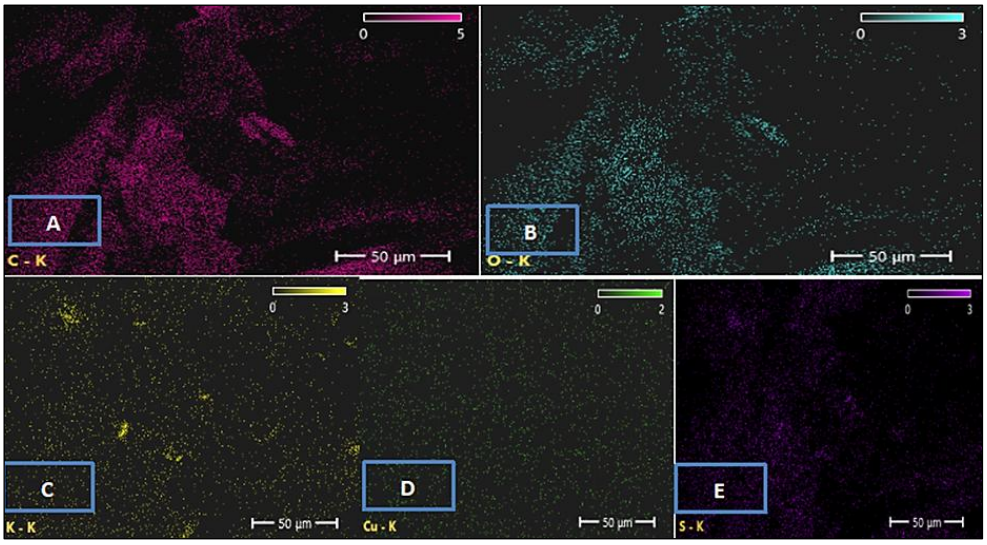


Figure 5: Elemental density-functional theory map for bee venom particles

The EDX Elemental Mapping

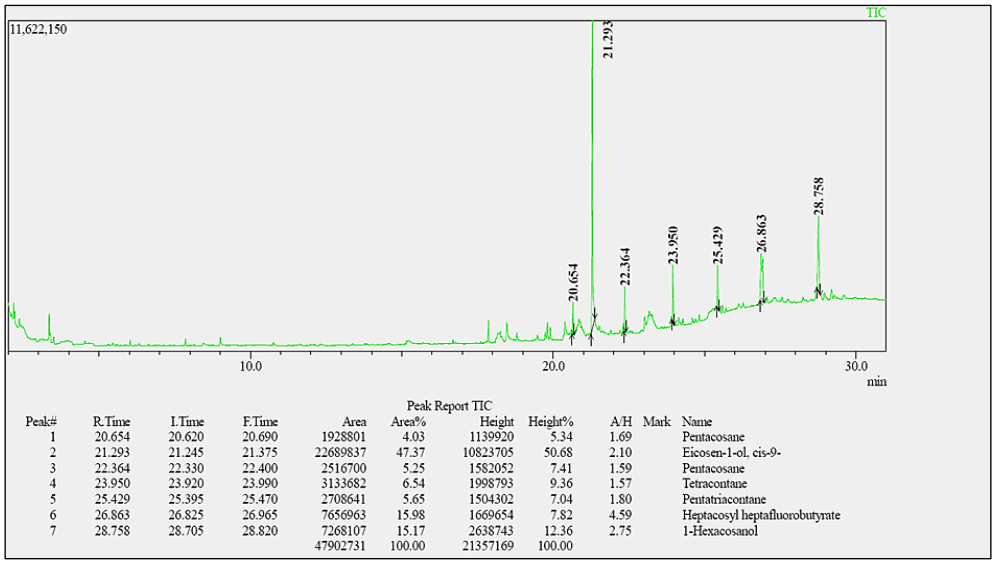
EDX mapping detected C, O, K, Cu, and S in the elemental composition of the bee venom chemical profile, which aligned with bee venom biochemistry and indicated a high content of organic and inorganic compounds (Figure 5).

EDS mapping was used to depict the dispersion of crucial elements in bee venom across a surface. The count map shows changes in the amounts of elements (in Figure 5, A for carbon, B for oxygen, C for potassium, D for copper, and E for sulfur) between the disorganized form and the amorphous particles

Gas Chromatography Mass Spectroscopy (GC MASS analysis)

GC-MASS analysis detected volatile and semi-volatile compounds in bee venom. Before analysis, they were isolated by an appropriate sample preparation technique. The mass spectrometer produces a characteristic fragmentation pattern for each of these compounds, so the resulting chromatogram (Figure 6) can discriminate between the components by their volatility. The peak report was analyzed and showed the presence of long-chain hydrocarbons, i.e., pentacosane and tetracontane, and fatty alcohols, i.e., cis-9-eicosen-1-ol and 1-hexacosanol, as major compounds with approximately 47.37% relative area represented in the chromatograms.

The test displays the main bioactive elements in bee venom, along with mass spectra taken at retention durations of 25, 26, and 28 minutes (m/z 275, 292, and 283, respectively). The mass peaks that correspond to these substances are pieces of phospholipase A2, melittin, and derivatives of fatty acids.



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	20.654	20.620	20.690	1928801	4.03	1139920	5.34	1.69		Pentacosane
2	21.293	21.245	21.375	22689837	47.37	10823705	50.68	2.10		Eicosen-1-ol, cis-9-
3	22.364	22.330	22.400	2516700	5.25	1582052	7.41	1.59		Pentacosane
4	23.950	23.920	23.990	3133682	6.54	1998793	9.36	1.57		Tetracontane
5	25.429	25.395	25.470	2708641	5.65	1504302	7.04	1.80		Pentatriacontane
6	26.863	26.825	26.965	7656963	15.98	1669654	7.82	4.59		Heptacosyl heptafluorobutyrate
7	28.758	28.705	28.820	7268107	15.17	2638743	12.36	2.75		1-Hexacosanol
				47902731	100.00	21357169	100.00			

Figure 6: GC-MS Test

Bee venom Antibacterial Activity

The present investigation was set to assess the antibacterial specificity of low-dose BV 0.1 mg/ml against a Gram-positive bacterium, *Staphylococcus aureus*, and a Gram-negative bacterium, *Pseudomonas aeruginosa*, and evaluate the antibiotic susceptibility profile of the bacterial isolates. (Table 2,3) We determined the sensitivity by using the Kirby-Bauer method on Muller Hinton agar. The most potent growth inhibitory effect was shown against the Gram-positive isolates, especially *Staphylococcus aureus* strains, with 48, 30, 25, 45, 44, 33, 35, 48,30,25,45,44,33,35,45 mm and 30, 23, 24, 28, 19, 27, 24, 30,23,24,28,19,27,24,28 mm inhibition zones against *P. aeruginosa* by bee venom. The effect was a clear inhibition zone, which illustrates the high sensitivity to this compound. The sensitivity to venom decreased for Gram-negative bacteria (30 mm), and both strains were MDR based on their antibiotic profiles.

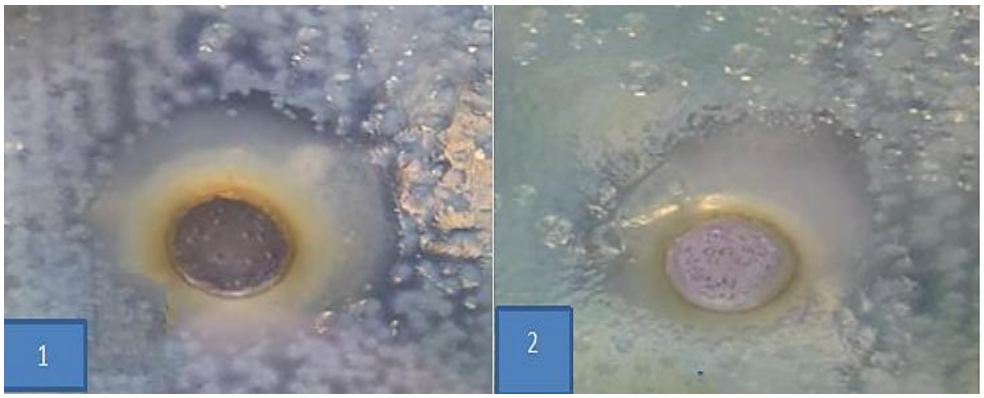


Figure 7: Antibacterial Activity of Bee Venom on Mueller Hinton Agar Plate

The average inhibition zone sizes (mm) for *S. aureus* and *P. aeruginosa* at 0.1 mg/ml of bee venom were 18 mm (A) and 14 mm (B), respectively, which showed significant antibacterial activity of the eight isolates compared to reference antibiotics using Kirby-Bauer diffusion assays on Muller-Hinton agar medium after incubating for 24 h at 37°C.

Antimicrobial Susceptibility Test (AST) Profiles *P. aeruginosa* and *S. aureus* Isolates

Eight clinical isolates of Staphylococcus aureus were evaluated for their antimicrobial susceptibility profile using the disk diffusion method in accordance with CLSI criteria. The antibiotics employed in the studies included cefoxitin (30 µg), oxacillin (1 µg), linezolid (30 µg), and clindamycin (2 µg); additionally, erythromycin

(15 µg) and trimethoprim-sulfamethoxazole [SXT; 1.25/23.75 µg] were evaluated for some collections of MRSA. CLSI 2024 classified the zone diameter (mm) as either susceptible or resistant. Table 1 detects that the majority of isolates were MDR (multidrug resistant), and cefoxitin and oxacillin recorded the highest resistance percentage. Erythromycin and clindamycin followed, indicating different levels of methicillin resistance (MRSA). While linezolid is still effective against all the studied isolates, susceptibility to trimethoprim-sulfamethoxazole differs between them (Table2).

Table 2: Antibiotic Susceptibility of Gram-Positive Bacteria, *S. aureus*

Antibiotic (Abbreviation)	Disk Conc. (µg)	Zone Diameter (mm) for 8 Isolates	Interpretation
Cefoxitin (FOX)	30	14, 15, 13, 12, 16, 14, 15, 24	7 R, 1 S
Oxacillin (OX)	1	10, 11, 9, 12, 13, 10, 11, 20	7 R, 1 S
Linezolid (LZD)	30	28, 26, 27, 25, 29, 28, 27, 30	8 S
Clindamycin (DA)	2	16, 14, 13, 12, 15, 13, 12, 25	6 R, 2 S
Erythromycin (E)	15	10, 9, 11, 8, 12, 10, 9, 22	7 R, 1 S
Trimethoprim–Sulfamethoxazole (SXT)	1.25/23.75	20, 18, 19, 17, 21, 18, 19, 26	6 S, 2 R

Eight clinical isolates of *Pseudomonas aeruginosa* were tested for antimicrobial susceptibility with a panel of antibiotics: amikacin (AKN), gentamicin (CN), levofloxacin (LEV), piperacillin/tazobactam combination (PRP), meropenem (MRP), ticarcillin/clavulanic acid combo (TTC), ATM, imipenem, and ceftazidime. Seven of eight isolates were resistant to all antibiotics except amikacin and ticarcillin/clavulanic acid. Three isolates were susceptible to amikacin, and one to ticarcillin/clavulanic acid. This resistance pattern indicates strong multidrug-resistant *P. aeruginosa*, making antimicrobial assignment difficult.

Table 3: The Antibiotic Susceptibility of Gram-Negative Bacteria *P. aeruginosa*

Antibiotic (Abbreviation)	Disk Conc. (µg)	Zone Diameter (mm) for 8 Isolates	Interpretation
Amikacin (AKN)	30	19,10.5,20,25, 12,10,6,5	5R, 3 S
Gentamycin (CN)	10	13,3,9,13,5,8,4,6	8 R
Levofloxacin (LEV)	5	4,10,14,16,8,10,11,8	6 R, 2I
Piperacillin (PRP)	100	15,10,12,8,14,10,1,6	6 R, 2 I
Meropenem (MRP)	10	12,13,7,9,8,14,10,21	7 R,1I
Ticarcillin/clavulanic acid (TTC)	75/10	19,7.5,12,8,10,13,6,14	1 S, 6 R,1I
Azetronam (ATM)	30	8,13,14,7,10.5,12,9,4	8R
Imipenem (IMI)	10	11,16,17.5,10,8,9,18,6	8R
Ceftazidime (CAZ)	30	5,12,8,10,7.5,14,9,10	8 R

Cytotoxicity of Breast Cancer (MCF-7) and Human Fibroblast Neonatal Cell Line (HdFn) Cells Treated with Bee Venom

Table 4 showed the results of the cell viability tests for the MCF-7 as well as HdFn normal cells after being exposed to different amounts of bee venom. The viability of the cells was tested at five different concentrations: 25, 50, 100, 200, and 400 µg/ml. Apitoxin had a clear dose-dependent toxic impact upon both MCF-7 tumor cells and HdFn normal cells. As the concentration went up from 25 µg/ml to 400 µg/ml, MCF-7 survival rates went down from 78% to 29%. At the same time, HdFn cell viability went down from 95% to 72%. Bee venom had a stronger cytotoxic impact on malignancies than on HdFn cells at all concentrations. This means that it was still somewhat selective in its toxicity toward cancer cells; at 400 µg/ml, 29% of MCF-7 cells were still alive, while 72% of HdFn cells were still alive.

Table 4: Dose Dependent Cytotoxicity of MCF-7 and HdFn Cells treated with Bee venom

Con. µg/ml	HdFn Mean ± Std. Deviation	MCF- 7	P value (P ≤ 0.05)
25	95.13900 ±.347[a]	78.31800 ±.7708[a]	0.0001*
50	93.67267 ±.4382[a]	66.43533 ± 1.0415[b]	0.0001*
100	88.96633 ±2.4533[b]	55.32433 ± 2.2352[b]	0.0001*
200	84.49067 ±.5044[b]	42.20667 ±2.2151[c]	0.0001*
400	72.33800 ±.6940[c]	29.20500 ±.4818[d]	0.0001*
P value (P ≤ 0.05)	0.000*	0.000*	

*Significant difference under p ≤ 0.05 by one-way ANOVA and T-test
Different small letters refer to significant differences within comparisons; similar letters refer to non-significant differences.

Cytotoxicity of Breast Cancer (MCF-7) and human Fibroblast Neonatal Cell Line (HdFn) Cells Treated with Doxorubicin Cancer Drug

Table 5 showed the results of the cell viability tests for the MCF-7 as well as HdFn normal cells after being exposed to different amounts of the cancer drug (Doxorubicin). The viability of the cells was tested at five different concentrations: 25, 50, 100, 200, and 400 µg/ml. The positive control (Doxorubicin) had a clear dose-dependent toxic impact upon both MCF-7 tumor cells and HdFn normal cells. As the concentration went up from 25 µg/ml to 400 µg/ml, MCF-7 survival rates went down from 90% to 42%. At the same time, HdFn cell viability went down from 95% to 72%. Doxorubicin had a stronger cytotoxic impact on malignancies than on HdFn cells at all concentrations. This means that it was still somewhat selective in its toxicity toward cancer cells; at 400 µg/ml, 42% of MCF-7 cells were still alive, while 72% of HdFn cells were still alive.

Table 5: Dose Dependent Cytotoxicity of MCF-7 and HdFn Cells Treated with Doxorubicin Drug

Concentration positive control /Con. µg/ml	**Mean ± SD** **HdFn**	**MCF-7**	**P-value**
25	95.37033 ± .578500[a]	90.70200 ±.997602[a]	0.0021*
50	93.13267 ±.467736[a]	77.73933 ±1.215461[b]	0.0001*
100	88.23300 ±2.640041[b]	67.12967 ±.918643[b]	0.0002*
200	77.85500 ±.334286[c]	55.70967 ±.928419[c]	0.0001*
400	72.76267 ± 1.646245[c]	42.59267 ±1.028949[c]	0.0001*
P-value	0.000*	0.000*	

*Significant difference under p ≤ 0.05 by one-way ANOVA and T-test
Different small letters refer to significant differences within comparisons; similar letters refer to non-significant differences.

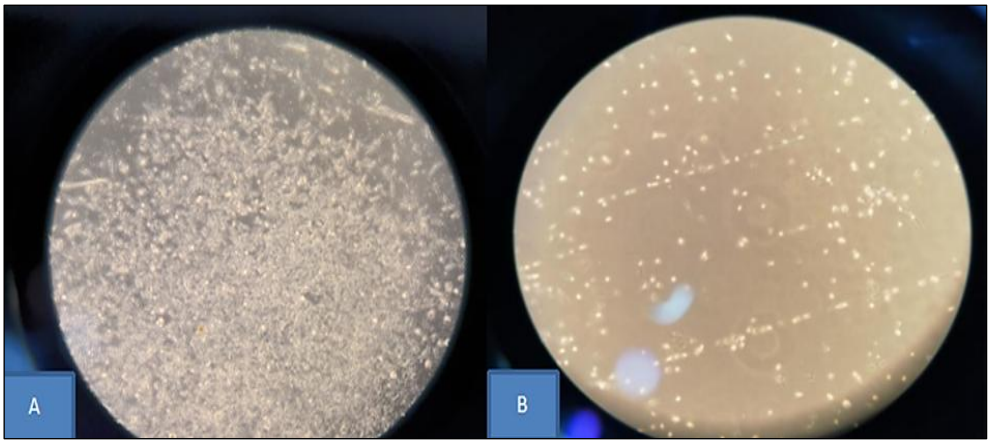


Figure 8: Light Microscopical Observation of the MTT Assay Induced Morphological Changes in MCF-7 Cells

A represented the no treatments on the breast cancer cell line. B. Bee venom at 400 µg/ml induces a cytopathic effect on the breast cancer cell, such as cell detachment from the culture, cell death, cell shrinking, nuclear condensation, and apoptosis.

Discussion

The UV-Vis spectroscopy study confirmed the aromatic amino acid content of bee venom, including tryptophan and tyrosine, with a strong absorption peak at around 279 nm (Figure 1). This finding is in line with the fact that bee venom contains a high amount of proteins, especially phospholipase A2 and major peptides like melittin. The intensity of the peak indicated the high concentrations of proteins with antibacterial and anti-inflammatory properties. Researchers have extensively used the 280 nm absorbance to evaluate the protein concentration, purity, and stability of bee venom. The detected peak provided further evidence that the venom's protein structure is intact, which is essential for its antibacterial film and other practical uses.

Figure 2 Bee venom FTIR analysis revealed: Bands I and II of Amide, which represent proteins and peptides: Typically, amide I may be seen at 1658.07 cm^{-3}, which is mostly caused by C=O vibrations that stretch in the peptide backbone. Amide II, which is formed by vibrations of N-H stretching and C-N stretching, is usually detected at around 1534.42 cm^{-1}. Results Figure 2 presents important markers for the amount of proteins and peptides, such as melittin, which is a main ingredient in bee venom. The stretching of N-H bonds, which consist of amine groups, is observed at 3402.86 cm^{-1}. The peak is often accompanied by peptides and proteins in the venom and is located between 3300 and 3500 cm^{-1}. C-H stretching vibration at 2933.50 cm^{-1} was detected, which corresponds to aliphatic chains. Located between 2850 and 2950 cm^{-1}, it suggests that fatty acids and other organic substances contain aliphatic hydrocarbons. The presence of C=O stretch (carboxyl groups) around 1700–1750 cm^{-2} showed that carboxylic acid compounds or esters were present. And lastly, the referenced phosphate groups include 1095.12 cm^{-1}. And these results were consistent with (24), who said that if phospholipids or nucleotides are found, bands about 1000–1250 cm^{-1} will be observed. These peaks show that bee venom contains many physiologically active peptides and enzymes, such as melittin, phospholipase A2, apamin, and others. They add to the complex biochemical makeup of bee venom.

From table 1 and figures 3 and 5, EDS analysis for the bee venom showed that C does exceed 60% and thus has been declared as organic material according to its main organic type being peptides and different proteins, even melittin with phospholipase A2. Oxygen (O) accounted for 33% in total, which suggests that it was present in these small molecules and also water as one of the major components of venom. The S content was 1.5%, illustrating that there are amino acids containing S, such as cysteine, in the bee venom proteins. Potassium (K) was found at 1.8% as well, indicating a role in venomous ionic balance and enzymatic processes. A low quantity of copper (Cu) at 3.4% was also found that could either be an environmental exposure or the restricted involvement of Cu in biological activity in the venom, which may be related to enzymatic activities reported for some toxic species (24).

The distribution of elements supports the biochemical make-up of bee venom and shows that its trace element concentration is affected by biological function and the environment in a big way. To illustrate the point, a newer study in 2019 found that melittin's sulfur content is essential for its pore-forming activity, which is vital for lysing bacterial cells. Phosphorus further enhances the antibacterial activity by binding to phospholipase A2 and aiding in membrane breakdown (25).

The varied shape is a result of the gathering method (Figure 4, A and B). To collect bee venom, one usually places a collecting device on a glass or plastic surface, stimulating the bees to release their venom. As it dries, the venom leaves behind deposits that are both uneven and fractured. These big, crooked bits are probably the end product of the drying and coagulation steps performed on the collecting plate, which caused the particles to agglomerate and become non-uniform. Some particles may appear amorphous because the venom components did not fully crystallize or because mechanical scraping disturbed particle homogeneity during collection. These shape inconsistencies, which are typical of manually harvested venom, may have an impact on the physical as well as chemical properties of bee

venom (26). In experimental settings, this variation in particle shape may impact the venom's dispersibility and surface area (10).

The EDX elemental mapping shows elemental distribution in the venom matrices per space, reflecting general homogeneity and degradation trends. It can be used to compare Apis venom from different species or to study the influence of environmental and storage conditions (Figure 5). Spatial data enhances the repeatability and standardization of medicinal formulations, as noted by past researchers (27). Bee venom in nanomedicine: nutrients are transferred through nanoparticles to the drug delivery system. EDS can be used to verify that elements do in fact integrate and that the surface now has a different composition. It calculates encapsulation efficiency as well as the presence of venom-derived components on the surface of the nanoparticles, which influences cellular uptake and therapeutic effects (28). In bee venom research, EDS mapping shows a certain degree of improvement in nanotechnology.

The GC-MS analysis of the venom sample, as shown in figure 6, revealed a complex mixture of long-chain hydrocarbons, alcohols, and ester derivatives that are similar to known Hymenoptera venoms known to be relevant for defining structure-bioactivity relationships important for drug discovery applications (18). Due to the hydrophobic nature and antibacterial and anti-inflammatory effects of the venom that could have originated from Hymenoptera venoms (identified from 2 peaks), they were presumable; some molecules identified in major amounts as C9-20:OH (Pentacosaine) refer to emollient and antioxidant properties and may exert potential anticancer activity (29). The scientists suggest that these identified high-molecular-weight alkanes, which include tetracontane and pentatriacontane, could aid in compound stability and transdermal delivery, making them possible candidates for use in controlled drug release and nanocarrier systems. The first two would improve membrane permeability and immunological regulation, respectively, while the last two are closely related to neuroprotection and wound healing (30, 31). The lipophilic nature of components found in bee venoms will continue to stimulate pharmacological applications in cancer and drug administration, particularly in the setting of ongoing studies into their mechanisms of action/therapeutic value.

There may be fatty acid derivatives, such as oleic acid, present, as shown by the peak at m/z 283 and a retention time of approximately 28 minutes. Phospholipase A2 activity, which breaks down lipid membranes and releases fatty acids, is believed to be the source of these components. Prior research demonstrating that fatty acids are involved in the cytotoxic venom effects provides credence to this (26). These three mass peaks provide further evidence that bee venom's harmful effects are due to its complex combination of peptides and lipids, the most physiologically relevant components. The outer membranes of Gram-negative bacteria, such as E. coli and Pseudomonas aeruginosa, make them more resistant to bee venom peptides. Pseudomonas' resistance, further enhanced by its ability to form biofilms and utilize efflux pumps, necessitates higher concentrations (32). One of the main ways that bee venom kills bacteria is by destroying their cytoplasmic membrane and stopping them from building biofilms. Therefore, bee venom shows promise as an antibacterial agent, particularly against Staphylococcus aureus and other Gram-positive bacteria, which could have therapeutic uses in the treatment of clinical and food-borne illnesses. Pseudomonas aeruginosa and other highly resistant bacteria may impair its efficiency; in these instances, synergistic treatments or greater dosages may be necessary (33). The results of the current study show that bee venom can be a potent natural antibacterial agent, warranting the incorporation of local bee venom with antibacterial films or gels in wound plaster and its potential medicinal uses in fighting resistant bacterial illnesses. To improve public health, the findings lend credence to the idea of using local bee venom to provide a sustainable and novel solution. (Figure 7)

The current results from Tables 2 and 3 indicate that all P. aeruginosa strains, except for three aminoglycoside-susceptible and one ticarcillin-clavulanic acid-susceptible isolate (95% CI = 10.8%), are extensively multi-resistant to the antimicrobials of choice for treating wounds in the case of burn infections and the appropriate therapeutic antibiotics in urinary tract infection patients. For example, Pseudomonas aeruginosa can cause a variety of diseases. In

hospital settings, exacted medical resources, this bacterium displays resistance to diverse drugs such as ceftazidime, imipenem, meropenem, aztreonam, piperacillin, and levofloxacin (34). Similarly, staphylococcal species (including Staphylococcus aureus) were resistant to clindamycin, erythromycin, trimethoprim-sulfamethoxazole, oxacillin, and cefoxitin as MRSA. Linezolid was active in vitro on all S. aureus isolates, and two of them were also clindamycin-susceptible. Linezolid is a member of the oxazolidinone class of antibiotics that inhibits bacterial protein synthesis by preventing 50S subunit assembly and the formation of functional initiation complexes (35). Staph. aureus is the most susceptible host, as this bacteriocin mainly targets Gram-positive bacteria, but P. aeruginosa has inherent resistance by virtue of its impermeable outer membrane and active efflux pumps. The team concluded that the resistance patterns in burn infections and urinary tract infections from multi-drug-resistant bacteria present a huge challenge for clinicians because of the fewer treatment options that are available.

The current study demonstrated that bee venom exerts a significant effect on cancer cell lines, with a mean and standard deviation of 29.20500 ± .4818 at high doses (400 µg/ml). In contrast, its effect on normal cells yielded a mean and standard deviation of 72.33800 ± .6940. Comparatively, compared to the results of the current study, the chemotherapeutic agent doxorubicin exhibited an availability ratio of 42% against breast cancer cells and 72% toward HdFn normal cells, highlighting the selectivity and efficacy of bee venom against tumor cells (Tables 4, Figure 8). One hypothesis of TNBC is that the antitumor effects of bee venom are regulated via the melittin peptide, which induces cell membrane disruption and apoptosis (36). One study after another has shown the bee venom as a selective cytotoxic poison, which means it is the one that has been targeted towards carcinogenic cells (MCF-7), rather than normal ones. This is a critical finding. As well as being an inhibitor of topoisomerase II, intercalating between DNA bases, and generating reactive oxygen species, Epirubicin HCL for its non-target effect is involved in the formation of reactive oxygen species. The wider diversity in the toxic profile of this approach can sometimes result in unwanted side effects, such as damage to healthy cells; despite that, the method works even against rapidly dividing cancerous cells (37). For example, some studies have also demonstrated melittin as having a range of pathways to inhibit cancer cell proliferation, which contributes to the anticancer effects of BV. Such mechanisms are mediated by mitochondrial damage, generation of reactive oxygen species (ROS), caspase activation, and programmed cell death (apoptosis) (38). Results of the study remind us that bee venom is an ideal candidate for a selective therapy against breast cancer, as it doesn't harm normal cells and rather specifically sensitizes them. More importantly, the selective toxicity of bee venom against triple negative breast cancer (TNBC) and human epidermal growth factor receptor 2 (a protein on the surface of cells that helps them grow and divide). It usually performs healthy cell functions. However, in certain cancers, HER2 is overproduced, making the cells grow uncontrollably and thus resulting in tumors. HER2-enriched subtypes provide a potential benefit compared with conventional chemotherapies targeting indiscriminately also healthy tissues. Companion studies support that this increased ratio could provide added benefits when used in combination with established chemotherapeutic agents, e.g., docetaxel and doxorubicin, to improve the efficacy of treatment while minimizing the total drug burden and associated side effects (39, 40). We have come to the conclusion that bee venom has the potential to be a beneficial and selective therapeutic agent. This is in contrast to standard chemotherapies such as epirubicin, which cause extensive damage.

Conclusion

The study shows the antibacterial activity of bee venom from local honeybee on multidrug resistant bacteria including Pseudomonas aeruginosa and Staphylococcus aureus. Its hyper intricate architecture and elemental structure suggest it to be potential for the synthesis of antibacterial films and gels for medicine application, suggesting the significance of natural resources. Interestingly, the effects of bee venom on MCF-7 and normal cell line HdFn compared to chemo drug doxorubicin are more positive (Fig.5), indicating that this sample had preferential activity on cancer cells, especially at higher concentrations 400 µg/ml substantiate their efficiency. In the context of biological

systems, a naturally occurring drug may be used in conjoint therapy with other drugs in order to mitigate toxicity. Limitation of the study: The weakness of this study is that no physicochemical determination was carried out on honey samples before performing biological activities. It is proven that bioactivity of honey is directly linked to the sugar and pH content, total polyphenolic compounds and flavonoid contents. Without such studies it is difficult to directly correlate the observations of biological effect with specific chemical forms. This will be the subject of future research incorporating comprehensive compositional profiling necessary to facilitate a stronger linkage between chemical composition and biological activity. The role of BV as a possible medicine will be stressed in the current study in Iraq that can be considered safe and legitimate natural source for the treatment at lower cost comparing with others traditional ways. Plants from Babylon may have unique properties that contribute to its high levels of bioactivity, which would be particularly relevant for local health issues such as infectious disease and cancer. This study might give an indication for the use of Iraqi honey bee venom in treatments as natural medicine, with low cost. The special plant species present around Babylon may add to its chemical diversity and hence potential bioactivity, which makes it more promising as a possible treatment against endemic health disorders like infectious diseases or cancer. Iraqi honey bee derivative has anti-tumoral effects for the topical application, the cancer adjuvant therapy or even as nutraceutical particularly, then they will be tried, clinically tested and accepted to grant its safety and efficacy to confirm their quality.

Abbreviations

AST: Antimicrobial susceptibility test, BV: Bee venom, EDS: Energy-dispersive X-ray spectroscopy, FTIR: Fourier transform infrared, GC MASS: Gas chromatography Mass spectroscopy, HdFn: Human fibroblast neonatal cell line, MCF-7: Breast cancer, MIC: minimum inhibitory concentration, UV: Ultra violet ray.

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The design, concept and manuscript are all the result of equal labor from all authors. All authors have read and agreed to the submitted version of the paper and consent to be accountable for all aspects of the work.

Conflict of Interest

The authors assert that they possess no identifiable competitive financial interests and personal ties that may have seemingly influenced the work presented in this study.

Declaration of Artificial Intelligence (AI) Assistance

The authors declare that they did not use AI-assisted tools (ChatGPT, OpenAI) during the writing process.

Ethics Approval

This study was conducted in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was ethically approved and registered as 38 College of Science-Women's biology-Babylon University-Iraq on 20/10/2024. Written informed consent was obtained from all participants before participation.

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