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The effect of honey bee (*Apis Mellifera*) venom as an bacterial and antifungal antagonist

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Honey Bee Venom (*Apis Mellifera*), pathogenic bacteria, anti fungi, bacterial inhibition, herbal alternative therapy, Rapid wound healing.

ABSTRACT

A smooth, colorless, sweet liquid with a bitter taste and a precise weight of 1.13 is the Honey Bee Poison. It is a complex complex of proteins, carbohydrates, amino acids, and volatile oils that cause stinging pain and heating damages bee venom. The toxin starts to be deposited shortly before its departure from the virgin in honey bee workers' worker bees. For the prevention of rheumatic fever, nerve and joint inflammation and irritation, bee venom may be used. The aim of this study The Effect of Honey Bee Venom (*Apis Mellifera*) On Pathogenic Bacteria. In the Abbasid district of Salah al-Din governorate, and from the apiary of the College of Agriculture, University of Baghdad, an apiary was discovered. The number of workers ranges from 2000 to 3000 workers in each cell. The extraction was carried out in two ways: the venom of the honey bee is extracted by using an insulin syringe, where the needle is implanted. Direct effect (without dilution) of samples on bacterial and fungi isolates using the cross-drilling method and Specify MIC and MFC. Natural products are known to be a renewable alternative with less problems than a wide variety of active compounds may create. BV includes a number of bioactive ingredients that play a critical role as antimicrobials, including melittin, apamin, and PLA2. BV trials may well contribute to the potential production of novel pharmaceuticals.

1. INTRODUCTION

The venom of honey bee (*Apis Mellifera*) is a pure, colorless, fragrant liquid with a bitter flavor, a basic weight of 1.13 and an array array of proteins, carbohydrates, amino acids, and volatile oils [1- 4]. Immediately before leaving the virgin, the poison starts to be contained in honey bee workers' worker bees. In spring and summer, the quantity of poison is high, and in fall and winter, it decreases [5- 8]. It improves if the proportion of protein substances in the diet of bees reaches carbohydrates. There are several active substances in bee venom, including meliltin, which is one of the strong inflammatory agents, and also Adolapin. Bee venom can be used to treat rheumatic fever, to treat nerve and joint inflammation and discomfort, to treat severe back and neck pain, to treat hepatitis C, to treat blood pressure [9]. The benefits of bee sting therapy are that no patient is available. Among the patients who were treated with bee venom, death resulted. Honey has properties that are anti-fungal, bacterial and viral. It raises the number of red

blood cells and facilitates the circulation of the blood. It stimulates the brain's nerve cells and delivers feedback messages to the subcerebral nervous cells [10^5]. By burning body fat in the affected region, it decreases weight. The susceptibility of bacterial infections to antimicrobial drugs has achieved troubling heights in many areas of the world. the study aims to know the effect of honey bee venom (Apis Mellifera) on pathogenic bacteria. The amount of toxin produced by the worker during her life is estimated at about 85 mg. Bee venom is one of the most complex compounds, as it is known that it mainly consists of three components: protein components: Hyaluronidase (an enzyme that diffuses the toxin), Phospholipase A and B and Mellitin, which have a large molecular weight, which gain the body immunity and are in it [11]. Antibodies. The peptide ingredients: Cycabin Peptide (MCD) mast cell destroying- peptide), Tertypin, Apamin, Procamine, small peptides, and active amines: Histamine (which promotes the release of more histamine through the existing histidine compound. Originally in animal tissues) dopamine noradrenaline norepinephrine Y Aminobutyric acid. The treatment of bee venom has been practiced since ancient times as it has been used in the treatment of arthritis and other inflammatory diseases, and bee venom contains many active substances, including meliltin, which is one of the strong agents against inflammation, as well as Adolapin, which is another strong substance as it inhibits the cyclic oxidation enzyme for that It has pain-relieving activity and accordingly, researchers were able to treat rheumatic fever, treat inflammation and pain in nerves and joints, treat chronic pain in the back and neck, treat hepatitis C, treat blood pressure, treat chronic headaches, insomnia and other diseases [12].

2. Material And Methods

2.1 Extract honey bee venom

Samples were collected from one of the apiaries in the Abbasid area of Salah al-Din Governorate and from the apiary of the College of Agriculture, University of Baghdad, for the purpose of extracting and collecting honey and honey and marking wax, and samples were collected with the help of the beekeeper. The number of workers in each cell ranges from 2000 to 3000 workers. The extraction was done in two ways the first way: by withdrawing honey bee venom using an insulin syringe, where the needle is inserted It was noticed that the collected quantity contains some impurities and when tested, contamination occurred in the hole to which the poison was added in the culture medium when the inhibitory effect was observed. This was observed for two strains after collection. It was preserved at grade 4 for the purpose of studying. The experiment was repeated in the initial study. Contamination was also observed, indicating a third of the samples collected during the study. The result of this method was neglected after extraction for 12 days. From my observation, I think that pollution is due to the collection and extraction of poison in the same apiary, whether in the University of Baghdad or in the Abbasid area. The second method: by collecting the venom from the poison gland directly, where the workers are collected in a laminate cover. The hood was sterilized using formaldehyde (formalin 1: 9) for the purpose of reducing and stopping the movement of the workman, making it easier to withdraw the poison through it.

Approximately 3.4 ml of each strain (three cells) was collected using this method, and it was tested directly on the pathogen, or after diluting it with distilled water for the purpose of use, it was kept at a degree of 4 until use.

No. 1 was assigned to the toxin disposed of the first strain No. 2 for the toxin extracted from the second strain

No. 3 for the toxin extracted from the third strain

Through the classification, we will know if these three strains are really different strains (classification is in progress due to the Institute of Natural History where they have many living creatures models for the

purpose of classifying them and I am waiting for my turn)

2.2 Effect of extracted honey bee venom on some pathogenic bacteria and fungi

The effect of the six extracted honey bee venom samples (1,2,3), honey and wax extracted from the same cells was studied on some bacterial and fungi isolates that were isolated and diagnosed in this study, and some isolates showed a clear variation in their pattern of susceptibility. Honey and wax are produced from the same cells for workers. Venom and melittin were screened against pathoginc bacterial and yeast strains which were *Klebsiella pneumoniae*, *Helicobacter pylori*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Lactobacillus spp*., *candida albicanse* and *candida tropicalis* by means of diffusion disc sensitivity tests. The antibacterial test was carried out in duplicate. For each strain, a particular antibiotic was used as a positive (maximum activity) control and H2O as a negative control. The inhibition area was calculated using a caliper to calculate the blocking area area [13- 15].

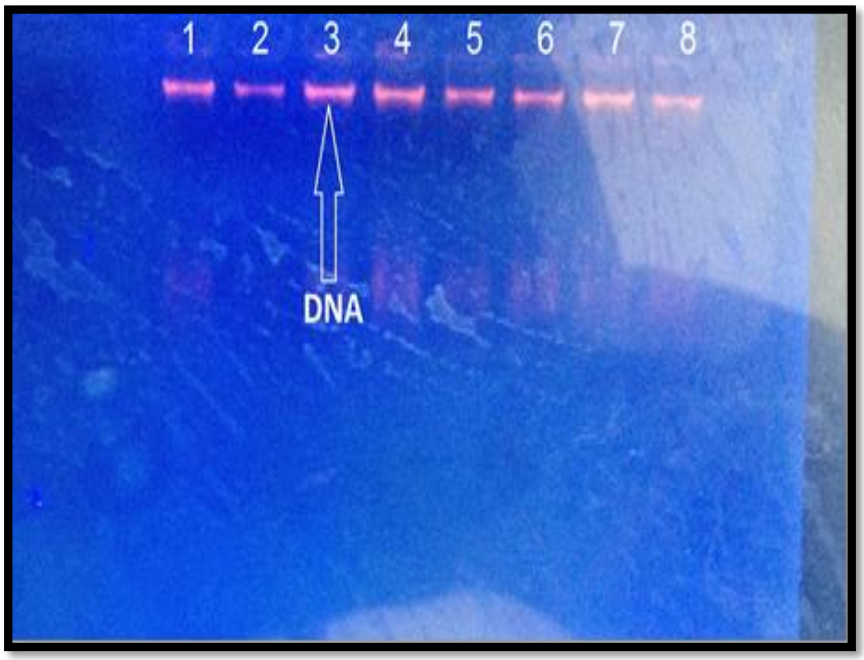
2.3 Statistical Analysis

Mean ± standard deviation (SD) was expressed as the findings. Statistical significance was identified as p-valu at 0.05, SPSS version 20 was used to analyze all the data of the study.

3. Results And Discussion

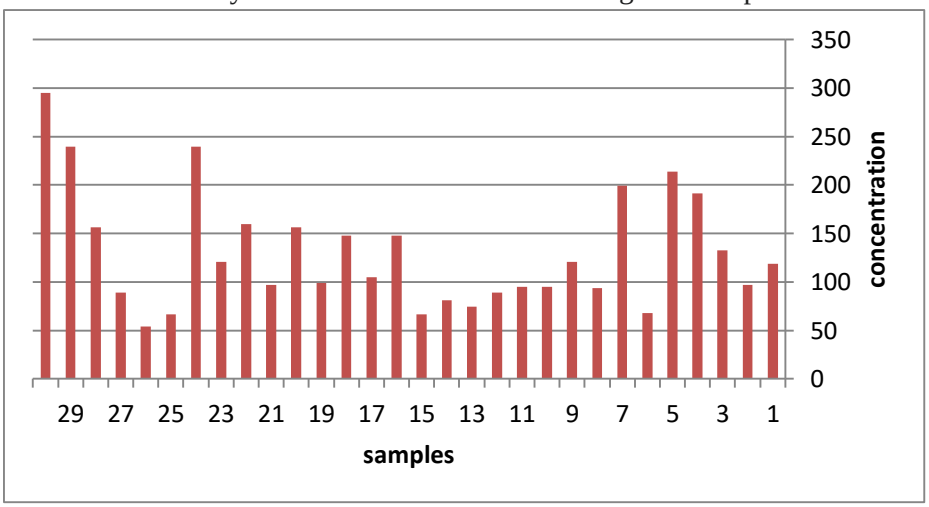
3.1 Direct effect (without dilution) of extracted honey bee venom samples on bacterial and fungi isolates using the cross-drilling method

Pass 1 ~ 2 ml of cell cultured bacteria into a 2 ml tube. Pellet bacteria at 13,000 rpm by centrifugation for 1 min, then discard the supernatant. The cell pellet with remnant supernatant is completely resuspended by tapping or actively vortexing. Add 200 µl of Buffer CL, 20 µl of Proteinase K and 5 µl of RNase A solution to the sample tube and blend vigorously with the vortex. For 10 ~ 30 min, incubate lysate at 56 ° C (preheated heat block or water bath). Add 200 µl of Buffer BL into the upper sample tube after full lysis, and blend thoroughly. Then incubate the mixture for 5min at 70 ° C. To extract un-lysed tissue particles, centrifuge the sample tube for 5 min at 13,000 rpm. Then move 350 ~ 400 µl of the supernatant carefully into a fresh 1.5 ml tube. Into the lysate, apply 200 µl of absolute ethanol and blend well by softly inverting 5 to 6 times or by pipetting. After blending, rotate the 1.5 ml tube quickly to extract drops from the inside of the lid. Without wetting the bottom, gently apply the mixture to the Spin Column (in a 2 ml Collection Tube), close the cap and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and put a 2 ml Collection Tube in the Spin Column. At 13,000 rpm, apply 700 µl of Buffer WA to the spin column without wetting the rim and centrifuge for 1 min. Dispose of the flow-through to use the Storage Tube again. Add 700 µl of Buffer WB to the spin column and centrifuge for 1 min at 13,000 rpm without wetting the rim. Discard the flow-through and put the column in a 2.0 ml collection tube, then centrifuge the membrane again for another 1 minute to dry. Discard the flow-through and the whole Collection Tunnel. Place the Spin Column directly on the membrane into a new 1.5 ml tube and 30-100 µl of Buffer CE. Incubate at room temperature for 1 min and then centrifuge at 13000 rpm to e for 1 min [18], [19].



Electrophoresis gel with isolation of genomic DNA from some bacteria and fungi isolated 1% agarose gel for 30 min at 5 V / cm. Then visualized under ultraviolet light after staining with ethidium bromide.

Purity and concentration of DNA using nano drop



concentration of DNA(ng/ul)

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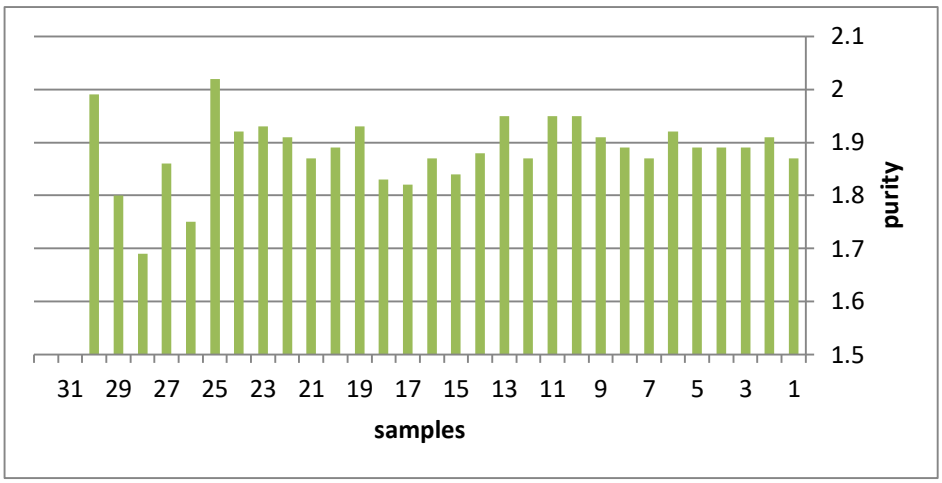
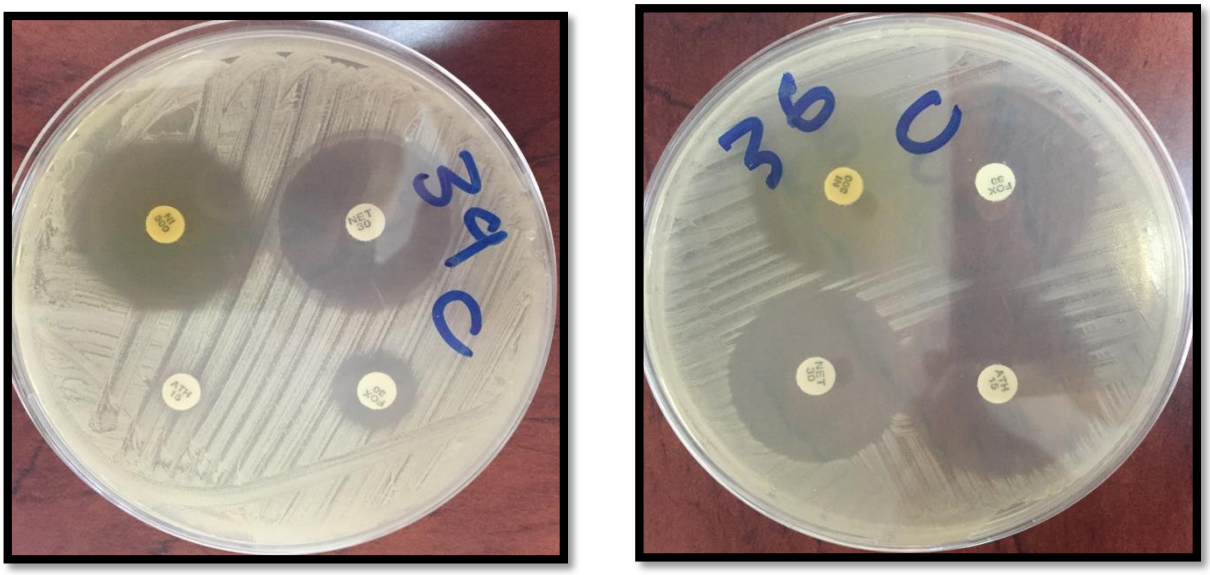


Purity of DNA 260/280



Antibiotic Susceptibility Variations in the latest sample of 60 isolated bacterial isolates.

Bacterial Isolates	Number of isolates
candida albicanse	19
candida tropicalis	3
K. pneumoniae	7
helicobacter pylori	3
Staphylococcus aureus	13
Staphylococcus haemolyticus	13

Lactobacillus spp.	2
Total	60

Sample	TS	TM	TGC	AK	Amp	C	TE	DXT	GAT	NET	FOX	NI	ATH
1	M	S	M	M	R	S	M	M	M	M	M	M	M
2	M	S	M	M	R	S	M	S	M	M	R	M	M
3	S	S	M	S	M	S	S	M	M	M	M	S	M
4	S	S	S	S	M	S	S	S	S	M	S	S	S
5	S	S	S	S	R	S	S	S	S	M	S	S	M
6	S	S	M	S	R	S	S	S	M	M	S	S	S
7	S	S	S	S	M	S	S	S	S	S	S	S	S
8	S	S	S	S	M	S	S	S	M	M	S	S	S
9	S	S	S	S	R	S	S	S	S	M	S	S	S
10	S	S	S	S	R	S	S	S	M	M	S	S	S
11	S	S	M	S	R	S	M	R	S	M	R	S	S
12	S	S	S	M	R	S	R	S	M	R	S	M	S
13	S	S	M	M	R	S	M	S	R	M	S	S	M
14	S	M	M	M	R	M	M	M	M	S	S	S	M
15	S	S	M	M	R	S	M	M	M	M	S	S	R
16	S	S	M	M	M	S	M	S	M	R	S	M	R
17	S	S	M	M	R	S	M	M	M	M	M	S	M
18	S	S	M	M	R	S	M	S	M	M	S	S	M
19	S	S	M	M	R	S	M	M	M	R	M	S	M
20	S	S	M	M	R	R	R	R	M	M	R	S	M
21	S	S	M	M	R	S	M	M	M	M	R	R	R
22	S	S	M	M	R	S	M	M	M	M	S	S	M
23	S	S	M	M	R	S	M	S	M	M	M	M	M
24	M	S	M	M	R	M	R	S	M	R	S	M	M
25	S	M	M	M	R	M	R	R	R	R	M	S	R
26	S	M	M	M	R	M	M	M	R	R	R	M	R
27	S	S	M	M	R	S	R	S	M	M	S	S	R
28	M	R	R	M	R	M	M	R	R	R	R	M	R
29	M	M	S	M	R	M	M	R	R	M	S	M	M
30	M	M	S	M	R	S	R	R	M	R	R	M	M
31	S	M	M	R	R	M	R	R	R	M	S	S	R
32	S	S	M	M	R	M	R	R	R	M	M	S	R
33	S	S	S	S	S	S	S	S	M	M	S	S	S
34	M	S	R	S	S	S	R	S	R	M	S	M	S
35	S	S	M	S	M	S	S	S	M	M	S	S	S
36	M	S	S	S	S	S	S	M	S	M	S	S	S
37	S	S	S	S	M	S	S	S	S	M	S	S	S
38	S	S	M	S	S	S	S	S	M	M	S	S	S
39	R	M	M	S	R	S	R	R	S	M	R	S	R
40	M	S	M	M	R	S	M	M	S	S	S	M	S
41	M	S	S	M	R	M	M	S	M	R	S	R	S
42	S	S	M	M	M	S	M	M	M	M	S	S	M
43	M	S	M	M	S	S	M	S	M	M	S	S	S
44	S	M	S	S	M	M	M	S	R	M	S	M	M
45	S	S	M	S	R	M	S	S	M	M	M	S	M
46	S	S	S	S	M	S	S	S	R	R	S	S	S

47	M	R	M	R	R	R	R	R	R	R	R	R	R
48	S	S	M	M	R	S	M	S	S	M	S	M	M
49	S	M	M	R	R	S	M	S	M	R	S	S	R
50	S	M	R	R	R	M	R	R	M	M	S	M	R
51	R	R	M	M	R	S	R	R	S	R	R	M	R
52	M	M	M	M	R	M	R	S	M	R	S	M	M
53	M	S	M	M	R	M	M	M	R	R	S	R	S
54	M	M	M	M	R	M	M	M	M	R	M	M	R
55	M	M	R	M	R	M	R	S	R	R	S	M	M
56	M	M	R	M	M	R	R	R	R	R	R	M	M
57	M	M	M	R	R	S	R	S	S	R	S	M	R
58	M	M	M	M	R	R	R	M	R	S	S	S	S
59	S	M	S	S	M	M	R	M	M	M	S	M	R
60	S	S	M	M	R	R	M	R	R	R	S	M	R
R	**2**	**3**	**5**	**5**	**43**	**5**	**19**	**14**	**16**	**20**	**11**	**4**	**18**
M	**19**	**17**	**39**	**35**	**12**	**17**	**26**	**16**	**32**	**36**	**9**	**23**	**22**
S	**39**	**40**	**16**	**20**	**5**	**38**	**15**	**30**	**12**	**4**	**40**	**33**	**20**

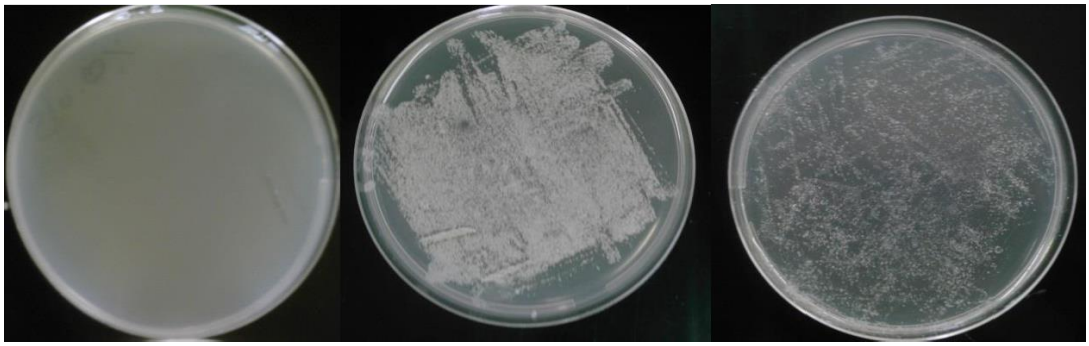
Direct effect (without dilution) of samples on bacterial isolates using the cross-drilling method

Inhibition zone diameter (ml)					the sample
S. haemolyticus	*S. aureus*	*K. pneumoniae*	*candida tropicalis*	*candida albicanse*	
31	29	29	33	35	Honey bee venom 1
31	29	29	33	37	Honey bee venom 2
30	29	31	34	35	Honey bee venom 3
25	27	25	22	22	Honey 1
23	26	24	22	22	Honey 1
25	25	25	25	23	Honey 2
19	20	22	21	20	Wax 1
20	22	22	20	20	Wax 2
20	22	24	20	23	Wax 3

0	**0**	**0**	**0**	**0**	**Distilled water**

Specify MIC and MFC

The minimum inhibitory concentration and lethal bacterial concentration were determined by the dilution method used



a b c

MFC –a

MIC –b

Control –c

Determination of MIC and MFC of samples against the used bacterial isolates using the dilution method

MIC

The values of MIC					**µg/ml**
S. haemolyticus	***S. aureus***	***K. pneumoniae***	***candida tropicalis***	*candida albicanse*	
245	245	234	244	250	**Honey bee venom 1**
250	250	234	239	250	**Honey bee venom 2**
240	245	234	240	250	**Honey bee venom 3**
1000	970	978	964	1000	**Honey 1**
1000	988	990	980	1000	**Honey 1**
998	970	995	1000	1000	**Honey 2**

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2000	2000	2000	2000	2000	**Wax 1**
2000	2000	2000	2000	2000	**Wax 2**
2000	2000	2000	2000	2000	**Wax 3**
0	**0**	**0**	**0**	**0**	**Distilled water**

MFC

The values of MFC					µg/ml
S. haemolyticus	***S. aureus***	***K. pneumoniae***	***candida tropicalis***	*candida albicanse*	
500	488	500	450	500	**Honey bee venom 1**
500	490	500	444	500	**Honey bee venom 2**
500	490	500	450	500	**Honey bee venom 3**
2000	1995	1990	2000	2000	**Honey 1**
2000	1994	1995	2000	2000	**Honey 1**
2000	1995	1995	2000	2000	**Honey 2**
498	499	5000	496	5000	**Wax 1**
496	499	5000	496	5000	**Wax 2**
498	495	5000	496	5000	**Wax 3**
0	**0**	**0**	**0**	**0**	**Distilled water**

The advantages of bee sting treatment are that there is no patient. Of the patients who were treated with bee venom, it was subjected to death, while many deaths are recorded annually due to the drugs used in the treatment of rheumatic fever, for example, and the affected area of the body by a bee sting tries to heal itself quickly in a way that activates the blood flow in it [20], [21], as bee venom has a strong sterilization property Of germs and viruses, and it can be considered the best antibiotic, equivalent to 1000 times the capacity of penicillin [22] and it is worth mentioning that the average person tolerates More than a thousand bee stings. And extracting bee venom has become easy thanks to the discovery of the electric shock that causes the stinging machine to emerge and its response to the secretion of venom, as several designs

appeared that work on this principle [23], [24], including the Benton machine consisting of a wooden board above which wires are fixed at close distances to each other. This plate is placed at the base of the brood box in The honey bee hive, which leads to the largest number of bees coming into contact with this machine, and when the bees are exposed to a weak electric current that does not exceed 3 volts, the bee stings the piece of nylon installed under the wires, so the poison settles on a glass plate placed directly behind the piece of nylon, and after the poison dries [25], it is scraped with a sharp mousse This design was modified and many other designs came to collect the toxin dry and others to collect the liquid toxin. Other researchers also discovered that honey eliminates the sticking of bacteria with each other in colonies, and this weakens their effect and makes them less resistant to antibiotics [26]. In addition, laboratory and clinical tests have proven that honey has anti-fungal, bacterial and viral properties. It increases the number of red blood cells, stimulates blood circulation, which helps increase activity, and the vitality of the body. It activates the nerve cells in the brain, and thus sends sensory signals that are transmitted to the sensory cells located below the brain. The body was exposed to more than one bite, which helps to damage the skin if the bites are in the same place, meaning that the skin becomes more resistant to temperatures and resistant to bacteria [27- 29]. It reduces weight, when the bee pinches the person, it secretes a substance called ionic saliva, which works to burn body fat in the affected area, as it burns approximately 99% of the fat in that area [30].

4. Conclusion

Natural products are known to be a renewable alternative with less problems than a wide variety of active compounds may create. BV includes a number of bioactive ingredients that play a critical role as antimicrobials, including melittin, apamin, and PLA2. BV trials may well contribute to the potential production of novel pharmaceuticals.

5. References

[1] Hider RC. Honeybee venom: a rich source of pharmacologically active peptides. Endeavour. 1988;12(2):60–65.

[2] Hegazi A, Abdou AM, Abd El-Moez, SI, Abd F. Evaluation of the antibacterial activity of bee venom from different sources. World Appl Sci J. 2014;30(3):266–270.

[3] Jenssen H, Hamill P, Hancock RE. Peptide antimicrobial agents. Clin Microbiol Rev. 2006;19(3):491–511.

[4] Wenhua R, Shuangquan Z, Daxiang S, Kaiya Z, Guang Y. Induction, purification and characterization of an antibacterial peptide scolopendrin I from the venom of centipede Scolopendra subspinipes multilans. Indian J Biochem Biophys. 2006; 43:88–93.

[5] Castro HJ, Mendez-Lnocencio JI, Omidvar B, Omidvar J, Santilli J, Nielsen HS, Jr et al. A phase I study of the safety of honeybee venom extract as a possible treatment for patients with progressive forms of multiple sclerosis. Allergy Asthma Proc. 2005;26(6):470–476.

[6] Fennell JF, Shipman WH, Cole LJ. Antibacterial action of bee venom fraction against a penicillin-resistant Staphylococcus and other microorganisms. Res Dev Tech Rep. 1967; 5:1–13.

[7] Fennell JF, Shipman WH, Cole LJ. Antibacterial action of Melittin, polypeptide from bee venom. Proc Soc Exp Biol Med. 1968;127(3):707– 710.

[8] Dani MP, Richards EH, Isaac RE, Edwards JP. Antibacterial proteolytic activity in venom from the endoparasitic wasp Pimple hypochondriaca (hymenoptera: Ichneumonidae) J Insect Physiol. 2003;49(10):945–954.

[9] Permual SR, Pachiappan A, Gopalakrishnakone P, Thwin MM, Hian YE, Chow VT et al. In vitro antibacterial activity of natural toxins and animal venoms tested against Burkholderia Pseudomallei. BMC Infect Dis. 2006; 6:100.

[10] Hegazi AG, EL-Feel MA, Abdel-Rahman EA, Al-Fattah A. Antibacterial activity of bee venom collected from apis mellifera carniolan pure and hybrid races by two collection methods. Int J Curr Microbiol App Sci. 2015;4(4):141–149.

[11] Bauer AW, Kirdy WM, Sherries JC, Turck M. Antibiotic susceptibility testing by standardized signal disk method. Am J Clin Pathol. 1966;45(4):493–496.

[12] Surendra NS, Jayaram GN, Reddy MS. Antimicrobial activity of crude venom extracts in honeybees (Apis cerana, Apis dorsata, Apis florea) tested against selected pathogens. Afr J Microbiol Res. 2011;5(18):2765–2772.

[13] Park JW, Jeon JH, Yoon J, Jung TY, Kwon KR, Cho CK et al. Effects of sweet bee venom pharmacopuncture treatment for chemotherapy- induced peripheral neuropathy: a case series. Integr Cancer Ther. 2012;11(2):166–171.

[14] Kim HW, Kwon YB, Ham TB, Roh DH, Yoon SY, Lee HJ. Acupoint stimulation using bee venom attenuates formalin-induced pain behavior and spinal cord fos expression. J Vet Med Sci. 2003;65(3):349–355.

[15] Kim KS, Choi US, Lee SD, Kim KH, Chung KH, Chang YC et al. Effect of bee venom on aromatase expression and activity in leukaemic FLG 29.1 and primary osteoblastic cells. J Ethnopharmacol. 2005;99(2):245–252.

[16] Leandro LF, Mendes CA, Casemiro LA, Vinholis AH, Cunha WR, de Almeida, R et al. Antimicrobial activity of apitoxin, melittin and phospholipase A_2 of honey bee (Apis mellifera) venom against oral pathogens. An Acad Bras Cienc. 2015;87(1):147–155.

[17] Cujova S, Bednarova L, Slaninova J, Straka J, Cerovsky V. Interaction of a novel antimicrobial peptide isolated from the venom of solitary bee colletes daviesanus with phospholipid vesicles and Escherichia coli cells. J Pept Sci. 2014;20(11):885–895.

[18] Ortel S, Markwardt F. Studies on the antibacterial properties of bee venom. Pharmazie. 1955; 10:743–746.

[19] Lariviere WR, Melzack R. The bee venom test: a new tonic-pain test. Pain. 1996;66(2-3):271–277.

[20] Nunez V, Arce V, Gutierrez JM, Lomonte B. Structuer and functional characterization of myotoxin I, a LYS49 Phospholipase A2 homologus from the snake Bothrops atrox. Toxicon. 2004;44(1):91–101.

[21] Mourelle D., Brigatte P., Bringanti L.D.B., De Souza B.M., Arcuri H.A., Gomes P.C., Baptista-Saidemberg N.B., Ruggiero Neto J., Palma M.S. Hyperalgesic and edematogenic effects of Secapin-2, a peptide isolated from Africanized honeybee (Apis mellifera) venom. Peptides. 2014; 59:42–52.

[22] Meng Y., Yang X.X., Sheng Y.X., Zhang J.L., Yu D.Q. A novel peptide from Apis mellifera and solid-phase synthesis of its analogue. Chin. Chem. Lett. 2012; 23:1161–1164.

[23] Gauldie J., Hanson J.M., Shipolini R.A., Vernon C.A. The structures of some peptides from bee venom. Eur. J. Biochem. 1978; 83:405–410.

[24] Shkenderov S., Koburova K. Adolapin—A newly isolated analgetic and anti-inflammatory polypeptide from bee venom. Toxicon. 1982; 20:317–321.

[25] Drici M.D., Diochot S., Terrenoire C., Romey G., Lazdunski M. The bee venom peptide tertiapin underlines the role of I(KACh) in acetylcholine-induced atrioventricular blocks. Br. J. Pharmacol. 2000; 131:569–577.

[26] Georgieva D., Greunke K., Genov N., Betzel C. 3-D Model of the bee venom acid phosphatase: Insights into allergenicity. Biochem. Biophys. Res. Commun. 2009; 378:711–715.

[27] Rybak-Chmielewska H., Szczęsna T. HPLC study of chemical composition of honeybee (Apis mellifera L.) venom. J. Apic. Sci. 2004; 48:103–109.

[28] Shipolini R.A., Callewaert G.L., Cottrell R.C., Doonan S., Vernon C.A., Banks B.E. Phospholipase A from bee venom. Eur. J. Biochem. 1971; 20:459–468.

[29] Blank S., Seismann H., Bockisch B., Braren I., Cifuentes L., McIntyre M., Rühl D., Ring J., Bredehorst R., Ollert M.W., et al. Identification, recombinant expression, and characterization of the 100 kDa high molecular weight Hymenoptera venom allergens Api m 5 and Ves v 3. J. Immunol. 2010; 184:5403–5413.

[30] Kettner A., Hughes G.J., Frutiger S., Astori M., Roggero M., Spertini F., Corradin G. Api m 6: A new bee venom allergen. J. Allergy Clin. Immunol. 2001; 107:914–920.