Egypt. J. Plant Prot. Res. Inst. (2021), 4 (4): 623 –630

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Antimicrobial activity of bee (Hymenoptera*:* Apidae) venom against pathogens of human and honey bee colonies

Esraa, A.M.A. Badawy[1]; El-Bassiony, M.N. [2]; Mahfouz, H.M.[2] and Mansour, H.A.M. [1]

[1] ***Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.***

[2]***Plant Production Dept., Fac. of Environ. Agric. Sci., Arish Univ., 45511, Egypt.***

ARTICLE INFO

Article History

Received: 17/10 / 2021

Accepted: 29 / 12 /2021

Keywords

Apis mellifera, antimicrobial activity , bee venom, bacterial strains , fungi strains and human .

Abstract:

Experiments were conducted in the Research Complex of Agriculture Faculty (Microbiology Research Laboratory), Cairo University-Giza, Egypt in 2019 season, to study the antimicrobial activity of a mix of bee venom collected from two hybrid, Carniolian bees *Apis mellifera carnica* and Italian bees *Apis mellifera ligustica* (*Hymenoptera*: *Apidae*) at different concentrations using a well diffusion assay against different bacterial and fungi strains which causes diseases of honey bee colony and human. Also, Minimum inhibitory concentration (MIC) of bee venom against fungal disease "Stone brood" caused by *Aspergillus flavus* NRRl 1957 affect honey bees colony. Data indicate the antimicrobial activity of the collected bee venom against different microorganisms that infect both human and honey bee colonies. The present study showed that antifungal effect has a dry bee venom against "Stone brood" caused by *Aspergillus flavus* that affect honey bee colonies. Moreover, the experiment detected the minimum inhibitory concentration (MIC) of the collected bee venom against fungus *Aspergillus flavus*. Moreover, the bee venom concentration at (40 and 80 mg/ml) showed an incomplete reduction of *Aspergillus flavus* growth rate while bee venom concentration at (≥ 320 mg/ml) recorded has the highest reduction rate. Thus, the highest concentration of bee venom showed the highest antifungal and antibacterial activity. Furthermore, the bacterial and antifungal activity of bee venom on different bacterial and fungi strains affecting humans were investigated too in the current study. Concentrations of bee venom were chosen according to previous MIC experiments studied in the current work (320 µg/ml). Bee venom showed a broad antibacterial activity against different G+ve bacterial strains of (*Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus MRSA* ATCC 43300, *Bacillus Cereus* ATCC 33018),and G-ve bacteria (*Salmonella typhimureum* ATCC 14028). In addition, it showed antifungal activity against (*Candida albicans* ATCC 10231 and *Aspergillus niger* NRRL 326) indicating bee venom had broad spectrum activity against a different variety of microorganisms. Finally, bee venom is an important economical product of honey bees colonies in the medical field and plant disease control. Therefore, the light should be shed on bee venom productivity and industry.

Introduction

The products of the honey bee *Apis mellifera* L. (*Hymenoptera*: *Apidae*) are of great concern in (Apitherapy) fields of medicine and pharmaceutical industry nowadays and many researches proved the importance of honey bee and honey bee products. These products such as honey, royal jelly, wax, venom, pollen and propolis are very important due to their nutritive value or important pharmacological activities, which influence different biological and medical aspects of human health. Successful treatments of the central and peripheral nervous system, such as back pain, limb pain, neuralgia, neuritis, articulates polyneuritis and ear inflammation (Munstedt and Bogdanov, 2009). According to Dong *et al.* (2007), the venom also contains mineral substances, volatile-organic acid, formic acid and some antibiotics. Venom is one of the products of honey bee which is an important component in the pharmaceutical industry of use of naturally available substances medicines. Bee venom has interesting pharmacological properties and is used in the treatment of various health condition such as rheumatism (Kim *et al.,* 2003).

Bee venom therapy is a part of apitherapy that utilizes bee venom in the treatment of health conditions. Apitherapy is the use of bee products, such as: honey bee, pollen grain, propolis, royal jelly, and wax and bee venom. It has been used since ancient times and in this modern age as an alternative therapy to treat several diseases like multiple sclerosis, Lyme disease, and chronic fatigue syndrome. Bee venom has a rich source of enzymes, peptides and biogenic amines and contains at least 18 active components (El-Bassiouny, 2007).

Pheromones secretion is considered as one of the main stimuli for inducing an aggressive attitude amongst defending worker bees (Gary, 1974), also the use of the bee venom for medical purposes is known to be a very old practice, especially for rheumatism and thirties. Hypocrites (400 B.C.) have mentioned about bee sting therapy used for arthritis. Nowadays bee venom (Epitoxen) is used for the treatment of naturopaths. It's also used in immunotherapy as a means for decreasing the sensitivity of allergic reactions in individuals who are hypersensitivity to bee stings.

Attention has been made to bee venom collection in abundance from two hybrid from honey bee colonies. In this working bee venom collecting device (VC-4FK) was used under local conditions of North Sinai in Egyptian apiaries ,to determine some factors affecting the bee venom collection from two hybrid honey bee colonies such as: Study the effect of bee venom collection from two hybrid against fungal diseases that affect honey bees colony. Stone brood caused by *Aspergillus flavus* (NRRL 1957) and different strains of fungal and bacterial microorganisms affecting human been.

Materials and methods

Experiments were conducted in the research complex of Agriculture Faculty (Microbiology Research Laboratory), Cairo University-Giza, Egypt in 2019 season, to detect the antimicrobial activity of a mix of bee venom collected from two hybrid, Carniolian bees *Apis mellifera carnica* and Italian bees *Apis mellifera ligustica* (*Hymenoptera*: *Apidae*) against different bacterial and fungi strains that causes diseases of honey bee colony and human been.

Venom preparation:

Bee venom was dissolved in sterile water as it must be freshly prepared to avoid protein denaturation. This experiment included:

1. Effect of bee venom against fungal diseases "Stone brood" that affect honey bees colony:

The current experiment was carried out at Microbiology Research Lab,(Faculty of Agriculture Laboratory), Cairo University and it is considered as the principle experiment in the present study where it detecting both minimum inhibitory concentration (MIC) of antifungal activity of bee venom against fungal diseases that affect honey bees colony such as: stone brood caused by *A. flavus.*

Briefly, the method follows well diffusion assay, where the well which is used in the experiment was 8mm and saturated with 100µl of bee venom at different concentrations(40,80,160, 320 mg/ml). *A. flavus* NRRl 1957 ,which was obtained from (Biofertilizers Unit "Cairo Mircen", was inoculated in Sabaroud Dextrose Agar (TSB, Oxoid ,Basingstoke, UK) at 25 ºC for 24-48hr.The MIC was defined as the lowest concentration of each bee venom sample at which visible inhibition of bacterial growth was induced and the diameter of each inhibition zone was measured by a regular ruler and presented in cm.

2. Antimicrobial and antifungal activity of bee venom on different bacterial and fungi strains affecting human been:

The current experiment was carried out at Microbiology Research Lab, Faculty of Agriculture Laboratory, Cairo University. Each bacterial strain of *Staphylococcus aureus* (ATCC 6538P), *S. aureus* MRSA (ATCC 43300), *Salmonella typhimureum* (ATCC 14028) were grown overnight in Mueller-Hinton Agar medium (TSB, Oxoid, Basingstoke, UK) at 37ºC/24-48 hr., while *Bacillus Cereus* (ATCC 33018) was incubated at 300c/24-48 hr. The *Candida albicans* (ATCC 10231) and *Aspergillus niger* (NRRL 326) were inoculated in Sabaroud Dextrose Agar at 250c/24-48 hr. The method follows well diffusion assay, where the well which is used in the experiment was 8mm and saturated with 100µl of bee venom sample at 320 mg/ml concentration. Concentrations of bee venom chosen according to previous MIC experiment studied in the current work (320 µg/ml). The inhibition zone of bacterial growth was observed, and the diameter of each inhibition zone was measured by a regular ruler and presented in cm.

Results and discussion

Effect of the collected bee venom on different microorganism:

The experiment detected the antimicrobial activity of the collected bee venom from the two bee hybrids against different microorganism of bacterial and fungi strains that affect diseases of both honey bee colony and human been to evaluate the effect of different concentrations of bee venom on their growth.

1. Effect of bee venom against fungal diseases affect honey bee colony "Stone brood" caused by *Aspergillus flavus*:

Data in Table (1) and Figures (1 and 2) showed minimum inhibitory concentration (MIC) of collecting bee venom against fungus *A flavus* as well as its antifungal activity against stone brood caused by *A. flavus* NRRL 1957 that affect honey bees' colony. The results showed that the collected bee venom at high concentrations (≥ 320 µg/ml) recorded the highest reduction rate against fungal cells of Stone brood caused by *A. flavus* NRRL 1957. On the other hand, the low bee venom concentration (40 and 80 µg/ml) showed the lowest inhibition of *A. flavus* NRRL1957 growth rate. Thus, indicated that the higher concentration of bee venom recorded the highest antifungal activity.

Table(1):The minimum inhibitory concentration (MIC) of tested honey bee venom against honey bee's colony fungal disease " Stone brood" caused by *Aspergillus flavus* NRRl 1957.

Disease affects honey bee colony	Microbial Type	Causative Microorganism	Bee venom (MIC)		Medium	Incubation Conditions
			Conc. (mg/ml)	Inhibition Zone (cm)		
stone brood	Fungi	Aspergillus flavus (nrrI 1957)	40	9	Sabaroud Dextrose Agar	25 o C /24 - 48h.
			80	13		
			160	20		
			320	25		

The minimum inhibitory concentration (MIC) of tested honey bee venom against honey bee's colony fungal diseases " Stone brood" caused by *Aspergillus flavus* NRRl 1957

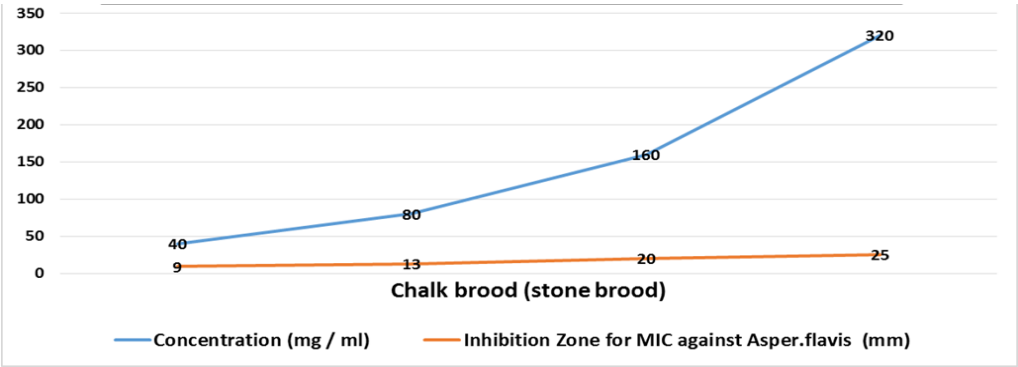

Figure (1): The minimum inhibitory concentration (MIC) of tested honey bee venom against honey bees colony fungal diseases "Stone brood" caused by *Aspergillus flavus* NRRl 1957.

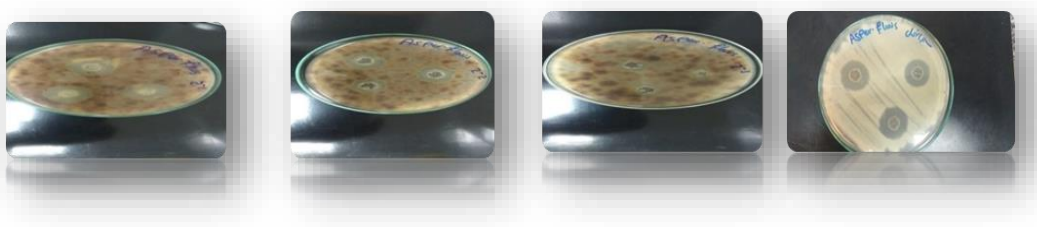

Figure (2): Show effect of bee venom against fungal diseases "Stone brood" caused by *Aspergillus flavus* (MIC).

2. Antimicrobial activity of bee venom against different bacterial and fungi strains affecting human been:

Results in Table (2) and Figures (3 and 4) showed the antimicrobial activity of bee venom against different bacterial and fungi strains affecting humans been using the highest concentrations of bee venom (320 µg/ml) chosen according to previous MIC experiment studied in the current work. In the current study, bee venom showed a broad antibacterial activity against different G+ve bacterial strains of (*Staphylococcus aureus* ATCC 6538P, *S. aureus MRSA* ATCC 43300, *Bacillus Cereus* ATCC 33018), and G-

ve bacteria (*S. typhimureum* ATCC 14028). In addition, it showed antifungal activity against (*Candida albicans* ATCC 10231 and *A. niger* NRRL 326) indicating bee venom broad spectrum activity against different variety of microorganisms.

Table (2): Effect of antimicrobial activity of bee venom Extracted from Italian hybrid on different bacterial and fungi strains affecting human been.

Different Pathogenic Microorganisms Name	Microbial Type	Bee venom Extracted		Medium	Incubation Conditions
		Concentration (mg/ml)	Inhibition Zone(cm)		
Staphylococcus aureus (ATCC 6538P)+	Bacteria	320	15	Mueller - Hinton Agar	37 o C /24 – 48 hrs.
Staphylococcus aureus MRSA+ (ATCC 43300)	Bacteria	320	17	Mueller - Hinton Agar	37 o C /24 - 48 hrs.
Bacillus Cereus+ (ATCC 33018)	Bacteria	320	20	Mueller - Hinton Agar	37 o C /24 - 48 hrs..
Salmonella - typhimureum (ATCC 14028)	Bacteria	320	23	Mueller - Hinton Agar	30 o C /24 - 48 hrs.
Candida albicans (ATCC 10231)	Fungi	320	25	Sabaroud Dextrose Agar	25 o C /24 - 48 hrs.
Aspergillus niger (nrrI 326)	Fungi	320	20	Sabaroud Dextrose Agar	25 o C /24 - 48 hrs.

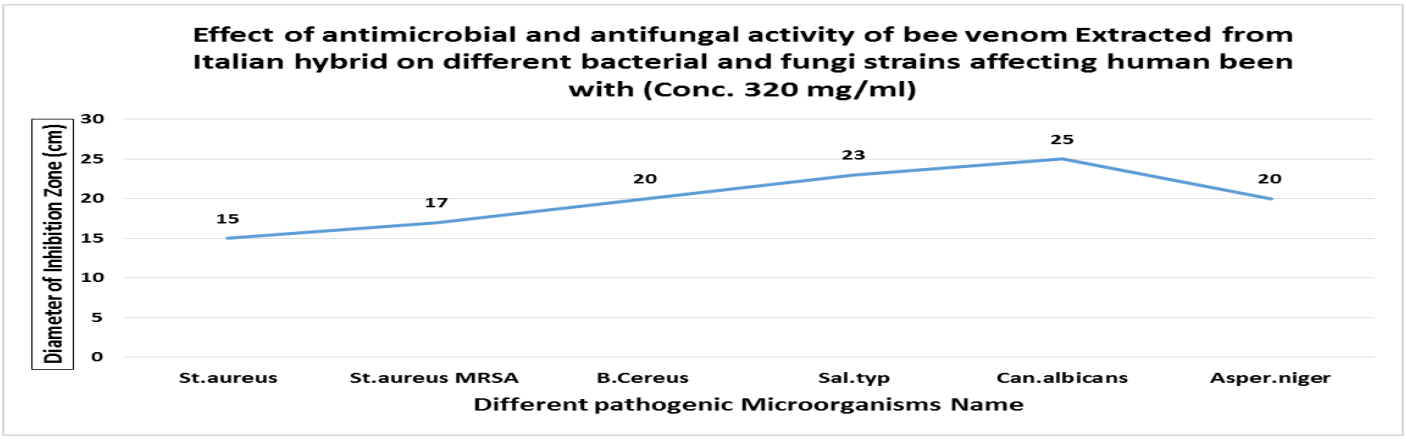

Figure (3): Effect of antimicrobial and antifungal activity of bee venom on different bacterial and fungi strains affecting human.

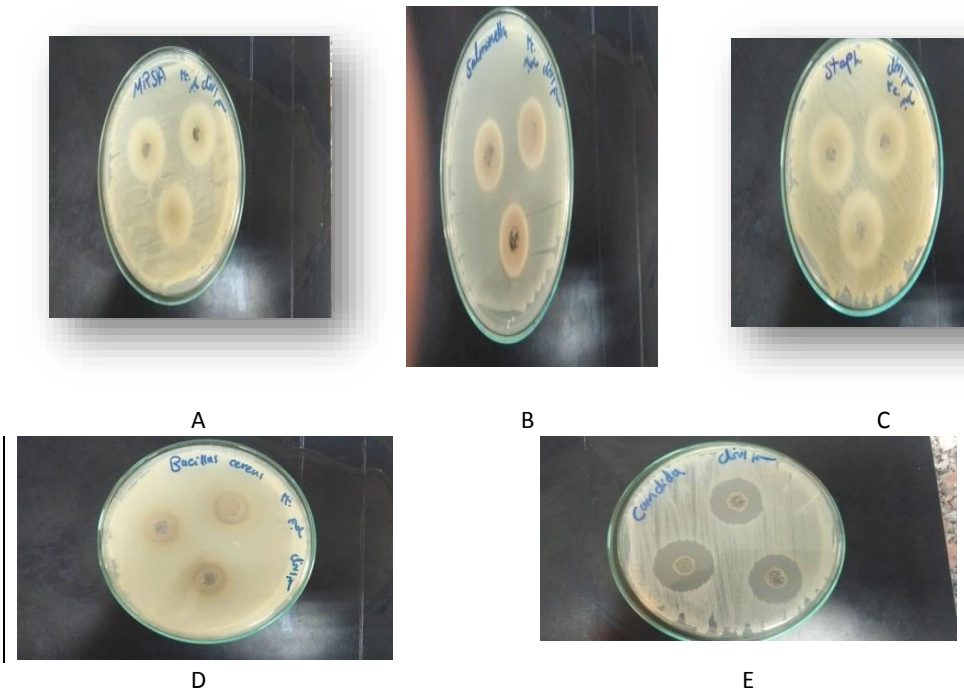

Figure (4): Antibacterial and antifungal activity of bee venom on different bacterial and fungi strains affecting human been such as:
A.B.V against *Staphylococcus aureus* MRSA (ATCC 43300).
B. B.V against *Staphylococcus aureus* (ATCC 6538P).
C.B.V against *Salmonella typhimureum* (ATCC 14028).
D.B.V against *Bacillus Cereus* (ATCC 33018).
E.B.V against *Candida albicans* (ATCC 10231).

References

Beven, L. and Wroblewski, H. (1997): Effect of natural amphipathic peptides on viability, membrane potential, cell shape and motility of mollicutes. Res. Micro.,148(2) 163-175.

Dong , Ju Son; Jae, Woong Lee; Young, Hee Lee; Ho, Sueb Song; Chong Kil Lee and Jin, Tae Hong (2007): Therapeutic application of anti – arthritis, painreleasing, and anti- cancer effect of bee venom and its constituent compounds. Pharmacology &Therapeutics, 115 (2):246-270.

El-Bahnasy, S.A. (2017): Studies on collection of bee venom from different strains and its effect on the activity of hive with referring to its antimicrobial activity M.Sc. Thesis, Fac. Environ. Agric. Sci., EL-Arish, University.

El-Bassiouny, A. M. (2007): Monitoring the reproductive individual in the oriental hornet *Vespa orientalis*. Arab Univ. J. Sci., 15 (2): 459-511.

Fennell, J.F.; Shipman, W.H. and Cole, L.J. (1968): Antibacterial action of melittin, a polypeptide from bee venom. Proc. Soc. Exp. Biol. Med.,127(3):707-710.

Gary, N.E. (1974): Pheromones that effect the behavior and physiology of honey bees, pheromones, Ed. M.C. Birch. Amsterolam: N. Holland, 200-201.

Han, S.M.; K.G. Lee; Park, K.Y. and Pak, S.C. (2013): Antimicrobial activity of honey bee venom against select infectious fish pathogens. North American Journal of Aquaculture, 75(3): 445-448.

Hegazi , A.G.; Abdou, A.M. and Abd Allah, F. (2014): Evaluation of the antibacterial activity of bee venom from different sources. World Applied Sciences Journal, 30(3): 266-270.

Hegazi, A. G.; El-Feel, M. A.; Abdel-Rahman, E.H. and Al-Fattah, M. A. A. (2015): Antibacterial activity of bee venom collected from *Apis mellifera* Carniolan pure and hybrid races by two collection methods. Int .J. of Current Microbiology and Applied Sci., 4(4): 141-149.

Hegazi, A. G.; Moharram, N. Z.; Abd-Allah, F. A.; Nour, M. S. and Khair, A.M. (2002): Antibacterial activity of different Egyptian honeys in relation to some bee products. Egyptian J. of Veterinary Science, 36 (3): 1-42.

Inoue, H.; Nakajima, T. and Okada, I. (1987): The venomous components of the worker and queen honey bees during their nutrition and the seasonal generation. Japanese of Sanitary- Zoology, 38: 211-217.

Kim, H.W.; Kwon, Y.B.; Ham, T.B.; Roh, D.H.; Yoon, S.Y. and, Lee, H.J. (2003): Acupoint stimulation using bee venom attenuates formalin-induced pain behavior and spinal cord fos expression. J .Vet. Med. Sci., 65(3):349-355.

Kondo, E. and Kanai, K. (1986): Bactericidal activity of the membrane fraction isolated form phagocytes of mice and its stimulation by melittin. Japan, J. Med. Sci. Biol., 39(1):9-20.

Kwon, Y.B.; Lee, H.J. ; Han, H.J.; Mar, W.C. ; Kang, S.K. ; Yoon, O.B.; Beitz, A.J. and Lee, J.H.(2002): Antinociceptive and anti-inflammatory effects on rheumatoid arthritis in rats. Life Sci., 71 (2): 191-204.

Marz, R.; Mollay, G.; Krell, R. and Zelger, J. (1981): Queen bee venom contains much less phospholipase than worker bee venom . Insect Biochem., I I : 685-690.

Matsuzaki, K. (1997): Molcular action mechanisms and membrane recognition of membrane-acting antimicrobial peptides, Yakugaku Zasshi, 117(5): 253-264.

Metwaly , A.A. (2016): Studies on honey bee venom. Ph.D. Thesis, Fac Environ. Agric. Sci., Cairo, Al-Azhar University.

Mohamed, W. F.(2017): Studies on honeybee venom production and its antimicrobial activity M.Sc. Thesis, Fac. Environ. Agric. Sci., EL-Arish, University.

Monk, J. D.; Beuchat, L. R. and Hathcox, A. K. (1996): Inhibitory effects of sucrose monolaurate, alone and in combination with organic acids, on *Listeria monocytogenes* and *Staphylococcus aureus*. J. Appl. Bacteriol., 81: 7-18.

Munstedt, K. and Bogdanov, S. (2009): Bee product and their potential use in modern medicin .J. of Api. Product and Api. Medical Sci.,1(3):57-63.

Nour, M.E.; Zakaria, M.E. and Abdel-Wahab , T.E. (2004): Electrophoretic studies on venom properties of the honey

bee (*Apis mellifera* L.). Bull. Ent. Soc. Egypt, 81: 43-46.

Omar, M.O.M. (1994): Some factors affecting bee venom extraction from honeybee colonies, Assiut J. Agric. Sci., 25: 139-148.

Oren, Z. and Shai, Y. (1997): Selective lysis of bacteria but not mammalian cells by diatereomers of melittin : Structure-Function study. Biochemistry, Feb., 36 (7) : 1826-1835.

Ortel, S. and Markwardt, F. (1955): Studies on the antibacterial properties of bee venom. Pharmazie, 10(12): 743-746.

Park, S.; Park, B.; Yun, S.; Kang, H.; So, B. and Yun, S. (2013): Antimicrobial activities of honey bee venom against pathogens isolated from clinical bovine mastitis in Korea Planta Med., 79 – PL 16.

Perumal, S. R. P.; Gopalakrishnakone, M. M.; Thwin, T. K.; Chow, H.; Bow, E. H.; Yap, T. W. and Thong, J. (2007): Antibacterial activity of snake, scorpion and bee venoms: a comparison with purified venom phospholipase A2 enzymes. J. Appl. Microbiol.,102(3):650-659.

Robinson, A. and Otis, W. (1996): Bee venom: Concern about variability, Am. Bee J., 136(8): 584-588.

Stocker, J.F. and Traynor, J.R. (1986): The action of various venoms on Escherichia coli. J. Appl. Bacteriol., 61 (5): 383-388.

Subbalakshmi , C.; Nagaraj, R. and Sitaram, N. (1990): Biological activities of C-terminal 15-residue synthetic fragment of melittin: design of an analog with improved antibacterial activity .FEBS Lette., 448(1) : 62-66.