**Journal of Biosciences and Medicines, 2016, 4, 95-104**
Published Online July 2016 in SciRes. http://www.scirp.org/journal/jbm
http://dx.doi.org/10.4236/jbm.2016.47010

# Antimicrobial Activity of Purified Toxins from Yellow Wasp *Polistes flavus* (Vespidae) against Certain Bacteria and Fungi

**Krishna Kumar Prajapati, Ravi Kant Upadhyay**

Department of Zoology, DDU Gorakhpur University, Gorakhpur, India
Email: rkupadhya@yahoo.com

Received 19 May 2016; accepted 17 July 2016; published 20 July 2016



## Abstract

**Yellow Wasp *Polistes flavus* venom toxins were isolated and purified on a Sepharose CL-6B 200 column. Purified proteins were investigated for its antibacterial and antifungal activity against 13 infectious microbial pathogens. Paper disc diffusion and serial micro-dilution assays were performed for the determination of inhibition zone (DIZ) diameters and minimal inhibitory concentration, respectively. Triton X-100 (0.1%) proved to be a good solubilizing agent for toxin/proteins. Higher protein solubilization was observed in the supernatant than in the residue, except TCA (tri-chloroacetic acid). The elution pattern of purified and homogenized sting glands exhibited two major peaks at 280 nm in fraction No. 41 - 61 and 81 - 101. The total yield of protein was 69.21% and specific activity was determined in each fraction. Molecular weights in protein fractions were ranging from 6 - 70 kD. MIC (Minimum Inhibitory Concentration) values were 12.3 µg/ml against *K. pneumonia* 12.3 µg/ml against *E. coli* and *L. acidophilus*, 24.6 µg/ml against *B. cereus*; 49.24 µg/ml against *S. aureus* and *M. luteus*. By agar disc diffusion method, the diameter of inhibition zones in mm in presence of yellow wasp toxins is at a concentration range of 98.56 - 6.9 µg/ml *E. coli* 18.36 ± 0.14, *Bacillus cereus* 14.566 ± 0.21, *L. acidophilus* 18.10 ± 0.21, *Micrococcus luteus* 18.76 ± 0.19, *S. aeurus* 17.36 ± 0.43, *Klebsiella pneuminiae* 19.56 ± 0.21, *Salmonella typhi* 19.96 ± 0.31, *Vibrio cholera* 21.83 ± 0.22, *Pseudomonas aeruginosa* 22.90 ± 0.09, *Aspergillus niger* 21.66 ± 0.16, *Candida albicans* 23.33 ± 0.26, *Rhizopus stolonifer* 24.96 ± 0.16 respectively. The antibacterial and antifungal activity of venom toxin may be due to action on cell membrane, its destruction and cell lysis. The wasp toxins may be used as strong biological agents to control microbes.**

## Keywords

**Wasp Venom Toxins, Peptides, Antimicrobial Activity, Inhibition Zone Diameter**

**How to cite this paper:** Prajapati, K.K. and Upadhyay, R.K. (2016) Antimicrobial Activity of Purified Toxins from Yellow Wasp *Polistes flavus* (Vespidae) against Certain Bacteria and Fungi. *Journal of Biosciences and Medicines*, **4**, 95-104.
http://dx.doi.org/10.4236/jbm.2016.47010

## 1. Introduction

Hymenopterans insects mainly wasps, honey bees, hornets inflict venom to maintain self-defense for protection of territory. Hymenoptera venom is a secreted from social poison glands of insects attached to a sting apparatus present in last segment of the body. It serves both as defensive substances against aggressors as well as weapon used to paralyze the victim during gaining food. After sensing little disturbance in the territory, wasps make lethal attack in large numbers and they inflict venom into the body of mammals mainly man and his pets [1]. Wasps react and respond very fast to make an attack on predators and mammals. It is also highly toxic to microorganisms. These inflict venom into the body of enemy within no time by opening venom apparatus and charge upon heavily for making self defense. Venom toxins have been evolved to capture prey and make defense against predators and/or microorganisms. Generally wasp envenomation occurs after little disturbance occurred in near vicinity of their hive. Chemically, the venom is a mixture of biologically active substances of high-medium, and small molecular weight with a variety of physiological function [2]. Wasp venom is a complex cocktail of several hundreds of different components and lethal toxins. It is a complex mixture of active amines (serotonin, histamine, tyramine, dopamine, noradrenaline and adrenaline), peptides (pain producing peptides such as kinins and chemotactic peptides like mastoparans or crabrolin) and proteins including many types of enzymes such as hydralases (proteases, hyaluronidase, phosphatases, nucleotidases, phospholypases A) as well as allergens [3] and toxins [4]. Wasp venom toxins possess a number of charged amino acids which are highly critical for their biological activity. The wasp venom is highly toxic to small mammals in which it causes tissue irritation, swelling and inflammation and pain. Venom toxins induce severe anaphylaxis in animals and man soon after stinging [5] and cause early and delayed hypersensitivity, inflammatory reaction, necrosis and toxic complications in mammals and even invertebrates animals [6]. Venom toxins of *Polybia paulista* showed genotoxic and mutagenic effects. Polyamine toxin from yellow wasp is potent open-channel blockers of ionotropic glutamate (IGlu) receptors that show selective ligand binding [7].

Wasp stinging impose multisystem changes and show wide range of biological effects such as intravascular hemolysis, rhabdomylysis, acute renal failure, cardiac involvement, hepatic dysfunction, thrombocytopenia and coagulopathy. Wasp venom also causes scanty micturation, generalization, swelling, and respiratory distress [8]. Wasp venom toxins cause significant serological changes [9] and impose multisystem changes and show wide range of activities such as intravascular hemolysis, rhabdomylysis. Wasp venom toxins cause acute renal failure, hepatic dysfunction and occasionally thrombocytopenia and coagulopathy. At cellular level a large group of toxins breach the normal barrier to free the movement of molecules across cell membrane. More specifically cytotoxins in large amount cause cytolysis, by which soluble molecules leak out of the cell. Wasp toxins cause hemolytic activity and damage nerve and blood cells [10]. Its low concentration causes mild damage to plasma membrane. Besides these toxins act as enzymes and hydrolyze membrane phospholipids [11] and insert themselves into membrane and form channel blockage by binding through which small molecules may pass [12].

The wasp venom is a toxic substance that causes severe inflammation, pain and allergic reactions after infliction in farmers, researchers, free dwellers, rural and urban people. After an intense attack in large numbers with multiple stings, patient generates fast immediate (anaphylactic) responses that show severe or even fatal illness. Wasp venom toxins generate toxic effects with multiple organ dysfunction followed by anaphylactic reaction [13]. It causes significant serological changes [9] and severely effect blood biochemical parameters and generates toxic effects in man [14]. Patients face serious physiological effects like rhabdomyolysis, hemolysis, cerebral disturbances, hepatic and renal dysfunctions after multiple stings. Although allergic reactions to hymenoptera stings are often considered as general model for the underlying principles of the allergic diseases, hence venom immunotherapy is highly applicable to release or cut down systemic side effects of allergens [15].

Hymenopteran insect venom is a rich source of bioactive compounds that are highly useful tools in neuroscience and pharmacological investigations. Components of wasp venom can be used as important pharmacological tools or powerful probes or diagnostic techniques for the elucidation of complex biological processes of pharmaceutical importance. Currently, various toxin structures are used to drug design suitable three-dimensional templates for creating small molecules, which might mimic interesting pharmacological properties. Wasp envenomation is a worldwide public health problem that has economic and social implications. Wasp venom also contains some peptides, which are antimicrobial [12] [16] and anticancer in nature [17] and may act like antibiotics [18] [19]. In the present study antibacterial activity of purified yellow wasp toxins were evaluated against seven bacteria by MIC, MBC and zone inhibition. No doubt wasp venom toxins are of very high pharmacological importance. Several insecticidal compounds belonging to the class of peptides or polyamines like compounds

have been purified and characterized from the venom of arachnids and hymenoptera. Few invertebrates' specific peptides neurotoxins that have been isolated from the venom of wasp which are used as good pest control agents and are also used as invaluable tools in neuropharmacology [20]. Toxin peptides can also become a good source of strong antimicrobials and can replace broad spectrum antibiotics which are highly toxic and show multiple biological effects and responsible for drug resistance in microbes [21].

## 2. Material and Method

### 2.1. Microbial Culture

*Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 11778), *Lactobacillus acidophilus* (ATCC 53103), *Micrococcus luteus* (ATCC 9341), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 15380) and *Streptococcus pneumoniae* (ATCC 12755), *Salmonella typhi* (*MTCC* 98), *Vibrio cholera* (MTCC 3906), *Pseudomonas aeruginosa* (*MTCC* 4996), *Aspergillus niger* (*MTCC* 1344), *Candida albicans* (*MTCC* 227), *Rhizopus stolonifer* (*MTCC* 2456) were maintained in the laboratory in Luria Broth (2% w/v) for four days at 37˚C before use, 100 µl of the overnight culture was mixed in the tests and control. Bacterial cultures were stored at 4˚C and sub cultured after every 8$^{th}$ day in solid agar plates.

### 2.2. Purification of Wasp Venom

The living yellow wasp *Polistes flavus* were collected from different region of Gorakhpur. They were immobilized by quick freezing at −20˚C. The venom reservoir *i.e.*, venom glands were taken out by last segment of abdomen region of wasp and homogenized in phosphate buffer saline (50 mM, pH 7.2) with the help of power homogenizer. The homogenate was centrifuged at 3000 g at 4˚C for 5 minutes and the supernatant was used as crude venom.

Field collected yellow wasp *Polistus flavus* 100 in number were anesthetized with chloroform, dissected in cold PBS (pH 6.9) and their sting apparatus with venom gland were taken out from its last segment. These were homogenized in a glass-glass homogenizer in five ml of different solubilizing buffer viz. Triton X-100, Phosphate buffer (50 mM, pH 7.2), 10% TCA (Trichloroacetic acid), EDTA + Tris [Hydroxy methyl Methyl amino] and Ethanol separately. Homogenate was centrifuged at 10,000 g at 4˚C for 5 minutes and supernatant was taken out and venom protein present in the supernatant was estimated by using Lowry's method [22]. Besides this, proteins (tissue) were solubilized in other solubilizing agents (Triton X-100, PBS, 10% TCA, and EDTA + Tris) in different combinations. Homogenate was centrifuged at 12,000 rpm for 30 min and proteins were estimated in supernatant according to Lowry's method (1951) [22].

### 2.3. Solubilization of Venom Gland Homogenate

Proteins were eluted on a Sepharose CL-6B 200 a double cavity gel filtration column [23] with sintered disc fitted in the bottom having a height of 1 meter in 25mm diameter. A known volume of toxins/proteins solubilized in PBS (pH 6.8), was loaded in the column and a flow rate between 20 - 24 ml/hr was maintained by a continuous buffer supply in a cold room. Eluted fractions were collected at a fixed time interval using a Pharmacia fraction collector and the values of protein concentration in different eluted fractions were plotted on graph; absorbance was determined at 280 nm. Column was tightly held by clips and held erect with a stand. The eluted fractions containing venom proteins were pooled and lyophilized to a desired concentration of venom proteins. Dialysis bag made of cellulose membrane was boiled for 10 minutes in a large volume of 2% (w/v) sodium bicarbonate and 1 mM EDTA (pH 8.0) and then rinsed the membrane thoroughly in distilled water. The membrane was then cooled and stored at 4˚C. Membrane was washed with distilled water inside and outside before use. The lyophilized venom protein was filled in the dialyzing bag and dialyzed again three changes of phosphate buffer (50 mM, pH 7.2) to remove the excess salt from the lyophilized protein venom solution of *Polistes flavus*.

### 2.4. Antimicrobial Assays

Wasp venom toxin was evaluated for antibacterial activity by agar disc diffusion method. Six mm sterile filter paper discs (Whatman No. 1) were coated with four different concentrations of yellow wasp toxin, prepared in

phosphate buffer saline (pH 6.9). Inoculum size was adjusted to $10^6$ colony-forming units (CFU/ml). It was spread evenly on agar plate surface by a sterile rubber pad. Each toxin was assayed in triplicate. Sterile distilled water was used as negative control. Tetracycline, ampicillin and ciprofloxacin were used for comparison. Plates were incubated for 24 h at 37˚C and diameter of inhibition zones were measured (NCCLS, 1993) [24].

Susceptibility tests in liquid medium were conducted according to Amsterdam *et al*. (1996) [25]. Wasp toxin was diluted by serial micro dilution method (up to $10^{-10}$) using Luria Broth, final concentrations ranged from 58.57 to 0.229 mg\ml and assays were done in triplicate. MIC values were the lowest concentration of yellow wasp toxins where no turbidity was observed in the culture flask after 24 hours incubation at 37˚C and it was standardized in terms of absorbance at 600 nm in a spectrophotometer. For the determination of minimum bacterial concentration (MBC) inoculum size was adjusted to $10^6$ CFU/ml in sterile agar plates and determined again after incubation at 37˚C for 24 h in all test and control discs. The lowest concentration at which no visible growth was obtained in agar plates was considered as MBC value. For evaluation of inhibition two parallel controls were set for each test extract. Bacterial growth was observed in presence of different quantities of wasp venom toxin as well as in its absence.

## 3. Results

Triton X-100 (0.1%) proved to be a good solubilizing agent for toxins/proteins. Higher protein solubilization was observed in the supernatant than in the residue, except TCA (**Figure 1**). The elution pattern of purified and homogenized sting glands exhibited two major peaks at 280 nm in fraction no. 41 - 61 and 81 - 101 (**Figure 2(a)**). Further concentration and fractionation of venom proteins again revealed two peaks at 640 nm, a minor one between fractions 46 - 51 and a major peak between fractions 60 - 101 (**Figure 2(b)**). Both peaks were eluted with 0.13 M NaCl PBS buffer (pH 6.9) and protein estimation was done for each fraction by using Lowry's method (**Figure 2(c)**). The total yield of protein was 69.21% and specific activity was determined in each fraction (**Figure 2(d)**). Molecular weights in protein fractions were ranging from 6 - 70 kD (**Figure 2(e)**).

MIC values were 12.3 µg/ml against *K. pneumoniae* 12.3 µg/ml against *E. coli* and *L. acidophilus*, 24.6 µg/ml against *B. cereus*; 49.24 µg/ml against *S. aureus* and *M. luteus*. The results suggest that *E. coli* and *B. cereus* were most susceptible to yellow wasp toxins while *K. pneumoniae* was least susceptible. MIC values for tetracycline and ampicillin were 14 µg/ml and for ciprofloxin 28 µg/ml against *K. pneumoniae* (**Table 1**). *Salmonella typhi* 24.69 µg/ml, *Vibrio cholera* 24.69 µg/ml, *Pseudomonas aeruginosa* 12.3 µg/ml *respectively*, *while MIC value obtained against fungal strains i.e. Aspergillus niger* 49.2 µg/ml, *Candida albicans* 12.3 µg/ml, *Rhizopus stolonifer* 6.9 µg/ml (**Table 1**).

By agar disc diffusion method the diameter of inhibition zones in mm in presence of yellow wasp toxins at a concentration range of 98.56 - 6.9 µg/ml *E. coli* 18.36 ± 0.14, *Bacillus cereus* 14.566 ± 0.21, *L. acidophilus* 18.10 ± 0.21, *Micrococcus luteus* 18.76 ± 0.19, *S. aeurus* 17.36 ± 0.43, *Klebsiella pneuminiae* 19.56 ± 0.21,

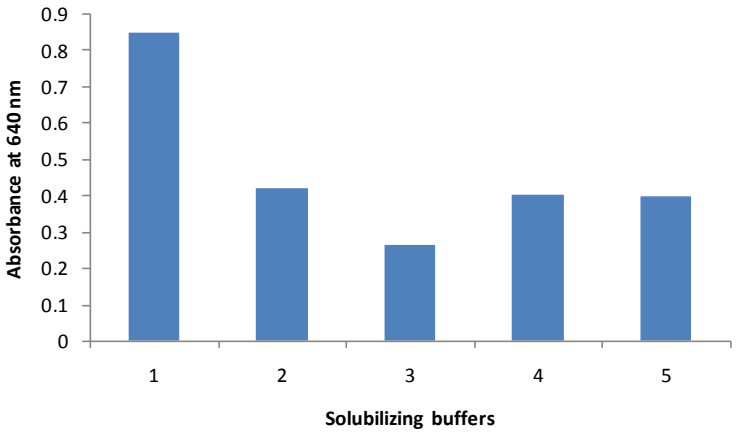


**Figure 1.** Solubilization of sting gland proteins of *Polistes flavus* in different buffers. Absorbance of solubilized protein was taken at 640 nm. Solubilizing buffers on X-axis are (1) Triton X-100, (2) Phosphate buffer, (3) 10% TCA, (4) EDTA+Tris and (5) Absolute Ethanol.

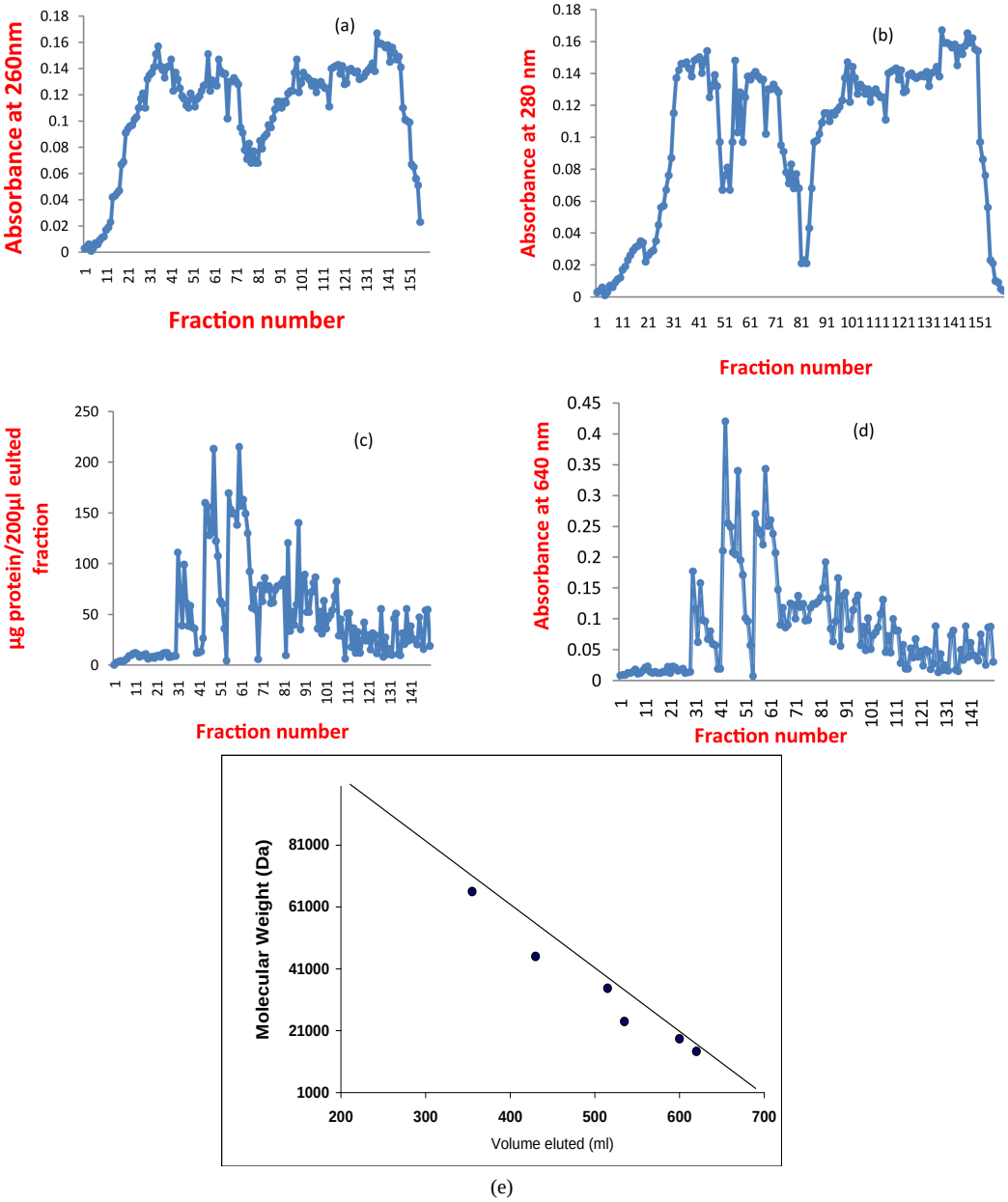


**Figure 2.** Elution pattern of PBS extractable proteins of *Polistes flavus* chromatoghraphed on Sepharose CL-6B 200 column. (a) absorbance at 260 nm., (b) absorbance at 280 nm., (c) absorbance at 640 nm., (d) µg protein/µl 200 fraction (e) standard protein chromatoghraphed on Sepharose CL-6B 200 column for determining the molecular weights of venom proteins/ peptides isolated from the *Polistes flavus*. Standard proteins used were bovine albumin mol. Weight 66,000, egg albumin mol. Weight 45,000, pepsin mol, weight 34,700, trypsinogen mol. Weight 24,000, beta lactoglobulin mol. Weight 18,400 and lysozyme mol. Weight 14,300. Elution volumes of unknown proteins were compared with log values on the X-axis for the estimation of molecular weights.

*Salmonella typhi* 19.96 ± 0.31, *Vibrio cholera* 21.83 ± 0.22, *Pseudomonas aeruginosa* 22.90 ± 0.09, *Aspergillus niger* 21.66 ± 0.16, *Candida albicans* 23.33 ± 0.26, *Rhizopus stolonifer* 24.96 ± 0.16 (**Table 2**).

## 4. Discussion

In the present study, antimicrobial activity of purified yellow wasp toxins was determined *in vitro* and compared with broad-spectrum antibiotics. From antimicrobial susceptibility tests it was found that antibiotics were found

**Table 1.** Antimicrobial activities of venom toxins isolated from *Polistes flavus* on different microbes and their corresponding MIC.

| S.N. | Name of organism | Toxin conc. range used (µg/mL) | Toxin MIC | Antibiotics MIC Controls | | | |
|---|---|---|---|---|---|---|---|
| | | | Test | Negative | Positive 1 | Positive 2 | Positive 3 |
| **1** | *E. coli* | 98.56 - 0.192 | 12.3 | - | 28 | 56 | 28 |
| **2** | *Bacillus cereus* | 49.24 - 0.096 | 24.6 | - | 56 | 28 | 28 |
| **3** | *L. acidophilus* | 98.56 - 0.192 | 12.3 | - | 14 | 28 | 28 |
| **4** | *Micrococcus luteus* | 49.24 - 0.192 | 49.24 | - | 56 | 56 | 112 |
| **5** | *S. aeurus* | 49.24 - 0.096 | 49.24 | - | 56 | 112 | 56 |
| **6.** | *Streptococcus pneumoniae* | 49.24 - 0.096 | 12.3 | | 14 | 28 | 28 |
| **6** | *Klebsiella pneumoniae* | 98.56 - 0.192 | 12.3 | - | 14 | 14 | 28 |
| **7** | *Salmonella typhi* | 98.56 - 0.192 | 24.69 | - | 28 | 56 | 28 |
| **8** | *Vibrio cholera* | 98.56 - 0.192 | 24.69 | - | 28 | 56 | 56 |
| **9** | *Pseudomonas aeruginosa* | 98.56 - 0.192 | 12.3 | - | 28 | 14 | 28 |
| **10** | *Aspergillus niger* | 197.12 - 0.385 | 49.2 | - | 56 Gsf | ND | ND |
| **11** | *Candida albicans* | 98.56 - 0.192 | 12.3 | - | 28 Gsf | ND | ND |
| **12** | *Rhizopus stolonifer* | 49.24 - 0.192 | 6.9 | - | 14 Gsf | ND | ND |

*Positive controls are 1) tetracycline, 2) ampicillin & 3) ciprofloxacin and T represents wasp toxins, negative control is DMSO, Gsf Griseofulvin, ND = not done.

**Table 2.** Zone of inhibition of wasp venom toxin isolated from *Polistes flavus* on different microbes and their corresponding IZD.

| S.N. | Name of organism | Toxin conc. range used (µg/mL) | Wasp toxin | Antibiotics IZD in mm (control) | | | |
|---|---|---|---|---|---|---|---|
| | | | IZD in mm | Negative | Positive 1 | Positive 2 | Positive 3 |
| **1** | *E. coli* | 98.56 - 0.192 | 18.36 ± 0.14 | | 15.66 ± 0.22 | 16.43 ± 0.28 | 9.86 ± 0.17 |
| **2** | *Bacillus cereus* | 49.24 - 0.096 | 14.566 ± 0.21 | ± | 13.90 ± 0.14 | 12.30 ± 0.18 | 9.33 ± 0.15 |
| **3** | *L. acidophilus* | 98.56 - 0.192 | 18.10 ± 0.21 | ± | 16.00 ± 0.29 | 10.30 ± 0.21 | 12.83 ± 0.26 |
| **4** | *Micrococcus luteus* | 49.24 - 0.192 | 18.76 ± 0.19 | ± | 17.66 ± 0.24 | 10.23 ± 0.19 | 14.13 ± 0.19 |
| **5** | *S. aeurus* | 49.24 - 0.096 | 17.36 ± 0.43 | ± | 15.23 ± 0.38 | 10.63 ± 0.25 | 11.16 ± 0.21 |
| **6** | *Streptococcus pneumoniae* | 49.24 - 0.096 | 15.30 | ± | 13.86 ± 0.11 | 9.1 ± 0.32 | 12.03 ± 0.34 |
| **7** | *Klebsiella pneumoniae* | 98.56 - 0.192 | 19.56 ± 0.21 | ± | 17.06 ± 0.21 | 14.23 ± 0.21 | 9.80 ± 0.18 |
| **8** | *Salmonella typhi* | 98.56 - 0.192 | 19.96 ± 0.31 | ± | 12.46 ± 0.19 | 13.63 ± 0.21 | 12.00 ± 0.24 |
| **9** | *Vibrio cholera* | 98.56 - 0.192 | 21.83 ± 0.22 | ± | 12.06 ± 0.22 | 12.36 ± 0.28 | 13.60 ± 0.23 |
| **10** | *Pseudomonas aeruginosa* | 98.56 - 0.192 | 22.90 ± 0.09 | ± | 13.80 ± 0.21 | 12.76 ± 0.15 | 14.66 ± 0.28 |
| **11** | *Aspergillus niger* | 197.12 - 0.385 | 21.66 ± 0.16 | ± | 17.36 ± 0.33 | ND | ND |
| **12** | *Candida albicans* | 98.56 - 0.192 | 23.33 ± 0.26 | ± | 18.73 ± 0.33 | ND | ND |
| **13** | *Rhizopus stolonifer* | 49.24 - 0.192 | 24.96 ± 0.16 | ± | 17.93 ± 0.30 | ND | ND |

*Values are expressed as mean ± SD ($N$ = 3) and values followed by same letter are not significantly different at the $p < 0.05$ determined by Duncan's Multiple Range Test. Negative control is DMSO, Positive control are 1) tetracycline, 2) ampicillin & 3) ciprofloxacin and T represents wasp toxins.

less active than the wasp venom toxins against the test bacteria and fungi. From the inhibition zone diameter assays the IZDs obtained were larger in case of wasp venom than antibiotics. Yellow wasp venom toxin (16.4 mg/ml) gave inhibition zone diameters of 18 - 23 mm while tetracycline, ampicillin, and ciprofloxacin gave inhibition

zone diameters in the range of 15 - 26 mm at a concentration of 8 mg/disc (**Table 1**). By agar disc diffusion method the diameter of inhibition zones in mm in presence of yellow wasp toxins at a concentration range of 6.9 - 49.24 µg/ml were 17.36 ± 0.43, 19.56 ± 0.21, 14.566 ± 0.21, 18.36 ± 0.14, 18.10 ± 0.21, 18.76 ± 0.19 for *S. aureus*, *K. pneumoniae*, *B. cereus*, *E. coli*, *L. acidophilus* and *M. luteus* respectively. Inhibition zone diameters in mm for tetracycline, ampicillin and ciprofloxacin against *S. aureus*, *K. pneumoniae*, *E. coli*, *L. acidophilus* and *M. luteus*, *Streptococcus pneumoniae* and *Bacillus cereus* were 15.23 ± 0.38, 10.63 ± 0.25, 11.16 ± 0.21; 17.06 ± 0.21, 14.23 ± 0.21, 9.80 ± 0.18; 15.66 ± 0.22, 16.43 ± 0.28, 9.86 ± 0.17; 16.00 ± 0.29, 10.30 ± 0.21, 12.83 ± 0.26; 17.66 ± 0.24, 10.23 ± 0.19, 14.13 ± 0.19 respectively (**Table 2**). In all the cases venom toxins displayed much better antimicrobial action against bacterial and fungal strains. The results suggest that 22.90 ± 0.09 was most susceptible to yellow wasp toxins while *B. cereus* and *S. aeurus* least susceptible (**Table 2**). Similar results were also obtained in Egyptian yellow wasp on the growth of *Aspergillus versicolor* [26].

Melittin, a highly toxic and immunogenic peptide isolated from honey bee, shows an MIC value between 0.5 - 0.03125 mg/ml against *B-pseudomallei* [27] [28]. It also exhibits powerful *in vitro* inhibitory activity against spirochetes [29]. Yellow wasp venom also contains few other peptides such as apamin, procamine, abcaecin and secapin [17] [30] [31]. Apamin constitutes less than 2% of venom dry weight; has few amino acid residues and shows neurotoxic properties. It acts as an inductor of convulsion in mice [32] but does not exert any influences on mammalian cells. Apamin also affects sodium and calcium channels in cells and influence nerve transmission [10] [33]. It also blocks the postsynaptic ion channels present in neurons of both vertebrates and invertebrates [34]. Most of the peptides isolated from yellow wasp venom strongly act upon bacterial cell membrane. These toxin peptides interact with lipid membrane, disrupt its integrity [10] [35] [36] and increase the diffusion of molecules from inside to outside of the cell [32] [37]. Few venom peptides which occur as monomer but in presence of salt these transform into tetramer and form helical structure [38] [39]. This tetramer structure forms trans-membrane pores in lipid bilayers and in lipid vesicles [40] [41]. It also acts like a detergent [42] [43] and results in heavy cell lyses [44], and disruption of membrane integrity. This might be due to hydrophilic nature of toxin peptides, and presence of basic amino acids in their active site region [35].

Similar antimicrobial activity is also reported in cationic peptides polybia-MPI [35], anoplin [35] [45] and their synthetic analogues [35] [46]. Anoplin shows membrane anchoring through a lipophilic amino acid derivative and slight changes in its structure shows improvement in biological activity [47]. Wasp venom toxins interact with voltage-sensitive sodium channels and generate action potentials in most of excitable tissues. Membrane depolarization causes voltage-dependent conformational changes that increase the permeability of sodium ions and can work as local anesthetics [48]. $\alpha$-Pompilidotoxin ($\alpha$-PMTX) isolated from the venom of solitary wasp *Anopolis samariensis* [49] greatly facilitates both excitatory and inhibitory synaptic transmission in the lobster neuromuscular synapse [49] as well as disrupts synchronous firing in rat cortical neurons [50]. $\alpha$-PMTX affects presynaptic neurons while carybdotoxins or apamin affects calcium ions activated potassium ion channels [49]. From the results it can be concluded that the purified yellow wasp toxin has displayed similar, better or worse anti bacterial activity against different bacterial and fungal strains in comparison to the three antibiotics tested. These could replace highly toxic antibiotics which can solve the problem of drug resistance.

## Acknowledgements

The authors would like to thanks the D. D. U. Gorakhpur University, Gorakhpur U.P. for providing research opportunity and for the use of laboratory facilities to carry out this study.

## Conflict of Interest

Authors have no conflict of interest. Authors are responsible for the content and writing of the paper.

## References

[1] Hardy, M.C., Cochrane, J. and Allavena, R.E. (2014) Venomous and Poisonous Australian Animals of Veterinary Importance: A Rich Source of Novel Therapeutics. *BioMed Research International*, **2014**, 671041. http://dx.doi.org/10.1155/2014/671041

[2] Ciszowski, K. and Mietka-Ciszowska, A. (2012) Toxicology of Hymenoptera Venoms. *Przeglad Lekarski*, **69**, 519-527.

[3] Rodríguez-Pérez, R., Monsalve, R.I., Galán, A., Perez-Piñar, T., Umpierrez, A., Lluch-Bernal, M., Polo, F. and Caballero, M.L. (2014) Cross-Reactivity between *Anisakis* spp. and Wasp Venom Allergens. *International Archives of Allergy and Immunology*, **163**, 179-184. http://dx.doi.org/10.1159/000358060

[4] Nadolski, J. (2014) Effects of the European hornet (*Vespa crabro* Linnaeus 1761) crude venom on its own species. *Journal of Venomous Animals and Toxins Including Tropical Diseases*, **19**, 4.

[5] Palgan, K., Bartuzi, Z. and Gotz-Zbikowska, M. (2014) Treatment with a Combination of Omalizumab and Specific Immunotherapy for Severe Anaphylaxis after a Wasp Sting. *International Journal of Immunopathology and Pharmacology*, **27**, 109-112.

[6] King, G.F. and Hardy, M.C. (2013) Spider-Venom Peptides: Structure, Pharmacology, and Potential for Control of Insect Pests. *Annu Rev Entomol.*, **58**, 475-496. http://dx.doi.org/10.1146/annurev-ento-120811-153650

[7] Nørager, N.G., Poulsen, M.H., Jensen, A.G., Jeppesen, N.S., Kristensen, A.S. and Strømgaard, K. (2014) Structure-Activity Relationship Studies of N-Methylated and N-Hydroxylated Spider Polyamine Toxins as Inhibitors of Ionotropic Glutamate Receptors. *Journal of Medicinal Chemistry*, **57**, 4940-4949. http://dx.doi.org/10.1021/jm5004705

[8] Jesmin, T., Muinuddin, G., Hossain, M.M., Rahman, M.H. and Mamun, A.A. (2014) Acute Renal Failure Following Wasp Sting. *Mymensingh Medical Journal*, **22**, 609-612.

[9] Sturm, G.J., Kranzelbinder, B., Schuster, C., Sturm, E.M., Bokanovic, D., Vollmann, J., Crailsheim, K., Hemmer, W. and Aberer, W. (2014) Sensitization to Hymenoptera Venoms Is Common, But Systemic Sting Reactions Are Rare. *The Journal of Allergy and Clinical Immunology*, **133**, 1635-1643. http://dx.doi.org/10.1016/j.jaci.2013.10.046

[10] Hider, R.C. and Dotimas, E.M. (1987) Honeybee Venom. *Biochimica et Biophysica Acta*, **911**, 285-293.

[11] Tonismagi, K., Samel, M., Trummal, K., Ronnholm, G. and Siigur, J. (2006) 1-Amino Acid Oxidase from *Vipera lebetina* Venom: Isolation, Characterization, Effects on Platelets and Bacteria. *Toxicon*, **48**, 227-237. http://dx.doi.org/10.1016/j.toxicon.2006.05.004

[12] Fennel, J.E., Shipman, W.H. and Cole, I.J. (1968) Antibacterial Action of Melittin, a Polypeptide from the Venom. *Experimental Biology and Medicine*, **127**, 707-710. http://dx.doi.org/10.3181/00379727-127-32779

[13] Xie, C., Xu, S., Ding, F., Xie, M., Lv, J., Yao, J., Pan, D., Sun, Q., Liu, C., Chen, T., Li, S. and Wang, W. (2014) Clinical Features of Severe Wasp Sting Patients with Dominantly Toxic Reaction: Analysis of 1091 Cases. *PLoS ONE*, **8**, e83164.

[14] Ahmad, S. and Upadhyay, R.K. (2011) Production of Polyclonal Antibodies against Indian Honey Bee (*Apis indica*) Venom Toxins and Its Efficacy in Reversal of Toxic Effects. *African Journal of Biotechnology*, **10**, 10991-11003.

[15] Blank, S., Seismann, H., McIntyre, M., Ollert, M., Wolf, S., Bantleon, F.I. and Spillner, E., (2013) Vitellogenins Are New High Molecular Weight Components and Allergens (Api m 12 and Ves v 6) of *Apis mellifera* and *Vespula vulgaris* Venom. *PLoS ONE*, **8**, e62009.

[16] Casteels, P., Ampe, C., Riviere, L., Van Damme, J., Elicone, C., Fleming, M., Jacobs, F. and Tempst, P. (1990) Isolation and Characterizatio of Abaecin, a Major Antimicrobial Response Peptide in the Honeybee (*Apis mellifera*). *European Journal of Biochemistry*, **187**, 381-386. http://dx.doi.org/10.1111/j.1432-1033.1990.tb15315.x

[17] Mishima, S., Ono, Y., Araki, Y., Akao, Y. and Nozawa, Y. (2005) Two Related Cinnamic Acid Derivatives from Brazilian Honeybee Propolis, Baccharin and Drupannin, Induce Growth Inhibition in Allografted Sarcoma S-180 in Mice. *Biological & Pharmaceutical Bulletin*, **28**, 1025-1030. http://dx.doi.org/10.1248/bpb.28.1025

[18] Hancock, R.E. (1997) Peptide Antibiotics. *Lancet*, **349**, 418-422. http://dx.doi.org/10.1016/S0140-6736(97)80051-7

[19] Hancock, R.E. and Scott, M.G. (2000) The Role of Antimicrobial Peptides in Animal Defenses. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 8856-8861. http://dx.doi.org/10.1073/pnas.97.16.8856

[20] Schwartz, E.F., Mourão, C.B., Moreira, K.G., Camargos, T.S. and Mortari, M.R. (2012) Arthropod Venoms: A Vast Arsenal of Insecticidal Neuropeptides. *Biopolymers*, **98**, 385-405. http://dx.doi.org/10.1002/bip.22100

[21] Ahmed, A. and Beg, A.Z. (2001) Antimicrobial and Phytochemical Studies on 45 Indian Medicinal Plants against Multi-Drug Resistant Human Pathogens. *Journal of Ethnopharmacology*, **74**, 113-123. http://dx.doi.org/10.1016/S0378-8741(00)00335-4

[22] Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein Measurement with Phenol Reagent. *The Journal of Biological Chemistry*, **193**, 265-275.

[23] Spier, R.E. (1982) Gel Filtration Column, Animal Cell Technology: An Over View. *Journal of Chemical Technology and Biotechnology*, **32**, 304-312. http://dx.doi.org/10.1002/jctb.5030320134

[24] NCCLS (National Committee for Clinical Laboratory Standards) (1993) Performance Standard for Antimicrobial Disc Susceptibility Tests. Approved Standard, National Committee for Clinical Laboratory Standards, Villanova, PA Publication M2-A5, USA.

[25] Amsterdam, D. (1996) Susceptibility Testing of Antimicrobials in Liquid Medium. In: Lorian, V. and Baltimore, M.D., Eds., *Antibiotics in Laboratoratory Medicine*, 4th Edition, Williams & Wilikins, Baltimore, 52-111.

[26] Aly, S.A. and Elewa, N.A. (2007) The Effect of Egyptian Honeybee Propolis on the Growth of *Aspergillus versicolor* and Sterigmatocystin Biosynthesis in Ras Chees. *Journal of Dairy Research*, **74**, 74-78. http://dx.doi.org/10.1017/S002202990600207X

[27] Perumal Samy, R.R., Gopalakrishnakone, P.P., Pachiappan, A.A., Thwin, M.M., Hian, Y.E., Bpow, H., Chow, V.T. and Tuck Weng, J.T. (2006) *In Vitro* Antimicrobial Activity of Natural Toxins and Animal Venoms Tested against *Burkholderia pseudomallei*. *BMC Infectious Diseases*, **6**, 100. http://dx.doi.org/10.1186/1471-2334-6-100

[28] Mueller, U., Reisman, R., Elliot, W., Steger, R., Walsh, S., Wypych, J. and Arbesman C. (1982) Study of Chemically Modified Honeybee Venom I. Biochemical, Toxicological and Immunological Characterization. *International Archives of Allergy and Immunology*, **68**, 312-319. http://dx.doi.org/10.1159/000233119

[29] Lubke, L.L. and Garon, C.F. (1997) The Antimicrobial Agent Melittin Exhibits Powerful *in Vitro* Inhibitory Effects on the Lyme Disease Spirochete. *Clinical Infectious Diseases*, **25**, 48-51. http://dx.doi.org/10.1086/516165

[30] Peak, M.L. and Oconnor, R. (1976) Procamine and Other Basic Peptides in Venom of the Honeybee (*Apis mellifera*). *Journal of Agricultural and Food Chemistry*, **22**, 51-61. http://dx.doi.org/10.1021/jf60191a002

[31] Siriwat, wongsiri, Saridwongsa, Wongsathuaythong, Methawee and Suwanagul (1987) Composition of Skin Tests for Honeybee Allergy Using Venom. In: Gopalakrishnakone, P. and Tan, C.K., Eds., *Progress in Venom and Toxin Research*, National University of Singapore, Singapore, 603-615.

[32] Neuman, W., Habermann, E., Amend, G., Apud Banks, B.E.C. and Shipolini, R.A. (1986) Chemistry and Pharmacology of *Honeybee venom*. In: Piek, T., Ed., *Venoms of Hymenoptera*: *Biochemical, Pharmacological and Behavioral Aspects*, Academy Press, London, 329-416.

[33] Edstrom, A. (1992) Venomous and Poisonous Animals. Krieger Publishing Company, Malabar, 210 p.

[34] Hider, R.C. (1988) Honeybee Venom, a Rich Source of Pharmacologically Active Peptides. *Endeavour*, **12**, 60-65. http://dx.doi.org/10.1016/0160-9327(88)90082-8

[35] Wang, K., Yan, J., Dang, W., Xie, J., Yan, B. and Yan, W. (2014) Dual Antifungal Properties of Cationic Antimicrobial Peptides Polybia-MPI: Membrane Integrity Disruption and Inhibition of Biofilm Formation. *Peptides*, **56**, 22-29. http://dx.doi.org/10.1016/j.peptides.2014.03.005

[36] Hoffman, D.R. (1996) Hymenoptera Venom Proteins. *Natural Toxins*, **2**, 169-186. http://dx.doi.org/10.1007/978-1-4613-0361-9_10

[37] Habermann, E. (1972) Bee and Wasp Venoms. *Science*, **177**, 314-322. http://dx.doi.org/10.1126/science.177.4046.314

[38] Brown, L.R., Lauterwein, J. and Wtithrich, K. (1980) High-Resolution H-NMR Studies of Self-Aggregation of Melittin in Aqueous Solution. *Biochimica et Biophysica Acta*, **622**, 231-244. http://dx.doi.org/10.1016/0005-2795(80)90034-3

[39] Thatham, A.S., Tuder, R.C. and Drake, A.F. (1983) The Effect of Counter Ions on Melittin Aggregation. *Biochemical Journal*, **211**, 683-686. http://dx.doi.org/10.1042/bj2110683

[40] Vogel, H. and Jahning, F. (1986) The Structure of Melittin in Membranes. *Biophysical Journal*, **50**, 573-582. http://dx.doi.org/10.1016/S0006-3495(86)83497-X

[41] Rex, S. and Schwarz, G. (1998) Quantitative Studies on the Melittin-Induced Leakage Mechanism of Lipid Vesicles. *Biochemstry*, **37**, 2336-2345. http://dx.doi.org/10.1021/bi971009p

[42] Ladokhin, A.S. and White, S.H. (2001) Detergent Like Permeabilization of Anionic Lipid Vesicles by Melittin. *Biochimica et Biophysica Acta*, **1514**, 253-260. http://dx.doi.org/10.1016/S0005-2736(01)00382-0

[43] Papo, N. and Shai, Y. (2003) Exploring Peptide Membrane Interaction Using Sutaa Plasmon Resonance, Differentiation between Pore Formation versus Membrane Disruption by Lytic Peptides. *Biochem*, **42**, 458-466. http://dx.doi.org/10.1021/bi0267846

[44] Liu, X., Chen, D., Xie, L. and Zhang, R. (2002) Effect of Honeybee Venom on Proliferation of K1735M2 Mouse Melanoma Cells *in Vitro* and Growth of Murine B16 Melanomas *in Vitro*. *Journal of Pharmacy and Pharmacology*, **54**, 1083-1089. http://dx.doi.org/10.1211/002235702320266235

[45] Libardo, M.D., Nagella, S., Lugo, A., Pierce, S. and Angeles-Boza, A.M. (2015) Copper-Binding Tripeptide Motif Increases Potency of the Antimicrobial Peptide Anoplin via Reactive Oxygen Species Generation. *Biochemical and Biophysical Research Communications*, **456**, 446-451. http://www.ncbi.nlm.nih.gov/pubmed/25482446 http://dx.doi.org/10.1016/j.bbrc.2014.11.104

[46] Jindřichová, B., Burketová, L. and Novotná, Z. (2014) Novel Properties of Antimicrobial Peptide Anoplin. *Biochemical and Biophysical Research Communications*, **444**, 520-524. http://www.ncbi.nlm.nih.gov/pubmed/24472551 http://dx.doi.org/10.1016/j.bbrc.2014.01.097

[47] Slootweg, J.C., van Schaik, T.B., Quarles van Ufford, H.L., Breukink, E., Liskamp, R.M. and Rijkers, D.T. (2013) Improving the Activity of the Antimicrobial Peptide Anoplin by Membrane Anchoring through a Lipophilic Amino Acid Derivative. *Bioorganic & Medicinal Chemistry Letters*, **23**, 3749-3752.

http://www.ncbi.nlm.nih.gov/pubmed/23719232
http://dx.doi.org/10.1016/j.bmcl.2013.05.002

[48] Strichartz, G.R., Zhou, Z., Sinnott, C. and Khodorova, A. (2002) Therapeutic Concentrations of Local Anaesthetics Unveil the Potential Role of Sodium Channels in Neuropathic Pain. *Novartis Foundation Symposia*, **241**, 189-201. http://dx.doi.org/10.1002/0470846682.ch13

[49] Konno, K., Hisada, M., Naoki, H., Itagaki, Y., Yasuhara. T., Nakata, Y., Miwa, A. and Kawai, N. (2000) Molecular Determinants of Binding of a Wasp Toxin (PMTXs) and Its Analogs in the $Na^+$ Channels Proteins. *Neuroscience Letters*, **285**, 29-32. http://dx.doi.org/10.1016/S0304-3940(00)01017-X

[50] Harsch, A., Konno, K., Takayama, H., Kawai, N. and Robinson, H. (1998) Effects of Alpha-Pompilidotoxin on Synchronized Firing in Networks of Rat Cortical Neurons. *Neuroscience Letters*, **252**, 49-52. http://dx.doi.org/10.1016/S0304-3940(98)00555-2