Assessment of the Antimicrobial Activity of Few Saudi Arabian Snake Venoms

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Abstract: Background: Venoms of two cobras, four vipers, a standard antibiotic and an antifungal, were evaluated comparatively, as antimicrobials. Methods: Six venom concentrations and three of the standard antibiotic and the antifungal were run in micro-dilution and diffusion plates against the microorganisms. Results: Echis pyramidum, Echis coloratus and Cerastes cerastes gasperetti highest venom concentrations gave significant growth inhibition zones (GIZ) with respect to a negative control, except Bitis arietans, whose concentrations were significant. The cobra Walterinnesia aegyptia had significant venom concentrations more than Naja haje arabica. The Staphylococcus aureus Methicillin Resistant (MRSA) bacterium was the most susceptible, with a highly (P < 0.001) significant GIZ mean difference followed by the Gram positive Staphylococcus aureus, (P < 0.001), Escherichia coli (P < 0.001), Enterococcus faecalis (P < 0.001) and Pseudomonas aeruginosa which, had the least significance (P < 0.05). The fungus Candida albicans was resistant to both viper and cobra venoms (P > 0.05). The antibiotic Vancomycin was more effective than snake venoms though, they were more efficient in inhibiting growth of the resistant Pseudomonas aeruginosa. This antibiotic was also inactive against the fungus, whilst its specific antifungal Fungizone was highly efficient with no antibacterial activity. Conclusions: These findings showed that snake venoms had antibacterial activity comparable to antibiotics, with a directly proportional relationship of venom concentration and GIZ, though, they were more efficient in combating resistant types of bacteria. Both venoms and the standard antibiotic, showed no antifungal benefits.

Keywords: Agar diffusion, antimicrobial, Echis pyramidum, fungizone, GIZ, venoms.

INTRODUCTION

Bacterial infections involving the multidrug resistant strains are among the top leading causes of death throughout the world. Healthcare system across the globe has been suffering from an extra-ordinary burden in terms of looking for the new and more potent antimicrobial compounds [1]. The majority of bacteria such as Pseudomonas, Klebsiella, Enterobacter, Acinetobacter, Salmonella, Staphylococcus, Methicillin Resistant S. aureus (MRSA), Enterococcus and penicillin-resistant Streptococcus pneumoniae (PRSP) vancomycin-resistant enterococci have developed several ways to resist antibiotics. Such bacteria are becoming a serious clinical problem throughout the world [2-6].

Natural products are important sources of medicinal compounds. A wide variety of organisms produce such bioactive compounds and some of these natural substances have been shown to be able to kill bacteria [6-10]. Snake venoms contain a great variety of biologically active proteins responsible for various pathological effects. Venoms include toxins which are highly potent compounds with selective and specific activities. They can be useful and valuable as pharmacological tools in drug research, as potential drug design templates and as therapeutic agents [11, 12]. In recent years, venoms and venom components from different venomous animals have shown potential antibacterial activity. This includes snake [13-15] and scorpion venoms [16-19]. To date, only a few studies have been made on the antimicrobial activities of snake venoms [9]. In 1948, Glaser investigated antibacterial activity of Crotalus venom [20] and then in 1968 Aloof Hirsch and his colleagues reported an antibacterial lytic factor from the venom of the cobra Hemachatus haemachatus [21]. Now it is fully documented that the snake venoms have a number of cytotoxic factors along with potent killing effect on bacteria as well as viruses [22-25]. Rideiro et al., [15] reported the presence of L-amino acid oxidase present in snake venom and displayed many important biological properties that included the bactericidal and virucidal activities. A new antibiotic vejovine has been discovered from the Vaejovis mexicanus scorpion venom and this agent has been proved to be highly effective on pathogenic bacteria [19]. Captopril, anti-hypertensive drug was designed based on the peptide inhibitor of angiotensin-converting enzyme from the venom of Bothrops jararaca snakes [26]. Echistatin and Ecarin are two bioactive molecules, isolated from Echis carinatus snake venom. Echistatin is one of the most potent disintegrin polypeptide which has platelet aggregation inhibitor activity and used as an anticoagulant while Ecarin is an enzyme used in the ecarin clotting.
time (ECT) test to monitor anticoagulation during treatment with hirudin [27-30].

In a recent study it was demonstrated that the venom from one of the world's most venomous snakes Mamba could be the key to a new breed of painkillers, may be more potent then morphine [31]. Several naturally occurring peptides have shown their promise towards the antimicrobial activity however the family Viperidae snake venoms have not been explored thoroughly, although they are a major source of so many active peptides [13, 32]. The antimicrobial peptides are electrically attracted to the negative charged groups of the cell surface, where they develop an α-helical conformation and these charged groups get accumulated on the membrane. This can result in the formation of transient pores, membrane perturbation and ultimately the cell lysis [33].

In this study we employed 4 crude venoms of the snakes of Viperidae and 2 crude venoms of Elapidae family for their effects on the growth of pathogenic microorganisms. Similar screening focusing on antimicrobial property has not been attempted previously among indigenous Saudi Arabian snakes.

MATERIALS AND METHODOLOGY

Materials

Snake venoms of two snake groups that belong to two families (Viperidae and Elapidae) were obtained from the Research Center, Prince Sultan Military Medical City (PSMMC), Riyadh, Saudi Arabia. The initial stocks of venoms were prepared in normal saline at a concentration of 10mg/mL and purified by passing through 0.22μm membrane filter. Further dilutions were made in sterile saline and used in this study. Freeze-dried microbial cultures of S. aureus (S. aureus) ATCC 25923, E. faecalis (E. faecalis) ATCC 29212, E. coli (E. coli) ATCC 25922, P. aeruginosa (P. aeruginosa) ATCC 27853 and Candida albicans (C. albicans) ATCC 66027 were purchased from Microbiologics, Inc. (St. Cloud, MN, USA). The laboratory strain of methicillin resistant S. aureus (MRSA) 12498 was obtained from the Department of Microbiology in PSMMC. Brain Heart Infusion broth (BHB), Sabouraud Dextrose broth (SDB), Brain Heart Infusion agar (BHA), Sabouraud Dextrose agar (SDA) were purchased from local company agents in Riyadh, Saudi Arabia. The antifungal (Fungizone) was originally bought from Gibco Life Technology, U.K. and the antibiotic (Vancomycin) was from Sigma, USA and were used accordingly.

Venoms

The initial stocks of venoms were prepared in normal saline at a concentration of 10mg/mL and sterilized by 0.22μm membrane filter and stored in -20°C until use.

Growth Media

All of the bacterial cultures with the exception of C. albicans were grown in BHB and the stocks prepared in 50% glycerol were stored at -40°C. C. albicans culture was grown in SDB and stored at -40°C as glycerol stock culture. The enumeration of C. albicans colonies and the growth inhibition were determined on SDA-plates prepared in the laboratory.

Microbial Inoculum

Microbial inoculum that would be used for antibacterial screening assays was prepared using the standard method of log-phase growth and were standardized to 0.5 McFarland unit and log dilutions were made in phosphate buffer saline (PBS) for each microorganism.

Agar Diffusion Procedure

Antimicrobial susceptibility tests were performed by the agar-diffusion method, which was based on Ouchterlony technique and slightly modified according to Barry and Brown [34]. Sterilized BHIA was melted in a microwave oven and then placed in a water bath set at 45°C. Once the temperature was stable, bottles containing agar were removed and 0.1mL of overnight grown bacterial cultures were added. Two to three Petri plates were poured for each microbe and once the agar was solidified, plates were transferred to the refrigeration temperature for 2-3 hours. A dedicated agar punch was used to cut holes in agar and 0.05 mL of various dilutions of venom (0.25, 0.5, 1, 2.5, 5, 10 mg/mL) was added to each well. Normal saline, used as a diluent was employed as a negative control. After leaving plates on the bench for an hour, they were transferred to the incubator. The zones of microbial growth inhibition were recorded after 48h of incubation.

Determination of Colony Forming Units (CFU) and Minimum Inhibitory Concentration (MIC)

24h microbe and once the agar was solidified, plates were transferred to the incubator. The zones of microbial growth inhibition were recorded after 48h of incubation.

Statistics

Means and standard deviations of the zone inhibition data were collected and calculated using Microsoft Excel. Statistical significance was determined by t-test and one-way ANOVA, employing GraphPad Prism, GraphPad Instat and SPSS packages.

RESULTS

Table 1 shows mean zones of microbial growth inhibition (in mm), which were obtained by reading the agar diffusion plates. Out of eight concentrations (0.0625, 0.125, 0.25, 0.5, 1, 2.5, 5, 10 mg/mL) that gave the minimum inhibitory concentrations (MIC), six most effective venom concentrations...
Table 1. Mean zones of microbial growth inhibition (in mm), which were caused by the six most effective venom concentrations of six snakes.

| Family Viperidae | E. pyramidum venom concentration - zone of Growth Inhibition (in mm) |
|------------------|---------------------------------------------------------------------|
|                  | 0.25 mg/mL  | 0.5 mg/mL  | 1 mg/mL  | 2.5 mg/mL | 5.0 mg/mL | 10 mg/mL |
| S. aureus        | 7.25        | 9.25       | 9.75     | 12.25     | 13.5      | 15       |
| S. aureus (MRSA) | 11          | 12         | 12.25    | 13        | 14        | 15.25    |
| E. faecalis      | 0           | 0          | 0        | 6.25      | 8         | 9        |
| E. coli          | 6           | 8.25       | 8        | 10.5      | 12        | 13.25    |
| P. aeruginosa    | 0           | 0          | 0        | 6         | 7.5       | 8.5      |

| E. coloratus venom concentration - zone of Growth Inhibition (in mm) |
|---------------------------------------------------------------------|
| S. aureus            | 7            | 10          | 11        | 12        | 12         | 13       |
| S. aureus (MRSA)     | 12           | 14          | 15.5      | 16.25     | 17.25      | 18.25    |
| E. faecalis          | 0            | 6.75        | 8         | 10.25     | 12.5       | 13       |
| E. coli              | 0            | 7           | 9         | 10.25     | 11         | 12.5     |
| P. aeruginosa        | 0            | 0           | 0         | 7         | 8          | 8.75     |

| C. gasperettii venom concentration - zone of Growth Inhibition (in mm) |
|---------------------------------------------------------------------|
| S. aureus            | 6.75         | 7.25        | 8.75      | 10        | 11         | 12       |
| S. aureus (MRSA)     | 14.25        | 10          | 11.5      | 13.25     | 14.5       | 15       |
| E. faecalis          | 0            | 0           | 0         | 0         | 8          | 10       |
| E. coli              | 0            | 0           | 0         | 6         | 6.5        | 7        |
| P. aeruginosa        | 0            | 0           | 0         | 0         | 0          | 0        |

| Bitis arietans venom concentration - zone of Growth Inhibition (in mm) |
|---------------------------------------------------------------------|
| S. aureus            | 0            | 0           | 6.5       | 7         | 7          | 7.5      |
| S. aureus (MRSA)     | 0            | 0           | 7         | 8.5       | 9.25       | 10       |
| E. faecalis          | 0            | 0           | 0         | 0         | 0          | 0        |
| E. coli              | 0            | 0           | 0         | 0         | 0          | 0        |
| P. aeruginosa        | 0            | 0           | 0         | 0         | 0          | 0        |

| Family Elapidae |
|-----------------|

| Family Elapidae | N. arabica venom concentration - zone of Growth Inhibition (in mm) |
|-----------------|---------------------------------------------------------------------|
| S. aureus       | 0            | 6            | 7.25        | 9.25       | 10         | 11       |
| S. aureus (MRSA)| 6.5          | 9.25         | 9.5         | 11.25      | 12.5       | 14.4     |
| E. faecalis     | 0            | 0            | 6           | 7          | 8          | 10.5     |
| E. coli         | 0            | 6            | 6           | 7          | 9.5        | 11.5     |
| P. aeruginosa   | 0            | 0            | 0           | 6.5        | 7          | 7        |

| W. aegyptia venom concentration - zone of Growth Inhibition (in mm) |
|---------------------------------------------------------------------|
| S. aureus          | 6.75         | 9.5          | 10          | 11.25      | 12         | 13.75    |
| S. aureus (MRSA)   | 13           | 14           | 15.75       | 16.5       | 18.5       | 20.5     |
| E. faecalis        | 0            | 7            | 8           | 10.5       | 12.5       | 13.25    |
| E. coli            | 0            | 7.25         | 9           | 10         | 11         | 12.25    |
| P. aeruginosa      | 0            | 6            | 6.75        | 7          | 7          | 8.25     |
Table 2. Paired T-test analysis of viper snake venoms concentration mean microbial zone of growth inhibition against control (Normal Saline).

| Viper snake venom concentration | Mean  | Std. Error Mean | t     | Significance (2-tailed) |
|---------------------------------|-------|-----------------|-------|-------------------------|
| E. pyramidum Venom 0.25mg       | 4.00  | 1.915           | 2.089 | 0.091                   |
| E. pyramidum Venom 0.5mg        | 4.83  | 2.227           | 2.170 | 0.082                   |
| E. pyramidum Venom 1mg          | 5.00  | 2.295           | 2.179 | 0.081                   |
| E. pyramidum Venom 2.5mg        | 7.83  | 1.973           | 3.969 | 0.011                   |
| E. pyramidum Venom 5.0mg        | 9.33  | 2.171           | 4.300 | 0.008                   |
| E. pyramidum Venom 10mg         | 10.00 | 2.338           | 4.277 | 0.008                   |
| E. coloratus Venom 0.25mg       | 3.17  | 2.104           | 1.505 | 0.193                   |
| E. coloratus Venom 0.5mg        | 6.33  | 2.261           | 2.801 | 0.038                   |
| E. coloratus Venom 1mg          | 7.33  | 2.578           | 2.845 | 0.036                   |
| E. coloratus Venom 2.5mg        | 9.17  | 2.197           | 4.172 | 0.009                   |
| E. coloratus Venom 5.0mg        | 10.00 | 2.324           | 4.303 | 0.008                   |
| E. coloratus Venom 10mg         | 10.83 | 2.469           | 4.388 | 0.007                   |
| Cerastes gasperettii Venom 0.25mg | 3.50  | 2.391           | 1.464 | 0.203                   |
| Cerastes gasperettii Venom 0.5mg | 2.83  | 1.833           | 1.545 | 0.183                   |
| Cerastes gasperettii Venom 1mg  | 3.50  | 2.247           | 1.557 | 0.180                   |
| Cerastes gasperettii Venom 2.5mg | 4.83  | 2.344           | 2.062 | 0.094                   |
| Cerastes gasperettii Venom 5.0mg | 6.50  | 2.335           | 2.784 | 0.039                   |
| Cerastes gasperettii Venom 10mg | 8.33  | 2.140           | 3.895 | 0.011                   |
| B. arietans Venom 1mg           | 2.17  | 1.376           | 1.574 | 0.176                   |
| B. arietans Venom 2.5mg         | 2.50  | 1.586           | 1.576 | 0.176                   |
| B. arietans Venom 5.0mg         | 2.67  | 1.706           | 1.563 | 0.179                   |
| B. arietans Venom 10mg          | 3.00  | 1.915           | 1.567 | 0.178                   |

were found to be the most relevant ranges of the six snake venoms, applicable for statistical analyses. The paired T-test was employed to verify the significance of venom concentration effect of all snake species with respect to control (saline). For the first viper snake *E. pyramidum*, the venom concentration range (0.25mg - 10mg) gave 4.00 mm - 10.00 mm mean growth inhibition zones (GIZ) for all five microbial species (Table 2).

Only concentration 2.5mg, 5.0mg and 10mg gave significant (P ≤ 0.011, P ≤ 0.008 and P ≤ 0.008, respectively) GIZ with respect to controls (Table 2).

For the second viper snake *E. coloratus*, the venom concentration range (0.25mg - 10mg) gave 3.17 mm - 10.83 mm mean GIZ for all five microbial species. The concentration 0.5mg, 1mg, 2.5mg, 5.0mg and 10mg gave significant (P ≤ 0.038, P ≤ 0.036, P ≤ 0.009, P ≤ 0.008 and P ≤ 0.007, respectively) GIZ with respect to controls (Table 2).

For the third viper snake *C. gasperettii*, the venom concentration range (0.25mg - 10mg) gave 3.50 mm - 8.33 mm mean GIZ for all five microbial species. The concentration 5.0mg and 10mg gave significant (P ≤ 0.039 and P ≤ 0.011, respectively) GIZ with respect to controls (Table 2).

For the fourth viper snake *B. arietans*, the only concentration range of its venom (1mg - 10mg) gave 2.17 mm - 3.00 mm mean GIZ for all five microbial species. All these concentrations (1mg 2.5mg, 5.0mg and 10mg) gave insignificant (P ≥ 0.176, P ≥ 0.176, P ≥ 0.179 and P ≥ 0.178, respectively) GIZ with respect to controls (Table 2).

With respect to the effect of elapid snake venom concentrations, the first one *N. arabica* venom concentration range (0.25mg - 10mg) gave 1.00 mm - 9.00 mm mean GIZ for all five microbial species. Out of six, only the concentrations 1mg, 2.5mg, 5.0mg and 10mg gave significant (P ≤ 0.032, P ≤ 0.007, P ≤ 0.006 and P ≤ 0.007, respectively) GIZ with respect to controls (Table 3).

The second elapid snake *W. aegyptia* venom concentration range (0.25mg - 10mg) gave 3.33 mm - 11.17 mm mean GIZ for all five microbial species. All six venom concentrations,
Table 3. Paired T-test analysis of elapid snake venoms concentration mean microbial zone of growth inhibition against control (Normal Saline).

| Elapid (Cobra) snake venom concentration | Mean | Std. Error Mean | t    | Significance (2-tailed) |
|-----------------------------------------|------|----------------|------|------------------------|
| *Naja arabica* Venom 0.25mg              | 1.00 | 1.000          | 1.000| 0.363                  |
| *Naja arabica* Venom 0.5mg              | 3.50 | 1.628          | 2.150| 0.084                  |
| *Naja arabica* Venom 1mg                | 4.83 | 1.641          | 2.945| 0.032                  |
| *Naja arabica* Venom 2.5mg              | 6.67 | 1.520          | 4.385| 0.007                  |
| *Naja arabica* Venom 5.0mg              | 7.83 | 1.721          | 4.552| 0.006                  |
| *Naja arabica* Venom 10mg               | 9.00 | 2.033          | 4.427| 0.007                  |
| *W. aegyptia* Venom 0.25mg              | 3.33 | 2.246          | 1.484| 0.198                  |
| *W. aegyptia* Venom 0.5mg               | 7.33 | 1.892          | 3.877| 0.012                  |
| *W. aegyptia* Venom 1mg                 | 8.33 | 2.108          | 3.953| 0.011                  |
| *W. aegyptia* Venom 2.5mg               | 9.00 | 2.160          | 4.166| 0.009                  |
| *W. aegyptia* Venom 5.0mg               | 10.00| 2.463          | 4.060| 0.010                  |
| *W. aegyptia* Venom 10mg                | 11.17| 2.738          | 4.079| 0.010                  |

Table 4. Tukey’s Multiple Comparison Test of microbial growth inhibition level caused by various snake venom concentrations and a control (Normal saline).

| Tukey’s Multiple Comparison Test       | Mean Difference | q    | P value    | 95% CI of diff       |
|---------------------------------------|----------------|------|------------|----------------------|
| *S. aureus* vs Control                | 8.958          | 14.80| P < 0.001  | 6.393 to 11.52       |
| *S. aureus* (MRSA) vs Control         | 12.38          | 20.45| P < 0.001  | 9.814 to 14.94       |
| *E. faecalis* vs Control              | 4.847          | 8.008| P < 0.001  | 2.282 to 7.413       |
| *E. coli* vs Control                  | 6.021          | 9.946| P < 0.001  | 3.455 to 8.586       |
| *P. aeruginosa* vs Control            | 2.986          | 4.933| P < 0.05   | 0.4208 to 5.551      |

except the first (0.25mg) gave significant (P ≤ 0.012, P ≤ 0.011, P ≤ 0.009, P ≤ 0.010 and P ≤ 0.010, respectively) GIZ with respect to controls (Table 3).

It was observed that GIZ had a directly proportional relationship with venom concentration for all experimental venom groups.

The Tukey’s Multiple Comparison Test was employed to determine the susceptibility of each microorganism compared to control (Normal saline) based on the GIZ (Table 4). *S. aureus* (MRSA) bacterium was the one with the largest (12.38 mm) Mean Difference and the highest (20.45) q level and a highly (P < 0.001) significant probability value. Next susceptible bacterium was *S. aureus* with 8.958 mm Mean Difference, q level of 14.80 and a highly (P < 0.001) significant probability value. Next were *E. coli* and *E. faecalis* bacterial species, with the same highly (P < 0.001) significant probability values. The least susceptible bacterium was *P. aeruginosa* with 2.986 mm Mean Difference, q level of 4.933 and a significant (P < 0.05) probability value.

Fig. (1) shows the comparative differences between effects of the elapid and viperid snake venoms, one antibiotic (Vancomycin), one antimycotic (Fungizone) and a negative control (normal saline) on growth of five pathogenic bacterial species and a fungus shown GIZ (in mm). The standard antibiotic (Vancomycin) was the most effective antibacterial agent in inhibiting the growth of four bacterial species (*S. aureus* (MRSA), *E. faecalis*, *S. aureus* and *E. coli*), in that order. All venoms were next to it in activity, though they were more efficient in inhibiting the growth of *P. aeruginosa* except the venom of the viper *C. gasperettii*. Both Vancomycin and venoms were unable to inhibit the fungal *Candida albicans* growth whilst its specific antifungal Fungizone (Amphotericin B) highly efficient with no antibacterial activity.

**DISCUSSION**

Venoms, especially those of snakes are a mixture of proteins and peptides including the nucleotides, free lipids and carbohydrates, which are bound to proteins [35]. They have consistently shown high levels of heterogeneity and intra and interspecies variation and this could be due to local adaptation for feeding on different prey [36]. The venoms obtained from the Viperidae family had long been recognized for their
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The Open Microbiology Journal, 2015, Volume 9

Fig. (1). Comparative differences between effects of snake venoms, antibiotics, antimycotics and normal saline (control). Inhibition of microbial growing of six pathogenic bacterial species and a fungus shown as mean growth inhibition zone (in mm).

complexity of molecular composition [36]. Several studies had described the antimicrobial effect of snake venoms, which enlightened the emergence of bio-active peptides as therapeutic alternatives to combat the antibiotic resistant microorganisms [37].

Six venom concentrations were chosen out of eight that showed the least concentrations that represented the MIC but, this choice was applied according to the analysis of the GIZ. Paired T-test analysis of the selected concentration ranges of the six snake venoms has clearly shown significant GIZ, with varying degrees. With respect to venoms of the vipers, *E. coloratus* was the most effective against the five microorganisms, reflecting the largest mean of GIZ and a significance of five rising venom concentrations. Venoms of *E. pyramidum* and *C. gasperettii* have got medium effects, presenting only three and two significant venom concentrations, respectively. The puff adder *Bitis arietans* venom has got the least effect and none of the six venom concentrations showed any significant GIZ. Previous studies have reported comparable results [5, 38].

On the other hand, venoms of the cobras showed to be very effective against the five microorganisms, with comparable significance to viper venoms and though the cobra *W. aegyptia* showed five significant rising venom concentrations, similar to that of *E. coloratus* (the first most effective viper venom) but, it exceeded it (the cobra exceeded the viper) in GIZ value. The second was the cobra *N. arabica* and though it had got a slightly less GIZ value than the second viper (*E. pyramidum*, with three significant venom concentrations), but, it got more significant venom concentrations (four) which, showed that this cobra venom was more effective than the second viper. One more venom concentration in the serial range presented a more dilute concentration that implies a more effective venom type, according to the MIC criteria and the directly proportional relationship between venom concentration and GIZ. This put the second cobra as a the third in effectiveness of the whole list of venoms. The group of cobra venoms appeared to be relatively more efficient as antimicrobial agents than viper venoms. Some previous studies [39] reported contradictory conclusions to our results though, some cobra venoms (*Ophiophagus hannah*) were also more active than those of the vipers. It was suggested that snake venom antimicrobial activity was due to enzymes such as PLA2 [9, 40, 41], which is also available in the venoms of cobras.

With respect to microorganism susceptibility, The Gram positive cocci *S. aureus* (MRSA) bacterium appeared to be the most sensitive to venoms, as having the largest mean difference and a highly significant probability value. Close to it was the second Gram positive cocci *S. aureus* in sensitivity to venoms, with the same highly significant probability value. Venom sensitivity of these Gram positive bacteria and other species has been reported before [42]. The Gram negative *E. coli* and the positive *E. faecalis* bacterial species, have also got the same highly significant probability but, their mean difference GIZ values were less, thus showing some relative resistance to venom action. The least susceptible bacterium was also the Gram negative *P. aeruginosa* which, had got a minimum of mean difference with a relatively significant value. Resistance of the Gram negative bacteria had been attributed to the outer membrane of the bacteria formed of lipopolysaccharides (LPS) which affected the uptake of antimicrobial peptides [43].

Levels of elapid and viperid snake venoms presented a fair pattern of effectiveness in comparative differences with the two standard treatment agents, the antibiotic (Vancomycin), and the antimycotic (Fungizone), employing GIZ parameters of selected three concentrations. Vancomycin as a specifically effective antibacterial agent, was the best in inhibiting the growth of *S. aureus* (MRSA), *E. faecalis*, *S. aureus* and *E. coli*, in that order, whilst all the venoms closely came next, with varying levels. Venoms showed to be more efficient than Vancomycin in inhibiting the growth of the more resistant *P. aeruginosa*, excepting the venom of *C. gasperettii*. Several works concerning comparisons of venoms and venom fraction with antibiotics had been re-
ported [14, 40]. With respect to the fungus Candida albicans, results were negative, hence they were removed from the analysis tables.

CONCLUSION
It was concluded here that snake venoms have comparable activity, if not more efficient than antibiotics, whilst cobra venoms appear to be relatively more efficient as antimicrobial agents than viper venoms. Until furthermore studies, the selected six snake venoms, within the employed ranges of concentrations, cannot be a suitable solution in treatment of fungi like Candida albicans.

CONFLICT OF INTEREST
The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS
The study was supported by Prince Sultan Military Medical City, Riyadh, Saudi Arabia. The authors wish to thank to Mr. Syed Ali for his help in this study.

REFERENCES
[1] Nafeesa A, Sheikh MA, Ibrahim Haque A, et al. Microbial resistance of Staphylococcus aureus against commonly used antibiotics. J Med Sci 2001; 1: 97-100.
[2] Ang YJ, Ezike E, Asmar BI. Antimicrobial resistance. Symposium series Society for applied microbiology. Ser Soc Appl Microbiol 2004; 3: 229-39.
[3] Dadgar T, Asmar M, Saiif A, et al. Antimicrobial activity of certain iranian medicinal plants against methicillin-resistant and sensitive. Asian J Plant Sci 2006; 5: 861-6.
[4] Zakaria ZA, Jais AMM, Mastura M, et al. In vitro anti staphylococcal activity of the extract of several neglected plants in Malaysia. Int J Phamacol 2007; 3: 428-31.
[5] Al-Ahmadi AJ, Fathi B, Jamshidi A, et al. Investigation of the antifungal effect of venom of the Iranian snake Echis carinatus. Ind J Vet Sci Technol 2010; 2: 93-100.
[6] Salama W, Geasa N. Investigation of the antimicrobial and hemolytic activity of venom of some Egyptian cobra. J Microbiol Antimicrob 2014; 6(1): 21-8.
[7] Wenhua R, Shuangquan Z, Daxiang S, et al. Induction, purification and characterization of an antibacterial peptide scolopendrin I from the venom of centipede Scolopendra subspinipes multilans. Ind J Biochem Biophys 2006; 43: 88-93.
[8] Jenssen H, Hamill P, Hancock REW. Peptide antimicrobial agents. Clin Microbiol Rev 2006; 19(3): 491-511.
[9] Permaul SR, Chappiapan A, Gopalakrishnan P, et al. In vitro antibacterial activity of natural toxins and animal venoms tested against Burkholderia pseudomallei. BMC Infect Dis 2006; 6(100): 1-16.
[10] Shittu LAJ, Bankole MA, Ahmed T, et al. Antimicrobial and anti-fungal activities of essential oils of crude extracts of Sesame Radiala 24 of fungi like Bra venoms appear to be relatively more efficient as antimicrobial agents than viper venoms. Until furthermore studies, the selected six snake venoms, within the employed ranges of concentrations, cannot be a suitable solution in treatment of fungi like Candida albicans.

[11] Wang Z, Zhang X, Li M, et al. Induction, purification and characterization of an antibacterial peptide scolopendrin I from the venom of centipede Scolopendra subspinipes multilans. Ind J Biochem Biophys 2006; 43: 88-93.
[12] Wenhua R, Shuangquan Z, Daxiang S, et al. Induction, purification and characterization of an antibacterial peptide scolopendrin I from the venom of centipede Scolopendra subspinipes multilans. Ind J Biochem Biophys 2006; 43: 88-93.
[13] Wang Z, Zhang X, Li M, et al. Induction, purification and characterization of an antibacterial peptide scolopendrin I from the venom of centipede Scolopendra subspinipes multilans. Ind J Biochem Biophys 2006; 43: 88-93.
[14] Permaul SR, Gopalakrishnan P, Thwin MM, et al. Antimicrobial activity of snake, scorpion and bee venoms: a comparison with purified venom phospholipase A2 enzymes. J Appl Microbiol 2007; 102: 650-9.
[15] Rideiro PH, Callejon DR, Baruffi MD, et al. L-amino acid oxidase isolated from Bothrops moojeni snake venom present selective cytotoxic activity against tumor. J Venom Anim Toxins Incl Trop Dis 2007; 13(1): 310.
[16] Haebelri S, Kuhn-Nentwng L, Schaller J, et al. Characterization of antibacterial activity of peptides isolated from the venom of the spider Cupiennius Salei. Toxicon 2000; 38: 373-80.
[17] Conde R, Zanudio FZ, Rodriguez MH, et al. Scorpion, an antimalarial and anti-bacterial agent purified from scorpion venom. FEBS Lett 2000; 471: 165-8.
[18] Torres-Larios A, Garrola GB, Zanudio FZ, et al. Hadrunir, a new antimicrobial peptide from the venom of scorpion Hadrurus aztecus. Eur J Biochem 2002; 267: 5023-31.
[19] Hernandez-Aponte CA, Silva-Sanchez J, Quintero-Hernandez V, et al. Vejovine, a new anti-bacterial from the venom of Vejovis mexicanus. Toxicon 2011; 57: 84-92.
[20] Glasser HRS. Bactericidal activity of Crotalus venom in vitro. Copeia 1948; 4: 245-7.
[21] Aaloof-Hirsch S, Devries A, Berger A. The direct lytic factor of cobra venom: Purification and chemical characterization. Biochem Biophys Acta 1968; 154: 53-60.
[22] Chaim-Matayas A, Borkow G, Ovadia M. Isolation and characterization of a cobra venom phospholipase A2 enzyme from Naja naja. Cell Mol Life Sci 2006; 63: 9030-41.
[23] Ang JY, Ezike E, Asmar BI. Antimicrobial resistance. Symposium series Society for applied microbiology. Ser Soc Appl Microbiol 2004; 3: 229-39.
[24] Siqueira JF, Jr., dos Santos KB, et al. Antimicrobial activity of snake, scorpion and bee venoms: a comparison with purified venom phospholipase A2 enzymes. J Appl Microbiol 2007; 102: 650-9.
[25] Rideiro PH, Callejon DR, Baruffi MD, et al. L-amino acid oxidase isolated from Bothrops moojeni snake venom present selective cytotoxic activity against tumor. J Venom Anim Toxins Incl Trop Dis 2007; 13(1): 310.
[26] Haebelri S, Kuhn-Nentwng L, Schaller J, et al. Characterization of antibacterial activity of peptides isolated from the venom of the spider Cupiennius Salei. Toxicon 2000; 38: 373-80.
[27] Conde R, Zanudio FZ, Rodriguez MH, et al. Scorpion, an antimalarial and anti-bacterial agent purified from scorpion venom. FEBS Lett 2000; 471: 165-8.
[28] Torres-Larios A, Garrola GB, Zanudio FZ, et al. Hadrunir, a new antimicrobial peptide from the venom of scorpion Hadrurus aztecus. Eur J Biochem 2002; 267: 5023-31.
[29] Hernandez-Aponte CA, Silva-Sanchez J, Quintero-Hernandez V, et al. Vejovine, a new anti-bacterial from the venom of Vejovis mexicanus. Toxicon 2011; 57: 84-92.
[30] Glasser HRS. Bactericidal activity of Crotalus venom in vitro. Copeia 1948; 4: 245-7.
[31] Aaloof-Hirsch S, Devries A, Berger A. The direct lytic factor of cobra venom: Purification and chemical characterization. Biochem Biophys Acta 1968; 154: 53-60.
[32] Chaim-Matayas A, Borkow G, Ovadia M. Isolation and characterization of a cobra venom phospholipase A2 enzyme from Naja naja. Cell Mol Life Sci 2006; 63: 9030-41.
[33] Ang JY, Ezike E, Asmar BI. Antimicrobial resistance. Symposium series Society for applied microbiology. Ser Soc Appl Microbiol 2004; 3: 229-39.
[34] Siqueira JF, Jr., dos Santos KB, et al. Antimicrobial activity of snake, scorpion and bee venoms: a comparison with purified venom phospholipase A2 enzymes. J Appl Microbiol 2007; 102: 650-9.
[35] Rideiro PH, Callejon DR, Baruffi MD, et al. L-amino acid oxidase isolated from Bothrops moojeni snake venom present selective cytotoxic activity against tumor. J Venom Anim Toxins Incl Trop Dis 2007; 13(1): 310.
[36] Haebelri S, Kuhn-Nentwng L, Schaller J, et al. Characterization of antibacterial activity of peptides isolated from the venom of the spider Cupiennius Salei. Toxicon 2000; 38: 373-80.
[37] Conde R, Zanudio FZ, Rodriguez MH, et al. Scorpion, an antimalarial and anti-bacterial agent purified from scorpion venom. FEBS Lett 2000; 471: 165-8.
[38] Torres-Larios A, Garrola GB, Zanudio FZ, et al. Hadrunir, a new antimicrobial peptide from the venom of scorpion Hadrurus aztecus. Eur J Biochem 2002; 267: 5023-31.
[39] Hernandez-Aponte CA, Silva-Sanchez J, Quintero-Hernandez V, et al. Vejovine, a new anti-bacterial from the venom of Vejovis mexicanus. Toxicon 2011; 57: 84-92.
Assessment of the Antimicrobial Activity

Bustillo S, Leiva AC, Merino L, et al. Antimicrobial activity of Bothrop alternatus venom from the Northeast of Argentina. Microbiologica 2008; 50: 79-82.

Kocholaty WF, Ledford EB, Daly JG, et al. Toxicity and some enzymatic properties and activities in the venoms of crotalidae, elapidae and viperidae. Toxicon 1971; 9: 131-8.

Samy PR, Gopalakrishnakone P, Bow H, et al. Purification, characterization and bactericidal activities of basic phospholipase A2 from the venom of Agkistrodon halys (Chinese pallas). Biochimie 2008; 90: 1372-88.

Wang Y, Hing J, Lai X, et al. Snake cathelicidin from Bungarus fasciatus is a potent peptide antibiotics. PLoS ONE 2008; 3: 3217.

Nair DG, Fry BG, Alewood P, et al. Antimicrobial activity of omawprin, a new member of the waprin family of snake venom proteins. Biochem J 2007; 402: 93-104.

Devine DA, Hancock REW. Cationic peptides: Distribution and mechanisms of resistance. Curr Pharm Des 2002; 8: 703-14.

Received: November 25, 2014
Revised: May 18, 2015
Accepted: May 26, 2015

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