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Purification of two Antibacterial Fraction of Vipera Lebetina Venom.

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Summary

In the present study the effects of vipera lebetina venom were tested against gram-positive and gram-negative bacteria. The crude venom gave a zone of inhibition against both groups of bacteria including *Staphylococcus aureus, Streptococcus faecalis* and *Escherichia coli.* Vipera lebetina snake venom was separated into three fractions by means of gel filteration sepadex G-100. The three fractions (F1, F2, F3) were injected and our observation showed the F1 fraction was non-toxic but had antibacterial activities. Antibacterial fraction F1 was refractionated by ion-exchange chromatography (DEAE-celullose) and two antibacterial components (F1-II and F1-III) were purified. The fractoins F1-II and F1-III were tested against *Staphylococcus aureus* and *Escherichia coli* and they showed antibacterial activities (the antibacterial activity of F1-II was stronger than F1-III). The purpose of this study was to purify the antibacterial components from vipera lebetina venom and to study their properties *in vitro.*

Key words: Antibacterial, Chromatography, Vipera lebetina, Venom

Introduction

There are many reports on snake venoms composition. Neurotoxins, cytotoxins, hemotoxins and myotoxins are found in snake venoms depending on the species. Previous investigators have described bioactive components from snake venom, however no systematic search for antibacterial component has been explained. Aloof and his co-workers in 1968 found the direct lytic factor from a cobra venom (*Hemachatus haemachatus*) had antibactarial effects against *Staphylococcus aureus* and

Escherichia coli. The antibacterial effects of viperia venoms as shown by Glaser (1948) also had effects with two different ratlle snake venoms on gram-positive organisms but there was little effect against *Bacillus subtilis*, and *Escherichia coli.*

In the present study we explained the antibacterial activity of 8 different snake venoms and purified two antibacterial protein component from vipera lebetina venom.

Materials & Methods

The antibacterial activity of eight different snake species (Naja.Naja oxiana, Echis-Carinatus, Vipera Lebetina, Pseuocerastes Persicus, Vipera latifii, Agkistrodon halys, Vipera raddei and Albicornuta) were tested. In the present study out of eight snake venom only vipera lebetina venom was selected for further study. All above mentioned snake venoms were obtained from herpentology and antivenin department of Razi Institute.

Five hundered milligrams of the vipera lebetina venom was dissolved in 5ml of Tris-HCl buffer (pH 8) and loaded onto a sephadex G-100 column (3x100cm). The material was eluted in three peaks with the same solvent at a flow rate 40ml/hr, optical density was monitored at 280 nm. The fractions were desalted and concentrated. The peptidic fraction F1 (The first eluted fraction) was dissolved in 5ml of sodium phosphate buffer and loaded onto DEAE-cellulose column (1x30cm) (Ion-exchange chromatography used for refractionation sodium phosphate gradient buffer, pH 7). Eluted peaks were desalted, lyophilized and stored. All column chromatography was performed at 4°C.

Cultures: Bacteria used in this study include *Staphylococcus aureus* ATCC 9144, *Streptococcus faecalis* NCTC 8043 and *Escherichia coli* ATCC 25922.

Disc -diffusion assay: The antibacterial effects of eight different snake species and antibacterial potent of vipera lebetina fraction were tested by disc diffussion assay (Bauer *et al.* 1966).

Toxicity test: The toxicity test of vipera lebetina fraction were performed *in vivo* and *in vitro.*

Results and Discussions

Antibacterial effects of crude venoms: The antibacterial activity of venoms of eight different snake species is shown in Fig 1. The venoms of eight snake species showed strong antibacterial effect specially against *S. aureus* (Fig. 1A) and had moderate effect against *E. coli* (Fig. 1B). In this study we purify only vipera lebetina venom which had significant antibacterial activity.

Purification of vipera lebetina antibacterial components: Two steps were required to purify the antibacterial components of vipera lebetina venom.

The Fig. 2A shows the chromatographic profile obtained from fractionating of vipera lebetina venom on column. Antibacterial activity was found in the first eluted peak (fraction No.1 "F1") as shown in Fig. 3.

Fractions containing antibacterial activity also exhibited a yellow colour which is commonly associated in snake venom.

The fractions F1, F2 and F3 were injected into mice through IV route. The F1 fraction was nontoxic as shown in table.1, while F2 was the toxic fraction of vipera lebetina venom. The toxicity test were done on F1 fraction by injection upto 200 μg into mice (18-20g) and non of them were killed. Therefore with these observation it can be concluded that F1 fraction is nontoxic and has antibacterial effect.

Ion-exchange chromatography of vipera lebetina venom fraction with antibacterial activity was resolved into four peaks.(Fig. 2B)

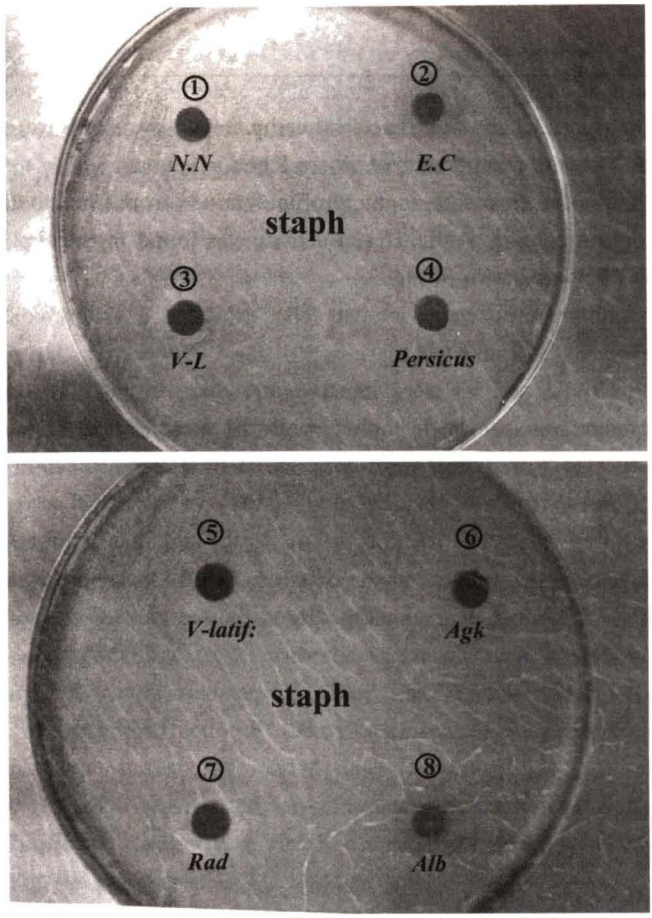


Fig. 1A: Antibacterial activity of eight different snake venoms species against *Staphylococcus aureus*.

1. Naja, Naja oxiana= N.N,
2. Echis - carinatus= E.C,
3. Vipera - lebetina=V.L
4. Pseuocerastes persicus = Persicus
5. Vipera - latifii= V-latifi
6. Agkistrodon=Agk
7. Vipera Raddei=Rad,
8. Albicornuta = Alb

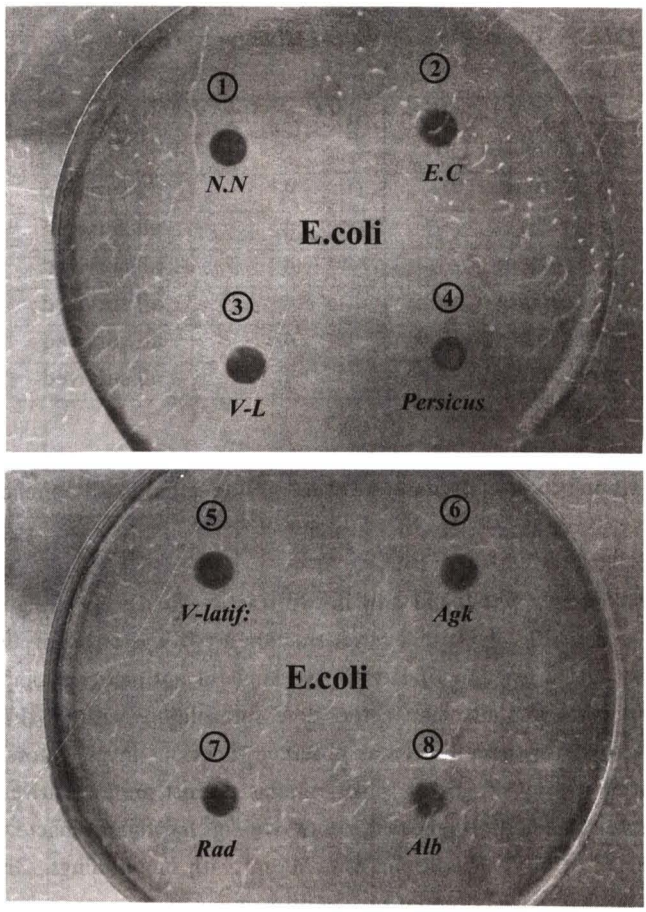


Fig. 1B: Antibacterial activity of eight different snake venoms species against *E.coli*.

1. Naja, Naja oxiana= N.N
2. Echis - carinatus= E.C
3. Vipera - lebetina= V.L
4. Pseuocerastes persicus= Persicus
5. Vipera - latifii= V-latifi
6. Agkistrodon= Agk
7. Vipera Raddei= Rad
8. Albicornuta= Alb

Table 1: Toxicity test of Vipera lebetina fractions *in-vivo*.

Amount of V.Lebetina Fractions (F1.F3)	NO. of Mice	Results
10μg	4	all survived
20μg	4	all survived
50μg	4	all survived
100μg	4	all survived
150μg	4	all survived
200μg	4	all survived

Note : An injection of 10 μg of F2 fraction was lethal to all animals.

The antibacterial activity was found only in two fractions and were designated F1-II and F1-III as seen in Fig. 4. Antibacterial fraction F1-II was stronger than F1-III. Compared antibacterial fraction F1-II with five kinds of antibiotics. Comparsions of tetracycline, nalidixic acid, amikacine, ceftizoxime, furasolidone with F1-II is shown in Fig. 5. Inhibition of growth by F1-II was observed. Previous investigators have also reported the antibacterial effects of snake venoms against gram positive and gram negative bacteria. One report showed cobra venom having antibacterial effects against *S. aureus* and *E. coli.* This report is coherent with our findings. One isolated report showed antibacterial potential of viperid venoms, and another study showed that L-amino acid oxidase purified from the venom of crotalus adamanteus had antibacterial activity against various gram negative organisms. Our results in the present study is in agreement with other investigators. our report shows that eight different snake venoms have antibacterial effects against *S. fecalis, S. aureus* and *E.coli.* The antibacterial activity of vipera lebetina venom was more active when compared with another snake venoms. In the present investigation only vipera lebetina venom was selected for study.

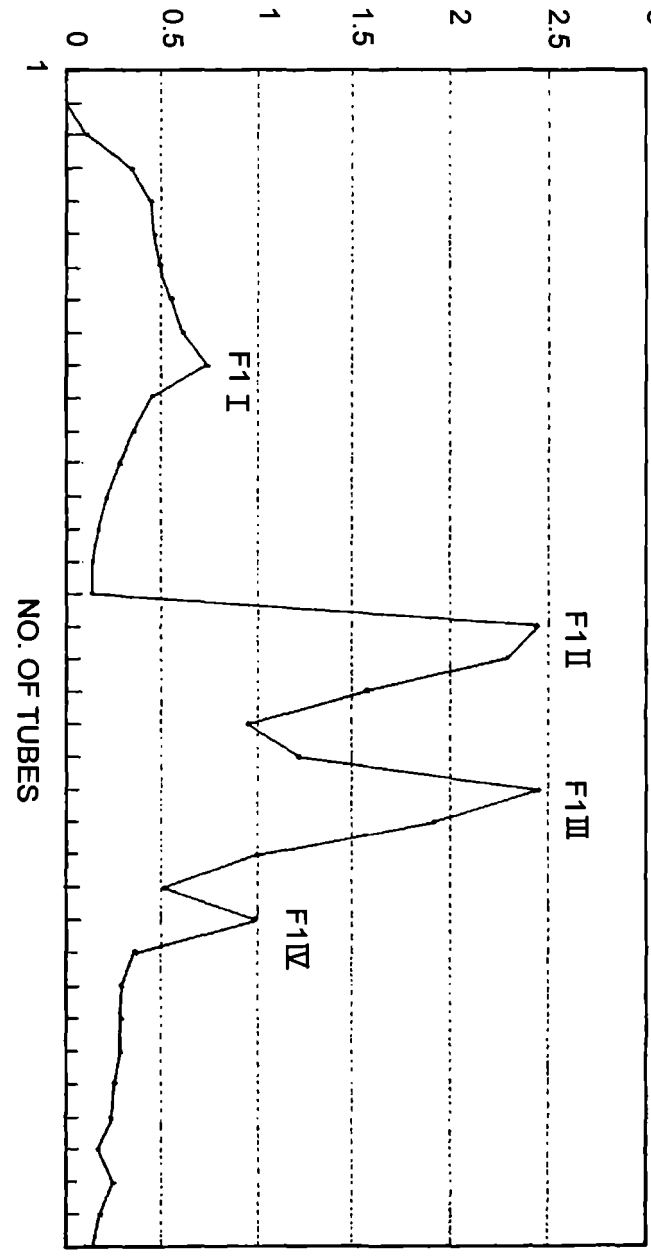
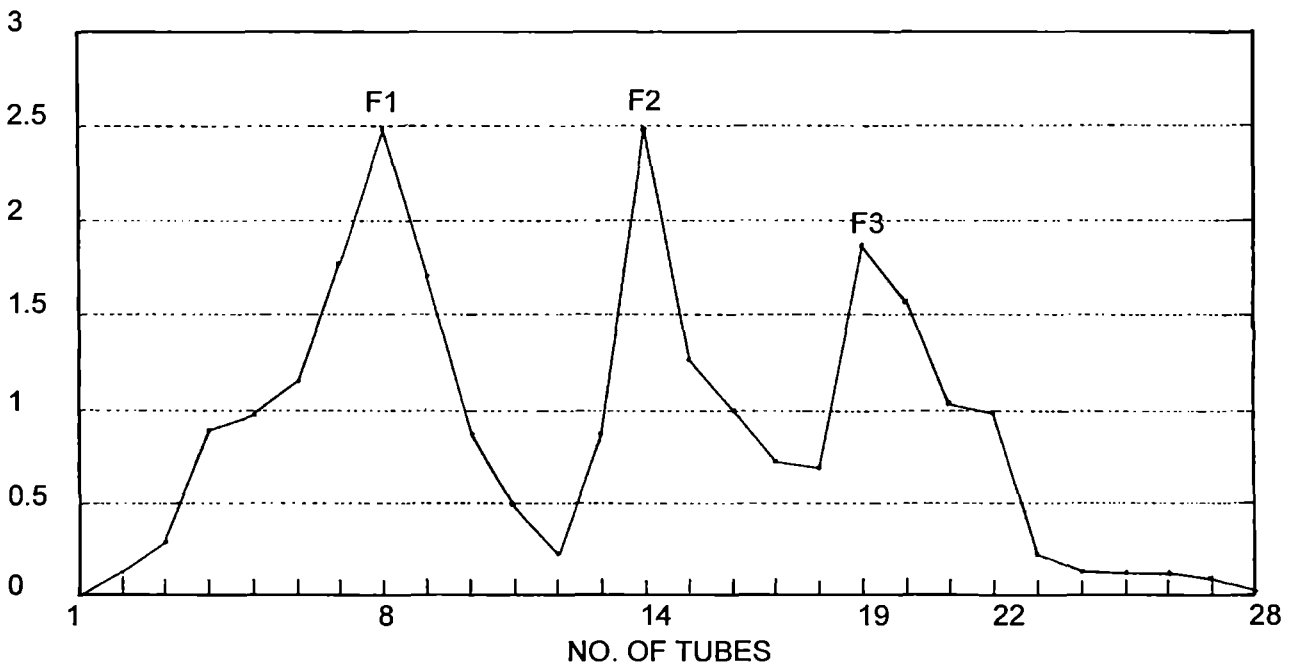


Fig-2A - FRACTIONATION OF F1
BY DEAE CELLULOSE
3
2.5
2
1.5
1
0.5
0
1
F1 I
F1 II
F1 III
F1 IV
NO. OF TUBES

Fig-2B - VIPERA LEBETINA VENOM

SEPHADEX G-100 GEL FILTERATION



Aloof-Hirsch *et al.* (1968) showed in the presence of 200g/ml DIF (direct lytic factor) the cultres of both *E. coli* and *S. aureus* grew at the same rate as an untreated control culture. They described that the growth of *S. aureus* was inhibited by DIF in concentrations 50μg/ml and higher. They also found that antibacterial properties of elapid was associated with direct lytic factor of H. haemachatus venom.

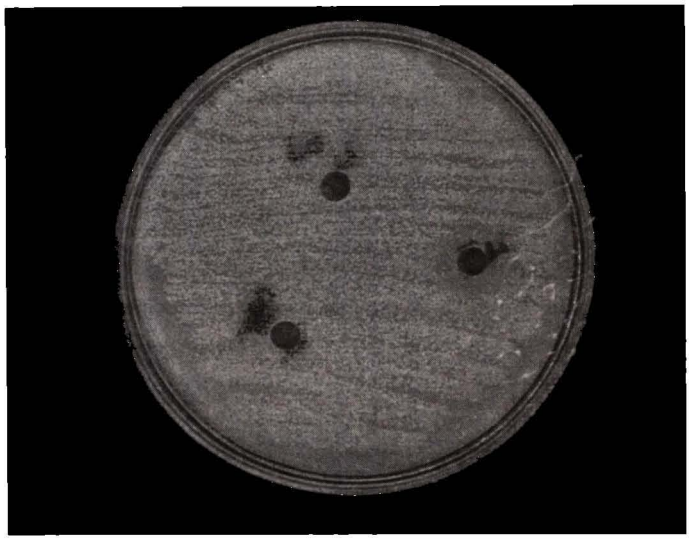


Fig. 3: The antibacterial effects of F1, F2, F3 against *Staphylococcus aureus* by disc diffusion.

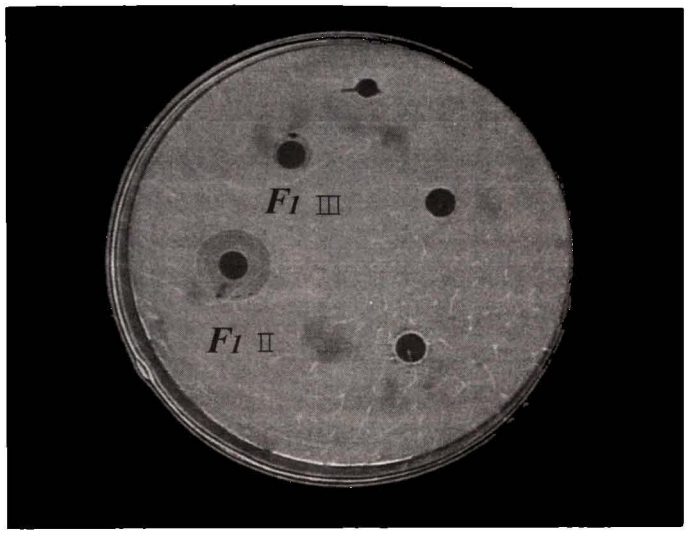


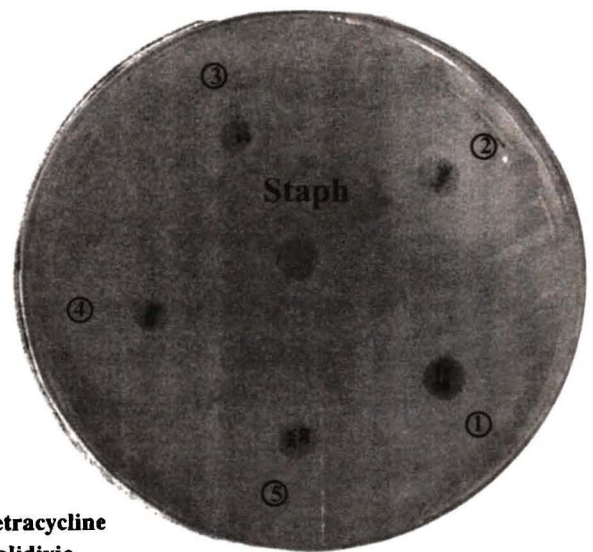
Fig. 4: The antibacterial effects of F1-II and F1-III against *Staphylococcus aureus*.

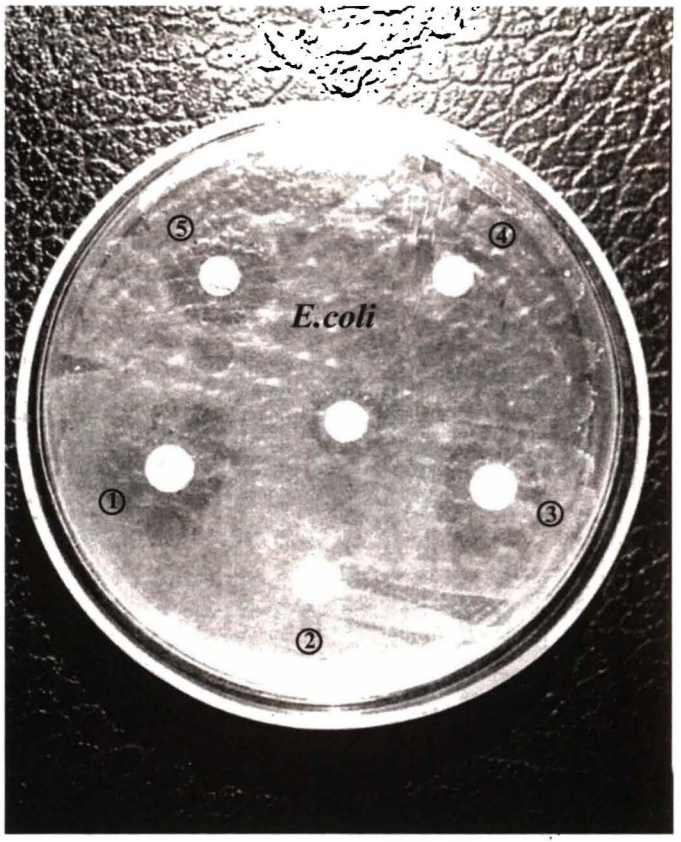
Antibacterial effects of viperid venoms have been studied by Glaser (1948), and Sharnes (1970). The minimal antibacterial effects of venoms from crotalus mitchellii pyrrhus and red diamond back rattlesnake against *E. coli, S. aureus* and *B. subtilis* has also been shown by Glaser (1948).

We observed marked effects of vipera lebetina venom against *S. aureus* and *S. fecalis* and minimal antibacterial effects against *E. coli*. We also found the antibacterial fractions of vipera lebetina venom was more active against *S. fecalis* and *S. aureus* and minimal effect against *E. coli*. Our findings is in correlation with other investigators.

Another report (Bradly *et al.* 1991) have shown the antibacterial effects of 30 different snake species against gram-positive and gram-negative bacteria. Stiles and coworkers found that a number of venom gave a zone of inhibition against both groups of bacteria including *Aeromonas hydropila*. They also observed two antibacterial components from the venom of an Australian elapid pseudechis australis which had potent antibacterial properties associated with L-amino acid oxidase activity. The antibacterial potancy of two protein fractions of Australian elapid pseudechis autralis were compared with tetracycline and they observed antibacterial fraction to be more effective. (Bradly *et al.* 1991) The two antibacterial protein fractions from vipera lebetina venom were also purified and the results showed the fractions having strong antibacterial activity against *S. aureus, S. fecalis* and moderate effect against *E. coli.* We also compared the potency of antibacterial protein (F1-II) with five different antibotics as shown in the Fig. 5.

Our observation showed the antibacterial fraction of viper alebetina venom to have stronger effects as compared with other antibiotics (Fig. 5). The above findings also reported previously by Stiles and co-workers found that the antibacterial fractions of snake venom were 17.5 to 70 times more effective as compared to tetracycline.





1. Tetracycline
2. Nalidixic
3. Amikacine
4. Ceftizoxime
5. Furazolidone

Fig. 5: comparsions of tetracycline, nalidixic acid, amikacine, ceftizoxim and furasolidone with F1-II (against *S. aureus* and *E. coli*).

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