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Screening Antifungal and Antibacterial Activity of Venom from Snake commonly Found in Iraq

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Abstract

To screen antimicrobial activity of Iraqi snake venom, which consist of a diverse range of proteins that identified as a potential source of therapeutics. It is unsurprising that snake venoms are being investigated for antimicrobial component due to antimicrobial resistance is becoming an increasingly sever issue.

In this study, antifungal and antibacterial activity were assessed against snake venom by using disc diffusion method with different concentrations of venom. The antifungal activity of Iraqi snake crude venom against nine different yeasts that isolated from women with vaginal infections (*Candida albicans, Candida glabrata, Candida tropicalis, Candida krusei, Candida kefyr, Candida parapsilosis, Candida incospicua, Candida sphaeriea, and Crypyococcus laurentii*), result showed that the crude venom dose not has a noticeable effect on all yeasts type, while in antibacterial activity the crude venom result showed the an effect against gram negative bacteria, *Proteus mirabilis* that isolated from patient with urinary tract infection, with inhibition zone about (10 mm and 8 mm) to (500 µg/ml, 250 µg/ml) of snake venom concentrations respectively.

It can be conclude that snake venom can be promising therapeutic agents against some microbial infections.

Key words: Snake venom; Antifungal; Antibacterial Activity.

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1. Introduction

Antimicrobial peptides are a small proteins, diverse group that have antimicrobial activity, and their function been recognized as important to the animal immune responce, and used as a first line of immune defense by many organisms, such as vertebrates, insects, plants, and bacteria [1]. Antimicrobial peptides from natural sources have attention in recent times Because of the development of antibiotic resistance in microorganisms, and for this reason many antimicrobial peptides have been isolated from different animals, specially snakes venoms [2].

Several studies were done on snake venoms and what contains, the result showed that snake venoms are complex mixtures of toxin, enzymes which have different activities on many biological system, and cytotoxins antiviral and antibacterial [3, 4].

phospholipase A2 and L-amino acid oxidase (LAAO), found in snake venoms had non-toxic and toxic antimicrobial activities [5]. Snake venoms are a rich source of bioactive compounds to different pharmacological activities because of their complex composition, which About 90% to 95% weight of the venom corresponds to proteins [6, 7]. Enzymatic activity was present in Many proteins isolated from snake venoms, such as aminotransferases, colinesterases, catalases, hyaluronidase, ATPases, β-glucosaminidases, phosphodiesterases, phospholipases A2, L-amino acid oxidase (LAAO), NAD nucleosidases, serineproteases, proteases, and metalloproteinases [7, 8]. Snake venoms and their bioactive derivatives can be promising therapeutic agent to some microbial infections and further investigations must be carried out to purification of biological active component in snake venom [9], antibacterial potential of *Bothrops jararaca* venom was great, and showing promising results even with resistant bacterial clinical isolates [10]. In this study, we investigated the preliminary screening of common Iraqi snake venom to 8 isolates of yeasts, and one isolate of gram negative bacteria, similar screening focusing on Antifungal and Antibacterial property have not been attempted previously among indigenous Iraqi snakes.

2. Materials and METHODS

2.1 Isolations

All clinical isolates were obtained from patients at Khanaqin General Hospital/ Diyala/ Iraq.

Clinical isolates obtained as vaginal swaps from women patients with yeasts infection. The vaginal swaps were streaked out on sabouraud dextrose agar with added antibiotics [11], and incubated for (24-48) hours at 37°C, to obtain adequate growth for the yeast, VITEK Yeast Biochemical Cards were used to identifying the yeasts isolated, by using the Vitek-2 compact system instrument, results printed. The yeast species were, (*Candida albicans, Candida glabrata, Candida tropicalis, Candida krusei, Candida kefyr, Candida parapsilosis, Candida incospicua, Candida sphaeriea, and Crypyococcus laurentii*). All isolates stored at 5°C.

Clinical isolate of Gram negative bacterium obtained from patient with Urinary tract infection (UTI), isolated on blood and MacConky agar, after staining by gram stain, the final identification done by Vitek-2 compact system by using VITEK G-ve Biochemical Card, result printed, the G-ve bacterium was *proteus mirabilis*. The

bacterium was streaked on to Muller-Hinton agar plates, [12] incubated overnight for growth and stored at 5°C.

2.2 Snake Venom collection

Common snake from Iraq-Iran border was caught to obtain the venom.

A sterile mug with rubber on its nozzle was used to collected the snake venom, by using special stick to catch the snake, the snake pushed on the mug nozzle coated with rubber, the venom secreted from snake tusks, the venom collected, transfered directly to the laboratory in ice bag, stored at 5°C to prevent proteolysis (Figure. 1).

Figure 1: Showded snake venom collection from common Iraqi snake

Thermoscience NanoDrop-2000 instrument (Germany), was used to estimated the venom protein, after the device was reset by de ionized water, a drop of venom putted, and the concentration of venom protein was (216.074 mg/ml).

A serial of snake venom dilution prepared by normal saline with NaCl 0.9 %, as (500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml). New solutions were prepared daily [10].

2.3 Evaluation of Antimicrobial Activity Through Agar Diffusion Method

- Filter paper was used to make discs, these discs immersed in different concentration of snake venom.
- Yeasts: yeasts colonies were collected from overnight growth on sabouraud dextrose agar with antibiotic, and suspended in 5ml of normal saline NaCl 0.9%, turbidity was adjusted to 0.5 in McFarland scale. 10 µl of yeast suspension spread on Mueller Hinton agar containing 2% glucose, and 0.5 µg/ml methylene blue. Discs were placed on it and incubated for (24-48) hours, as recommended by Clinical Laboratory Standards Institute [13].
- G-ve bacterium: *proteus mirabilis* colonies were collected and suspended in 5ml normal saline (0.9 NaCl) turbidity was adjusted to 0.5 in McFarland scale [12]. 10 µl of the bacterial suspension spread on

Mueller Hinton agar and left standing for 3 min. Diffusion discs saturated with crude snake venom solution with five different concentration were added to petri dishes, incubated for 24 hours, [13].

- An inhibition zone around the diffusion discs measured in millimeters. Each condition was tested in triplicate.

3. Results and Discussions

In order to evaluate the antifungal and antibacterial activity of common Iraqi snake venom, we used the discs diffusion method with different concentrations of crude venom.

In Table. 1, Results have showed no antifungal effects against (*Candida* spp. and *Crypyococcus laurentii*), as shown in (Figure. 2), in contrast, the crude venom was effective against gram negative bacterium *proteus merabilis*: maximum inhibitory zones were 10 mm in 500 µg/ml , and 8 mm in 250 µg/ml, venom concentration as shown in (Figure. 3).

Table 1: Inhibition zone around discs containing different concentration of crude snake venom. Results are expressed in mm of diameter (mean of 3 independent experiments).

Yeasts species	**500 µg/ml**	**250 µg/ml**	**100 µg/ml**	**50 µg/ml**	**25 µg/ml**
Candida albicans	-	-	-	-	-
Candida glabrata	-	-	-	-	-
Candida tropicalis	-	-	-	-	-
Candida krusei	-	-	-	-	-
Candida kefyr	-	-	-	-	-
Candida parapsilosis	-	-	-	-	-
Candida incospicua	-	-	-	-	-
Candida sphaeriea	-	-	-	-	-
Crypyococcus laurentii	-	-	-	-	-
Gram negative bacteria					
Proteus mirabilis	10mm	8mm	-	-	-

Diameter ≥ 8 mm was regarded as susceptible, according to our experience with susceptibility methods due to break point was not a available to interpret test results for snake venoms, [14]. The enzymes L-amino oxidase (LAAO), and phospholipase A_2 (PLA_2) are common antimicrobial components that have been isolated from snake venoms [15], PLA_2 isolated from *Agkistrodon halys* showed significant inhibition against *Proteus vulgaris, Proteus mirabilis* and *Staphylococcus aureus* [16]. Other study done on Bungarus fasciatus and *Ophiophagus hannah* which a part from enzymatic proteins, antimicrobial peptides purified from these snake venom, showed potent antimicrobial activity against many strains of gram negative bacteria [17, 18]. Antimycobacterial activity found in a small peptide vgf-1, that isolated from *Naja atra* [19].

The ineffectivity of snake venom against yeasts could be due to the yeasts cell wall that composed of (3-glucan, i-glucan, manoprotein and a fibrillar layer) [20]. The antimicrobial mechanism of snake venoms under electron microscopy showed that the venoms involves cell surface membrane destroy and subsequent cell contents leakage [16], which this leakage of cell membrane lead to cell death which still very much unclear. There were many factors that effected snake venom antimicrobial mechanism such as net charge of the protein, amino acid sequence, salinity of environment, three-dimensional structure and bacterial membrane composition [21]. The different proteins and peptides of different snakes venoms can have shown different mechanisms of cell membrane disruption that showed differences of susceptibility among the varieties of bacteria, viral, and fungi, strains. In other study reported that purified LAAO exhibited significantly higher inhibition than crude venoms [22]. In summary this study results showed the activity of snake venom against gram negative bacteria, further studies needed to screen the snake venom activity against other type of gram negative and gram positive bacteria and other pathogenic microbe.

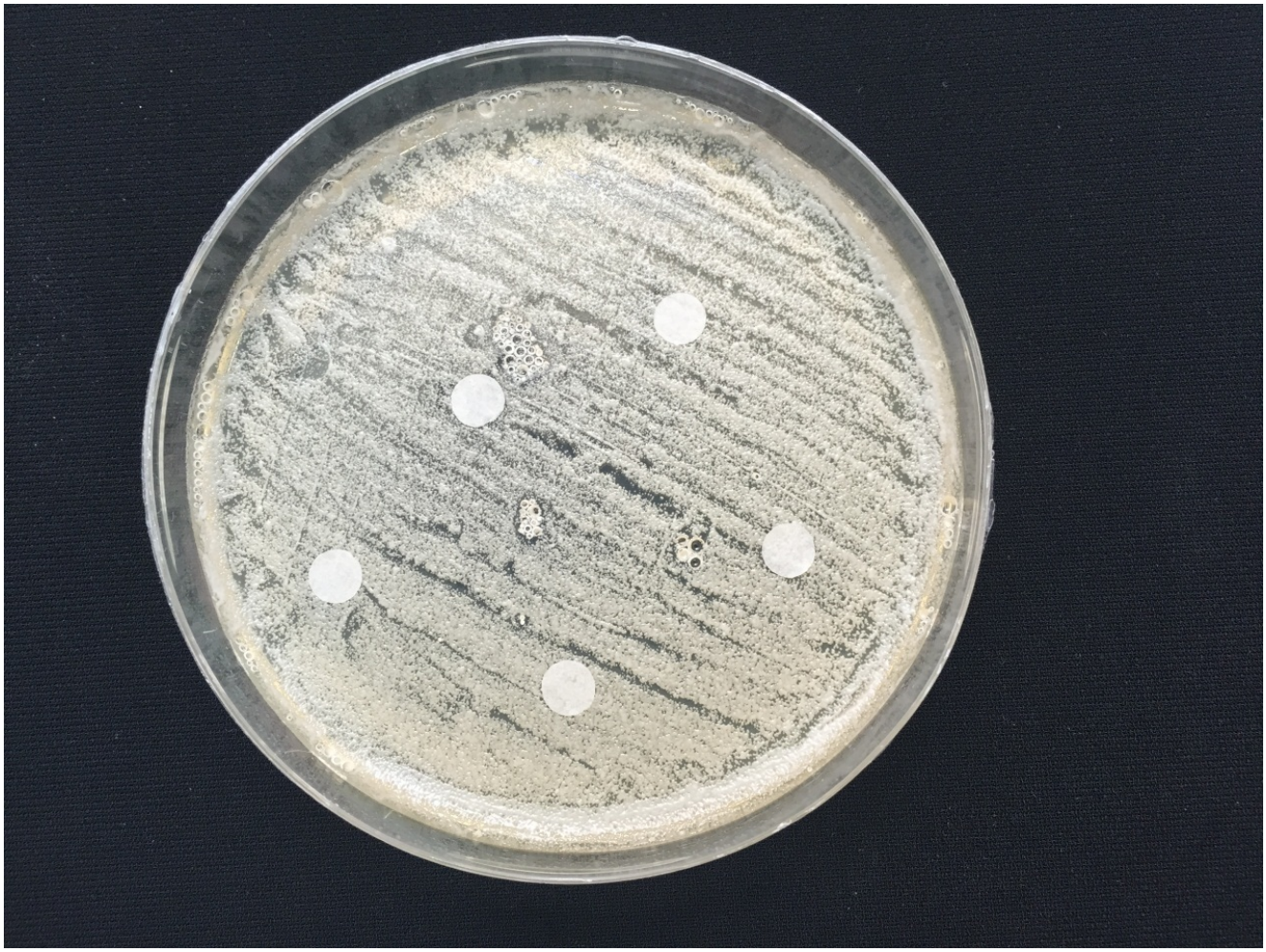

Figure 2: Discs diffusion methods showed, there were no effectivity of five different concentration of snake crude venom against clinical isolates of yeasts, from women with vaginal yeasts infections, (*Candida albicans, Candida glabrata, Candida tropicalis, Candida krusei, Candida kefyr, Candida parapsilosis, Candida incospicua, Candida sphaeriea, and Crypyococcus laurentii*). There were no inhibition zone around discs.

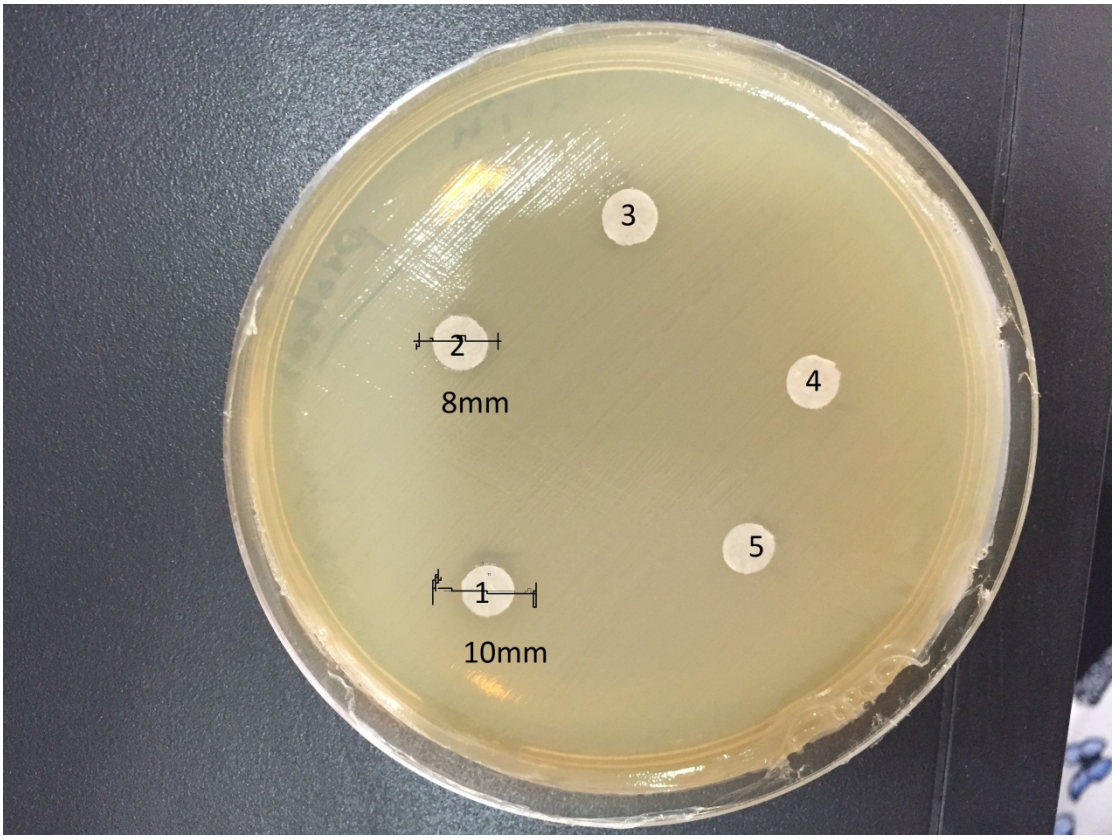

Figure 3: Discs diffusion methods with five different concentration of crude snake

venom against *proteus mirabilis* bacteria, showded:

1. snake venom 500 µg/ml =10 mm inhibition zone.
2. snake venom 250 µg/ml = 8 mm inhibition zone.
3. snake venom 100 µg/ml = 0 no inhibition zone.
4. snake venom 50 µg/ml = 0 no inhibition zone.
5. snake venom 25 µg/ml = 0 no inhibition zone.

4. Conclusion

Snake venom can be promising therapeutic agents against some microbial infections.

References

[1]. Gomes, V. M., Carvalho, A. O., Cunha, M., Keller, M. N., Bloch, J. R. C., Deolindo, P., Alves, E. W. (2005). Purification and characterization of a novel peptide with antifungal activity from Bothrops jararaca venom. Toxicon, 45, 817-27.

[2]. Sachidananda, M. K. , Murari, S. K. , Channe gowd,A. D. (2007). Characterization Of An Antibacterial Peptide From Indian Cobra (*Naja naja*) Venom. J. Venom. Anim. Toxinscl. Trop.Dis, V.13, n.2:446-461.

[3]. Mollmann, U., Gutsche, W., Maltz, L., Ovadia, M. (1997). Activity of cytotoxin P4 from the venom of the cobra snake *Naja nigricollis* on Gram-positive bacteria and eukaryotic cell lines. Arzneimittelforschung. 47(5):671-3. PMID:9205785.

[4]. Borkow, G., Marco, D., Ovadia, M. (2008). Isolation and partial characterization of an antiviral proteolytic fraction from the venom of *Echis Carinatus Sochureki*. The Open Biology Journal. 1:21-26.

[5]. Chellapandi, P., Jebakumar, S. (2008). Purification and antibacterial activity of Indian cobra and viper venoms. Electronic Journal of Biology. 4(1):11-16.

[6]. Neves, M. (2008). Avaliação da atividade antifúngica *in vitro* do veneno das serpentes amazônicas *Bothrops* atrox (LINNAEUS, 1758) crotalus durissus ruruima (HOGE, 1965). UEA.

[7]. Cunha, E., Martins, O., (2012). Principais compostos químicos presente nos venenos de cobras dos gêneros *Bothrops*, *Crotalus*—uma revisão. *Revista Eletrônica de Educação e Ciência*, 2, 21-26.

[8]. Caproni, P. (2009). Ação da Bothropstoxina-1 e do veneno total de *Bothrops jararacussu* irradiados sobre o sistema imune. 60 p.

[9]. Shebl, R. I., Mohamed, A. F., Ali, A. E., and Amin, A. M., (2012). Antimicrobial Profile of Selected Snake Venoms and Their Associated Enzymatic Activities. British Micribiological Research J., 2(4): 251-263.

[10]. Cendron, L. H., Bertol, C.D., Fuentefria, D. B., Calegari, E. M., Dallegrave, E., Moura, D. J., Moura, K., Marques, M. G., Rossato, L. G., (2014). Broad Antibacterial Activity of *Bothrops jararaca* Venom against Bacterial Clinical Isolates. Advances in Microbiology, 4: 1174- 1187.

[11]. Espinel-Ingroff, A., Barchiesi, F., Hazen, K., Martinez-Suarez, J., Scalise, G. (1998). Standardization of antifungal susceptibility testing and clinical relevance. Med. Mycol. 36 (suppl. 1): 68-78.

[12]. Queiroz, S. (2010) Identificação da atividade antimicrobiana no veneno da serpente *Bothrops moojeni* em bactérias gram negativas. 9 p.

[13]. CLSI, (2009) Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard. 1-76.

[14]. Barry, A., and Brown, S. (1996). Fluconazole disk diffusion procedure for determining susceptibility of *Candida* species. J. Clin. Microbial. 34: 2154-2157.

[15]. Stiles, B. G., Sexton, F. W., and Weinstein, S. A. (1991). Antibacterial effects of different snake venoms: purification and characterization of antibacterial proteins from *Pseudechis australis* (Australian King Brown or mulga snake) venom. Toxicon, 29:1129-1141.

[16]. Samy, R. P., Gopalakrishnakone, S., Bow, H., and Chow, V. T. K. (2008). Purification, characterization and bactericidal activities of basic phospholipase A_2 from the venom of *Agkistrodon halys* (Chinese pallas). Biochimie, 90:1372-1388.

[17]. Wang, Y., Hing, J., Lai, X., Yang, H. And Liu, R. (2008). Snake cathelicidin from *Bungarus fasciatus* is a potent peptide antibiotics. PloS ONE, 3: e3217-e3217.

[18]. Chen, X. X., Yu, G. Y., Zhan, Y., Shen, J. H., and Lee, W.H.(2009). Effects of the antimicrobial peptide OH-CATH on *Escherichia coli*. Zool. Res., 30: 171- 177.

[19]. Xie, J. P., Yue, J., Xiong, Y. L., Wamg, W. Y., Yu, S. Q, and Wang, H. H. (2003). In vitro activities of

small peptides from snake venom against clinical isolates of drug-resistant *Mycobacterium tuberculosos*. Int. J. Antimicrobol. Agents, 22: 172-174.

[20]. Cassone, A., Simonetti, *N.,* Strippoli, V. (1973). ltrastructural changes in the wall during germ-tube formation from blastospores of *Candida albicans; J. Gen. Microbial.* 77417- 426.

[21]. Nair, D. G., Fry, B. G., Alewood, P., Kumar, P., and Kini, R. M. (2007). Antimicrobial activity of omwaprin, a new member of the waprin family of snake venom proteins. Biochem. J., 402: 93-104.

[22]. Samy, R. P., Gopalakrishnakone, P., Thwin, M. M., Chow, T., Bow, H., Yap, E. H., Thong, T. W. (2007). Antibacterial activity of snake, scorpion and bee venom: A comparison with purified venom phospholipase A_2 ENZYMES. J. Applied Microbiol., 102: 650- 659.