Black cobra (*Naja naja karachiensis*) lysates exhibit broad-spectrum antimicrobial activities

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It is hypothesized that animals living in polluted environments possess antimicrobials to counter pathogenic microbes. The fact that snakes feed on germ-infested rodents suggests that they encounter pathogenic microbes and likely possess antimicrobials. The venom is used only to paralyze the rodent, but the ability of snakes to counter potential infections in the gut due to disease-ridden rodents requires robust action of the immune system against a broad range of pathogens. To test this hypothesis, crude lysates of different organs of *Naja naja karachiensis* (black cobra) were tested for antimicrobial properties. The antimicrobial activities of extracts were tested against selected bacterial pathogens (neuropathogenic *Escherichia coli* K1, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*), protist (*Acanthamoeba castellanii*), and filamentous fungus (*Fusarium solani*). The findings revealed that plasma and various organ extracts of *N. n. karachiensis* exhibited antimicrobial activity against *E. coli* K1, MRSA, *P. aeruginosa*, *S. pneumoniae*, *A. castellanii*, and *F. solani* in a concentration-dependent manner. The results of this study are promising for the development of new antimicrobials.

Keywords: Infectious diseases, Antimicrobials, Black cobra, *Acanthamoeba*, Fungi, Protists

Introduction

Antimicrobial resistance presents a significant challenge to human and animal health. This is particularly important for developing countries where drug-resistant microbes are prevalent.^{1–3} For example, it is estimated that around 180 000 cases of multiple drug-resistant-tuberculosis (MDR-TB) occur annually in South-East Asia with more than 80% of these in Bangladesh, India, Indonesia, Myanmar, and Thailand.⁴ *Streptococcus pneumoniae* is one of the most common causative agents of pneumonias in children and adults in Asia.⁵ Multiple drug-resistant *Klebsiella* spp., *Pseudomonas aeruginosa*, *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Acinetobacter* species have given new dimensions to the problem of hospital-associated infections.^{6–9} In addition, protists such as *Acanthamoeba* are now recognized as a source of microbial infections.¹⁰ *Acanthamoeba* has been shown to act as a reservoir for microbial pathogens including viruses (*Mimivirus*), bacteria (*Aeromonas*, *Coxiella*, *E. coli*, *Legionella*, *Vibrio*, etc.), protists (*Cryptosporidium*), and yeast/fungi (*Cryptococcus*).^{11–13} Apart from its role as the

Trojan horse of the microbial world, *Acanthamoeba* can produce blinding keratitis and fatal granulomatous amoebic encephalitis^{14,15} and showed resistance to a variety of anti-amoebic agents.¹⁶ Thus there is an urgent need to identify novel antimicrobials to counter pathogens.

Previously, we hypothesized that animals living in polluted environments possess antimicrobials to counter infections.¹⁷ In support, our studies identified potent antimicrobial properties in the brain lysates of cockroaches and locusts that intrigued the scientific community.¹⁷ Here, we tested this hypothesis further by examining other animals for antimicrobial activities.

The fact that snakes feed on germ-infested rodents (by swallowing the whole rodent) suggests their exposure to pathogenic microbes. The venom is used only to paralyze the rodent, but the ability of snakes to counter potential infections in the gut due to disease-ridden rodents requires robust action of the innate immune system against a broad range of pathogens.¹⁸ In many cases, the response (i.e., non-specific leucocytes, antimicrobial molecules, and the complement system) is highly effective,¹⁸ however this is a relatively untapped area of research that has the potential to provide pharmaceutical drug-leads for much needed antimicrobials. In this study, the

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plasma and lysates of various organs of black cobra were dissected out and tested for broad-spectrum antimicrobials against Gram positive and negative bacteria, protists, and fungal pathogens.

Materials and Methods

Snake sample and organ lysate preparation

The black Pakistani cobra (*Naja naja karachiensis*) is commonly found in southern parts of Pakistan.¹⁹ Snakes were provided routinely by M. Z. A. Khanzada, Dow Medical University of Health Sciences, Karachi, Pakistan. Snakes were anesthetized using chloroform and terminally bled through cardiac puncture. Blood was collected in EDTA vacutainers and subsequently plasma was separated through centrifugation at $1500 \times g$ for 10 minutes. Next, snakes were dissected aseptically and their various organs such as lungs, liver, gut, stomach, gallbladder, kidneys, and testicles were collected. Each organ was weighed, homogenized (Tekmar homogenizing mixer), and sonicated (Branson Sonifier 450) in sterile distilled water, in 1:1 ratio as previously described.¹⁷ Following sonication, tissue lysates were centrifuged at $12\,000 \times g$ for 30 minutes at 4°C. The supernatant containing soluble lysates were collected and filtered using a 0.2 µm pore size filters. All filtrates were stored at -80°C until tested. The concentrations of dissolved proteins were estimated using Bradford method.

Microbial cultures and growth conditions

Bacteria used in this study included neuropathogenic *E. coli* K1, MRSA, *P. aeruginosa*, and *S. pneumoniae*. *E. coli* K1 strains RS218²⁰ and MRSA²¹ were isolated earlier from CSF and blood samples of neonatal meningitis and sepsis patients, respectively. *Streptococcus pneumoniae* was isolated from the blood culture of a pneumonia patient and *P. aeruginosa* was isolated from pus sample. All bacterial isolates are deposited in the departmental microbial collection and available upon request. All bacteria were grown aerobically at 37°C in nutrient broth except *S. pneumoniae*, which was grown on sheep blood agar plates and brain-heart infusion (BHI) broth.

Acanthamoeba castellanii, a keratitis isolate, belonging to the T4 genotype was purchased from American Type Culture Collection (ATCC 50492). *Acanthamoeba castellanii* trophozoites were grown in the PYG medium [0.75% (w/v) proteose peptone, 0.75% (w/v) yeast extract, and 1.5% (w/v) glucose] at 30°C in T-75 tissue culture flasks. To obtain amoebae in the trophozoite forms, the culture medium was refreshed 15–20 hours prior to experiments. *Fusarium solani* was purchased from First Fungal Culture Bank of Pakistan (FCBP0055) and grown aerobically on potato dextrose agar (PDA) plates at 30°C for 5–7 days. Conidiospores were collected by scraping the surface of fungal colonies in phosphate buffered saline

(PBS) and hyphae were removed by filtering suspension through sterile gauze sieve. Spores in the filtrate were washed in PBS through centrifugation and used for experiments.²²

Antibacterial assays

Antibacterial assays were performed as described previously.²³ Briefly, approximately 10^6 colony forming units (cfu), suspended in 10 µl were incubated with various concentrations of snake plasma or different organ lysates (0.25, 0.5, 0.75, 1.0, 1.25 mg/ml; final volume adjusted to 200 µl) and incubated at 37°C for 2 hours. After this incubation, bacterial cultures were 10-fold serially diluted and plated on nutrient/BHI agar plates and incubated further at 37°C overnight. Bacteria incubated with PBS alone served as negative control. For 100% kill, bacteria were incubated with appropriate antibiotics, 100 µg/ml gentamicin for *E. coli* K1 and *P. aeruginosa*; 100 µg/ml vancomycin for *S. pneumoniae* and MRSA. Next day, colonies were enumerated. The percentage bactericidal effects were determined as follows: $100 - [(cfu \text{ in lysates/original inoculum}) \times 100]$.

Amoebicidal assays

Amoebicidal assays were performed in 24-well plates by incubating 10^6 *A. castellanii* trophozoites with lysates of various organs of black cobra (total volume was made up to 500 µl using PBS) and incubated at 30°C for 48 hours. Amoebae incubated with PBS alone served as controls. Following incubation, amoebae were counted microscopically using a hemocytometer. The percentage amoebicidal effects were determined as follows: $100 - [(amoebae \text{ count in lysates/amoebae count in control}) \times 100]$.

Fungicidal assays

To determine the fungicidal effects of various organs lysates of black cobra against *F. solani*, assays were performed similar to amoebicidal assays with minor modifications. Briefly, 10^6 conidiospores were incubated with different organ lysates in PBS in 500 µl at 30°C for 24 hours. *F. solani* colonies in PBS alone were considered as control. Following incubation, spores were diluted and plated on PDA plates and incubated at 30°C until visible colonies appeared (~48 hours). The percentage fungicidal effects were determined as follows: $100 - [(fungal \text{ colonies in lysates/fungal colonies in control}) \times 100]$.

Results

Black cobra plasma exhibited potent bactericidal activities against all bacteria tested except *S. pneumoniae*

To determine the bactericidal activity of black cobra plasma against *E. coli* K1, MRSA, *P. aeruginosa*, and *S. pneumoniae*, 10^6 cfu were incubated with different concentration of snake plasma. The results showed that snake plasma exhibited potent bactericidal

Figure 1 Black cobra plasma exhibited potent antibacterial activities against *Escherichia coli* K1, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa* but not *Streptococcus pneumoniae*. Various concentrations of black cobra plasma was incubated with approximately 10^6 cfu of *E. coli* K1 (A), MRSA (B), *P. aeruginosa* (C), and *S. pneumoniae* (D) at 37°C for 2 hours in 200 μ l. For positive control, *E. coli* K1 and *P. aeruginosa* were incubated with 100 μ g/ml gentamicin, while MRSA and *S. pneumoniae* were incubated with 100 μ g/ml vancomycin. Bacteria incubated with PBS alone served as negative control. After incubation, bacterial colonies were enumerated and percentage bactericidal effects were determined in the Materials and Methods section. The data are presented as the mean $\pm$ standard error of three independent experiments performed in duplicate. *P*-values were calculated by comparing results of PBS alone with different plasma concentrations using student's *t*-test. (*) indicates *P* < 0.0001.

activity against all bacteria tested, except *S. pneumoniae* (Fig. 1). For *E. coli* K1, 25 and 50% snake plasma produced $85\% \pm 3$ and $93\% \pm 1.8$ bactericidal activities, respectively (Fig. 1A). Similarly, 25 and 50% snake plasma showed $90\% \pm 5.5$ and $93\% \pm 7.5$ bactericidal activities against MRSA, respectively (Fig. 1B). As low as 12.5% plasma showed $98\% \pm 0.87$ killing of *P. aeruginosa* (Fig. 1C). Surprisingly, none of the snake plasma concentrations tested showed any effect on the viability of *S. pneumoniae* (Fig. 1D).

Lysates of black cobra organs exhibited selective bactericidal activities

To determine the bactericidal activities of internal organs of black cobra, tissue lysates of different organs (lungs, liver, intestine, stomach, gallbladder, kidneys, and testicles) equivalent to protein concentration of 0.25, 0.5, 0.75, 1.0, and 1.25 mg/ml were incubated with *E. coli* K1, MRSA, *P. aeruginosa*, and *S. pneumoniae*. In the case of *E. coli* K1, among different organs tested, liver, gallbladder, intestine,

stomach, and testicles lysates showed moderate to low level of bactericidal activities (Fig. 2A). Only liver lysate showed concentration-dependent bactericidal activity, where 0.75, 1.0, and 1.25 mg/ml lysate showed $13\% \pm 7.1$, $32\% \pm 7.0$, and $49\% \pm 9.6$ bacterial kill, respectively (Fig. 2A). Lungs and gallbladder showed potent bactericidal activities at 0.5 mg/ml. Moderate bactericidal activities ($32\% \pm 5.3$) were observed at 1.25 mg/ml (Fig. 2B). The lysates of lungs, intestine, stomach, and gallbladder showed significant antibacterial activities against *P. aeruginosa* at 0.25 mg/ml (Fig. 2C). For *S. pneumoniae*, lysates of lungs, gallbladder, and testicles showed dose dependent bactericidal activities (Fig. 2D).

Lysates of black cobra lungs and gallbladder exhibited potent anti-fungal and anti-protist activities, respectively

To study the fungicidal and amoebicidal activities of black cobra organ lysates, 10^6 conidiospores of *F. solani* and 10^6 *A. castellanii* were incubated with

Figure 2 Lysates of various internal organs of black cobra showed antibacterial activities against *Escherichia coli* K1, MRSA, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*. Various internal organs of black cobra were collected and their lysates were prepared. Lysate equivalent to protein concentration of 0.25, 0.5, 0.75, 1.0, and 1.25 mg/ml, were incubated with $\sim 10^6$ cfu of *E. coli* K1 (A), MRSA (B), *P. aeruginosa* (C), and *S. pneumoniae* (D) at 37°C for 2 hours in 200 μ l. For positive controls, *E. coli* K1 and *P. aeruginosa* were incubated with 100 μ g/ml gentamicin, while MRSA and *S. pneumoniae* were incubated with 100 μ g/ml vancomycin. Bacteria incubated with PBS alone served as negative control. After incubation, bacterial colonies were enumerated and percentage bactericidal effects were determined as described in the Materials and Methods section. The data are presented as the mean $\pm$ standard error of three independent experiments performed in duplicate. *P*-values were calculated by comparing results of PBS alone with different plasma concentrations using student's *t*-test. (*), (**), and (***) indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Figure 3 Black cobra lung lysate exhibited potent activity against *Fusarium solani* conidiospores. Approximately, 10^6 *F. solani* conidiospores were incubated with 1.25 mg/ml lysate of black cobra lungs, liver, gallbladder, and stomach in PBS at 30°C for 48 hours. Next, fungal colonies were enumerated and percentage fungicidal activity was calculated as described in the Materials and Methods section. *Fusarium solani* spores incubated in PBS alone served as control. Among various organ lysates tested, lung lysates showed optimal fungicidal effects. The data are presented as the mean $\pm$ standard error of three independent experiments performed in duplicate. *P*-values were calculated by comparing results of PBS alone with different organ lysates using student's *t*-test. (*) indicates $P < 0.001$.

Figure 4 Potent anti-protist activity of black cobra gallbladder against *Acanthamoeba castellanii* trophozoites. Approximately, 10^6 amoebae were incubated with 1.25 mg/ml lysate of black cobra lungs, liver, gallbladder, and stomach in PBS at 30°C for 48 hours. After incubation, *A. castellanii* trophozoites were counted microscopically using a haemocytometer and percentage amoebicidal activity was calculated as described in the Materials and Methods section. *A. castellanii* incubated in PBS alone served as control. Note that the gallbladder lysates showed optimal amoebicidal activity. The data are presented as the mean $\pm$ standard error of three independent experiments performed in duplicate. *P*-values were calculated by comparing results of PBS alone with different organ lysates using student's *t*-test. (*) indicates $P < 0.001$.

Table 1 Known antimicrobial activities of various organs of selected vertebrates and possible active factors

Organs	Animal	Antimicrobial activity	Active factor/s	References
Lung/Gills	<i>Gadus morhua</i>	G+, G–	ND	33
	<i>Siniperca chuatsi</i>	G+, G–	ScBD peptide	34
	<i>Crocodylus niloticus</i>	G+	ND	32
	<i>Corvus corax</i>	G+	ND	32
	<i>Gallus gallus domesticus</i>	G–	Gal-7, Gal-9 peptide	35
	<i>Meleagris gallopavo</i>	G+	ND	32
	<i>Mus musculus</i>	G+, G–	mBD-1 peptide	36
		G–	mBD-3 peptide	37
	<i>Rattus norvegicus</i>	G+	Surfactant containing fraction	38
	<i>Squalus acanthias</i>	G+, G–, <i>Candida albicans</i> , <i>Paramecium caudatum</i>	Squalamine	39
Liver	<i>Sebastes schlegeli</i>	G+, G–	SsPGRP-L2 (long chain of peptidoglycan related proteins)	40
	<i>Oryzias latipes</i>	G–	Medaka beta-defensin peptide	41
	<i>Limnonectes fragilis</i>	G+, G–	Cathelicidin like peptide	42
	<i>C. corax</i>	G+	ND	32
	<i>G. g. domesticus</i>	G–	Gal-7 peptide	35
	<i>M. musculus</i>	G+, G–	mBD-1 peptide	36
	<i>S. acanthias</i>	G+, G–, <i>C. albicans</i> , <i>P. caudatum</i>	Squalamine	39
Gallbladder	<i>G. morhua</i>	G+, G–	ND	33
	<i>S. acanthias</i>	G+, G–, <i>C. albicans</i> , <i>P. caudatum</i>	Squalamine	39
Stomach	<i>Xenopus laevis</i>	G+, G–, <i>C. albicans</i>	PGQ (24 amino acids peptide with amino-terminal glycine and carboxylterminal glutamine)	43
	<i>Bufo gargarizans</i>	G+, G–, <i>C. albicans</i> , <i>Cryptococcus neoformans</i> , and <i>Saccharomyces cerevisiae</i>	Buforin 1, 2, 2b, and histonins peptides	44
	<i>L. fragilis</i>	G+, G–	Cathelicidin like peptide	42
	<i>Rana catesbeiana</i>	G+, G–, <i>C. albicans</i> , <i>C. neoformans</i> , and <i>S. cerevisiae</i>	bPaAP, bPcAP - peptide	45
Intestines	<i>C. corax</i>	G+	ND	32
	<i>G. g. domesticus</i>	G–	Gal-7 peptide	35
	<i>S. acanthias</i>	G+, G–, <i>C. albicans</i> , <i>P. caudatum</i>	Squalamine	39
Kidney	<i>G. morhua</i>	G+, G–	ND	33
	<i>S. schlegeli</i>	G+, G–	SsPGRP-L1 (long chain of peptidoglycan related proteins)	40
	<i>Myxine glutinosa</i>	G+, G–	HFIAP 1, 2, and 3 peptides	46
	<i>S. chuatsi</i>	G+, G–	ScBD peptide	34
	<i>C. corax</i>	G+	ND	32
	<i>G. g. domesticus</i>	G–	Gal-7 peptide	35
	<i>M. musculus</i>	G–	mBD-3 peptide	37
	<i>M. musculus</i>	G+, G–	Cryptdin 1 and 2 peptides	47
	<i>Sus scrofa domesticus</i> (extracellular matrix)	G+, G–	ND	48
Reproductive organs	<i>S. chuatsi</i>	G+, G–	ScBD peptide	34
	<i>G. morhua</i>	G+, G–	ND	33
	<i>Sparus aurata</i>	G+, G–	SaBD (propeptide of 66 amino acids)	49
	<i>L. fragilis</i>	G+, G–	Cathelicidin like peptide	42
	<i>G. g. domesticus</i>	G–	Gal-7 peptide	35
	<i>M. musculus</i>	G+, G–	mBD-1 peptide	36
	<i>S. acanthias</i> (testes)	G+, G–, <i>C. albicans</i> , <i>P. caudatum</i>	Squalamine	39
	<i>R. norvegicus</i> (testes)	G–	DEFB-21, 24, and 27 peptides	50
	<i>G. g. domesticus</i> (ovary)	G–	Gal-4 and Gal-7 peptide	35
	<i>M. musculus</i> (ovary)	G+, G–	mBD-1 peptide	36
Skin	<i>Pleuronectes americanus</i>	G+, G–	Pleurocidin peptides	51
	<i>S. aurata</i>	G+, G–	SaBD (propeptide of 66 amino acids)	49
	<i>X. laevis</i>	G+, G–	Pexiganan peptide	52
	<i>Rana tigerina</i>	G+, G–	Tegrin 1, 2, 3, and 4 peptides	53
	<i>Litoria raniformis</i>	G+, G–	ND	54
	<i>L. fragilis</i>	G+, G–	Cathelicidin like peptide	42
	<i>X. laevis</i>	G+, G–, <i>C. albicans</i>	Meganins	55
	<i>G. g. domesticus</i>	G–	Gal-7 peptide	35

Table 1 Continued

Organs	Animal	Antimicrobial activity	Active factor/s	References
Blood/serum/ plasma	<i>Conger conger</i>	G–	ND	56
	<i>Ictalurus punctatus</i> (leucocytes)	G+, G–	Antimicrobial peptide (MW = 655 Da)	57
	<i>G. morhua</i>	G+, G–	ND	33
	<i>Tiliqua rugosa</i>	G–	ND	56
	<i>Alligator mississippiensis</i>	G+, G–, <i>Naegleria gruberi</i>	ND	58
	<i>Crocodylus siamensis</i>	G–, <i>C. neoformans</i> and <i>Aspergillus niger</i>	ND	59
	<i>Bufo marinus</i>	G–	ND	56
	<i>Gallus gallus</i>	G–	ND	56
	<i>G. g. domesticus</i>	G–	Gal-7 peptide	35
	<i>G. gallus</i> (leucocytes)	G+, G–	Gallinacins (Gal-1)	60
	<i>G. gallus</i> (heterophils)	G+, G–	CHP-1 and 2 peptides	61
	<i>M. gallopavo</i> (heterophils)	G+, G–	THP-1 peptide	61
		G+	THP-2 and THP-3 peptides	

G+: Gram positive bacteria; G–: Gram negative bacteria; ND: not determined.

lysates of lungs, liver, gallbladder, and stomach. The results revealed that only lungs lysate showed potent anti-fungal activities ($74\% \pm 5.76$) against *F. solani* spores, while liver, gallbladder, and stomach lysates had no effects ($8.4\% \pm 5.6$, $5.3\% \pm 4.0$, and $19.15\% \pm 12.2$, respectively) (Fig. 3). For *A. castellanii*, gallbladder lysate showed potent amoebicidal activity ($99\% \pm 1.02$), whereas lung and stomach lysates showed limited amoebicidal activities ($21\% \pm 4.65$ and $20\% \pm 5.76$, respectively) (Fig. 4).

Discussion

Widespread acquired resistance of bacteria against currently available antibiotics led the efforts to discover and design new antimicrobial agents. Previously, our group reported the discovery of antimicrobial activity from cockroaches and locusts.¹⁷ Both of these insects live in filthy environments and likely use these antimicrobials to protect their vital organs from invading bacterial pathogens. Given that snakes such as black cobra feed on germ-infested rodents, it was tempting to speculate that they possess antimicrobials to counter infections. A literature search revealed no reports on antibacterial activities of snake blood, plasma, or internal organs, albeit there are many reports on the antibacterial activity of snake venoms.^{24,25} Here for the first time, we show bactericidal activities in the blood/plasma as well as various body organs of the black cobra. Snake plasma showed potent activities against all bacteria tested except *S. pneumoniae*. Plasma, as high as 50%, failed to produce any antibacterial effect. Regardless, the remaining Gram negative and positive bacteria tested were effectively killed by the snake plasma suggesting the presence of robust antimicrobial molecules.

Lysates of snake lungs exhibited potent bactericidal activities. This is consistent with previous studies,

which showed that surfactant proteins and peptides of vertebrate lungs possess antibacterial properties (Table 1), but importantly fungicidal activity was also observed. In addition to surfactant proteins and peptides, these activities might be induced by microbicidal factors of the innate immune system such as lysozyme and lectoferrin.²⁶ It is noteworthy that lysates of the liver and gallbladder showed broad spectrum antibacterial activity against all bacteria tested. Gall bladder serves as a storage organ for bile, which is secreted by the liver. Bile is composed of proteins, ions, pigments, cholesterol, and various salts, collectively known to disrupt biological membrane and have selective antimicrobial activity against Gram positive bacteria.²⁷ Our results are consistent with previous findings that show that liver and gall bladder lysates of fish, frogs, birds, and mice possess microbicidal activities against Gram positive and negative bacteria (Table 1), but we also noted amoebicidal activity. Compounds such as squalamine, peptidoglycan-associated protein, and defensin-like peptides have been suggested to be responsible for antibacterial activity (Table 1). Future studies will determine whether similar factors are responsible for antimicrobial activities of snake plasma or identify novel bioactive molecules.

To test our hypothesis further, we tested antimicrobial activities of different internal organs of leaf-nose viper (*Eristicophis macmahonii*), a snake endemic to Pakistan–Iran border region. Our preliminary findings show that organs lysates of the leaf-nose viper exhibited antimicrobial activities similar to black cobra, suggesting that it is an important area for further research. Among other reptile species, the serum of American Alligator (*Alligator mississippiensis*),^{28–30} plasma of Siamese crocodile (*Crocodylus siamensis*),³¹ and tissue extracts of Nile crocodile (*Crocodylus niloticus*)³² have been shown to possess

antibacterial as well as antiviral and anti-protist activities.

In summary, for the first time we report antimicrobial activity from plasma and internal body organs of the black cobra. Although the identification and characterization of active compounds in snake plasma and organ lysates will determine their usefulness as potential antimicrobials, the results of this study are promising and suggest that animals living in polluted environments and/or feeding on germ-infested organisms are a potential source of antimicrobials. The broad spectrum activities against bacterial, fungal, and protist pathogens suggest that the black cobra and other snakes may serve as a novel source of antimicrobial compounds.

Disclaimer Statements

Contributors NK conceived the study. MS and RS designed and conducted all experiments under the supervision of NAK. MS, JI, and NAK contributed to the writing of the manuscript. All authors approved the final manuscript.

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Conflicts of interest None.

Ethics approval Not required.

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