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Investigating antibacterial potential of hippasa spider silk

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Abstract
Present study was designed with an aim to evaluate anti-bacterial potential of spider silk of *Hippasa holmerae* against gram negative bacteria *E. coli.* Extracts of spider silk were prepared in three different solvent – ethanol, methanol and acetone; and antimicrobial activity was carried out. Spider silk inhibited the growth of the *E. coli*. Effective zone of inhibition against the bacteria *E. coli* for the *Hippasa holmerae* silk extracts were observed as- in Methanol extract 10 mm zone of inhibition, Ethanol extract have 8 mm zone of inhibition and for Acetone extract 12 mm of inhibition after 24 hours. Whereas Methanol extract showed 10 mm zone of inhibition, Ethanol extract 9 mm zone of inhibition and Acetone extract 13 mm zone of inhibition after 48 hours. Amoxicillin reactivity was observed for *E. coli* as 9 mm and 10 mm zone of inhibition after 24 and 48 hours respectively.

Keywords: Spider silk, antimicrobial activity, *Hippasa holmerae, E. coli*. amoxicillin

Introduction
The pathogens are becoming resistant more quickly than the discovery of new drugs. The pharmaceutical industry is currently facing the trouble in developing effective antimicrobials for prevailing infectious diseases. It's a challenge for medicinal industry to develop biologically safer antibiotics. The antimicrobials derived from natural sources have always been regarded superior to synthetics in the scientific community because they occupy a broad range of biologically relevant and bio-friendly therapeutic chemicals. Spider silk components are a source of hope to overcome, as the spider silk showed bioactive nature.

The unique character of spider is production of silk. All spider produces silk throughout their life, but not necessary that all of them build a web. Silk gland and spinneret are the part of spider's morphology where the silk is manufactured to release. Spider abdomen bears appendages that have been modified into spinnerets that extruded silk from up to six of gland. In nature, spiders use their silk for several applications such as for web construction, wrapping of prey, protection of their eggs and offspring and as a lifeline, dragline which ensures their safe escape from predators.

Spider silk primarily consists of proteins that possess large quantities of nonpolar and hydrophobic amino acids like Glycine, Alanine and large amount of pyrrolidine. These amino acids maintain the moisture of spider silk and prevent it from drying out. Silk becomes acidic due to presence of these amino acids and microbes are unable to grow on it due to its acidic nature. Acidic environment prevents the biofilm formation and proves a challenging environment for bacterial growth (Cotter and Hill, 2003; Dagorn *et al*., 2013) [2, 3].

Spider silk is a remarkable material in nature; showing levels of strength, flexibility, lightness that are unmatched by man-made materials (Vollrath and Porter, 2006) [7]. Another notable feature of spider silk is its longevity. Spider webs often remain in the environment long after the spider has expired. The longevity of spider silk indicates that the material has some properties of resisting decomposing by microorganisms. (Esam J. Al-Kalifawi and Yasamine Jumaa Kadem 2019) [4].

The family Lycosidae consists of 2421 species of spiders belonging to 124 genera which are known for the unique eye pattern and typical egg sac carrying behavior. Keswani *et al* (2012) [5] reported 133 species belonging to 19 genera from India. Phartale *et al* (2016) [6]. *Hippasa holmerae* belongs to the family Lycosidae and commonly called as wolf spider, funnel web spider or tunnel spider. Wolf spiders are active spiders that may be found by day or night. The

body and legs are patterned with subtle hues of brown, gray and black which form excellent camouflage against the dead leaves and debris on the ground where they are found. Wolf spiders vary greatly in size. The smallest may measure only 3 millimeters in body length, while most lycosids are larger, reaching up to 30 millimeters.

Escherichia coli organism is Gram-negative bacillus from the family Enterobacteriaceae. *E. coli* comprises of non-pathogenic commensal isolates that forms part of the normal flora of humans and various animals. In humans, they are the major aerobic organism residing in the intestine. Several variants or pathotypes of *E. coli* have been described that cause infections of the gastrointestinal system while other pathotypes cause infections outside the gastrointestinal system. According to Al-Kalifawi (2017) [1], the Al-Ankabut's home extract (distilled water, ethanol and acetone) used against bacteria. Acetone has effective antibacterial activities for *Enterobacter cloacae*, *Escherichia coli* and *Pseudomonas aeruginosa.* Whereas distil water extract and ethanol extract of Al-Ankabut's home has no effect against tested isolates.

Amoxicillin is commonly used to treat a wide variety of bacterial infections. This medication is a penicillin-type antibiotic. It works by stopping the growth of bacteria.

Spider silk has been observed in nature to resist bacterial attachment on its surface, indicating that it contains microbe-repellent properties. Considering the antibacterial characteristic of silk of spider an attempt is made to explore antimicrobial activity of silk of *Hippasa holmerae* against bacteria *E. coli.*

Material and Methods

Hippasa holmerae spiders were selected because these spiders spin webs with large quantity of silk as well as they are abundantly found in the outskirt of Amravati, (MS) in the months between august to February. Webs were collected from different locations, from the places of their abundance.

These webs were washed with distilled water and then oven dried. Followed by drying web was weighed. Extract was prepared using three different solvents i.e. Ethanol, Methanol, Acetone.

Ethanol, Methanol and Acetone were tested for their antibacterial nature against *Escherichia coli* (gram-negative).

100 mg of web was dissolved in 1 ml of Ethanol, Methanol and Acetone separately for a week. The proportion of extract was 1:10. Extract made was centrifuged at 4000 rpm for 30 minutes. Extracts of web in different solvents showed different color appearance of supernatant.

Bacterial culture used for testing antibacterial activity of *Escherichia coli* (Gram-negative) Bacterial cultures were collected from Department of Microbiology PKV Akola.

Amoxicillin is a drug commonly used in the treatment of *E. coli* infection, therefore the amoxicillin tablet (500 mg) was used to compare antibacterial activity. Amoxicillin 100 mg was dissolved in 1 ml distill water to make equivalent concentration i.e. 1:10.

Agar gel was prepared. Inoculation was done in laminar airflow. Agar liquid was poured in petri plates and kept undisturbed for a while until it gets solidified into gel. Then using micropipette 5µl desired bacteria was inoculated. Spreading was done using 'L' shaped loop. A place was marked on the petri plate; a punched filter paper was deep into the extract in a cavity block and kept on the mark place. Incubation was done in incubator at 37 °C, after 24 hours and 48 petri plates were observed for zone of inhibition for all the three solvents.

Observations & Results

For control three solvents Ethanol, Methanol and Acetone were used without web. There was no effect observed of these solvents on the growth of the bacteria after 24 hours or later. The growth was uncontrolled and no inhibition of growth was observed. Ethanol, Methanol and Acetone extract prepared with *Hippasa holmerae* web showed clear zone of inhibition for bacteria *E. coli* (gram negative).

The results of the Methanol extract, Ethanol extract and Acetone extract against *E. coli* shows zone of inhibition 10 mm, 8 mm and 12 mm after 24 hours respectively. The petri plates were continued in incubator at 37 °C, and observed after 48 hours. Methanol extract shows 10 mm zone of inhibition. Ethanol extract shows 9 mm zone of inhibition and Acetone extract shows 13 mm zone of inhibition after 48 hours against *E. coli.*

For Amoxicillin extract *E. coli.* has 9 mm and 10 mm zone of inhibition after 24 and 48 hrs. of incubation respectively.

Table 1: Show the solvents (web extract) and diameter in mm

Solvents (web extract)	Diameter in mm	
	24 hours	48 hours
Methanol	10 mm	10 mm
Ethanol	8 mm	9 mm
Acetone	12 mm	13 mm
Amoxicillin	9 mm	10 mm

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