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Analyzing the antimicrobial potential of *Nephila pilipes* and *Stegodyphus sarasenorum* spider's silk on *E. coli*

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Abstract
Study was intended to assess antimicrobial activity of silk of spider *Nephila pilipes* and *Stegodyphus sarasenorum* on bacteria. Silk extracts were prepared separately in three different solvents Acetone, Ethanol and Methanol. Zone of inhibition was observed for bacteria *E. coli* after 24 hours. Apparent inhibition of bacterial growth was observed. For *E. coli* maximum zone of inhibition observed with silk extract of *N. pilipes* in Methanol extract 18 mm, followed by Acetone extract shown 14 mm diameter and 10 mm antimicrobial activity was found in Ethanol extracts. Maximum zone of inhibition observed with *S. sarasenorum* silk extract in Acetone 14 mm, Methanol 12 mm and Ethanol extract has shown the diameter 6 mm.

Keywords: Antimicrobial activity, web, *Nephila pilipes*, *Stegodyphus sarasenorum*, *E. coli*

Introduction
Spiders from order Araneae are air-breathing arthropods belongs to Subphylum Arachnids and rank seventh in total species diversity. They found worldwide on every continent except for Antarctica. As per report of World Spider Catalog (2019) [11] Version 20.5 taxonomists recorded worldwide there are 48,386 spider species, from 120 families. Spiders differ from other arthropods anatomically, having fused cephalothorax and abdomen, joined by a small cylindrical pedicel. The abdomen without appendages but bears one to four (usually three) pairs of movable spinnerets, which produce silk, Each spinneret has many spigots, each of which is connected to one silk gland. All spiders produce silk of different types and uses for variety of functions like building a web, capturing or wrapping prey, arresting a fall and making egg cases. Spiders silk is one of the most versatile materials in nature with great strength and flexibility. It contains protein fibers that have many advantages and functions. Spiders produce silk from seven different glands with varying mechanical and biomedical properties. These silks are produced in specialized glands on the spinnerets, which are located on the spider's abdomen. Spider silk consists of very large proteins (>200 KDa) (Altman *et al*., 2003) composed mostly of the non-polar and hydrophobic amino acids glycine or alanine.
Spider silk is mainly known for its remarkable mechanical properties and unique molecular structure. Silk can be considered as a potential biomaterial for the development of surgical sutures and tissue scaffold formations because of its desirable biomedical properties like slow biodegradability, biocompatibility, wound healing and nerve regeneration. All spiders do not spin the web, some only secrete silk to protect egg sacs or form simply drag line. Many spiders spin large webs, with different size and shape.
There are many ancient legends of application of spider silk in medicine. In the 18th century spider silk were used to cover the wounds by peasants of Carpathian Mountain (Heimer, 1988; Newman, 1995) [5, 9]. Spiders silk as therapeutic compound points out many dermatological applications like antihemorrhagic, antipyretic and advancement of cell regeneration, in 17th century (Newman, 1995) [9]. Heimer (1988) [5] stated that micro-organisms could not grow on the silk due to the acidic properties of the silk, suggesting that the spider silk may be preventing the formation of biofilms by microorganisms or may be bacteriostatic in nature.
Irrespective of the environment most of the spider webs stay in their environment for years. Being made up amino acids that can be easily assimilated by bacteria for growth, it is impressive that spider silk is so recalcitrant. In addition to glucose, amino acids are particularly valuable source of nutrition for bacteria as they can be used as pre-formed building block for protein synthesis and can also sole source of carbon, nitrogen or energy source (Moses *et al*., 2012) [7].

There are records indicating that spider silk has historically been used by humans for a variety of purposes. Peoples of the Carpathian Mountains are reported as using sections of the tubular shaped webs of *Atypus* spiders as topical bandages to heal wounds. This was believed to be beneficial due to the antiseptic properties of the spider silk.

Nephila pilipes is the golden orb-web spider. It found in China, Taiwan, Japan, India, Philippines, Sri Lanka, Myanmar, etc. The *Nephila pilipes* produces large, asymmetric, golden orb-web with a hub usually nearer top. These spiders invest very little energy in capturing prey by building larger sized webs. Webs of the young spiders are more circular than that of the adult and hungry spiders (Saravanan 2006) [10].

Stegodyphus sarasenorum are also known as social spider. It is native to India, Nepal, Sri Lanka and Myanmar (Karsch 1892, WSC 2016) [6]. This spider exhibit communal predation and feeding where individual live in large cooperatively build nest or retreat constructed of silk woven using leaves, twigs and sheet webs for capturing prey (Chakraborthy *et. al*. 2009) [2]. It has been observed that in life cycle of this spider at final instar stage female devotes her life for the spiderlings to use her body fluids and then dies (Deshmukh 2017) [3].

The natural world is a good source of therapeutic products that are able to inhibit the growth of microorganisms. Many of the microorganisms, especially bacteria are becoming resistant to many antibacterial agents. Study was intended to assess antimicrobial activity of silk of spiders *Nephila pilipes* and *Stegodyphus sarasenorum* on bacteria *E. coli*. Silk extracts were prepared separately in three different solvents Acetone, Ethanol and Methanol.

Material and method

Collection of spider's web

Spider silk of *N. pilipes* from family Nephilidae and *S. sarasenorum* from family Eresidae were collected from different locations, from the places of their abundance. Spider's fresh silk was collected with the help of brush and forceps. Silk was kept in polythene bags of 50 micron, maintaining aseptic conditions.

Bacterial culture

E. coli. Bacterial culture was procured from Department of Microbiology Bhartiya Mahavidyalaya, Amravati in the form of nutrient broth.

Broth cultures

Desired bacteria *E. coli* suspended in a liquid nutrient medium called Luria broth in an upright flask from which large amount of bacteria were cultured.

Extract preparation

Spider's silk was washed using distilled water and oven dried. Followed by drying silk was weighed. Extract was prepared using three different solvents i.e. Ethanol, Methanol, Acetone. 1 gm. silk dissolved in 10 ml of Ethanol, Methanol and Acetone separately for a week. Extract made was centrifuged at 4000 rpm for 30 minutes.

Inoculation

Agar gel was prepared and 5µl desired bacteria was inoculated. Spreading was done using 'L' shaped loop. Wells were made using sterile borer under aseptic conditions. Extracts were loaded on marked wells with the help of micropipette this process was carried out in flame zone of burner in a laminar air flow to avoid any kind of contamination and incubated at 37 ºC for 24 h. After 24 hours results were observed. The zone of inhibition was measured and expressed in millimeters.

Results and Discussions

Spiders are cosmopolitan and production of silk is one of their unique characteristic. In nature it has been observed that no spider web has been infected with any type of microorganism in any season, this study was planned to analyze antimicrobial potential of spider silk from *N. pilipes* and *S. sarasenorum* as their silk is abundantly available in our region.

Antimicrobial activity of silk of *N. pilipes* and *S. sarasenorum* was observed on *E. coli.* The extracts of spider's silk were prepared using three solvents Acetone, Methanol and Ethanol. Initially all these solvents (Ethanol, Methanol, and Acetone) were tested against *E. coli* for antimicrobial activity but there was no any activity observed. Extract of silk in distilled water also do not show any zone of inhibition.

Extracts of silk of *N. pilipes* and *S. sarasenorum* prepared by using three solvents, Ethanol, acetone, methanol, by taking concentration 1:10 (1 gm web in 10 ml solvent each). After inoculation the silk extracts were placed on the inoculated plate of *E. coli* and then incubated for 24 hour, results were observed after 24 hour.

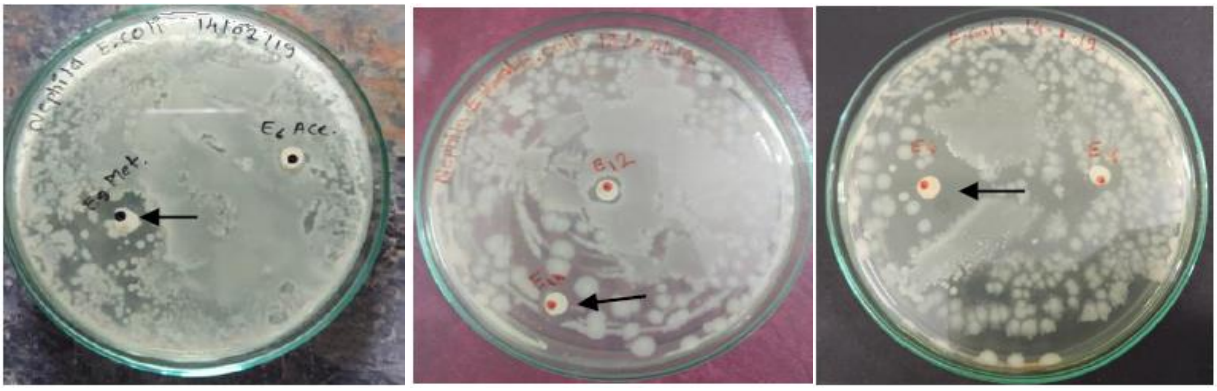
Zone of inhibition was observed for *E. coli*. Extract of *N. pilipes* silk in Methanol resulted in maximum activity i.e. 18mm in diameter as a zone of inhibition which was followed by Acetone extract as 14 mm and 10 mm in Ethanol. For *S. sarasenorum* silk extract in Acetone show maximum activity 14 mm in diameter followed by Methanol extract the zone of inhibition was 12 mm and Ethanol extract has resulted in minimum activity *i.e.* 6 mm.

Maximum zone of inhibition was observed in Methanol extract of *N. pilipes* for *E. coli* i.e. 18 mm. Extract in Acetone of silk *N. pilipes* and *S. sarasenorum* showed 14 mm of zone of inhibition.

Similar results were observed by Mohamed *et al.* (2012) [8] observed 10 mm of diameter of inhibition zone for *Bacillus subtilis* and 9 mm of diameter of inhibition zone for *Escherichia coli* in Acetone web extract. They also mentioned that in optimization, the maximum inhibition zone on the *Bacillus subtilis* was 15 mm at a time of 48 hours and concentration of 0.035 g/ml. and the maximum diameter of inhibition zone on the *Escherichia coli* was 12 mm at a time of 48 hours and concentration of 0.035 g/ml. The antimicrobial activity of Al-Ankabut's home (*Tegenaria Domestica*, Spider's web) extract on *Enterobacter cloacae*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Streptococcus sp*., *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus* was studied by Esam and Kadem (2017) [4], according to them the results show the acetone extract has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 12 mm for *Enterobacter cloacae*, 8 mm for *Escherichia coli* and *Pseudomonas aeruginosa*, 10 mm for *Klebsiella pneumonia* and *Proteus mirabilis.* 14 mm for *Bacillus subtilis*, 16 mm for *Staphylococcus aureus*, and 12 mm for *Streptococcus spp.* The antimicrobial activity of distil water extract and ethanol extract of Al-Ankabut's home has no effect against tested isolates, similar results were observed

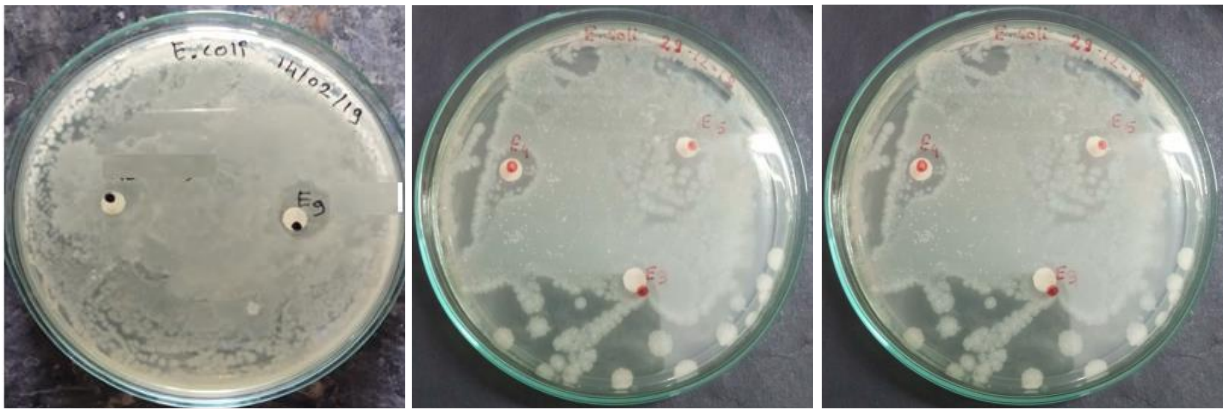
in our investigation ie no antimicrobial activity was observed in Acetone, Ethanol and Methanol (without spider silk), and extract of silk in water also not showed antimicrobial activity, indicating that spider silk is having antimicrobial potential.

Antibacterial Activity of *N. philipes* silk extract on *E. coli*



Acetone extrac-14mm Ethanol extrac-10 mm Methanol extrac-18 mm

Antibacterial Activity of *S. sarasenorum* silk extract on *E. coli*



Acetone extract- 14 mm Ethanol extract- 6 mm Methanol extract- 12 mm

Table 1: Zone of inhibition observed as - diameter in mm for silk extracts

Solvents	*N. pilipes*	*S. sarasenorum*
Acetone	14 mm	14 mm
Ethanol	10 mm	06 mm
Methanol	18 mm	12 mm

Conclusion

The results obtained show that spiders silk had significant potential as an antibacterial activity. The extract of both spiders silk prepared in Acetone have shown similar antimicrobial activity. This study will be the base for further investigations on advance purification.

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