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Medicinal prospects formajor ampullate silk of giant wood spider, *Nephila pilipes*

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Abstract
Spiders are well known for the silk they secrete. Amongst various spider species, *Nephila* is known for its long lasting and big size orb web having elastic but tough silk. Silk is an essential element in the life of orb weavers like *Nephila pilipes*. Silk appears in variety of physico-chemical forms from the body of this single individual. Spider silk is a protein-based material that does not appear to cause any allergic or inflammatory reaction. This remarkable fiber is very durable and can resist degradation in a wide variety of environments. Major ampullate silk of *Nephila pilipes* possesses the properties like water absorbance, supercontraction and antibacterial nature. Thus, this silk of *Nephila pilipes* could be potential source as antibacterial agent. It can be usedin bandages for advanced dressing of slow healing wounds. Major ampullate silk of *Nephila pilipes* canalso be used for manufacture of surgical threads, clinical masksand antibacterial cloths for small children.

Key words: Spider, *Nephila pilipes,* Major ampullate silk, Antibacterial, Biomaterial.

Introduction

We often take pride in our ability to create materials that are superior to ones created by nature. Yet some of the materials that nature creates out form everything designed by the human mind (Becker *et al.*, 2003). Spider silk is one of them, which have fascinated men for a long time.The production of silk is widespread among animal kingdom but it is particularly associated with phylum arthropoda. Certain insects like some lepidoptera, hymenoptera and neuropteran are capable of silk production. This ability is usually restricted to a single stage in their life span such as to build cocoon prior to pupation. In contrast, spiders have capacity to produce silk throughout their life. Silk production from the abdominal silk glands is unique to spiders which have made them successful in any habitat on the earth surface. Spiders depends on their silk for variety of life activities and functions like shelter, prey capture,dispersal, safety lines etc.Spider silk also contributed significantly in human welfare. An antibiotic property, light in weight, biocompatible and biodegradable nature of the spider silk motivates researchers for their use in medical science.

Material and Methods

Major ampullate silk samples were taken from the frame thread of the orb web of *Nephila pilipes*. For studying supercontraction, shrinkage of silk in pure water was calculated with formula given by Shao and Vollrath(1999).Water absorbance property of major ampullate silk threads of *Nephila pilipes*were observed. Antibacterial activity of major ampullate silk of *Nephila pilipes* were tested for bacteria like *Escherichia coli,Staphylococcus aureus, Pseudomonas aeruginosa* etc. Antimicrobial assay was performed using agar plates. Major ampullate silk threads (~ 1 mg) were first gently washed in sterilized distilled water and placed on the surface of inoculated agar. This process was carried out in flame zone of burner in a laminar air flow to avoid any kind of contamination. Plates were incubated for 24 hours at 37 ^{0} C and examined to observe zone of inhibition.

Results and Discussion

Nephila pilipes construct comparatively very big web in the woods of India. Dragline silk of *Nephila pilipes* as well as the main structural elements of *Nephila pilipes* web i.e. foundation lines and radii are having yellowish golden tinge. This silk is secreted from major ampullate glands.

A common feature of major ampullate silk of *Nephila pilipes* is its ability to shrink when submerged in water. Dragline or major ampullate silk thread get immediately supercontracted in contact with water. It was observed that major ampullate silk of *Nephila pilipes* shrank about 47 % (Table- 1; n= 05). Once a thread has shrunk to its maximally contracted state, it can no longer supercontract.

There are manmade polymers which exhibit supercontraction in organic solvents or when heated, but virtually none of which will supercontract in pure water at room temperature (Lewis, 1992). But a common feature of typical dragline silk is their apparent ability to supercontract when submerged in a solvent such as water (Work, 1977). Supercontraction property shows that dragline filaments are very sensitive to moisture. It causes the fiber to swell and increases its extensibility. The property of dragline silk of *Nephila pilipes* to supercontract immediately in water indicates that the elements of spider silk are oriented along the fiber axis. This is confirmed with the help of AFM images of dragline silk (Amaley *et al.*, 2015). Supercontraction generates substantial stress in silk when it is restrained, such as in the frames and radii of orb webs. Thus, supercontraction provides an advantage to the spider by tightening the web whenever the humidity is very high by contraction of the attachment lines and the framework of the web. This could potentially help

to maintain the structure and function of web (Savage *et al.*, 2004).

After being removed from water, the weight of major ampullate silk threads becomes nearly four times and after blotting to remove surface water droplets, it weighed nearly twice the original weight (Table – 2;n= 05). This result clearly indicates that of major ampullate silk of *Nephila pilipes* is able to absorb its own weight in water.Major ampullate silk of *Nephila pilipes* possesses very important properties of water absorbance and supercontraction. These properties make it excellent material for bandage and wound dressing. A good material for a bandage might have good water absorbance to soak up the dampness around the wound. This provides less favourable conditions for microbial growth. Heimer (1988) documented that, there is a tradition of pleasant using *Atypus* spider silk as a bandage and wound dressing.

Antibacterial activity of major ampullate silk was studied after 24 hours of incubation. Plate- 1 showed the results of antimicrobial activity of silk for different bacteria. After 24 hours of incubation, a clear zone of inhibition appeared around the major ampullate silk sample of *Nephila pilipes* for *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Diameter of clearing zones to the nearest millimeter were measured (Table- 3). The antibacterial property was observed in one year old silk also.

Testing of antimicrobial activity of *Nephila pilipes* silk gave strong evidence supporting the view that spider silk has antibacterial properties. Many times bacteria like *Staphylococcus, Pseudomonas* and *Escherichia coli* exist in air and dust (Griffin, 2007). There are number of chances that bacteria like *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* may be present in the forest soil. This could explain why *Nephila* silk had an inhibitory effect on these bacteria. This antimicrobial property might have evolved in silk in order to resist microbial decomposition. Reduced decomposition might be advantageous if it decreases the energetic cost of web maintenance and/or the level of harmful microbes to which spider is exposed (Wright and Goodacre, 2012). Such situation is beneficial for the orb weaver like *Nephila pilipes* which do not construct web daily, rather their webs are long lasting.*Nephila pilipes* generally sit at the hub of web, which is made up of dragline or ampullate silk. So, another benefit of this silk being antimicrobial is that this spider gets protected from microbes which may have adverse effect on the health of spider. One year old major ampullate silk also showed antimicrobial effect. This indicates that the antimicrobial properties are maintained for several days, probably, more than one year. Before inoculation on agar plates, silk threads of *Nephila pilipes* were gently washed in distilled water. In nature also, spider webs are frequently subject to rain and being wetted by rain. So, it is expected that antimicrobial properties of this spider silk threads would be maintained in wetted silk also.Spider silk has shown potential for use as a biomaterial. If fused to antimicrobial peptides (AMPs), recombinant spider silk could be used for medical applications and reduce the need to use conventional antibiotics to battle infections (Floderus L.S., 2016).

Conclusion

Major ampullate silk of *Nephila pilipes* possesses the properties like water absorbance, supercontraction and antimicrobial activities.This silk of *Nephila pilipes* inhibits the growth of bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*, which is the common cause of wound infections. So, it may be used as excellent material of bandage for wound dressing. Bacteria mentioned above are also important pathogens in nasocomial infections. So, this silk can be recommended to use for preparation of clinical masks used in hospitals. This high quality bio-material may be used for preparation of antibacterial cloths for small children and also for manufacture of surgical threads. Major ampullate silk of *Nephila pilipes* could be potential source as antibacterial agent. For this, recombinant DNA technologies can be applied for large scale production of silk.

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Table - 1: Supercontraction of major ampullate silk of *Nephila pilipes* (n= 05)

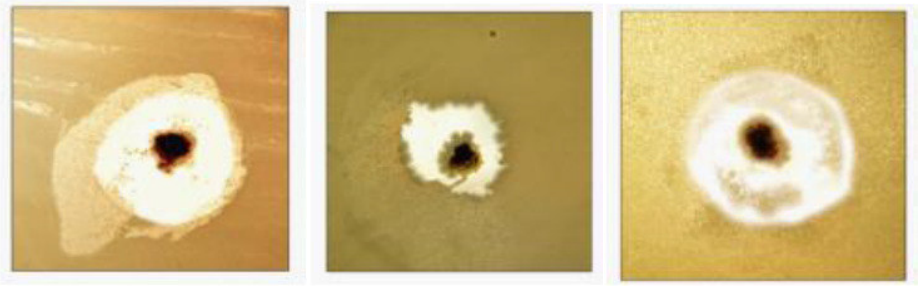
Sr. No.	Initial length of silk thread (L_0) in mm	Length of contracted silk thread (L) in mm	$Shrinkage(\%) = \frac{(L_0 - L)}{L_0} \times 100$
1	20	11	45
2	20	10	50
3	20	10	50
4	20	11	45
5	20	11	45
		Average	**47 %**

Table–2: Water absorbance capacity of major ampullate silk of *Nephila pilipes* (n=05)

Sr.No.	Weight of dragline silk (mg)		
	Dry	Wetted	After blotting
1	200	790	420
2	200	810	410
3	200	760	390
4	200	780	420
5	200	820	420
	Average	**792**	**412**

Table- 3: Antibacterial activity of major ampullate silk of *Nephila pilipes* after 24 hours of incubation.

Bacteria tested	Diameter of inhibition zone (in mm)
Escherichia coli	13
Staphylococcus aureus	11
Pseudomonas aeruginosa	14



a b c

Plate- 1: Inhibition zone for major ampullate silk of *Nephila pilipes* in the lawn of –
a. *Escherichia coli;* b. *Staphylococcus aureus;* c. *Pseudomonas aeruginosa.*

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