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Research Article

MAJOR AND MINOR AMPULLATE SILK GLAND PROTEINS OF *NEPHILA PILIPES* FROM KARNATAKA

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ABSTRACT
Spider silk is predominantly composed of structural protein called spider fibroins or spidroins. Spiders spin high performance silks through the expression and assembly of tissue restricted fibroin proteins. Spider silks are composite protein biopolymers that have complex microstructures. Protein concentration and estimation of molecular weight was carried out for major and minor ampullate silk gland of *Nephila pilipes* by Lowry's method and SDS-PAGE analysis. The Lowry method revealed that *protein* concentration was more in the major ampullate gland (33.5924µg) compared to minor ampullate gland (29.8084µg). SDS-PAGE revealed similar bands in the range of 35 to 40 KDa in weight.

Key Words: *Nephila, Major Ampullate Silk Gland, Minor Ampullate Silk Gland, SDS-Page.*

INTRODUCTION
Spiders spin high performance fibers with diverse biological functions and mechanical properties. Spider silk is an intriguing biomaterial that is light weight, extremely strong and elastic and exhibits mechanical properties comparable to the best synthetic fibers produced by modern technology (Hinman *et al.,* 2000). Silk fibers are protein based biopolymer filaments secreted by specialized abdominal glands connected the spinnerets, ducts or spigots and are used in different combination to produce structures for prey capture, reproduction and locomotion (Gosline *et al.,* 1986). Spider silk is spun near ambient temperatures and pressure using water as the solvent, which gives rise to an environmentally biodegradable material (Asakura, 1994). The different silk types are protein based polymers that are members of the spider silk protein super family and display restricted expression in seven morphologically distinct silk glands. These distinct abdominal glands are thought to have evolved from a single type of gland, and have subsequently diverged in their anatomy, luminal contents and morphology (Vollrath, 1992).
Based upon the differentiated amino acid composition of the luminal contents, the silk proteins within each gland are proposed to be assembled to create specific fibers with particular functions. To date most research has focused on the major ampullate gland which manufactures dragline silk constituents. Drag line silk is well known for its combination of high tensile strength and elasticity, which leads to a fiber with extraordinary toughness (Gosline, 1986). Spiders use dragline silk to create web anchors, as well as safety lines for survival. The minor ampullate gland, which shows morphological similarity to the major ampullate synthesizes web radii filaments and temporary capture silk. The present study is on the Indian spider Nehila, a preliminary study intended to compare the protein in the major and minor ampullate silk glands by Lowry's method and SDS-PAGE analysis.

MATERIALS AND METHODS
Spider Collection and Handling
Spiders were collected from different regions of Karnataka in India. Collection was done early in the morning from January 2012 to October 2012 and identified up to species. A sample size, n=10/ were utilized for the present analysis.

Spider Dissection

Spiders were euthanized with ethyl aetate. The abdomen (opisthoma) was separated from the cephalothorax and submerged and dissected in spider ringer solution 1x Ringer: 13g NaCl, 0.5 KCl, 0.89g CaC_{12}, 1.04g $MgC_{12}.6H_2o$ buffered with tris base (100 nM, pH=7.2) under a stereomicroscope (Dicko *et al.*, 2005). The tissues of the alimentary canal and blood vascular system were removed to make the glands clear.

Protein Estimation and Molecular Weight by SDS PAGE

The major and minor ampullate gland proteins of Nephila were separated by SDS following Yigit *et al*., (2004). Protein concentration was measured by Lowry method (1951) using bovine serum albumin as standard at 660nm. Electrophoretic separation of proteins was performed using 12% acrylamide gel with 1 mm thickness following dissociating and discontinuous buffer system. Protein bands were visualized by Commessei blue staining protocol.

RESULTS

There was a significant difference in the protein concentration of the two glands of *Nephila pilipes*. Major gland the concentration was 33.5924 and the concentration in the minor gland was 29.8084.

SDS PAGE showed two prominent bands for both the major and minor ampullate silk glands of *Nephila pilipes*. The molecular weight was in the range of 35kdaltons.

Table 1: Protein content in two different glands of *Nephila pilipes*

S.No.	1	2	3	4	5	6	7	Major ampullate gland	Minor Ampullate Gland
Conc. µg/tube	0	10	20	40	80	120	160	33.5924	29.8084
O.D @ 660nm	0.000	0.115	0.2	0.39	0.55	0.68	0.9	0.286	0.276

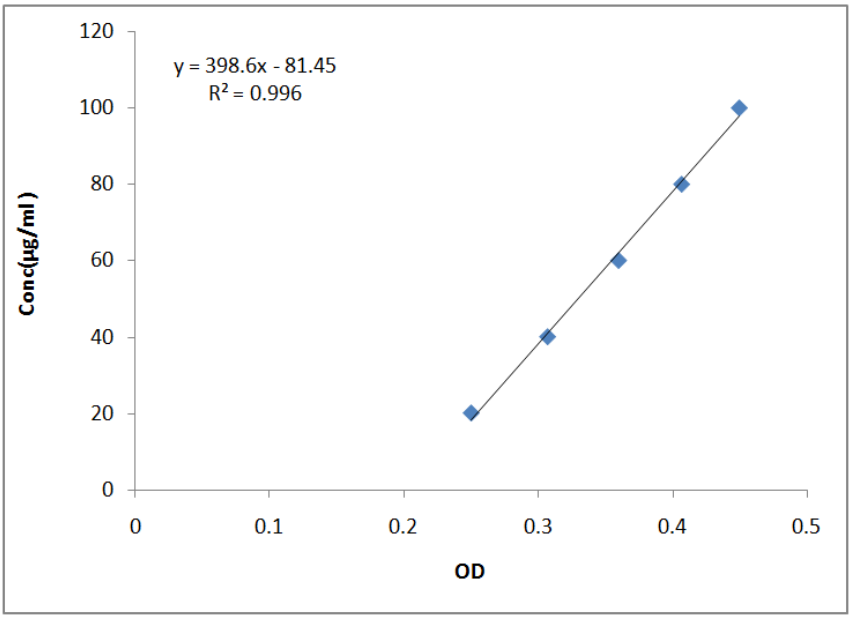

Figure 1: Standard Graph

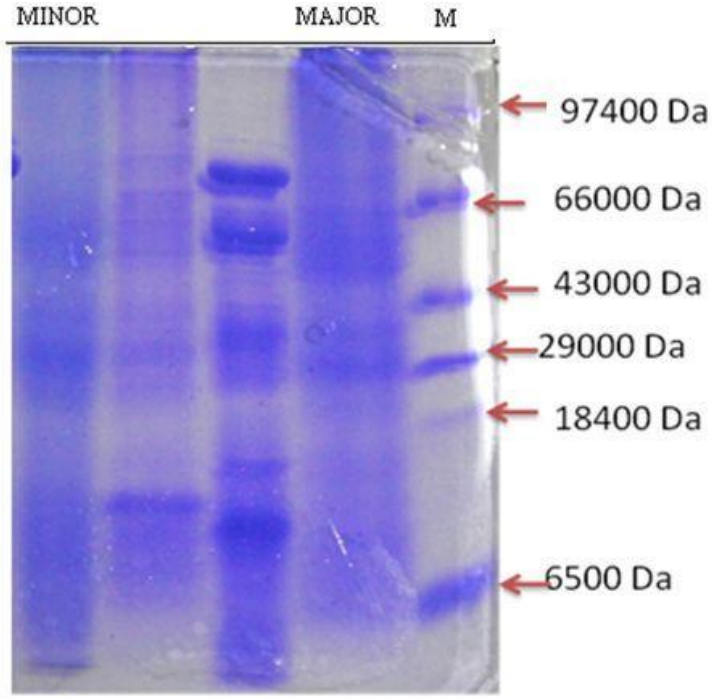

Figure 2: SDS PAGE of major and minor ampullate silk glands of *Nephila clavipes*

DISCUSSION

In this study the difference between the concentration of major and minor ampullate silk protein of Nephila *pilipes* species of Karnataka have been done. The major ampullate silk gland protein of *Nephila* was more than that of minor ampullate silk gland protein *(major* 33.5924µg/tube and minor 29.8084 µg /tube). The band in the SDS-PAGE for major and minor silk glands showed a band of molecular weight in the range of 35KD. Major and minor silk glands though have the same protein their function is different The concentration of protein is more in major ampullate gland and less in minor ampullate gland throwing insight in their functional variation.

REFERENCES

Alexander S, Bernhard S, Vollrath F, Eberhard U, Frank G and Klaus W (2005). Characterization of Protein components of Nephila Clavipes Dragline silk. *Biochemistry* **44** 4727-4736.

Casem ML, Turner D and Houdin K (1999). Protein and amino acid composition of silkbfrom cob weaver. Lactrodetus Hesperus (black widow spider). *International Journal of Biology and Macromolecules* **24** 103-108.

Lombardi SJ and Kaplan D (1990). The amino acid composition of major ampullate gland silk of Nephila clavipes. *Journal of Arachnology* **18** 297-306.

Hinman MB, Jones JA and Lewis RV (2000). Synthetic spider silk: a modular fiber. *Trends Biotechnology* **18** 374-379.

Gosline JM, Demont ME and Denny MW (1986). The structure and properties of spider silk. *Endeavous* **10** 31-43.

Vollrath F (1992). Spider webs and silk. *Scientific American* **266** 70-76.

Asaura T and Kaplan D (1994). Silk production and processing. *Encyclopedia Agricultural Sciences* **4** 1-11.

Yigit and Benli (**2008)**. The antibacterial activity of hemolymph of spider, *Agelena labyrinthica* (Araneae: Agelenidae*). Journal of Forestry Faculty* **8**(2) 120–124.