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***Research Article** (Open Access)*

PROTEOMIC ANALYSIS OF *STEGODYPHUS SARASINORUM* SPIDER SILK

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ABSTRACT
Spider silk is world strongest biopolymer made up protein. It was very difficult to solubilize spider web silk completely. The molecular weight of spider silk protein was determined to be ~600 kDa, under its natural unreduced state.
The proteomic analysis of Indian spider silk was not done yet. So, our aim is to solubilize the *Stegodyphus sarasinorum* web silk and analyzed it molecular weight and perform MALDI TOF TOF for partial sequencing of the protein. For a comprehensive proteomic analysis of *Stegodyphus sarasinorum* spider silk we treated it with different Hofmeister series chemicals. Partially solubilization achieved by using Lithium thiocyanate and proceeded for proteomic studies. In Present study we found at reducing condition the silk protein had a molecular weight ~74.1 kDa and made up of 664 amino acids

Keywords: *Spider, Stegodyphus sarasinorum, Proteomic analysis, MALDI*

INTRODUCTION
Spider silk exhibit various applications in field of materials science due to its impressive tensile property, characteristics of intriguing torsional and a water-induced physical response that is (Zemlin 1968, SavageGuerette & Gosline 2004, Lefèvre *et al.* 2014, Lefèvre & Auger 2016b). Spider silk is very attractive for biomedical uses as it shows antimicrobial potential, wound healing, biodegradability, immunogenicity, cancer treatment and low toxicity with cell adhesion and growth (Küppers 2011, Wright & Goodacre 2012, Kumari *et al.* 2013, Nagal & Singla 2013, Kundu *et al.* 2014, TottenWongpinyochit & Seib 2017). Spider silk is used for biochemical sensing (Tow *et al.* 2015). In addition, a spider silk protein, spidroins processed in various colloidal and physical states like gels, films, capsules, emulsions, foams, porous systems, non-woven fiber and fibers (Lefèvre & Auger 2016a). Therefore, so many opportunities are going to be open to find out the advantageous qualities of spidroins (Lefèvre & Auger 2016b). Biotechnological procedures also promise to design spidroin inspired sequences that possibly producing spidroin like materials with customized properties (HumenikSmith & Scheibel 2011, Huang *et al.* 2017).
There was very scanty work on the Proteomic analysis of Indian spider web silk protein. Current study focused on the proteomic analysis of Indian spider *Stegodyphus sarasinorum* web silk.

MATERIALS AND METHODS
The spider species were collected from North Maharashtra region during 2014-2017. The *Stegodyphus sarasinorum* (SS) spider species was identified from Zoological Survey of India (ZSI), Western Regional Centre, Pune. The spider web silk was collected by using spider silk harvester with few modifications (Work & Emerson 1982) the collected silk was stored at -20°C up to the use.
The Hofmeister series chemicals Lithium bromide (Sigma), Lithium thiocyanate (Sigma), Lithium Perchlorate (Sigma), 1,1,1,3,3,3-Hexafluoro-2-propanol (Sigma) and Guanidine thiocyanate (SRL chemical, Mumbai) were used to solubilize spider web silk proteins (HardyRömer & Scheibel 2008). For dialysis Spectra/Por Float-A-Lyzers G2, Molecular weight cut-off (MWCO) 3.5-5.0 kDa (Sigma) was used. Size exclusion chromatography was conducted using Sephadex$^{(R)}$ G-25 (Sigma).

The two-two ml fractions were collected and there absorption was taken at 280 nm using Systronics UV-Visible spectrophotometer-118. The fraction showing highest absorption were proceed further for HPLC analysis using C18 column and the mobile phase of water and acetonitrile as solvents A and B in 0.1% formic acid, respectively. Solvent B was used at the following concentrations and times: 99% for 5 min, 99% to 50% for 20 min, 50% to 1% for 20 min, 1% for 10 min, return to 99% for 5 min and 99% for 5 min (Larracas *et al.* 2016a).

The sample further proceeds for determined the Molecular weight of protein by using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The 12% separating and 3 % stacking gel was selected. Gel was stained by using Coomassie Brilliant Blue R-250 and Silver nitrate using protocol explained by Bull (2012), Matsuhira and Osaki (2015) and Larracas et al. (2016a). All chemicals used for both the studies are of analytical grade reagent (Sigma). MALDI TOF TOF analysis was conducted by Sandor Lifesciences, Hyderabad as a consultancy.

RESULTS

The spider web silk is strongest polymer found in the nature, and this polymeric protein is not solubilized in any polar or non-polar solvent also difficulty solubilized in acids and alkalis which denature the protein present in it. The spider web silk protein was not solubilize in Lithium bromide and Lithium Perchlorate while Lithium thiocyanate, 1,1,1,3,3,3-Hexafluoro-2-propanol and Guanidine thiocyanate it get solubilize but maximum solubilization found in Lithium thiocyanate.

The solubilized material was dialyzed Spectra/Por Float-A-Lyzers G2, also for purification and excess salt removal Sephadex$^{(R)}$ G-25 from Sigma Aldrich was used. The second fraction showed highest absorption at 280 nm. The purity of protein was checked by using HPLC. After 16 min run a large single peak was observed which indicates that the protein was isolated and pure. Estimated protein was 0.11 ± 0.03 Gm/Gm Lowry *et al.* (1951).

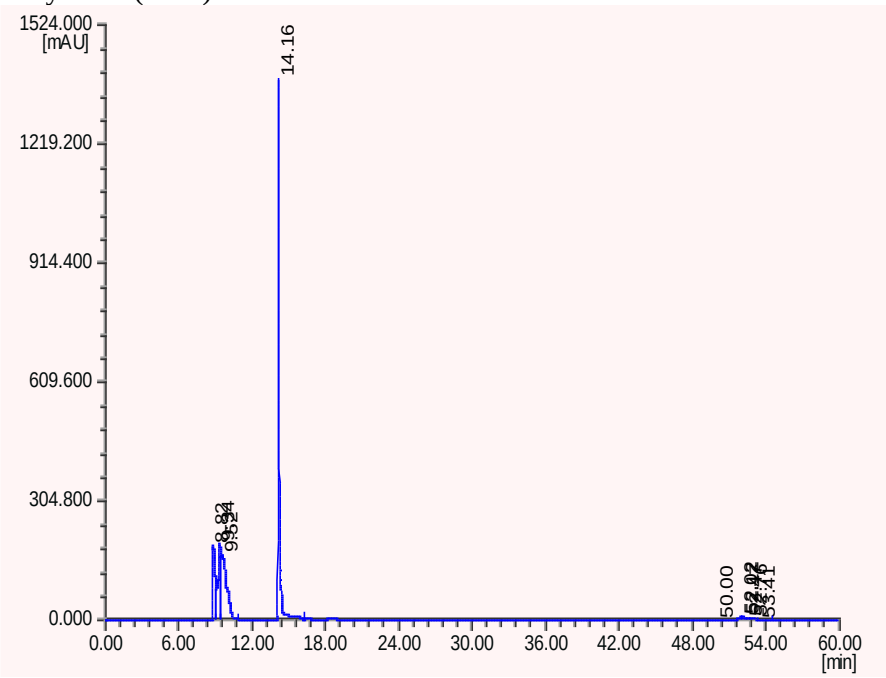

Figure 1: HPLC analysis for purified silk protein

To find out the molecular weight of isolated protein, SDS PAGE was performed and find out the molecular weight of SS web protein, it was 74.1 KD and the sample is proceed for the sequence analysis using MALDI TOF TOF from Sandor Lifesciences, Hyderabad.

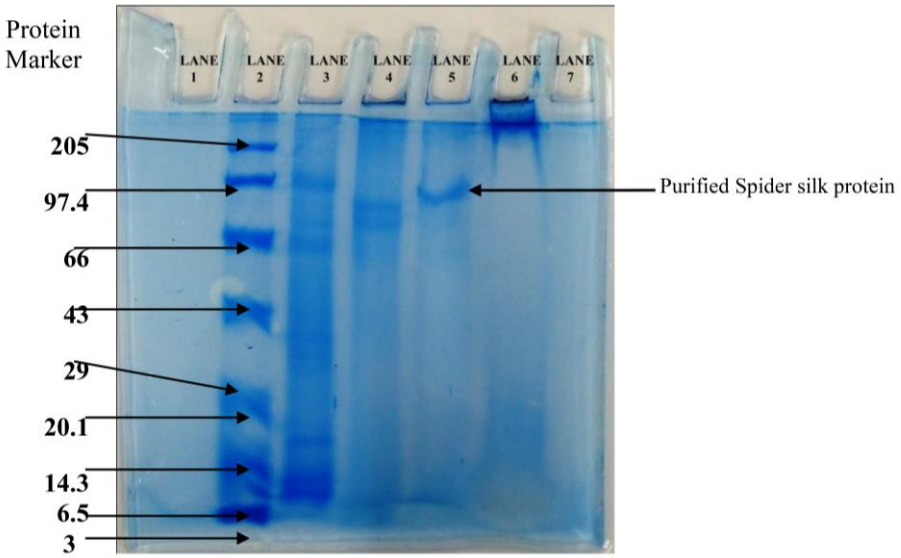

Figure 2: SDS PAGE of Spider silk protein

From left to right lane 1 Blank, Lane 2 protein Marker, Lane 3 Silk gland Protein, Lane 4 Guanidine thiocyanate (GITC) Soluble Silk, Lane 5 Lithium thiocyanate Soluble Silk, Lane 6 HFIP soluble Silk.

The MALDI TOF analysis was performed by the Sandor Lifesciences, Hyderabad and the PMF search was conducted using Mascot Distiller for getting the sequence of amino acid for the SS web protein. The protein made up of 664 amino acids. Sequence was blast at http://www.uniprot.org and it shows the sequence was closely similar to the *Stegodyphus mimosarum* spider silk protein and it was 58.6% identical to the *Stegodyphus mimosarum* spider silk protein accession number A0A087T6M7 and Score: 304. The MALDI MS and MASCOT Distiller sequence using MALDI MS was as follow,

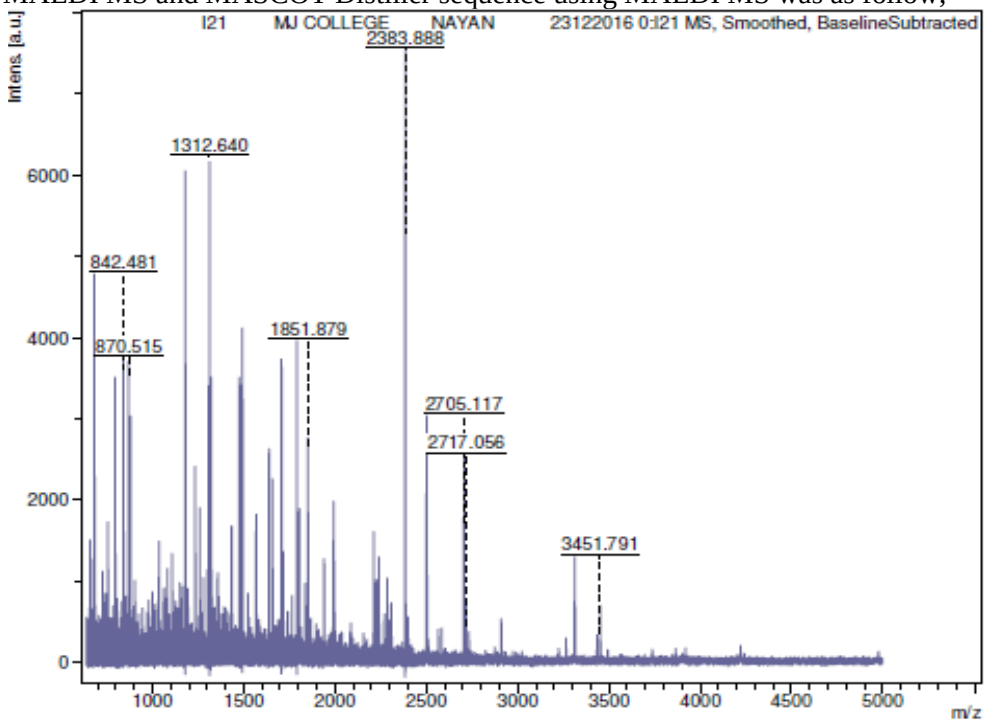

Figure 3: MALDI MS of SS spider silk protein

The MALDI TOF of SS silk was given as follow. After this the MS/MS analysis of 4 fragments were tested and the sequence was as follow which was predicted by using Bruker Daltoniks Biotools software from Sandor Lifesciences, Hyderabad. The sequence obtain by MS/MS was
For1312.748 M/Z= SHFTRQLAKPK
For 1475.691M/Z = DQQQDSLNCVLR
For 1657.775 M/Z = NMDEAHIDQRWK
For 1851.945 M/Z = SSSEGADPSVRPLDPIK

DISCUSSION

As per HardyRömer and Scheibel (2008) spider web silk dissolved in the Hofmeister series chemicals as those showing Salting in and Salting out effect. We use Lithium bromide, Lithium thiocyanate, Lithium Perchlorate, 1,1,1,3,3,3-Hexafluoro-2-propanol and Guanidine thiocyanate were used. We found Lithium bromide, and Lithium Perchlorate were fail to solubalize the spider web silk while spider web silk solubilized poorly in 1,1,1,3,3,3-Hexafluoro-2-propanol and Guanidine thiocyanate. The saturated solution of Lithium thiocyanate can solubilize spider web silk nicely. The protein contain was determine by Lowry et al. (1951) method. The SS silk have good protein contain and medicinal properties so it proceeded for purification. The Solubilized web silk was passed through size exclusion chromatography. It was observed that the Protein was eluted out in second fraction and it was confirmed by HPLC method explained by Larracas *et al.* (2016b). The molecular weight was determined by using SDS PAGE electrophoresis and it found 74.1 KD Web silk. The sample was further processed for MALDI and the MALDI data was interpreted using MASCOT Distiller which gives a 664 amino acid sequence. While at the same time Peptide mass fingerprinting (PMF) search was conducted at Protein Prospector v 5.20.0 against 572 uniprot Spidroin protein molecules. *Stegodyphus mimosarum* spider silk protein accession number A0A087T6M7 seems 58.6% identical and Score: 304. It suggests that the Indian spider species *Stegodyphus sarasinorum* web silk had molecular weight 74.1 KD and the PMF search showing 32% similarity with *Stegodyphus mimosarum* spider silk protein.

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