Comparative biochemical and structural analysis of silk fibers from weaver ants and paper wasps and their potential applications

Nasiha M I[1*], *Sajitha* N[2], *K A* Rasheed[1]

[1] Post Graduate and Research, Department of Zoology, Government Victoria college Palakkad, India
2 Department of Zoology, Arignar Anna Government arts and science College, Puducherry, India

Abstract: Silks are natural fibrous proteins comprises of specific sequences of amino acid that can be secreted into solidified fibres. These are naturally occurring polymers and have garnered profound attention for their mechanical properties, biodegradability and biocompatibility. These characters of silk made them stand out for a range of suitable biomedical applications. Present study researched and compares their biochemical composition, structural details, and thermal stability of silk fibres extracted from the nests of Paper Wasps (Polistes sp.) and Weaver Ants (*Oecophylla* sp). Fourier Transform Infrared Spectroscopy (FTIR) and Thermogravimetric Analysis (TGA), were exploited to shed light on the structural and thermal properties of the fibres. Results reveal that silks from Paper Wasps shows higher protein concentration (44.7%) and superior thermal stability compared to Weaver Ant silk (23.4%). These two silks reveal structural characteristics akin to *Bombyx mori* silk, with distinct β-sheet and α-helical conformations, indicating their potency for advanced applications in tissue engineering, nanotechnology, and drug delivery systems. This study highlights the untapped potential of these alternative silk sources in the biomedical field, which paved the way for further delve and its utilization.

Keywords: *Silk fibroin, Biomedical, FTIR, TGA, Biomaterials, Tissue engineering, Nanotechnology*

1. Introduction

Silks are naturally occurring biopolymers and known for their remarkable mechanical properties. These silks are primarily composed of proteins called fibroins and produced by animals which belongs to the class Arthropoda. These proteins showcase specific structural and chemical properties, making them exemplar for diverse biological functions such as building protective shelters, biocompatibility capturing prey [1]. Traditionally, silk derived from Bombyx mori has been extensively studied and utilized in biomedical applications due to its slow degradability, mechanical strength and biocompatibility [2]. However, other

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[*] *Corresponding Author: nasihami5@gmail.com*

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sources of silk, particularly insects from Hymenopterans groups like ants and wasps, remain unexplored despite of their potential properties.

Hymenopteran silks, those produced by Weaver Ants (*Oecophylla* sp.) and Paper Wasps (*Polistes* sp.), differ radically in their molecular structure and biochemical composition from silks of moths and spiders which exhibit β-sheet crystalline character [3]. The principal molecular structure of social hymenopteran silk is α helical proteins assembled into a tetrameric coiled coil conformation, a fundamentally different design to the β- sheet crystalline that dominate the silkworm cocoon. These silks are conferred with toughness and stability which contributes a capitivating area for biomedical applications [1]. Larval salivary glands of weaver ant produce a silk which is primarily used for nest construction and shows inimitable conformational and biochemical characteristics that maintain its potential as a natural scaffold for cell growth [4]. They also exhibit amide bonds, such as C-H and C-O bonds which attribute to their random coil and β-sheet conformation in their protein structure. Conversely, specific amino acid composition like glycine, proline, alanine, serine and high nitrogen content around 11% which overt significant strength and thermal stability [5].

Recent researches elevating the significance of substitute silk sources for tissue engineering and biomedical applications. They act as promising candidates for applications ranging from drug delivery systems to artificial tissue scaffolds with their ability to manoeuvre the morphology and molecular structure of silks, combined with their incomparable biocompatibility [6]. Regardless of these advancements, silks from both of these insects remain unexplored and demands more focus.

This study intended to elucidate the biochemical and structural characters of two different silk from Paper wasp and weaver ant by employing FTIR and TGA. Further investigation in this field is necessary to unravel the most of the biochemical and structural qualities of the silk fibre of these two different species to propagate the same for economic utility.

2. Materials and Methods

2.1 Sample Collection and Preparation

Silk fibers were collected from the nests of Weaver Ants (*Oecophylla sp.*) were sourced from their natural habitat, and nest of Paper wasp (*Polistes sp.*) were collected from buildings and hidden corners of house and construction site, wooden areas etc. Samples were carefully isolated without disturbing the insect colonies. The collected silk fibres were purified with distilled water to get rid of impurities and kept in a dry environment for analyses.

Fig. 1. Weaver ant nest

Fig. 2. Paper wasp nest

2.2 Biochemical Analysis

2.2.1 Protein Estimation

Lowry method [7] is about 10 times more sensitive than the biuret method. Aliquots of protein solution and samples of different concentration pipette out in a test tube and the total volume made up to 4ml with distilled water. To each test tube 5.5ml of alkaline mixture mixed well and incubated at room temperature for 15 minutes. 0.5ml of Folin-Ciocalteu reagent is added and mixed rapidly. After 30 minutes absorbance is measured at 650nm.

2.2.2 Lipid Estimation

Lipid extraction was done by using Bligh and Dyer method [8]. Silk fibers (100 mg) were prime with distilled water, keep the sample in warm water and blow and a chloroform-methanol (2:1) mixture was added. The resulting solution is subjected to centrifuge, generally 3 layers are seen. A clear layer of chloroform containing all the lipids, a coloured aqueous layer of methanol and a thick pasty interface are seen. The methanol layer is discarded and the lower layer is carefully collected free of interphase either by sucking out with a fine capillary or by filtration through glass wool.

2.2.3 Nucleic Acid Estimation

In this process RNA concentrations were estimated using Orcinol [9] and DNA by Diphenylamine methods [10]. DNA was determined by adding diphenylamine reagent, and then subjected to heating and absorbance was measured at 595nm. RNA was determined by mixing samples with orcinol reagent and then heated in a boiling water bath for 15 minutes. Then measure the absorbance spectra at 665 nm.

2.3 Structural and Thermal Analysis

2.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is used for analyzing chemical bonds present in the organic materials It is the sophisticated tool used to identify the secondary structures of silk proteins by and well-known instrument JASCO FTIR-4100. The secondary structure and conformational characteristics of the films (10 cm^2) prepared as were analysed by placing the thin films directly on the holder of the instrument. The frequency of absorption was in the region of 4000 to 400 cm^{-1} at room temperature. Infrared spectra were recorded from 32 scans per sample with resolution of 4 cm^{-1}. The structure of protein was confirmed by the presence of amide bond including of β-sheet and α-helices.

2.3.2Thermogravimetric Analysis (TGA)

Thermogravimetric analysis gives information on the thermal stability of the material. In TGA, a change in weight in relation to temperature is continuously recorded under controlled heating rate The Thermo gravimetric analyses of the films were carried out using Perkin Elmer STA-6000 instrument (USA). Each sample (5 mg) was heated from 40 ^oC to 500 ^oC at a heating rate of 10^oC/min. The inert atmosphere was provided by nitrogen at a flow rate of 200 ml/min. TGA is widely employed in research and testing to determine characteristics of polymers, to determine degree of degradation and moisture content of materials, solvent residues and composition of blends etc. Thermal degradation and stability were analysed by recording the weight loss pattern [11].

3. Results

3.1 Biochemical Composition of Silk Fibers

3.1.1 Protein Content

The protein content of silk fibers was estimated using Lowry's method [7]. Results revealed that the silk from Paper Wasp nests contained a significantly higher protein concentration (44.7%) compared to the silk from Weaver Ant webs (23.4%) (Table 1) (Fig.3). This indicates that Paper Wasp silk is richer in protein components and amino acid composition.

Table 1. Protein concentration in silk fibres

Silk fibre (1g)	Concentration of proteins(mg) for 5replica	Average	Percentage concentration
Silk from ant web	447 448 447.5 447 447	447.3	44.7%
Silk from wasp nest	234.5 234 234.5 235	234.4	23.4%

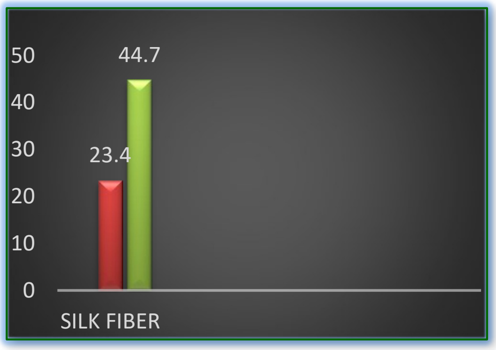

Fig. 3. Protein concentration silk fibre

3.1.2 RNA and DNA Content

Nucleic acid concentrations were found to be minimal in both silk fibers. RNA content was 0.015% in Weaver Ant silk and 0.020% in Paper Wasp silk. DNA content was 0.014% in Weaver Ant silk and 0.018% in Paper Wasp silk (Table 2 and Table 3) (Fig. 4 & 5). These findings suggest limited genetic material present in the silk fibers.

Table 2. RNA Concentration in silks

Silk fibre (1g)	Concentration of RNA (mg) for 5replica	Average	Percentage concentration
Silk from ant web	0.0015 0.0016 0.0015 0.0015 0.0016	0.00154	0.015%
Silk from wasp nest	0.0020 0.0020 0.0021 0.0020 0.0020	0.00202	

Table 3. DNA Concentration in silks

Silk fibre (1g)	Concentration of DNA for 5replica	Average	Percentage concentration
Silk from ant web	0.0015 0.0014 0.0015 0.0015 0.0015	0.00148	0.014%
Silk from wasp nest	0.0018 0.0018 0.0019 0.0019 0.0018	0.00184	0.018%

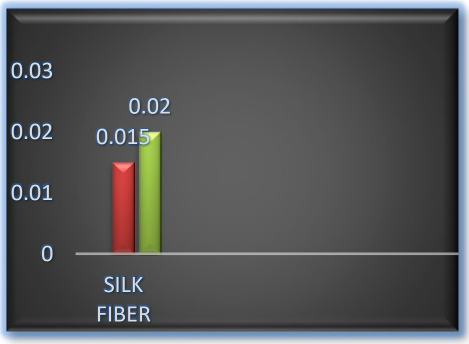

Fig. 4. RNA concentration

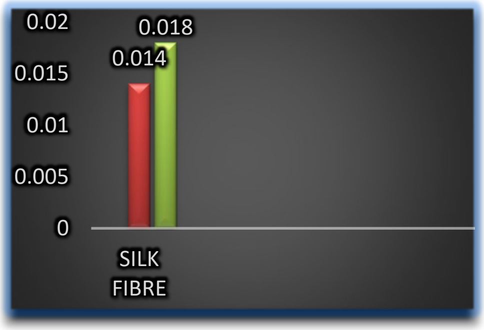

Fig. 5. DNA Concentration

3.1.3 Lipid Content

Lipid estimation showed that Paper Wasp silk contained a significantly higher lipid concentration (5%) compared to Weaver Ant silk (1%) (Table 4) (Fig. 6). This suggests that the lipid-rich silk from Paper Wasp nests could play a role in its structural and functional properties.

Table 4. Lipid Concentration in Silk Fibers

Silk fibre (1g)	Concentration of lipid (mg) for 5replica	Average	Percentage concentration
Silk from ant web	50 49 50 51 50	50	5%
Silk from wasp nest	10.6 10.0 10.0 10.5 10.0	10.2	1%

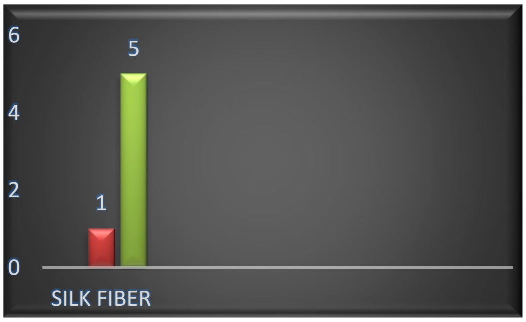

Fig. 6. Lipid concentration in silk fibres

3.2 Structural Analysis of silk fibres

Fourier Transform Infrared Spectroscopy (FTIR) revealed distinct secondary structures in the silk fibers. The Weaver Ant silk exhibited strong β-sheet conformations, as indicated by the amide I band at 1641.88 cm⁻¹ and the amide II band at 1532 cm⁻¹(Fig.7). In contrast, Paper Wasp silk displayed α-helical conformations with the amide I band at 1631 cm⁻¹ (Fig. 8) and other characteristic bands indicating the presence of hydroxyl and methyl groups.

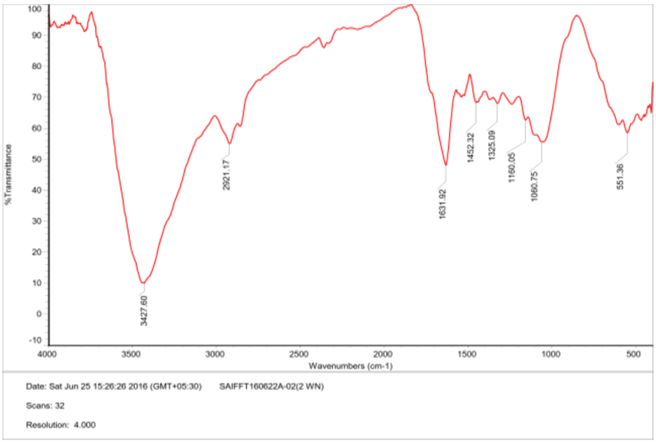

Fig. 7. FTIR of ant silk

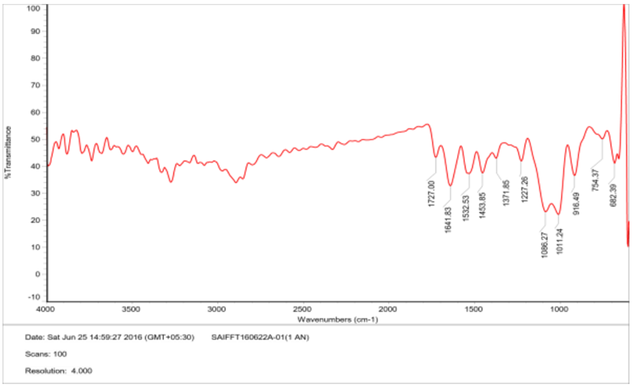

Fig. 8. FTIR of wasp silk

3.4 Thermal Stability Using TGA

Thermogravimetric Analysis (TGA) demonstrated that Paper Wasp silk had superior thermal stability compared to Weaver Ant silk (Fig. 9). Weight loss was significantly lower in Paper Wasp silk across all temperature ranges. At 700°C, the residual weight of Paper Wasp silk was approximately 47%, while that of Weaver Ant silk was only 5%. The maximum degradation temperatures were 319.75°C for Paper Wasp silk and 302.81°C for Weaver Ant silk, further indicating the enhanced thermal stability of the former.

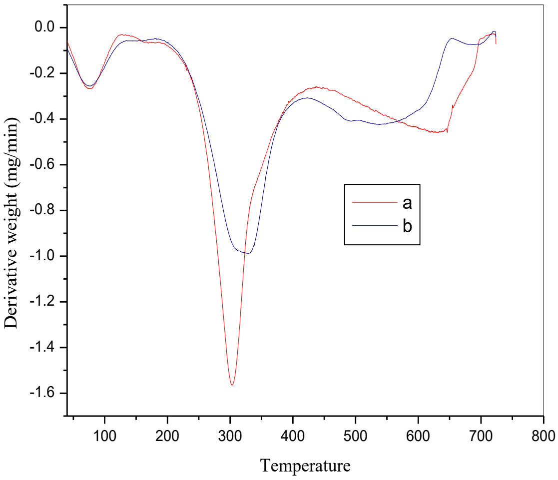

Fig. 9. Comparison of TGA in two silks

4. Discussions

Silks are unique biopolymers with remarkable mechanical, chemical, and biological properties, making them a valuable resource for biomedical and industrial applications. While most studies spotlighted on silk from *Bombyx mori* and spiders, this study unravel the potential abilities of silk fibres from Weaver Ants (*Oecophylla sp.*) and Paper Wasps (*Polistes sp.*). The results revealed notable differences in their structural characteristics, thermal stability and biochemical composition, providing insights into their potentiality and adaptability for diverse applications.

4.1 Biochemical Composition

The considerable higher protein content in Paper Wasp silk (44.7%) compared to Weaver Ant silk (23.4%) suggests as a biomaterial for applications which requires high protein integrity, such as drug delivery systems and tissue scaffolds. Previous studies emphasis on protein concentration, eminent nitrogen and amino acid content, including glycine, alanine, serine, and proline, content in wasp silk contribute to its strength and stability [5]. Conversely, the mediocre protein content in Weaver Ant silk demonstrates its use primarily as a structural component in web construction, and emphasizing its suitability for lightweight applications.

On the other hand, the lipid content plays critical role for influencing the mechanical and chemical properties of silk, was appreciably higher in Paper Wasp silk (5%) than Weaver Ant silk (1%). Lipids are known to augment silk's flexibility and resilience, making Paper Wasp silk a healthier candidate for applications which requires dynamic mechanical properties [12]. These results line up with the findings on lipid-rich pedicels in wasp nests, which ensure structural integrity and predator resistance [5].

The minimal concentrations of RNA and DNA in both silk types point out limited genetic material incorporation, which is consistent with their functional roles as structural proteins. However, the slightly higher nucleic acid content in Paper Wasp silk could be endorsed to its more complex silk production process, involving higher cellular activity.

4.2 Structural Characteristics

Fourier Transform Infrared Spectroscopy (FTIR) revealed distinct secondary structures of the silk fibers. In ant silk, Amide I and II bands were noticed at the frequencies of 1641.88 cm^{-1} and1532 cm^{-1}, respectively (Fig. 8). A similar type of amide I band was reported in the Mulberry silk at a frequency of 1621 cm^{-1}, and this band may arise due to the vibration of the C=O bond in the backbone of the silk protein [13, 14]. This may be responsible for the β-sheet conformation of the ant's silk. The second amide band at the same frequency has been reported in the silk of spider and B. mori. This band appears in the spectrum because of the C-N stretching and N-H bending vibrations of protein fibres [13, 14]. In this case, β-sheet conformation has been assigned to this band. Amide bands of the ant's silk contribute the same conformation like silks from both *Bombyx mori* and spider, and the β-sheet confers the remarkable tensile strength and rigidity of silk protein [6].

In FTIR spectrum of paper wasp the amide I band at a frequency of 1631 cm^{--1} and another band at 3427 cm^{-1} explicit the presence of hydroxyl group. The amide I band in the wasp silk endow with α-helical conformations. Chawla et al. [15] found the hydroxyl group and secondary structures such as α-helical, β-sheet and coiled coil in the silk of paper wasp. The silk of paper wasp is more flexible and elastic than silk of ant because of the occurrence of amide band and hydroxyl group. Indian wasp silk can be considered for next generation of organic electronics or advanced biomaterials and it could be ideal for applications which necessitate elasticity, such as flexible films or artificial ligaments. Studies propose that weaver Ant silk may be more suitable for applications which demand rigidity, such as rigid scaffolds or biosensors due to its structural differences

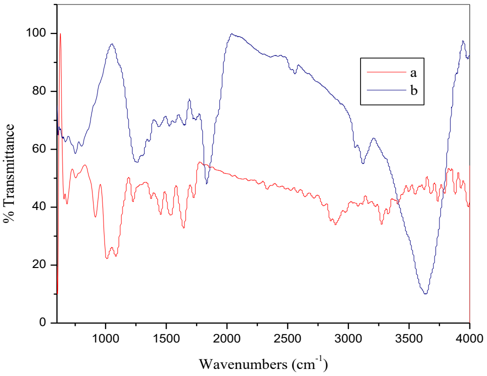

Fig. 10. Comparison of FTIR in two silks

4.3 Thermal Stability

The thermal properties of the silken mat from weaver ant and nest of paper wasp are studied by using TG-DT at temperature below 100 ºC (Fig. 9). The weight loss of the samples occur, which is mainly due to the loss of water. It was found to be 6 % and 4 % in the silk of ant web & wasp nest respectively. The temperature in range of 250 °C to 300°C, the weight loss experienced by ant silk fiber & silk fiber from wasp nest were 16% and 6% respectively (Fig. 9). However, both the samples experienced maximum weight loss in the temperature in range of 300-350°C. Compared to ant silk fiber, the weight loss experienced by silk from wasp nest is minuscule and was about 10% of its weight loss. The weight of the sample reached at about 5% when the temperature increased to 700°C. Unlike ant silk, the residual weight of silk fiber of wasp nest was around 47% at the same temperature. This may be an indicative of the higher thermal stability of latter than former. The temperature of maximum degradation was shown by ant silk was at 302.81°C. Anyhow, the silk fibre from wasp nest showed maximum loss of weight at 319.75°C. silk from paper wasp endure molecular structure, making it more suitable for thermal insulation or bioresorbable materials in extreme environments [11].

Weaver Ant act as potential entrant for nanotechnology applications such as electro spun nanofibers and biosensors due its β-sheet-rich structure and sensible thermal stability [16, 17]. Versatile characters of paper wasp silk such as high protein, lipid content, superior thermal stability, made them compatible for tissue engineering, tissue scaffolds, flexible biomaterials and drug loading [6].

5. Potential Applications

Studies revealed that characteristics of silk from both weaver ant and paper wasp align with advanced biomedical applications Recent studies by Kandhavadivu et al. [17] found that weaver ant silk have high chemical resistance and its hollow nature paved the way for wound healing and drug delivery system. It shows that 74% healing in 100µl concentration of nanoparticle coated ant silk protein. Case of drug delivery it shown that 1.36% in one hour and 7.89% in 12 hour which proven its ability to withhold the drug and its subsequent delivery. With its β-sheet-rich structure and sensible thermal stability come up as a potential candidate for nanotechnology applications such as electron spun nanofibers and biosensors [16].

Another study by (S.Chawla et al.[15] shows the structural conformation of paper wasp silk by protein modelling its deducted that it has α-helical structure along with the presence of β-sheets. It contains hydroxyl groups of polyphenols. The arrangements of coiled coils and β-sheets open up new path to use the Indian paper wasp for next generation organic electronics or advanced biomaterials for tissue engineering drug delivery system and tissue scaffold.

6. Conclusion

This study underscores the untapped potential of alternative silk sources from Hymenoptera species. Paper Wasp silk, with its biochemical richness and structural versatility, shows promise for applications requiring high strength and thermal stability. Weaver Ant silk, while less robust, offers unique properties that may be advantageous for lightweight and nanotechnology applications. Further studies are needed to explore the scalability and specific applications of these silks in biomedical and industrial fields.

7. Declaration

Ethics and consent to participate: Approved by Department council
Availability of data and materials: Included in the manuscript
Competing interest: There is no competing interest
Funding: The research is not supported by external funding source
Author’s contribution: Concept, supervision and final draft (Auth:3),
Experimentation and first draft (Auth:1), Data analysis (Auth:2)
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