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FATTY ACID PROFILE OF WEB SILK FROM PAKISTAN

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ABSTRACT

The oil from the spider silk was extracted through soxhlet apparatus using n-hexane with 4.46% yield. Fatty acid profile was measured by gas chromatography (GC) coupled with flame ionized detector (FID). Esterification of the oil was carried out with methanol by using boron trifluoride (BF_3) as catalyst. The GC FID study showed the Caproic acid ($C_{8:0}$) 0.259%, Capric acid ($C_{10:0}$) 1.5198%, Lauric acid ($C_{12:0}$) 1.1475%, Myristic acid ($C_{14:0}$) 3.0656%, Palmitic acid ($C_{16:0}$) 38.7852%, Palmitoleic acid ($C_{16:1}$) 0.4718%, Stearic acid ($C_{18:0}$) 0.0628%, Oleic acid ($C_{18:1}$) 23.1243%, Linoleic acid ($C_{18:2}$) 4.6999%, Arachidic acid ($C_{20:0}$) 1.268%, Linoleinic acid ($C_{18:3}$) 3.4311%, Gadoleic acid ($C_{20:1}$) 4.2798%, Behenic acid ($C_{22:0}$) 5.7898% and Lignoceric acid ($C_{24:0}$) 6.6668% respectively. The oil of spider silk contains both lower molecular weight and higher molecular weight fatty acids. FT-IR spectrum illustrates the presence of CH_2 (Anti sym str alkane) at 2920 cm^{-1}, C-H /CH_3 free (Sym str. alkane) at 2853 cm^{-1} and C=O (str. Carboxylic acid) at 1736 cm^{-1} and peak at 969 cm^{-1} showed the presence of trans-fat.

1.1 INTRODUCTION

Spiders are a cause of plotting dread and numerous mythologies, but it is extraordinary to see that regardless of environment the spiders are found almost in every kind of habitation range.[1] Spiders have a life span of years and are famous for their sturdiness.[2] Spiders are among the most common specie found on the earth with such a great diversity.[10] They are fortified with nature’s astounding material known as “Spider silk”. Silk production is evolution in arthropods.[7] Spider silk suggest promising venues for the next decade of research on fascinating creation and its snare.[3 & 4] About 47,188 species of spiders have been acknowledged, out of which 41,000 species are categorized as silk producing[5 & 6], and evolution of spiders has only left 4600 species that produce orb web. The webs of spiders are especially made-up to catch their prey, in addition to that spider web is also a home and protection for spiders.[13] Composition of silk may differ because of habitat, food, and life style, some spiders produce different kinds of silk at a same time.[14] Spiders not only helps in decreasing the number of pests and harmful insects but also stabilizes the ecosystem and food chain. Size of spider vary.[12] Web range from < 2cm (for example *Anapidae)* to almost 2m (for example *Caerostris darwini*) in diameter.[9&11] Spiders can produce up to seven different kinds of silk.[15 & 16] Spider silk is a commercially desired biomaterial due to possession of extraordinary properties such as high tensile strength, utmost elasticity, extreme hardness bio medical, bio-engineering, environment cleaning and therapeutic applications. It is five times stronger than steel and two times that of Kevlar.[17] Mechanical properties of silk can be correlated with its composition, glycine rich regions and polyalanine segments are the basic factors which influence high tensile strength and extensibility, which is why some spider silk types can stretch up to 140% of their original length without breaking.[19] Studies have revealed that at very low temperature (-196 °C) strength of silk improved by 64% as related to strength at room temperature.[20] Unique and extraordinary mechanical properties makes spider silk superior than the man-made artificial fibers.[21] It is used in manufacturing of rust free panels on boats, motor vehicles bullet proof jackets, furthermore, biodegradable bottles, bandages surgical thread, earth quake proof construction material, ropes of elevators, bridges and pillar might also be the potential items made out of the spider silk.[18]

MATERIALS AND METHODS

Web silk was procured from Bhatta Chowk Bedian Road Mananwala Lahore Cantt, during November 2017. Sample was cleaned manually and washed using distilled water. Sample was dried under shade and different pickets of web silk were made and kept in closed container for further processing. Oil was extracted through Soxhlet extractor by using n-hexane as an extracting solvent. Extraction of oil was carried out for 3 hours till the clear n-hexane changes its color. Yellowish orange colored oil was obtained, with 4.46% yield.

The n-hexane extracted material (oil or fatty acid), was taken in a test tube (2 g) and 5 mL of methanol and 1 mLof BF_3 was added. It was sealed with cap and heated on water bath for 4 hours. After 4 hours the resulting material was the esterified oil. This was extracted with n-hexane and organic layer was dried over water bath and then concentrated for GC-FID for fatty acid profile evaluation. A PEG capillary column (25m x 0.2 mm id) was used for fatty acid. The column was operated with temperature programming from 150 to 200 °C. The injection and detector temperature were maintained at 250 to 300 °C respectively (Flow gas carrier gas Nitrogen) was 20 ml/min at split ratio of 1:50. Identification of the component was based on their retention time as compared with those obtained from methyl esters of known fatty acids, analyzed under similar conditions.

RESULTS AND DISCUSSION

The orange colored oil from the spider silk was extracted through soxhlet apparatus using n-hexane with 4.46% yield with fatty smell. The chromatographic report of GC-FID is given below.

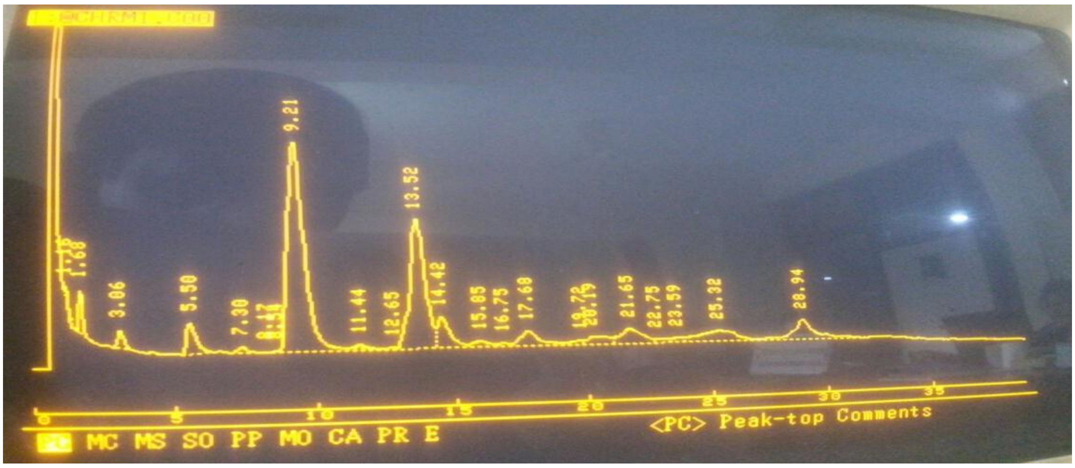

Fig 1: GC-FID chromatogram of esters of oil from web silk.

Table 1: Fatty acid profile of web silk analysed by GC-FID.

Peak. No	Time	Area	Hight	Id. No	Conc % of fatty acids	Name of fatty acids	Formula
1	1.16	654	115	$C_{8:0}$	0.259	Caproic acid	$C_8H_{16}O_2$
2	1.68	3837	480	$C_{10:0}$	1.5198	Capric acid	$C_{10}H_{20}O_2$
3	3.06	2897	197	$C_{12:0}$	1.1475	Lauric acid	$C_{12}H_{24}O_2$
4	5.5	7739	342	$C_{14:0}$	3.0656	Myristic acid	$C_{14}H_{28}O_2$
5	9.21	97908	2408	$C_{16:0}$	38.7852	Palmitic acid	$C_{16}H_{32}O_2$
6	11.4	1191	46	$C_{16:1}$	0.4718	Palmitoleic acid	$C_{16}H_{30}O_2$
7	12.65	159	9	$C_{18:0}$	0.0628	Stearic acid	$C_{18}H_{36}O_2$
8	13.52	58374	1494	$C_{18:1}$	23.1243	Oleic acid	$C_{18}H_{34}O_2$
9	14.42	11864	352	$C_{18:2}$	4.6999	Linoleic acid	$C_{18}H_{32}O_2$
10	15.85	3201	71	$C_{20:0}$	1.268	Arachidic acid	$C_{20}H_{40}O_2$
11	17.68	8661	168	$C_{18:3}$	3.4311	Linoleinic acid	$C_{18}H_{30}O_2$

12	21.65	10804	171	$C_{20:1}$	4.2798	Gadoleic acid	$C_{20}H_{38}O_2$
13	25.32	14616	133	$C_{22:0}$	5.7898	Behenic acid	$C_{22}H_{44}O_2$
14	28.94	16830	242	$C_{24:0}$	6.6668	Lignoceric acid	$C_{24}H_{48}O_2$

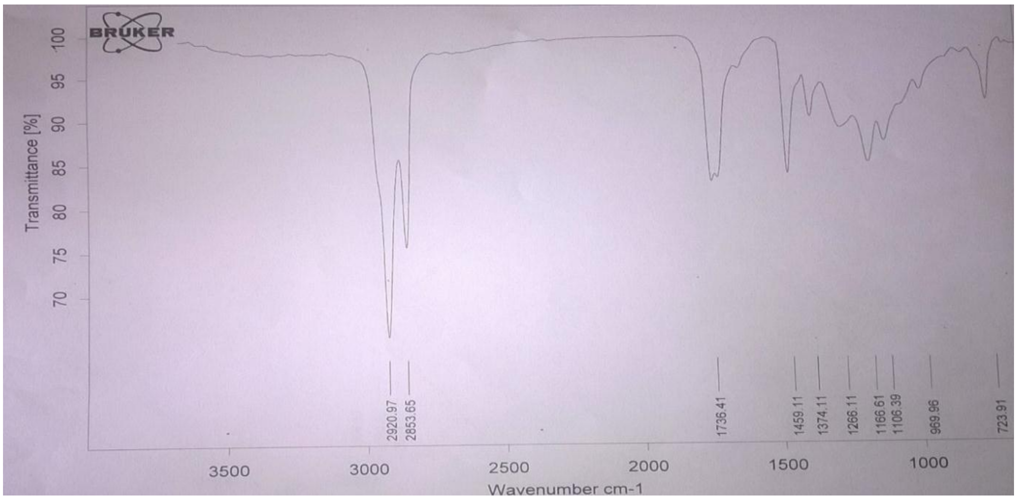

Fig. 2: FT-IR spectrum of oil of web silk.

Table 2: FT-IR Analysis of oil from web silk.

Sr. no.	Frequency (wavenumber) cm^{-1}	Functional group
1.	2920	Anti sym str. CH_2 (alkane)
2.	2853	Sym str. C-H /CH_3 free (alkane)
3.	1736	C=O str. (Carboxylic acid)
4.	1459	C-H Scissoring
5.	1374	CH sym. Rocking
6.	1266	Ester C-O stretch
7.	1166	Ester Stretching/ CH in plane
8.	1106	In plane CH bending
9.	969	Trans RCH=CHR (bending)
10.	723	CH out of plane def/ CH_2 Long chain

DISCUSSION

The present study contains first GC-FID characterization of the silk lipid of spider. Previously complete characterization of silk lipid through GC-MS and 1 methoxyalkanes from the silk of linyphiid spider *Linyphia triangularis* has also been characterized.[39] Cuticular lipids of *Tegenariaatrica* contain high percentile of alkanes which are also present other insects but cuticular lipid of *Anelosimus eximus* consists of very unusual propyl esters of alkanes together with long chain fatty acids. In previously methods identification of methyl ethers with up to two methyl branches, with modification of replacing the final $LiAlD_4$ reduction step by substitution with cyanide has been used.[8] Fatty acid profile was measured by gas chromatography (GC) coupled with flame ionized detector (FID). Esterification of the oil was carried out with methanol by using boron trifluoride (BF_3) as catalyst. The GC FID study showed the caproic acid ($C_{8:0}$) 0.259%, capric acid ($C_{10:0}$) 1.5198%, lauric acid ($C_{12:0}$) 1.1475%, myristic acid ($C_{14:0}$) 3.0656%, palmitic acid ($C_{16:0}$) 38.7852%, palmitoleic acid ($C_{16:1}$) 0.4718%, stearic acid ($C_{18:0}$) 0.0628%, oleic acid ($C_{18:1}$) 23.1243%, linoleic acid ($C_{18:2}$) 4.6999%, arachidic acid ($C_{20:0}$) 1.268%, linoleinic acid ($C_{18:3}$) 3.4311%, gadoleic acid ($C_{20:1}$) 4.2798%, behenic acid ($C_{22:0}$) 5.7898% and lignoceric acid ($C_{24:0}$) 6.6668% respectively. The oil of spider silk contains both lower molecular weight and higher molecular weight fatty acids. About 3 major peaks are visible in FT-IR graph, study illustrates the presence of Anti sym str. CH_2 (alkane) at 2920 cm^{-1}, sym str. C-H /CH_3 free (alkane) at 2853 cm^{-1} and C=O str. (Carboxylic acid) at 1736 cm^{-1}. Thus FT-IR analysis proves that fatty acids with symmetrical and anti-symmetrical alkane chains are present and peak at 969 cm^{-1} shows that the presence of trans fat.

N.clavipes silk contain 1-methoxyalkanes with four methyl branches. Methyl group is present at the end of alkyl chain usually at 1, 5- configuration. This configuration of methyl groups is prominent in all di, tri or tetramethyl alkyl chains, with the exception of 1-methoxy-2, 28-dimethyltriacontane. That is only one with a methyl group at C-2. A prominent structural feature of the ethers from the silk of *L. triangularis*, which are less branched. No other ether occurs in both species, which are not closely related. The biosynthesis of the 1-methoxyalkanes, which have so far not been described from organisms other than spiders, can be assumed to start from the alkyl end with the combination of methylmalonate as a replacement for of malonate units, leading to the methyl branches, as has been found in insects.[26-28]

Patterns indicate that the biosynthesis of hydrocarbon and ether chains are not closely related. In arthropods odd number hydrocarbon chains are predominant on surface.[29-30] While contrast to that *N.clavipes* contain even number hydrocarbon chains dominantly. This fact is in contrast to the point of directly uptake of hydrocarbons by spiders because dominant alkenes of flies that are used as diet are odd-numbered 2-methyl alkanes.[34-38] In the GC-FID chromatogram it has been proved that Palmitic acid ($C_{16:0}$) 38.7852% and Oleic acid ($C_{18:1}$) 23.1243% both are abundant amount with highest yield, and both of them are even numbered hydrocarbon chains.

1-alkanols and 1, 3-diols are reported in recent study, 1-alkanols have often been found in arthropod cuticle, the 1, 3-diols are not known from any other arthropod so far.[30-36] Fatty acids and wax esters frequently occur in the cuticle of arthropods. The function of lipids in silk of spider is still not extricated. Large amount of lipid percentile is present on the surface of silk fiber. But long time extraction results in more yield of lipid indicating that lipid is also present in core fiber The chief function may be ordinance of the water content of the silk or safeguard of the protein-rich silk against deprivation, e.g., by microbes or chemical agents. The lipids may also be used for communication, because males can identify the webs of females by contact.[37] Most of the lipid produced by different types of silk by different spiders are still unknown as well as the origion of lipid.[38] Freshly wanned dragline silk from another Nephila species comprises of only comparatively low amounts of lipids (Vollrath, F., and Schulz, S., unpublished data), signifying that most of it is present in other silk types or is dropped from the spider while walking on the web. While, the cuticle lipids of the spider comprises of only small amounts of ethers and are fore mostly hydrocarbons, it is possible that the hydrocarbons are transported from the cuticle during walking, while the ethers originate from another source, e.g., glands. 1Methoxyalkanes have now been found in silk from three spider families: Araneidae, Linyphiidae, and Theridiidae. The reason for the production of more elaborate ethers as lipids instead of the more widespread alkanes is not clear. Probably some degree of polarity on one end of the molecule is needed for good interaction with the proteinaceous fiber or other silk compounds. On the other hand, attempts to determine the position of double bonds in unsaturated alcohols or ethers failed. No characteristic ions useful for the location of the double bond could be detected in the mass spectra.[8]

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