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ORIGINAL ARTICLE

GC–MS analysis of compound from extract of *Samia ricini* fed on *Heteropanax fragrans* leaves

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ABSTRACT
Samia ricini is a multivoltine, polyphagous insect reared by the farmers of Northeast India particularly in Assam. Pupae and pre-pupae of S. ricini are considered as delicacy and a good source of protein, minerals, vitamins, and lipids. However, the literature on the fatty acid profiling is scanty. Therefore, the present study was designed to evaluate the presence of fatty acid, its components and chemical nature of the S. ricini fed in Heteropanax fragrans leaves. The GC-MS analysis of the extract of silkworm fed on H. fragrans detected 14 compounds with Z,Z-6,28-Heptatriactontadien-2-One with highest peak ratio of 10.940% and 1,3-Dioxolane, 4,5-Dibutyl-2,2-Bis(Difluoromethyl), Cis with lowest peak ratio of 0.271%. The presence of various bioactive compounds may be because of feeding the silkworm with H. fragrans. Investigation of the biological activities of the isolated compounds is necessary to know exactly which of the compounds is responsible for its acclaimed beneficial properties for the development of silkworm and their consumption by humans.
Keywords: *Kesseru, fatty acid profile, bioactive components, GC-MS, silkworms.*

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INTRODUCTION

Sericulture has been a part of Indian culture and traditions and it has become an important agro based rural industry. Northeastern part of India is rich in seri biodiversity being a natural abode for several sericigenous insects and their host plants [19]. Out of all varieties, *Samia ricini,* Eri silkworm is one of the most domesticated, exploited, and popular non mulberry silkworms which are largely reared by the farmers of North-eastern part of India, particularly in Assam [20]. Ericulture is preferred mostly because it is domesticated and can be reared indoors, it has low gestation period and being polyphagous it feeds on numerous host plants. In Assam, Ericulture is associated with the culture and tradition of the tribal people. It helps to generate income and provide nutritional support as the pupae of eri silkworm is also considered delicacy which is consumed by the tribal inhabitants of the region.

The consumption of insects as food has been reported since time immemorial. Many tribal communities India consume silkworm pupae [14]. The tribal inhabitants of northeast India use numerous insects as food including the pre-pupae and pupae of eri silkworm [18]. Insects have very high crude protein content, and they are reported to be high source of protein, essential amino acids, carbohydrate, vitamins essential for human consumption and they contribute majorly to the food security and livelihoods in the developing countries [21]. Silkworm pupae have been put on the list of 'novel food resources managed as common food' by the Ministry of Health, P. R. China [24]. Information on consumption of silkworm pupae in some countries is available, but there are limited data on the nutrient composition. Studies had been done on the pupae of *B. mori* as a human food source due to its high nutritional value [13] and silkworm pupal oil is also known as a exploitable food resource that lowers cholesterol, improves memory and it has a good antioxidant capacity. However, the food plants of polyphagous insects are not equally rich in all kinds of nutritional value. The types of food plants consumed by the silkworm have profound effect on its survival, rate of food intake, digestion and assimilation of nutrients which directly influences the growth and development of silkworms [9]. The quality of leaves is the most important factor which highly

influences the larval growth and quality of cocoons produced by silkworm [2]. Longvah *et al.,* [10] also reported the pupal oil of eri silkworm is a good source of protein, fat, minerals, and essential amino acids. Dietary intervention and epidemiological studies have shown that fatty acid intake plays a key role in human health [22]. However, the research and literature available on the fatty acid and profile of eri silkworm is scanty therefore the present work tries to analyze the fatty acid profiling of eri silkworm larvae reared using different host plants.

MATERIAL AND METHODS

Rearing of silkworms: Disease free layings of *S. ricini,* Kokrajhar race were collected from Directorate of Sericulture, CSB, Kokrajhar. The rearing was done using standard procedures as described by Sarkar, [20]. The larvae were fed on *Ricinus communis* (castor) leaves for three times a day till it reached second instar. After second instar of silkworms were fed using *Heteropanax fragrans* (Kesseru).

Protein Carbohydrate Analysis: The protein and carbohydrate were analyzed by using the standard protocol of Lowry method and Anthrone method respectively. The hemolymphs of the fifth instar 3rd day silkworm have been taken by pricking the prologs for the test.

Sample preparation: Matured fifth instar larvae were taken for the experiment. The silkworms were taken and washed using warm water. Excess water was removed by spreading on white paper. The cleaned silkworms were then transferred to a petridish and dried overnight in an oven at 60^0C. The dried silkworms were then homogenized and stored for further use.

Lipid Extraction: Lipid extraction was done using the method described by Folch *et al.,* [4]. 2.25gm of sample was taken and homogenized with 2:1 Chloroform Methanol mixture using a magnetic stirrer for 30mins. The mixture was filtered and kept in a separating funnel, 20ml of distilled water was added and the mixture was swirled gently. The mixture was then let to stand overnight till two layers were formed. Then upper methanol layer was discarded. The lower layer was collected and evaporated in a rotary evaporator till the solution was nearly dry.

Preparation of Fatty acid methyl ester (FAME): The lipid extract was mixed with 10% BF3 in methanol in a conical flask and kept in oven at 83^0C for 6 min. The solution was then transferred to separating funnel with an addition of 4 ml Hexane. Then 4 ml of saturated NaCl brine solution was added and shaken vigorously for 5 min. The lower salt was discarded, and the upper FAME was collected for the analysis.

Fatty Acid analysis: The fatty acids were analyzed by GC–MS system (Perkin Elmer (USA) make GCMS instrument, Model: Clarus 680 GC & Clarus 600C MS comprising a liquid auto-sampler), by using Helium (99.99%) as carrier gas (i.e., mobile phase) at a flow rate of 1 ml/min. The temperature was maintained at 70-290^0C. The FAME sample was injected to GC-MS Clarus 680. The total run time was ~39 min. The data obtained from GC-MS was analyzed by using Library Software: Turbomass NIST 2008 MS library. The mass spectrum of the unknown compound was compared with the spectrum of known components already stored in the NIST library. The identification of the compounds is done based on the retention time, peak area, molecular formula and molecular weight.

Statistical Analysis: All the results were expressed in mean±Standard deviation, n=3. The significance test was done by using ANOVA at $p \leq 0.05$ level using MS Excel.

RESULTS

Protein and Carbohydrates Analysis:

Table1: Hemolymph protein and Carbohydrate of *S. ricini* fed on *H. fragrans*

Hemolymph of Silkworm fed on *H. fragrans* plant	Total Protein (mg/ml)	Total Carbohydrate (mg/ml)
	19.94±0.399	15.21±0.172

GCMS analysis:

GCMS analysis of the extract of silkworm fed on *H. fragrans* detected 14 compounds namely Tetradecanoic Acid, 10,13-Dimethyl-, Methyl Ester (C1), L-(+)-Ascorbic Acid 2,6-Dihexadecanoate (C2), Heptadecanoic Acid, 16-Methyl-, Methyl Ester (C3), Methyl 5,12-Octadecadienoate (C4), Octadecane, 1,1-Dimethoxy-(C5), 11,14,17-Eicosatrienoic Acid, Methyl Ester (C6), Z,Z-6,28-Heptatriactontadien-2-One (C7), Methyl 18-Methylnonadecanoate (C8), Tricosanal (C9), 3-Methyl-2-(2-Oxopropyl)Furan (C10), 1,3-Dioxolane, 4,5-Dibutyl-2,2-Bis(Difluoromethyl)-, Cis-(C11), Glycidyl Oleate (C12), Linolenic Acid, 2-Hydroxy-1-(Hydroxymethyl) Hexa-2,4-Dienoic Acid (C13), 1,3-Dioxolane, 4-Heptyl-5-Methyl-2,2-Bis(Trifluoromethyl)-, Trans-(C14). The retention time, peak area, height, molecular weight and formula of the identified compounds are presented in Table 1. The GCMS cromatograms are presented in Fig.1. The 2 D structures of the identified compounds are shown in Fig.2.

Table 2: GC-MS properties of compound identified from *S. ricini* extract fed in *H. fragrans* leaves:

Sl no.	Compound name	Retention time	Area (%)	MW (g/mol)	MF
1	Tetradecanoic Acid, 10,13-Dimethyl-, Methyl Ester	28.597	3.341	270	$C_{17}H_{34}O_2$
2	L-(+)-Ascorbic Acid 2,6-Dihexadecanoate	29.938	6.948	652	$C_{38}H_{68}O_8$
3	Heptadecanoic Acid, 16-Methyl-, Methyl Ester	31.243	7.117	298	$C_{19}H_{38}O_2$
4	Methyl 5,12-Octadecadienoate	31.383	6.917	294	$C_{19}H_{34}O_2$
5	Octadecane, 1,1-Dimethoxy-	31.518	1.506	314	$C_{20}H_{42}O_2$
6	11,14,17-Eicosatrienoic Acid, Methyl Ester	31.703	7.501	320	$C_{21}H_{36}O_2$
7	Z,Z-6,28-Heptatriactontadien-2-One	32.599	10.940	530	$C_{37}H_{70}O$
8	Methyl 18-Methylnonadecanoate	33.719	1.273	326	$C_{21}H_{42}O_2$
9	Tricosanal	33.969	1.713	338	$C_{23}H_{46}O$
10	3-Methyl-2-(2-Oxopropyl) Furan	34.179	1.060	138	$C_8H_{10}O_2$
11	1,3-Dioxolane, 4,5-Dibutyl-2,2-Bis (Difluoromethyl)-, Cis-	36.060	0.271	322	$C_{13}H_{20}F_6O_2$
12	Glycidyl Oleate	36.800	0.832	338	$C_{21}H_{38}O_3$
13	1,3-Dioxolane, 4-Heptyl-5-Methyl-2,2-Bis (Trifluoromethyl)-, Trans	37.141	0.547	322	$C_{13}H_{20}F_6O_2$
14	Pentadecanoic Acid, 14-Bromo-	39.337	0.847	320	$C_{15}H_{30}O_2$

DISCUSSION

Heteropanax fragrans is considered as good food plant for Eri silkworm in terms of grainage and silk quality. In the present study we analyzed the protein, carbohydrate and fatty acid composition of the silkworm fed on *H. fragrans*. The GCMS study revealed the silkworm extract fed on *H. fragrans* have many such components which have pharmacological activities like Tetradecanoic Acid, 10,13-Dimethyl-, Methyl Ester (hexadecanoic acid, methyl ester) Antibacterial and antifungal [3]. 9,12-octadecadienoic acid (Z,Z)-, methyl ester have Anti-cancer properties [23], anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant, antileukotriene D4 [1]. L-(+)-Ascorbic Acid 2,6-Dihexadecanoate have antioxidant [6], anti-allergic, anti-anemic, anti-anxiety, antibacterial, anti-bronchitic, anti-cancer, anti-carcinogenic, anti-cataract, anticoagulant, anticonvulsant, anti-diabetic, anti-diarrheic, anti-fatigue, anti-fertility, anti-gastric, anti-inflammatory, anti-malarial, antioxidant, anti-stress, antiulcer, anti-atherosclerotic, anti-cold, anti-glaucomic, anti-hepatic, anti-hypertensive, anti-plague, anti-proliferant, anti-protozoal, antiseptic, anti-stroke, anti-tuberculic, anti-tumer, cns stimulant, chelator, chemopreventive, cytochrome p450 inducer, deodorant, dermal, detoxicant, flavor, hypolipidimic, neuroprotective, neurotransmitter, termiticide, antiviral activity [16], anti-inflammatory and anti-nociceptive properties [15]. The 11,14,17-Eicosatrienoic Acid, Methyl Ester is an omega 3 unsaturated fatty acid ester which have antiarthritic, anti-coronary and anti-inflammatory activities [7]. Z, Z-6,28-heptatriactontadien-2-one has vasodilatory activity [11]. The 3-methyl-2-(2-oxopropyl) furan were also found which is recorded to have antipyretic, anti-inflammatory, hepatoprotective properties on *Mallotus tetracoccus* [15]. Pentadecanoic acid, 14-Bromo has lubricants, adhesive agents and antioxidant properties [12]. The Glycidyl oleate was found in very less amount which might be because of heating the sample to 60^0C for overnight. The glycidyl oleate could be more susceptible to thermal oxidation and instability [8]. Trace amount of Tricosanal was also found which is identified as an anti-inflammatory agent that is non-steroidal in nature. In addition to anti-inflammatory actions, they have analgesic, antipyretic, and platelet-inhibitory actions. They act by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase, which converts arachidonic acid to cyclic endoperoxides, precursors of prostaglandins. Inhibition of prostaglandin synthesis accounts for their analgesic, antipyretic, and platelet-inhibitory actions; other mechanisms may contribute to their anti-inflammatory effects (NCBI). The methyl-18-methylnonadeanoate was isolated and its biological activities were not known or documented.

CONCLUSION

In the present study, 14 components were identified by GCMS analysis. The presence of various bioactive compounds may be because of feeding the silkworm with *H. fragrans*. The isolation of individual

photochemical constituents and subjecting it to biological activity will give fruitful results. Further studies are needed to undertake its bioactivity, chemotaxonomy properties, and toxicity profile. There is the need to investigate the biological activities of these isolated compounds to know exactly which of the compounds is responsible for its acclaimed beneficial properties for the development of silkworm and their consumption by humans.

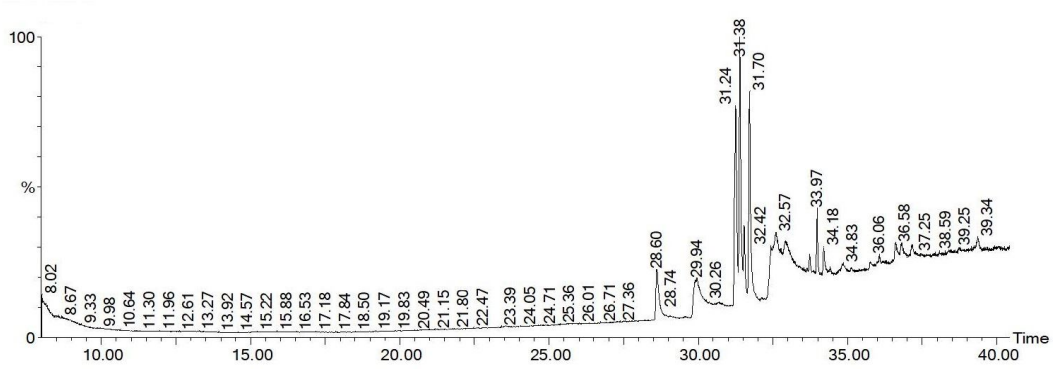

Fig.1 GC-MS Chromatogram of silkworm *S. ricini* fed on *H. fragrans* leaves

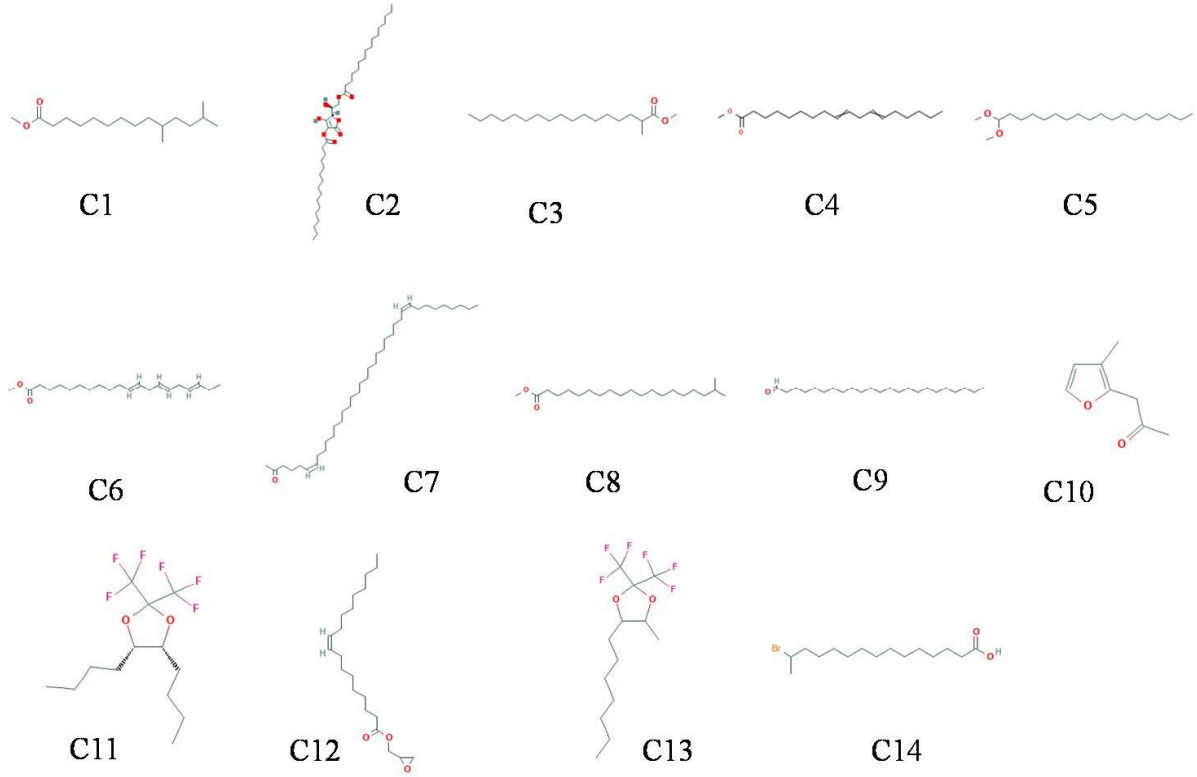

Fig. 2 Structures of the GC–MS identified compounds from the *S. ricini* extract fed on *H. fragrans* leaves

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