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# Evaluation of proximate compositions and profiling of nutritional aspects in pupae of tasar silkworm *Antheraea mylitta* (Drury) as potential for food and feed resources

**S. Ananta, K. Jena*, S. Das, J. Singh, Chakrapani, A. Sinha and K. Sathyanarayana**

Central Tasar Research and Training Institute, Central Silk Board, Ranchi-835 303, India

***Corresponding Author Email :** jenakb2010@gmail.com ***ORCiD:** https://orcid.org/0000-0003-3058-3692

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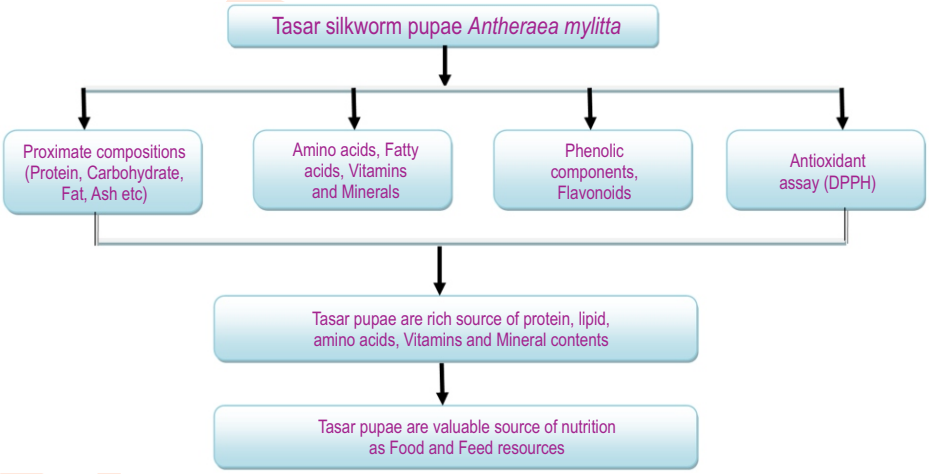
## Abstract

**Aim:** To investigate the proximate compositions, amino acids profile, phenols, flavonoids, fatty acids and minerals in Tasar silkworm pupae and assessment of biological potential of pupae extracts.

**Methodology:** Biochemical composition such as proteins, fat, carbohydrate, chitin, ash, moisture and minerals were analyzed in Tasar silkworm pupae. The amino acid, vitamins, phenolics and flavonoid, fatty acids and free radical scavenging assay were evaluated.

**Results:** The pupae powder contained 11.23% moisture, 60.67% crude protein, 23.83% fat, 3.29% carbohydrate, 5.1% ash and 3.87% chitin on a dry matter basis. Further, the pupae protein contained 19 amino acids, including all of the essential amino acids. Similarly, the pupae powder contained both water and fat soluble vitamins with higher content of tocophero l(13.23 µg 100 $gm^{-1}$), phenolics compounds majorly ferulic acid (1317.5 µg $gm^{-1}$) and p-coumaric acid (730.96 µg $gm^{-1}$), flavonoids catechin (40.771 µg $gm^{-1}$) and myricetin (12.466 µg $gm^{-1}$) at higher levels and fatty acid with higher palmitic acid content (6.565 mg $g^{-1}$). Mineral analysis indicated higher magnesium (208 mg 100 $gm^{-1}$), calcium (47.79 mg 100$gm^{-1}$) and iron (37.72 mg 100 $gm^{-1}$) contents. Further, pupae extract exhibited free radical scavenging potential in a concentration-dependent manner.




**Interpretation:** The results of the study indicate tasar pupae as a renewable and sustainable source for preparation of animal feeds.

**Key words:** *Antheraea mylitta*, Antioxidants, Tasar pupae

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## Introduction

India is a home land to all varieties of silk such as Mulberry, Eri, Tasar (Tropical and Temperate) and Muga. Among other silkworms, at all stages of life cycle Tasar silkworm (*Antheraea mylitta*) has higher body weight (egg: 9-10 mg; larva: 35-50 gm; pupa: 9-16 gm; cocoon: 10-14 gm and above). Tropical Tasar silkworm (*A. mylitta* Drury) is a polyphagous, vanya silkworm found in wild conditions feeding on trees belonging to *Terminalia* species (mainly *Terminalia arjuna* and *Terminalia tomentosa*) and *Shorea robusta*, as well as other secondary and tertiary plants (Barsagade, 2017). About 44 ecoraces of *A. mylitta* feed on naturally growing plant species available over the regions of Jharkhand, Odisha, Chhattisgarh, Bihar, Madhya Pradesh Andhra Pradesh, Uttar Pradesh, West Bengal and Maharashtra states in India (Ojha *et al.,* 2009). In the last decade, Indian tasar industry has achieved significant growth with production ranging from 1590 MT during 2011-2012 to 2689 metric ton during 2020-2021 (Annual Report, Central Silk Board, 2021), and proved to be an ideal tool for self-employment and poverty alleviation in the tribal and women populace. Recent findings suggest that silkworm pupae serve as rich source of valuable nutrients such as proteins, fatty a carbohydrates, vitamins, minerals and antioxidants, etc. (Sadat *et al.,* 2022). Due to high nutritional values, silkworm pupae are also consumed as food by humans in China, India, Japan, Thailand and some other countries. Silkworm pupae are included as 'novel food resource managed as common food' in the Ministry of Health of P.R. China's list (Zhu, 2004).

Further, owing to higher protein level, silkworm pupae have been used as feed/feeders for poultry, fish and pigs (Sheikh *et al.,* 2018).Unsaturated fatty acids, particularly Omega-3 fatty acids, are abundant in silkworm pupae oil and play a crucial role in improving human health (Kumar *et al.,* 2020). However, little information is available on the nutritive value of tasar silkworm (Ghosh *et al.,* 2020). Several researches have demonstrated that the bioactive molecules present within the silkworm pupae can be extracted using organic solvents that scavenge free radicals and are used to assess their antioxidant activity, to prevent aging by many oxidants (Gutierrez *et al.,* 2006; Lee *et al.*, 2021). The active components found in silkworm pupae have a wide range of pharmacological effects, including anticancer, antioxidant, hepatoprotective, antibacterial, antiapoptotic, and immune-modulatory effects. This opens up more possibilities for the use of silkworm pupae in biomedical research industries (Sadat *et al.,* 2022; Kumar *et al.,* 2015). Therefore in view of the above, an attempt was made to generate additional information on the nutritional composition of pupae of the silkworm *A. mylitta*.

## Materials and Methods

Tasar pupae were collected from the the Central Tasar Research and Training Institute, Ranchi, Jharkhand. The pupae were washed properly and dried in hot air oven (at 55°C), ground to powder form and stored for future use. The dried pupae powder was analyzed for the proximate constituent viz. moisture, ash and fat contents were estimated by protocols of the Association of the Official Analytical Chemists (AOAC, 2006). Protein content was determined by Kjeldahl method (AOAC, 2006). Carbohydrate was estimated by anthrone method (Dubois *et al.,* 1956). Chitin was extracted by defatting the samples followed by deproteinization (10% NaOH) and demineralization (7% HCl) as mentioned by Battampara *et al.* (2020). The energy content (kcal) of silkworm pupae was determined as the sum of protein, carbohydrate and fat by the Atwater system (Feás *et al.*, 2012).

Total phenolic content (tannin) was estimated by Folin-Denis method (Thimmaiah, 2006). Elemental minerals such as calcium, iron, sodium, magnesium, manganese, zinc and copper were analyzed through ICP-OES (Avio 200, Perkin Elmer, USA) at Odisha University of Agriculture and Technology, Bhubaneswar. Phenolic acids, flavonoids and vitamins profiling were conducted through LC-MS/MS (Waters Acquity UPLCH class system fitted with TQDMS/MSsystem), amino acid acids were analyzed by UPLC-MS/MS (Waters UPLCH class system fitted with TQDMS/MS) system and fatty acids profiling were analyzed through GC-MS/MS (Varian4000) at Indian Institute of Horticulture Research, Bangalore. Free radical scavenging potential of the fluorescent compound was analyzed by DPPH radical scavenging assay as described by Blois (1958) with slight modifications.

**Statistical analysis:** All the experiments were conducted in three replication; mean and standard deviation were calculated using MS-Excel.

## Results and Discussion

The proximate composition provides information about the nutrient level and gross energy values of feeds and feedstuff (Jobling, 2001). The percentage of crude protein content by dry weight was 60.67% (Table 1), which is comparable to Muga (59.66%) and *B. mori* silkworm (60.7%) pupae (Sengupta *et al.,* 1995; Sheikh *et al.,* 2018). Insects are a good source of nutrients and among them high protein content (79%) has been reported in the edible insect *Gonimbrasia* (Ramos-Elorduy *et al.,* 1997). High percentage of crude protein in tasar pupae is indicative of its prospective use as a protein source. The percentage of moisture content was found to be 11.23% (Table 1), which was slightly higher than *B. mori* (7.18%) pupae as reported by Bose and Mazumdar (1990). The findings of the present investigation showed that the ash percentage in tasar pupae was 5.1% (Table1) whereas ash content in *B. mori* pupae was 5.8% (Sheikh *et al.,* 2018). The ash content reflects the amount of minerals present. As compared to other by-products, silkworm pupae meal contains low mineral content (3-10%) (Rashmi *et al*., 2023).

The result showed that tasar pupae contain 23.83% of fat (Table 1). However, in *B. mori* the lipid content was 25.75-32.2% (Bose and Mazumdar, 1990; Sheikh *et al.*, 2018; Tomotake *et al*., 2010). The result indicates that the pupae powder contains both

**Table 1:** Proximate composition of *Antheraea mylitta* pupae

| Proximate components | |
|---|---|
| Moisture (%) | 11.23±0.28 |
| Protein (%) | 60.67±0.27 |
| Fat (%) | 23.83±0.29 |
| Carbohydrate (%) | 3.29±0.59 |
| Ash (%) | 5.1±0.1 |
| Chitin (%) | 3.87±0.06 |
| Tannin (%) | 0.067±0.001 |
| Energy (kcal 100 $gm^{-1}$) | 470.31 |

Values are mean ±S.D.

**Table 2:** Fatty acid profiling of Tasar silkworm *Antheraea mylitta* pupae. Fatty acid profiling was done once using GC-MS/MS method

| Fatty acids | | mg g $dw^{-1}$ | %Peak area |
|---|---|---|---|
| Lauric acid/dodecanoic acid | C12:0 | 0.043 | 0.237 |
| Myristic acid/tetradecanoic acid | C14:0 | 0.181 | 1.348 |
| Palmitoleic acid/(9Z)-hexadecenoic acid | C16:1 | 0.075 | 0.944 |
| Palmitic acid/hexadecanoic acid | C16:0 | 6.565 | 65.071 |
| Linolenic acid | C18:3 | 0.505 | 9.512 |
| Linoleic acid | C18:2 | 0.191 | 2.507 |
| Stearic acid | C18:0 | 1.682 | 18.376 |
| Eicosanoic acid | C20:0 | 0.053 | 0.881 |
| Behenic acid/Docosanoic acid, methyl ester | C22:0 | 0.077 | 1.123 |

**Table 3:** Amino acid composition of pupae of Tasar silkworm, *Antheraea mylitta*.

| Amino acids | Content (mg $gm^{-1}$) |
|---|---|
| **Non essential** | |
| Glycine | 0.0146±0.001 |
| Alanine | 5.066±0.252 |
| Serine | 5.279±0.092 |
| Proline | 3.439±0.042 |
| Cysteine | 1.23±0.011 |
| Asparagine | 0.164±0.020 |
| Aspartic acid | 3.520±0.280 |
| Glutamic acid | 4.025±0.411 |
| Arginine | 2.588±0.019 |
| Citrulline | 0.363±0.001 |
| Tyrosine | 2.741±0.093 |
| **Essential** | |
| Leucine | 3.853±0.004 |
| Lysine | 2.097±0.009 |
| Valine | 2.519±0.092 |
| Threonine | 1.060±0.023 |
| Leucine | 3.853±0.004 |
| Methionine | 2.178±0.666 |
| Histidine | 1.100±0.044 |
| Phenyl alanine | 1.759±0.008 |
| Tryptophan | 0.036±0.000 |

Values are means of three replicates ±S.D.

**Table 4:** Vitamin profiling of pupae of Tasar silkworm *Antheraea mylitta*.

| Vitamins | (Content) µg 100 $gm^{-1}$ |
|---|---|
| **Water soluble** | |
| Niacin | 0.43±0.109 |
| Pyridoxin | 0.54±0.010 |
| Pantothenicacid | 2.34±0.143 |
| Biotin | 0.6±0.016 |
| Thiamine | 7.68±0.128 |
| Riboflavin | 1.57±0.027 |
| Folic acid | 0.027±0.002 |
| Cyanocobalamine | 0.032±0.004 |
| **Fat soluble** | |
| Vitamin D1 | 0.007±0.000 |
| Vitamin D2 | 0.261±0.018 |
| Tocopherol | 13.23±0.794 |
| Vitamin K2 | 0.051±0.005 |
| Vitamin K1 | 0.033±0.004 |

Values are means of three replicates ±S.D.

saturated and unsaturated fatty acids. Among different fatty acids, palmitic acid (6.565 mg $g^{-1}$), stearic acid (1.682 mg $gm^{-1}$) and linolenic acid (0.505 mg $g^{-1}$) were at higher concentrations when compared with other fatty acids. According to Dietary Guidelines Advisory Committee (1977), the intake of saturated fatty acids (SFA) intake should be kept as low as possible with a recommendation of less than 10% of calories because high intake of SFA may pose cardiovascular risk (Astrup *et al.*, 2020). The value obtained for saturated fat content was well within the permissible limit. The chitin content in tasar pupae was 3.87% (Table 1) whereas chitin content of *B. mori* was 3.5-4.7% (Avazova *et al.*, 2020). The amount of chitin varies between the races of the insects. Chitin content of tasar pupae is almost similar to that of *B. mori.* The percentage of tannin in pupae of tasar silkworm was 0.067% (Table 1), which was lower in comparison to *B. mori* 2.04% (Omotoso, 2015). Tannins are the anti-nutrients that reduce protein digestion and absorption. The lower percentage of tannin in tasar pupae indicates that it is a good nutritional source. Further, the carbohydrate content was 3.29% in Tasar pupae (Table 1). On the other hand, 1.2, 1.32 and 1.8% carbohydrate was reported in Eri, Muga and *B. mori* pupae (Mishra *et al*., 2003). *A. mylitta* contains higher percentage of carbohydrate as compared to Eri, Muga and *B. mori.* Carbohydrates are good source of energy and are required alongside fat and proteins as important component in our diet.

The energy content was 470.31kcal in tasar silkworm pupae. Among different amino acids, the majority were serine (5.279 mg $g^{-1}$), alanine (5.066 mg $g^{-1}$), glutamic acid (4.025 mg $g^{-1}$), leucine (3.853 mg $g^{-1}$), aspartic acid (3.520 mg $g^{-1}$), proline (3.439 mg $g^{-1}$), tyrosine (2.741 mg $g^{-1}$), arginine (2.588 mg $g^{-1}$), valine

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**Table 5:** Mineral content of Tasar silkworm, *Antheraea mylitta* pupae

| Minerals | Content) (mg 100 $g^{-1}$) |
|---|---|
| Na | 32.31 |
| Ca | 47.79 |
| Mg | 208.7 |
| Mn | 0.56 |
| Fe | 37.72 |
| Cu | 1.02 |
| Zn | 24.88 |

**Table 6:** Phenolic profiling of *Antheraea mylitta* pupae

| Phenolic composition | Content (µg $g^{-1}$) |
|---|---|
| Benzoic acid | 3.93±0.069 |
| p-hydroxy benzoic acid | 28.12±0.11 |
| Salicylic acid | 67.96±0.532 |
| 3-Hydroxy benzoic acid | 31.75±0.10 |
| t-Cinnamic acid | 36.78±0.432 |
| 2,4-dihydroxybenzoic acid | 45.21±0.594 |
| Gentisic acid | 45.93±4.188 |
| Protocatechuic acid | 23.38±0.839 |
| p-Coumaric acid | 730.96±4.625 |
| o-Coumaric acid | 3.23±0.044 |
| Vanillic acid | 7.69±0.359 |
| Gallic acid | 17.64±0.072 |
| Caffeic acid | 55.81±1.05 |
| Ferulic acid | 1317.5±24.595 |
| Syringic acid | 3.15±0.018 |
| Sinapic acid | 0.29±0.014 |
| Ellagic acid | 0.09±0.002 |

Values are means of three replicates ±S.D.

(2.519 mg $g^{-1}$), and methionine (2.178 mg $g^{-1}$) (Table 3). The silkworm *A. mylitta* had higher contents of valine, leucine, methionine and phenylalanine contents than *B. mori* (Paul and Dey, 2014). A relatively higher content of essential amino acids observed in Tasar pupae clearly indicates prospects of these silk industrial byproducts as a good supplement to amino acid enriched food. Vitamins and minerals are micronutrients required in trace amount for maintaining the metabolic functions of the cells. Tasar pupae contain a variety of vitamins (Table 4). Among the vitamins, tocopherol (13.23 µg $100^{-1}$ g) level was higher compared to other vitamins. Similarly, the presence of multivitamins in silkworm pupae was earlier reported by Wu *et al.* (2021). The mineral composition indicates Tasar pupae is a rich source of minerals, including sodium (32.31 mg $100^{-1}$g), calcium (47.79 mg $100^{-1}$g), magnesium (208.7 mg $100^{-1}$ g), manganese (0.56 mg $100^{-1}$ g), iron (37.72 mg $100^{-1}$ g), copper (1.02 mg $100^{-1}$ g) and zinc (24.88 mg $100^{-1}$ g) (Table 5), which are important nutrients. Variation in mineral contents of *A. pernyi* pupa (Zhou and Han, 2006) may be due to difference the in geographical location, seasons, and also the diet on which silkworm feed. Based on flavan nucleus, flavonoids are low molecular weight polyphenolic substances.

**Table 7:** Flavonoid profiling of *Antheraea mylitta* pupae

| Flavonoids | Content (µg $g^{-1}$) |
|---|---|
| Umbelliferone | 0.013±0.000 |
| Apigenin | 0.721±0.02 |
| galangin | 0.002±0.000 |
| Naringenin | 6.535±0.042 |
| Kaemperol | 0.021±0.001 |
| Luteolin | 0.254±0.028 |
| Fisetin | 0.002±0.000 |
| Eriodictyol | 0.002±0.000 |
| Catechin | 40.771±1.660 |
| Epicatechin | 0.691±0.014 |
| Hesperetin | 1.330±0.022 |
| Quercetin | 4.243±0.188 |
| Epigallocatechin | 0.697±0.047 |
| Myricetin | 12.466±0.367 |
| Rutin | 0.279±0.024 |

Values are means of three replicates ±S.D.

The polyphenolic components of pupae were ferulic acid (1317.5 µg $g^{-1}$), p-coumaric acid (730.96 µg $g^{-1}$), salicylic acid (7.96 µg $g^{-1}$), caffeic acid (55.81 µg $g^{-1}$), gentisic acid (45.93 µg $g^{-1}$), 2,4-dihydroxybenzoic acid (45.21 µg $g^{-1}$) and t-cinnamic acid (36.78 µg $gm^{-1}$) (Table 6). Similarly, the flavonoid profiling indicated the presence of catechin (40.771 µg $g^{-1}$), myricetin (12.466 µg $g^{-1}$), naringenin (6.535 µg $g^{-1}$) and quercetin (4.243 µg $g^{-1}$) (Table 7). Flavonoids and phenolic compounds exhibit a wide range of antibacterial, antioxidant, anti-inflammatory, antiviral and antiallergic actions (Tungmunnithum *et al.,* 2018). Antioxidants protect cells/tissues from oxidative damages by scavenging free radicals, and consequently prevent diseases (Pari and Amarnath, 2004). The antioxidant potentials of different pupae extracts were studied by assay, which is based on the reduction of DPPH radicals. In the present study, DPPH radical scavenging assay revealed dose-dependent stronger antioxidant properties of the pupal extract (Fig. 1). This suggests that the scavenging potential of the extract is directly proportional to the concentration of the sample ($P<0.05$). In addition to this, pupae also contained phenolic and flavonoid compounds (Table 6, 7). Polyphenols and flavonoids have potential to scavenge free radical (Anibarro-Ortega *et al.,* 2020; Mardani Ghahfarokhi and Farhoosh, 2020; Rashmi *et al.,* 2018). Thus, in the present study, the DPPH scavenging potential may be due to the presence of these polyphenols and flavonoids in the pupal extract.

The present study revealed that tasar pupae contain high percentage of protein, lipid, crude fibre and a number of minerals & vitamins. Besides its primary contribution (Silk production) to the tasar silk industry, it can be used for edible/feeder purposes thus paving the way for a significant contribution to the beneficiaries of the society. The pupae are the byproducts during the reeling process but if utilized for nutrition purpose, the rural

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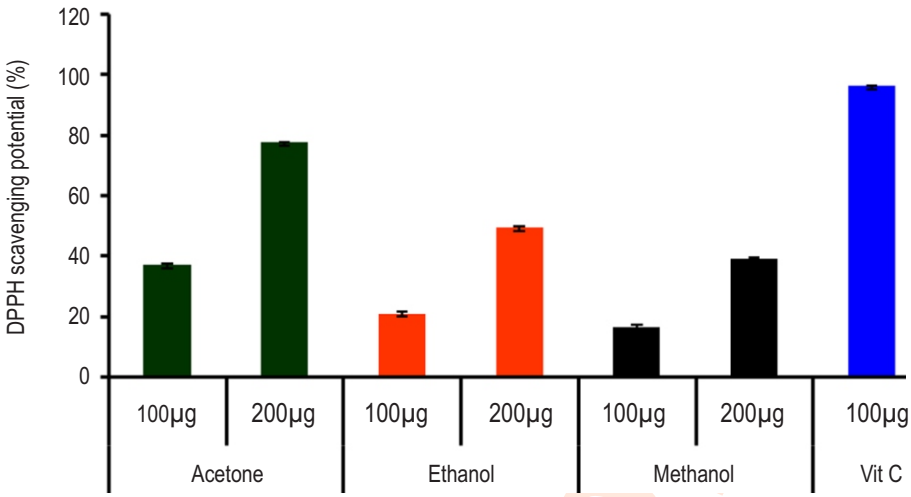


**Fig. 1:** DPPH scavenging potential of pupaeof Tasar silkworm, *Antheraea mylitta*. Values are means of three replicates.

women associated with reeling can generate additional income. Thus, there is a need to promote tasar culture to generate additional income for people, particularly tribals and women, and also to increase the production and availability of a quality, cheaper alternative source of protein, antioxidant and energy.

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**Authors' contribution: S. Ananta:** Carried out biochemical analyses and antioxidant assay, interpreted the results and data analyses; **K. Jena:** Suggested the study, planned design, interpreted the results, data analyses and approved the final manuscript; **Chakrapani and A. Sinha:** Carried out few biochemical analyses; **S. Das, J. Singh and S. Kutala:** Language editing, interpretation of results and data analyses.

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