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Loungu (Carpenter worm): Indigenous Delicious Insects with Immense Dietary Potential in Nagaland state, India

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Carpenter worms of genus *Cossus* (Lepidoptera: Cossidae) are common wood-boring insects that can cause significant damage to several economically important plant species across the globe. Nevertheless, these worms are a popular delicacy among the indigenous population of Nagaland state of India since age old days. The carpenter worms (locally known as '*Loungu*') are culturally significant during the *Te-l Khukhu* festival of Southern Angami region, annually held during July. The direct larval consumption is also cited for medicinal value. Rearing of carpenter worm is gaining popularity in hill tracts of Nagaland, because of its potential as a viable source of income for the rural population. The present study aimed to determine the eventual nutritional value of the larva by approximating its nutritional potential for the first time. Proximate analysis presents a significantly higher value of crude fat (37%), crude protein (48%), crude fibre (12.90%) and an appreciable calorific value. Close correlation between increased polyphenol value with its higher antioxidant capacity and pigment content is strongly evident. Nevertheless, the larva also provides appreciable quantities of dietary minerals reflected in terms of higher zinc and iron content. Analysis of thin layer chromatography undertaken in the study interestingly identified some of the essential amino acids, *viz.*, methionine, lysine, leucine, histidine, threonine etc. This is the first report pioneering other detail studies to establish the significant value of carpenter worm larvae as an exotic dietary supplement among the indigenous Naga population, thereby providing more impetus for its promotion and commercialization.

Keywords: Economic potential, Edible insect, Indigenous food, Mineral elements, Nutritional potential
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Carpenter worms (Lepidoptera: Cossidae) are common wood-boring insects that can cause significant damage to several economically important plant species across the globe[1]. Carpenter worms, *Cossus* spp. are considered as important pest of oaks and other hardwood trees like birch, willow, maple, elm, poplar, sycamore etc., but the insect discernibly exhibits regional host preferences[2]. Since, the larvae construct large tunnels in the bark, sapwood, and heartwood of living hardwoods, the name of carpenter worm has become epithet. They survive solely on the pith of these perennial trees, especially oaks (*Quercus serrata*). Nevertheless, these larvae are popular high-end exotic delicacy among the indigenous population of Nagaland state of India from the olden days (Fig. 1). Owing to its very high market price (up to Rs. 10,000/kg), they are considered as '*the saffron of insects*' by the residents of the state[3]. Characterized by its strong odor, the larval consumption is also cited for medicinal value in certain ailments[4]. A bath with boiled water of the larva is considered as an excellent balm for muscle aches and joint pains[5]. Pliny, a Roman naturalist cum writer, in his '*Historia Naturalis'* repeatedly referred to Oak (*Quercus* spp.) feeding

Fig. 1 — Carpenter worms (edible stage)

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Cossus larvae as a dish highly appreciated by the Romans[6]. Three species of *Cossus* (*C. chinensis, C. cossus* and *C. hunanensis*) are consumed in China as dietary food with medicinal value[7]. Few species of genus *Cossus* are also eaten as a medicinal and nutritional supplement in Japan, Korea and Australia[8]. In general, insects are not just an alternative source of food, but they are superior in many ways. Insect meat is also rich in mineral contents than other meats. Zinc deficiency is a core public health problem, especially for child and maternal health. Zinc is a component of about 100 enzymes which catalyze activation, cell division, and immune action[9]. Iron is a component of haemoglobin and myoglobin which function as oxygen carrier and acts as a cofactor of various enzymes[10]. Copper is a component of various oxidizing enzymes which contributes to metabolic oxidation–reduction reactions[11].

Insect-meat is an excellent source of nutrients[12]; thus their consumption becoming a popular trend in Western cultures[13,14] though less frequent in India. Entomophagy is however reasonably higher in northeast India[15]. In Nagaland state of northeast India, the carpenter worms are mostly confined to oak growing areas in Southern Angami regions of Kohima and neighboring Phek district with increased preference for lower altitude and warmer areas. Its vernacular name in Tenyedie dialect is '*Loungu'* and in Lotha dialect is '*Tsungra'*[16]. The use value (UV) of the carpenter worm larva among seven tribal communities of Nagaland has been documented and the UV value was in the range of 0.7 to 0.8[16]. The pinkish red larva of this worm serves as high-end delicacy with seasonal availability from July to February every year. The tradition of harvesting these worms has been practiced since time immemorial. Carpenter worm also bears cultural significance during the annual *Te-l Khukhu* millet festival of Southern Angami region celebrated during July. The only festival dedicated to girls, this was celebrated by young head-shaven damsels in the olden days. During the festive preparations, men cut oak trees and collect such worms whereas the women folk catch snails and grind millets. The foods are served in *Nakhwu* (a type of peepal leaf) rolled into cone shape and fixed with thin bamboo sticks. Carpenter worms are overwhelmingly oak-scented and their body fat is extremely sweet and tasty. They are cooked in various ways - poached until dry and crispy, or fried in oil and onion. Local ingredients like bamboo shoots, fresh ginger leaves and green chillies enhance the flavours. However, besides its high utility and preference, nutritional composition/dietary value of these carpenter worms have not been studied so far. In this backdrop, this study is primarily aimed to evaluate the nutritional value of the collected larva and generate knowledge on its food potential in the Nagaland for the first time.

Materials and methods

Study area and location

The study was conducted in Biochemistry, Plant physiology and Entomology section of the ICAR Research Complex for North eastern Hill Region, Umiam, Meghalaya (India) from 2015-2018.

Collection of market samples of carpenter worms

The fresh carpenter worms (edible stage) were sourced from twenty vendors from different local markets of Kohima, viz., Super market, New Market, BOC market, Kezieke market, High School junction market, Razhu point market etc. The collected samples were then transported to the laboratory, freeze-dried and stored at -20^{o}C till the analysis.

Preparation of the extracts

Initially, ten larvae from each twenty different vendors were selected and were grounded in mortar and pestle for biochemical analysis. One gram of the powder from each grounded sample was extracted for 12 h in 80% methanol, homogenized and clarified by centrifugation at 10000 rpm for 15 min at 4°C. The supernatant was collected for estimation of Total antioxidant capacity (TAC) and phenols, respectively.

Proximate analyses of carpenter worms larvae

Official methods of analysis of the AOAC[17] were used to study the proximate composition of crude fat, crude protein and crude fibre contents. Defatted samples were used for crude protein, crude fibre and total energy. Each test sample was replicated twenty times for determination of the nutrient content.

Crude Fat

Two gram of the sample was weighed accurately into labeled thimbles and fat was extracted with 80 mL of petroleum ether by boiling at 40-60^{o}C and refluxed in Soxhlet apparatus (Socsplus - SCS 06E, Pelican Equipments, Chennai, India) for 3 h. The defatted sample was removed and after recovery of the solvent, the ether extract left in the flask was then dried in the oven at 60°C for 30 min to remove any residual solvent. It was cooled in desiccators and weighed. The weight of the ether extract was determined by difference and

calculated as a percentage of the weight of sample analyzed.

Crude Protein

Total crude protein content was determined by micro Kjeldahl method using Elite EX, Kelplus automatic nitrogen analyzer (Pelican Equipments, Chennai, India). Initially, 100 mg sample was mixed with catalyst mixture (copper sulphate: potassium sulphate: selenium: 10:50:1) and digested with 10 mL concentrated H_2SO_4. The digest was distilled and ammonia released was captured in 4% boric acid solution. Titration was done with 0.1 N HCl and the resulting N_2 content was multiplied by factor 6.25 to convert into crude protein content.

Crude Fibre

Two gram of the defatted sample was weighed into a 250 mL conical flask and 200 mL of 1.25% conc. H_2SO_4 was added and the mixture was boiled under reflux for 30 min. The solution was filtered with Whatman filter paper. The residue was washed completely with boiling water until it was not any more acidic when checked on pH paper. The residue was transferred into a 250 mL beaker and 200 mL of 1.25% NaOH was added and boiled for 30 min in a digestion apparatus. Afterwards, it was filtered and rinsed with distilled water till the filtrate become neutral. The residue was transferred into a crucible and dried in an oven at 100°C for 8 h. The dried residue was weighed and ashed in a muffle furnace (NSW-101, NSW India) at 600°C and the loss of weight was determined. The difference (loss) in weight of the dry residue upon ignition was taken as the amount of crude fiber.

Estimation of Total Energy

Energy contents were estimated using a calorimeter (IKA Calorimeter System C2000 Basic, USA) in which gross energy was determined by measuring the heat of combustion.

Estimation of Total Carbohydrates

Determination of total carbohydrates was determined using phenol-sulphuric acid method based on the absorbance of a colored aromatic complex formed between phenol and carbohydrate at 490 nm[18]. The defatted sample (100 mg) was extracted with 10 mL (v/v) 80% ethanol thrice at 85°C in a water bath. The supernatants were collected and evaporated till ethanol was removed. The pellet was used in determining starch content. Ethanol-free extract was then made to 10 mL with distilled water. To 0.3 mL of sample, 1 mL of 3% phenol was added followed by 3 mL of conc. H_2SO_4, vortexed, and incubated at room temperature. Absorbance was measured in a spectrophotometer (Shimadzu UV-1700, Japan) and using sucrose as the standard, total carbohydrates content was calculated in mg/g dry weight**.**

Estimation of Total Phenols

Phenol content was determined with Folin-Ciocalteau reagent by the method described by Singleton et al.[19]. For that, 50 µL of extract was initially made up to 3 mL with 80% methanol, and to which 1 mL of DMSO and 1 mL of 10% Folin-Ciocalteau reagent was added and mixed. Finally 3 mL of 7% Na_2CO_3 was added after 3 min, and the tubes were incubated for 2 h at room temperature. Absorbance was measured at 760 nm in a spectrophotometer (Shimadzu UV-1700, Japan), and the phenolic content were calculated from the standard curve of gallic acid (50-300 ug/mL) and expressed as mg gallic acid equivlents (GAE)/g dry weight.

Estimation of Ascorbic Acid content

Vitamin C content was determined by the method described by Sadasivam and Manickam[20]. Two gram of the powder was extracted for 12 h with 4% oxalic acid. The extract was filtered and used for the analysis. To 10 mL of the extract, a few drops of bromine water was added until the solution became coloured, confirming the completion of the oxidation of ascorbic acid to dehydroascorbic acid. The final volume was then made to 50 mL with 4% oxalic acid. 2 mL of sample aliquots was made up to 3 mL of distilled water. To this 1 mL of 2,4-Dinitrophenylhydrazine reagent was added and 1-2 drops of 10% thiourea was added to remove excess bromine. The reaction mixture was mixed and incubated at 37°C for 3 h. 5 mL of 80% sulphuric acid was then added to dissolve the osazone crystals formed. A standard curve was plotted using different concentrations of Ascorbic acid (20-120 µg/mL). Absorbance was measured at 540 nm in a spectrophotometer (Shimadzu UV-1700, Japan). The total phenolic content was expressed in mg/g dry weight.

Estimation of Total Antioxidant Capacity

Total antioxidant capacity was estimated with phosphomolybdenum reagent by the method described by Prieto *et al.*[21]. To 0.3 mL of sample extract, 3 mL of phosphomolybdenum reagent solution (0.6 M sulphuric acid, 28 mM potassium phosphate and 4 mM ammonium molybdate) was

added. The tubes were then incubated at 95°C for 1 h. After cooling at room temperature the absorbance was measured at 695 nm in a spectrophotometer (Shimadzu UV-1700, Japan). A standard curve of ascorbic acid was plotted (20-100 µg/mL) to calculate the values. Total antioxidant capacity was expressed in mg Ascorbic acid equivalents (AAE)/g dry weight.

Determination of Amino acids

For identification of amino acids using Thin Layer Chromatography (TLC) in ethanolic larval extracts, the chromatogram was developed in Silica Gel $60F_{254}$ (Merck) in a standardized solvent system of *n*-butanol: acetic acid: water: 40:10:10); 0.1% ninhydrin in acetone was used in identification of spots[22]. The results also aligned with the respective standard Rf values of the acids.

Mineral element analysis

Oven-dried larval tissue (0.5 g) was first soaked for 12 h in 5 mL of conc. HNO_3. To the digest, 0.5 mL of di-acid mixture of conc HNO_3: perchloric acid (10:4) was added for complete digestion on a hot plate (350°C) for 8 h. The cooled samples were then made up to 50 mL with distilled water and vacuum filtered with Whatman No. 47 filter paper (0.25 µM pore size). The sample was analyzed using ICP-OES (Thermo Scientific ICP-iCAP 6300 Duo).

Estimation of Carotenoids content

Carotenoids pigment content in fresh larval tissue were extracted by homogenizing 0.5 g of larval tissue in 15 mL of 80% acetone in a pre-cooled pestle and mortar at 4°C. The extract solution was filtered through Whatman No. 42 filter paper and the volume of the extract made to 25 mL with the solvent. Absorbance was recorded at 645, 663 and 480 nm using a spectrophotometer (Shimadzu UV-1700, Japan). The pigment content is expressed in mg/g on fresh weight basis using the formula according to Davies *et al*.[23].

$$\text{Carotenoids} = (A_{480} + 0.114 \times A_{663} - 0.638 \times A_{645}) *(V/W)*(1/1000)$$

Wherein, V is the total volume of the extract, W is the weight of the tissue taken, and A_{663}, A_{645} and A_{480} is the optical absorbance values recorded, respectively.

Estimation of anthocyanin content

About 0.5 g of fresh larval tissue was homogenized by grinding in 20 mL of Propanol: HCl: H_2O (18:1:81 on v/v) and incubation in boiling water for 1.5 min. The tubes were further incubated in the dark at 25°C for 24 h. The extract were centrifuged for 40 min at 5000 g and the supernatant collected for recording the absorbance at 535 and 650 nm, respectively, in a spectrophotometer (Shimadzu UV-1700, Japan). The A values at 535 nm were corrected for scattering (S) using the A values at 650 nm (A_{650}) using Rayleigh's formula. Thus, corrected A_{535} nm is considered for actual anthocyanin calculation, since there is less or no absorption by anthocyanin at 650 nm[24]. Total anthocyanin was calculated using the following formula

$$\text{Corrected } A_{535} = A_{535} - A_{650}$$

$$\text{Anthocyanin (mg/g)} = (\text{Corrected } A_{535}) \times \text{Total volume} \times (1/W) \times (1/1000)$$

Wherein, W is the weight of larval tissue taken, V is volume of larval extract.

Statistical analysis

Data on different nutritional parameters were recorded by using different formulae (as per the nutrient concerned) as mentioned in the 'materials and methods' section. Mean values and standard error were calculated using SPSS 21.0 software for windows (Armonk, NY: IBM Corp.).

Results and discussion

The carpenter worms are consumed during larval stages, usually mature late larval instars. The proximate composition and biochemical values showed interesting information on the nutritional potential of mature larvae of carpenter worms viz., total Fat (37±0.58%), total crude protein (48±0.68%), total crude fibre (12.90±0.36%), energy value (504.4±0.56 kcal /100 g), total carbohydrates (defatted) (8.58±0.16 g/100 g), total phenols (25.2±0.42 mg GAE/g), total antioxidant capacity (6.29±0.21 µg AAE/g), total ascorbic acid (0.336±0.01 mg/100 g), total anthocyanins (674.1±1.87 µg/100 g) and total carotenoids (25.2±0.24 µg/100 g) (Table 1). The larvae were found to be rich in fat and

Table 1 —Proximate and biochemical values of Carpenter worms

Nutrient parameters with units	Mean (±SE)
Total Crude Fat (%)	37.00±0.58
Total Crude Protein (defatted) (%)	48.00±0.68
Total Crude Fibre (defatted) (%)	12.90±0.36
Energy Value (kcal /100 g)	504.4±0.56
Total Carbohydrates (defatted) (g/100 g)	8.58±0.16
Total Phenols (mg GAE/g)	25.20±0.42
Total Antioxidant Capacity (µg AAE/g)	6.29±0.21
Total Ascorbic Acid (mg/100 g)	0.336±0.01
Total Anthocyanins (µg/100 g)	674.1±1.87
Total Carotenoids (µg/100 g)	25.2±0.24

crude protein with an appreciable amount of fibre and other mineral elements.

The fat content of the carpenter worm at 20.1% was much higher than what has been reported for silkworm *A. pernyi* [25]. Edible insects contain on average 10 to 60% of fat in dry matter, and it is usually higher in the larval stages than in adults[26,27]. Makkar *et al*.[28] reported the proximate values in edible insects including silkworm pupae (*Bombyx mori* L.) and mealworm larvae (*Tenebrio molitor* L.) where the crude protein was estimated at 60.7 to 52.8%, and ether extracts of fats ranged from 25.7 to 36%. Similarly among the majority of the edible insects of south western Nigeria, the variations in fat content ranged from 1.5 to 31%[29]. The energy value of edible insects depends on their composition, mainly on the fat content, which is also generally higher in larval stages than adults[30]. Ramos-Elorduy *et al*.[31] had reported the calorific value in the range from 293 to 762 kcal per 100 g of dry matter in 78 different insects. In carpenter worm larva, the crude fat content of 37% represent an energy value of 504 kcal per 100 g, thereby indicating an appreciable source of dietary calories.

The crude protein content of insects is on average 40−75% on dry matter basis[32]. The value of crude protein in carpenter worm larvae was estimated at 48%; the value is at par with spent silkworm pupae[33] and at comparable values with the pupae of eri silkworm, muga silkworm[34], larvae of giant mealworm (*Zophobas morio*) and common mealworm (*T. molitor* L.)[35]. It can also be compared very well with those of conventional protein feed supplements such as soybean meal and fishmeal at 46.8% and 60.2%, respectively[36]. Longvah *et al*.[37] estimated the protein content of fresh eri silkworm prepupae and pupae at around 16%, and the value was as high as 75% in defatted samples. In general, high protein insect species are reported to have lower energy content[26]. Similar to this observation, higher crude protein values (>50%) and lower crude fat content at 20% and 16% was observed in pupae of eri silkworm and muga silkworms, respectively[34]. Thus at 48% crude protein content and 37% crude fat content, the carpenter worm larvae exhibits a balanced and appreciable value of both protein and fat content.

The most common form of fibre in insects is chitin, which is mainly derived from their exoskeleton. The crude fibre content of defatted carpenter worms at 12.90% is comparably better than that of edible insect *O. monocerus* (10.5%), eri silkworm (3.25%), muga silkworm (3%)[34,38], and the value was comparable with the edible larvae of yellow mealworm (*T. molitor* L.) and giant mealworm (*Z. atratus*) at 18 and 17%, respectively[26]. Crude protein content and fibre content in fifteen dried edible insects of south western Nigeria were also ranged from 6 to 29% and 1.0 to 3.4%, respectively[29]. Carpenter worm larvae also exhibited a carbohydrate content of 8.5 g % of defatted sample. Both pupae of muga and eri silkworms reportedly contain lower carbohydrate values of 1.2 and 1.6%, respectively[34].

The larval extract of carpenter worm was higher in total phenol content and total antioxidant capacity. According to Kahkonen *et al*.[39], the presence of polyphenols indicative of the presence of antioxidative function, due to their inherent high redox potentials which make them efficient reducing agents, hydrogen donors and singlet oxygen quenchers. Significant correlation has been established in antioxidant activity and phenolic content in plant extracts[40]. As it is known, animals including insects are not able to produce carotenoids de novo but they take them only with the food as plants is the source of all carotenoids for animals. The animals are only able to transform some of them by oxidation[41]. Lepidoptera chiefly store the carotenoids present in their food plants unchanged[42]. Apart from imparting red and yellow coloration to many insects, carotenoids also provide protection to cells from damage due to photo-oxidation, but this property of carotenoids is still unknown in insects. A high anthocyanins content and carotenoids pigments content in the larva also accounted for its reddish pink colour; the pigmentation also correlates with its polyphenol content and its antioxidant property. The results, therefore, iterate the potential of the larva as an important source of natural dietary antioxidants. The presence of ascorbic acid in the larvae also highlights the significance of ascorbic acid in all plant feeding insects. Dietary ascorbic acid needed for normal growth, moulting, and fertility in many insects, and ascorbic acid is probably an essential growth factor[43]; its omission from diet caused retarded growth and heaviest mortality at the moult from the fourth to the fifth instar[44,45]. Although the value in the study is insignificant for dietary benefits, some biosynthesis activity is, thereby indicated for its growth and development.

The mineral composition of the carpenter worm larvae was also determined (Table 2). Zinc, calcium

Table 2 —Mineral content of Carpenter worms

Mineral elements (mg/100 g*)	Mean (±SE)
Calcium	29.71±0.41
Iron	4.05±0.18
Potassium	279.19±1.33
Magnesium	61.91±0.37
Boron	0.058±0.00
Copper	0.814±0.01
Zinc	7.41±0.19
Phosphorus	219.21±0.79
Sulphur	73.98±0.71

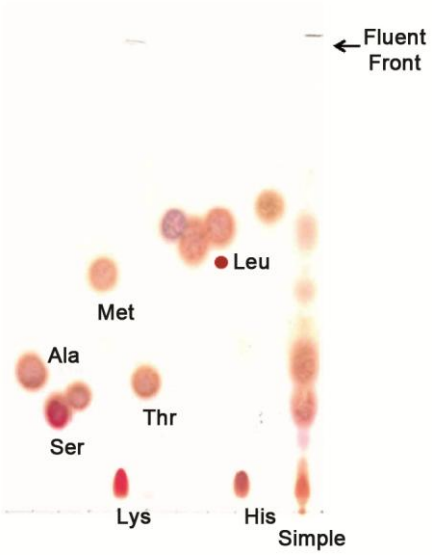

Fig. 2 — TLC plate developed in Ninhydrin for amino acids identification

and copper content of carpenter worm were higher than that of beef, goat, sheep pork and a variety of poultry birds[46]. Five edible insects of Korea, viz. *Tenebrio molitor L., Oxya chinensis sinuosa, Bombyx mori L., Protaetia brevitarsis seulensis*, and *Verlarifictorus asperses* is also reported to have comparable values of Zn, Fe and Cu without much variations[47].

Insect proteins are reported to be 77 to 98% highly digestible with essential amino acids representing 46–96% of the total amount of amino acids; the deficiency of tryptophan and lysine is noted in only a few cases[30]. The TLC chromatogram determined the presence of the essential amino acids methionine, lysine, leucine, histidine, and threonine (Fig. 2). Carpenter worm larvae is, thereby a nutritionally viable source of food because of its good protein and energy source, with adequate antioxidant potential. Also, the variations in its nutritional value will change according to the different forms of preparation and processing before consumption. The analyses were performed in starved conditions; a higher dietary value is, therefore, expected in its natural form.

Conclusion

This study reports the nutrient composition of carpenter worm larvae (*Cossus* spp.) for the first time, and concludes that these larvae have significant amounts of protein and fats, and exhibit antioxidant potential due to its good phenolic and pigments contents, with an appreciable source of dietary elements. This study, therefore, establishes the significant value of carpenter worms as an exotic dietary supplement among the indigenous Naga population. Further investigations are required to qualitatively determine the medicinal properties of the larvae to add more value to its promotion and commercialization.

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Conflict of interest

The authors declare that they have no conflict of interest.

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