

Nostochopsis lobatus Wood em. Geitler (Nostocales), Edible Algae in Northern Thailand

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ABSTRACT

The blue-green alga, Nostochopsis lobatus Wood em. Geitler, consists of a dark green colony with a mucilaginous sheath out-growth and is attached on rocks. It is usually found together with Cladophora spp. and Microspora spp. in some shallow rivers or streams in certain water resources, especially in the Nan River of Nan Province in northern Thailand. Scattered branches with the lateral branch stretching parallel and upward to the main filament in a polysaccharide sheath are their unique descriptive characteristics. N. lobatus can rapidly grow under clear water during both the cool-dry season and the hot-dry season in which water quality appears to be clean to moderate. Local people have prepared it as a salad dish called “Yum Lon”. The study of the bioactive compounds and other important compounds in N. lobatus showed a high polysaccharide content of 160.02 DP on BG11 medium, 76.77 DP in the samples of the Nan River and in some pigments; chlorophyll a: 8.26 mg/g cell dw, carotenoid: 0.339 mg/g cell dw, phycocyanin: 61.58 mg/g cell dw and allophycocyanin: 65.38 mg/g cell dw. Moreover, N. lobatus was found to contain a high protein content of 19.10 % dw, which is as high as freshwater fish. It was found to be 6,405 mg/100g cell dw of calcium, which was as high in content as the small fish, when eaten whole. In addition, this algae showed high content levels of certain vitamins and minerals, especially selenium, a well-known antioxidant (37 μg/100g dw). Thus, N. lobatus could come to be seen as an appropriate food source, which could grow well on semi-solid BG11 media on the laboratory scale. Furthermore, these algae should be considered as a source of supplemental food, as a therapeutic agent or for use as an ingredient in cosmetics in the future.

Keywords: Nostochopsis lobatus, edible algae, northern Thailand, polysaccharide, bioactive compounds.

1. INTRODUCTION

Macroalgae are macroscopic algae that can be viewed by the naked eye. In freshwater, they grow as a slime colony, in mats, sheets or filaments. Some macroalgae develop their morphology as a higher plant [1]. In lotic ecosystems, they are primary producers in the food chain which also produce oxygen. Local people have been using freshwater macroalgae
for food since ancient times. Presently, algae are gaining interest as a nutritionally functional food source. Cyanobacteria are known as a protein-rich food source. Moreover, some species of cyanobacteria showed novel bioactive compounds that are not found in higher plants and traditional therapeutic sources [2].

In northern Thailand, and especially in the Nan River of Nan Province, it was found that *Nostochopsis lobatus* Wood em. Geitler abundantly occurs from the cool-dry season to the hot-dry season when the velocity, turbidity and some nutrients in the streams and rivers are optimum. The common name is “Lon”. It forms the slimy colony on the rocks or cobbles under water. People in these areas have used it to prepare local dishes in the style of salads with traditional seasoning [3].

According to interviews conducted with local people, as well as in local markets surveys, *N. lobatus* has also been used as a medicinal ingredient, e.g. Lon is used to decrease fever. Some villagers consume Lon to relieve pain from stomach ulcers. This folk wisdom inspired Peerapornpisal et al. [3] to study the use of nutri-pharmaceuticals to relieve peptic ulcers, dyspepsia, rheumatoid arthritis, and hypertension. From this study, *N. lobatus* was of great interest as a potential source of food, as a food supplement and as a pharmaceutical product. However, *N. lobatus* from northern Thailand has not yet been studied in terms of their morphology or for their nutritional values. Therefore, this investigation will reveal their morphology, as well as many other valuable components.

2. MATERIALS AND METHODS

2.1 Algal Investigation

*Nostochopsis lobatus* Wood em. Geitler was individually collected from substrates; i.e. rocks, cobbles, and tree branches in water. Specimens were kept in plastic boxes at low temperatures (0-4°C). Morphological identification and classification were carried out in the laboratory. Samples were identified following the relevant books and publications. Photographs of each species were taken using a Light Olympus Normaski Microscope.

The water samples from each site were measured for temperature, current velocity, specific conductance, pH, DO, BOD, turbidity, nutrients (nitrate nitrogen, ammonium nitrogen and soluble reactive phosphorus) according to Eaton et al. [4]. In terms of water quality, Wetzel [5], Lorraine & Vollenweider [6], the Guidelines of Standard Surface Water Quality of Thailand [7], AARL PC-Score [8], [9] were followed.

2.2 Nutritional Value

The nutritional values e.g. lipid, protein, carbohydrate, fiber, vitamins, elements and calcium were investigated by the Central Laboratory (Thailand) Co. Ltd. based on AOAC[10].

2.3 Polysaccharide Investigation

*N. lobatus* which was cultured in BG11 broth and BG11 semi-solid agar were evaluated for their polysaccharide content, total sugar content following the phenol-sulfuric method [11]. Reducing sugar was determined by the dinitrosalicylic method (DNS method) [11]. The molecular weight of polysaccharide was analysed according to FAO/WHO [12].

2.4 Pigment Investigation

*N. lobatus* samples were separately rinsed under tap water and dried at 60°C. The determination for the presence of chlorophyll a was done in ethanol after Becker [13],
phycocyanin and allophycocyanin content was extracted in 0.05 M phosphate buffer (pH 6.8) and was measured using the simultaneous equations of Bennett & Bogorad [14] with the extinction coefficients from Bryant et al. [15]. Carotenoid content was extracted using ethanol and transferred into a solution of diethyl ether/NaCl (1:1) following de Quirós & Costa [16].

2.5 ABTS (2,2'-azino-bis 3-ethylbenzothiazoline -6-sulfonic) Radical Cation Decolorization Assay

The antioxidant activity was measured by ABTS radical cation decolorization assay according to Re et al. [17]. The stock solution of 7 mM ABTS and 2.45 mM potassium persulfate was mixed and kept for 16 hours in the dark. The solution was diluted with deionized water and ethanol to give 0.7 ± 0.2 absorbance at 734 nm. The aqueous and ethanolic algal extract was diluted to an appropriate concentration. Ten μl of algal extract was added with 1 ml of ABTS radical cation solution and mixed by Vortex mixer. The absorbance was measured at 734 nm. The activity was calculated as follows:

\[
\text{% inhibition} = \left[ 1 - \frac{\text{Ab sample}}{\text{Ab control}} \right] \times 100
\]

IC50, which stands for the concentration required for 50% scavenging activity, was then calculated from the above equation.

3. RESULTS AND DISCUSSION

3.1 Algal Investigation of Algae Ecology and the Utilization of Nostochopsis lobatus Wood em. Geitler

Nostochopsis lobatus Wood em. Geitler

Mucilaginous thalli were found attached on cobbles or stones under the water surface.
level throughout the rivers in the upper regions of Thailand, especially in Nan River. Mucilaginous thalli were found to be present in both stagnant and moving water. Thalli were spherical or sub-spherical and up to 5.5 cm in diameter with lobate smooth mucilaginous mostly formed in older thalli. The solid colony formed when young and became the hollow colony when reaching maturity, with blue-green, green or brown coloration. The mucilage was homogeneous, colorless and had a soft sheath.

The descriptive characteristics revealed the following: uniserated trichomes with equal diameter, barrel-shape, ellipsoidal or lengthy blue-green cells illustrated an inner colony and were arranged irregularly and were bent, and were richly branched at the lower part. Lateral branching with T-type formed as short, bent and ended parallel to the main filament. Cells in the branching zone displayed a barrel shape and were up to 10 μm broad. Heterocyst were mostly placed laterally (seesile) or terminally (pedicellate) on 2-3 lateral branched cells (Figure 2).

**Figure 2.** *Nostochopsis lobatus* Wood em. Geitler showed fresh samples, branch and heterocyte illustrated by microscope.
(a) Fresh samples of *N. lobatus*
(b) True branching (T-type) of *N. lobatus* under light microscope
(c) Lateral heterocyte (one arrow)
(d) Pedicellate heterocyte (two arrows)
Scale bar = 20 μm

Our survey of edible freshwater algae, “Lon” or *N. lobatus* in Nan River found that this alga were present from the cool-dry season until the hot-dry season. The optimum physical conditions included high transparency (<9 FTU), moderate velocity (1-2m/s), low temperatures (20-25°C) and suitable substrates as cobbles and stones. The water quality was classified and water qualities in all sampling sites are shown in Table 1.
3.2 Nutritional Value and Polysaccharide Investigation of Nostochopsis lobatus Wood em. Geitler

*N. lobatus* has been used as a popular local food. It is prepared as salad dish called “Yum Lon” (Figure 3). *N. lobatus* contained high nutrients as shown in Table 2. They are comprised of calcium as high in content as the small fish, which are eaten whole [18]. In addition, this alga showed high levels of vitamin and mineral content, especially magnesium, manganese, iron, selenium and niacin. Only 200 g dw of *N. lobatus* contained significant levels of magnesium; 265.4/100 g dw (Table 2), whereas a range of only 65-350 mg/d would meet the nutritional requirements of humans [19]. One hundred grams dw of this alga would be enough to meet the daily human requirement levels of manganese and iron (Table 2), for which the recommended intake levels are 1.5-2.6 mg/d and 7-27 mg/d, respectively [19].

Niacin is a one type of vitamin B (B3). It is water-soluble and cannot be stored in the human body. This vitamin can be lost from the body through urine. A continuous supply of niacin in the diet is important and required. Severe deficiency of niacin in the diet can cause Hartnup’s disease. Consumption of only 200 g dw of *N. lobatus* would provide close to the recommended daily intake levels of niacin for children (6-8 mg/d) [19] (Table 2).

*N. lobatus* also contained high selenium content (Table 2), which is close to the recommended intake level [19]. Male fertility requires selenium for testosterone biosynthesis and normal development of spermatozoa [20] but, low selenium status has been

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Appearance</th>
<th>Conductivity (µS/cm)</th>
<th>DO (mg/l)</th>
<th>BOD (mg/l)</th>
<th>NO₃ (mg/l)</th>
<th>NH₄⁺ (mg/l)</th>
<th>PO₄³⁻ (mg/l)</th>
<th>Water quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baan Tha Khum</td>
<td>**</td>
<td>231</td>
<td>7.2</td>
<td>2.0</td>
<td>1.8</td>
<td>0.07</td>
<td>0.54</td>
<td>Moderate</td>
</tr>
<tr>
<td>Baan Na Tao</td>
<td>**</td>
<td>241</td>
<td>7.5</td>
<td>2.2</td>
<td>1.7</td>
<td>0.09</td>
<td>0.07</td>
<td>Moderate</td>
</tr>
<tr>
<td>Baan Had Pah Khon</td>
<td></td>
<td>112</td>
<td>6.8</td>
<td>1.9</td>
<td>0.8</td>
<td>0.01</td>
<td>0.40</td>
<td>Clean-Moderate</td>
</tr>
<tr>
<td>Baan Num Yao</td>
<td>***</td>
<td>198</td>
<td>7.4</td>
<td>0.4</td>
<td>0.6</td>
<td>0.08</td>
<td>0.06</td>
<td>Clean-Moderate</td>
</tr>
</tbody>
</table>

* Rare (<10% of 1 m² in studied area), ** Moderate (≤ 10-50% of 1 m² in studied area), *** Plenty (>50% of 1 m² in studied area)

![Figure 3. Yum Lon (local salad): (a) Ingredients, (b) Ready to eat Yum Lon.](image-url)
associated with a significantly greater incidence of depression and other negative mood states, such as anxiety, confusion, and hostility [21-23]. Therefore, *N. lobatus* could be seen as an appropriate food or supplemental food source for human beings. In addition, *N. lobatus* contains high protein content, which is as high as freshwater fish [18].

### Table 2. Nutritional values of *Nostochopsis lobatus* Wood em. Geitler.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid</td>
<td>0.64 %</td>
</tr>
<tr>
<td>Protein</td>
<td>19.10 %</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>31.94 %</td>
</tr>
<tr>
<td>Fiber</td>
<td>2.05 %</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1.07 mg/100g dw</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.12 &quot;</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.07 &quot;</td>
</tr>
<tr>
<td>Niacin</td>
<td>2.48 &quot;</td>
</tr>
<tr>
<td>Calcium</td>
<td>6405 &quot;</td>
</tr>
<tr>
<td>Sodium</td>
<td>136.9 &quot;</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.5 &quot;</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.3 &quot;</td>
</tr>
<tr>
<td>Magnesium</td>
<td>265.4 &quot;</td>
</tr>
<tr>
<td>Manganese</td>
<td>4.5 &quot;</td>
</tr>
<tr>
<td>Iron</td>
<td>114.9 &quot;</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.65 &quot;</td>
</tr>
<tr>
<td>Selenium</td>
<td>37 μg/100g dw</td>
</tr>
</tbody>
</table>

In terms of evaluating the degree of polymerization (DP), cultivated *N. lobatus* on BG-11 semisolid agar showed the highest DP (160.02). *N. lobatus* in BG-11 broth revealed 89.41 DP, whereas the natural sample displayed the least DP (76.77). The levels of DP higher than 20, indicated the presence of a high molecular of weight of sugar or polysaccharides. This exploitation implied that *N. lobatus* should certainly be the subject of pre-biotic research in further studies.

### 3.3 Pigment investigation of *Nostochopsis lobatus* Wood em. Geitler

*N. lobatus* was found to have high pigment content levels; 8.82 mg/g cell dw of chlorophyll *a*, 0.339 mg/g cell dw of carotenoids, and especially phycocyanin and allophycocyanin, which were present at 64.58 mg/g cell dw and 65.38 mg/g cell dw, respectively. Natural samples taken from Nan River showed high contents of phycocyanin as immobilized cells when cultured under optimal conditions (cultured in BG-11, at 25±1°C, light intensity of 80 μm m<sup>-2</sup>s<sup>-1</sup> and 15:9 h light:dark cycle) of the control of Pandey [24] and Pandey [25]. The free culture cells of Pandey [24] and Pandey [25] showed 46.53 and 46.62 mg/g dw of phycocyanin, respectively, which was lower than the amounts found in *N. lobatus* taken from Nan River. These pigments are valuable pigments, which
can be used in fluorescence resonance energy transfer (FRET) assay, the labeling of proteins, fluorescence microscopy, as well as being used as biomarkers and as food colorants. From this result, *N. lobatus* would be a good source of phycocyanin and allophycocyanin, which could be seen as a reliable development within the relevant industries, if the concerned research will be undertaken.

3.4 Antioxidant activity of *Nostochopsis lobatus* Wood em. Geitler

The villagers in these areas believe that *N. lobatus* could be used for aphthous ulcer treatment, to decrease fever and pain from gastric ulcers. From the previously research, Peerapornpisal [3] found the therapeutic agents and antioxidant activities of this alga which confirmed this folk wisdom. These results indicated that *N. lobatus* exhibited anti-gastric ulcer activity, as well as anti-inflammatory and antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay. In the present study, the ABTS radical scavenging activity was also measured. The ethanolic extract clearly revealed a higher activity than the aqueous extract (Table 3) and this finding agreed with various previous reports [27, 28]. The aqueous extract showed complacent activities, as did *Padina minor* [26]. Although, the aqueous extract from our study showed lower activity than the aqueous extract that was derived from an Indian enhancement culture of *N. lobatus* [24, 25], the ethanolic extract did exhibit much higher activity than the aqueous extract from an unenhanced Indian *N. lobatus* [24, 25]. In addition, our findings also higher than those ethanolic extracts of some reported marine algae; *Halimeda macroloba*, *Sargassum binderi* and *Turbinaria conoides* [27].

Table 3. The 50% inhibition concentration (IC50) for ABTS

<table>
<thead>
<tr>
<th>Solvent</th>
<th>ABTS IC&lt;sub&gt;50&lt;/sub&gt; (mg/ml)</th>
<th>Maximum antioxidant capacity (μM of trolox equivalent per gram of wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aqueous extract</td>
<td>25.79</td>
<td>30</td>
</tr>
<tr>
<td>ethanolic extract</td>
<td>5.36</td>
<td>250.3</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

Local people have utilized this variety of algae in many ways over a long period of time by inheriting the knowledge passed down from previous generations. However, taxonomic studies have not yet been done. This report revealed the morphology, nutritional values and bioactive compounds of *N. lobatus*, which have been used as traditional food in Northern Thailand. The data on high pigment levels, polysaccharide content and the high activity of antioxidants indicated that *N. lobatus* could be a potential candidate for various nutritional applications.

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