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Two Endemic Species of Macroalgae in Nan River, Northern Thailand, as Therapeutic Agents

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Abstract: Two endemic species of macroalgae in the Nan River, Northern Thailand, *Cladophora glomerata* Kützing or "Kai", a branched filamentous alga in the Chlorophyta, and *Nostochopsis lobatus* Wood em. Geitler or "Lon", a mucilaginous colonial alga in the Cyanophyta, were selected for study as therapeutic agents. The anti-gastric ulcer, anti-inflammatory, analgesic, anti-oxidant, and hypotensive activities suggested their value as therapeutic agents. They may be employed as nutri-pharmaceuticals or food supplements for relieving chronic diseases and disorders including peptic ulcers, dyspepsia, rheumatoid arthritis, hypertension, etc. However, further detailed studies of these pharmacological activities are required to validate their therapeutic potentials.

Keywords: *Cladophora glomerata* Kützing, *Nostochopsis lobatus* Wood em Geitler, macroalgae, gastric ulcer, anti-oxidant.

Introduction

Macroalgae are the algae that can be viewed with the naked eye in the field. In freshwater, they grow often as slime colonies in mats, and as sheets or filaments. They are the important organisms in lotic ecosystems, both for their oxygen production and as primary producers in the food chain. In some freshwater ecosystem, macroalgae occur in filamentous forms covering the whole area of a river bed. The local people have been using these algae as local food for many years.

In northern Thailand, especially in Nan River, Nan Province, and Mekong River in Chiang Khong District, Chiang Rai Province, two genera of macroalgae occur in abundance in the dry season. The common names of these algae are "Kai" and "Lon". The people in these areas use them to make many kinds of locally consumed food. Moreover, there has been research to process various foods from Kai such as crisps, baked goods, paste, and noodle, to obtain more market products for the people in these areas[1].

Some of these algae have been used as medical ingredients, e.g. Lon is used to decrease fever. Some villagers consume Kai to calm down stomach ulcer. This local wisdom inspired the researcher in this project to study the therapeutic agents from these algae. Positive results from this research will confirm the value of these algae. If we are able to find useful potential medicine in these algae, the active compounds must be further studied and their mechanism of action elucidated.

Materials and Methods

Algal Samples and Preparation

Peerapornpisal *et al.* [1]. found that Kai consists of two genera: *Cladophora* spp. and *Microspora* spp. whilst Lon consists of one genus: *Nostochopsis* spp. In this investigation, we selected Kai as *Cladophora glomerata* Kützing, a green algae in the Division Chlorophyta and Lon as *Nostochopsis lobatus* Wood em. Geitler, a blue - green algae in the Division Cyanophyta.

C. glomerata and *N. lobatus* were collected from Nan River at Paka Subdistrict, Tha Wang Pha District, Nan Province, Thailand in December 2004. The samples were separated into two portions, one for identification using morphological features. The relevant books e.g. Desikachary [2], Whitford and Schumacher [3], Prescott [4] and John *et al.* [5] were followed. The amount of each species was estimated by the use of quadrats including dry weight determination, and the attachment to substratum was investigated in the field. The other portion was used to investigate for the presence of therapeutic agents.

Therapeutic Agent Investigation

Algal material and extraction

C. glomerata and *N. lobatus* were, separately, washed in tap water, shade-dried and powdered. The dried

powder was then extracted with 95% ethanol, evaporated *in vacuo* at 55 °C and lyophilized to obtain a dried ethanolic extract of *C. glomerata* and of *N. lobatus* which from now on are referred as CGE and NLE respectively.

Experimental animals

Male Sprague-Dawley rats weighing 40-60 g and 150 - 200 g as well as male Swiss albino mice were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakhon Prathom Province, Thailand. The animals were acclimatized for at least 7 days in an animal room where the temperature was maintained at 22 ± 3°C and there was a 12 h light-dark cycle. The food was supplied by Perfect Companion Co. Ltd., Samut Prakan Province. The animals had free access to food and water unless stated otherwise. All animals received humane care in compliance with the ethical guidelines for the use of animals issued by the National Research Council of Thailand 1999.

Pharmacological experiments

Restraint water immersion stress-induced gastric ulcers in rats

The CGE and NLE dissolved in water were administered orally to rats which had fasted for 48 hours. Sixty minutes later, rats were restrained individually in stainless steel cages and immersed up to their xiphoid in a water bath maintained at 22 ± 2° C, according to the method of Takagi *et al.* [6] After 5 h of this exposure, the rats were sacrificed and examined for gastric ulcers. After each rat was sacrificed, the stomach was removed, opened along the greater curvature and the glandular portion of the stomach was examined. The length in mm of each lesion was measured under a dissecting microscope and the sum of the length of all lesions was designated as the ulcer index.

Ethyl phenylpropiolate-induced ear edema in rats (EPP)

The experiment was performed to investigate the ability of an agent in inhibiting increased vascular permeability leading to edema in an inflammatory process.[7] Ear edema was induced by topical application of EPP. EPP at a dose of 1 mg/20 µl/ear was applied locally to the inner and outer surfaces of both ears of each rat. The CGE and NLE dissolved in acetone were applied in the same manner in a volume of 20 µl before the irritant. The thickness of each ear was measured with vernier calipers.

Acetic acid-induced writhing response in mice

A writhing response was produced by an injection of 0.75% acetic acid at 0.1 ml/10g body weight into the peritoneal cavity of mice, according to the method of Koster *et al.*[8] .The number of writhes, a response consisting of contraction of an abdominal wall, pelvic rotation following by hind limb extension, was counted during observation for 15 minutes beginning from 5 minutes after the acetic acid injection.

Blood pressure of pentobarbital anesthetized rats

Rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.). The trachea was intubated in order to prevent airway obstruction. The CGE and NLE dissolved in normal saline were given via a cannula inserted in the external jugular vein. Blood pressure was recorded from common carotid artery using a pressure transducer connected to a Grass polygraph. Blood pressure was expressed as the mean arterial blood pressure (MABP).

Scavenging activity against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical

The scavenging activity of the test sample or glutathione against the DPPH radical was measured according to the method of Hou *et al.*[9] Each 0.3 ml of the test sample (dissolved in MeOH or DI water) was added to 0.1 ml of 1 M Tris-HCl buffer (pH 7.9), and then mixed with 0.6 ml of 100 mM DPPH in methanol for 20 min, in the absence of light. The absorbance at 517 nm was determined. Deionized water was used as blank. The scavenging activity against the DPPH radical was calculated as follows:

$$\text{Activity (\%)} = 100 \times [A517_{blank} - A517_{sample}] / A517_{blank}$$

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

Results

Morphological Structure of Two Endemic Species of Macroalgae

Cladophora glomerata Kützing (Fig. 1.)

It is green or light green, filamentous in form, attached on rock or cobble in the bed of shallow rivers. Microscopically, thalli are composed of joined cylindrical cells, with lengths of 6 – 20 µm and widths of 4 - 10 µm and with dichotomously branching filaments. Branches are tufted, arising singly, arbuscular, the branches becoming irregular in old algae. Branches are narrowed towards tips, cell walls are thick and usually lamellate. The chloroplast is in a parietal network with numerous pyrenoids.

Nostochopsis lobatus Wood em. Geitler (Fig. 2)

In nature, this species looks as a soft colony attached on the surface of rock or cobble in the bed of shallow

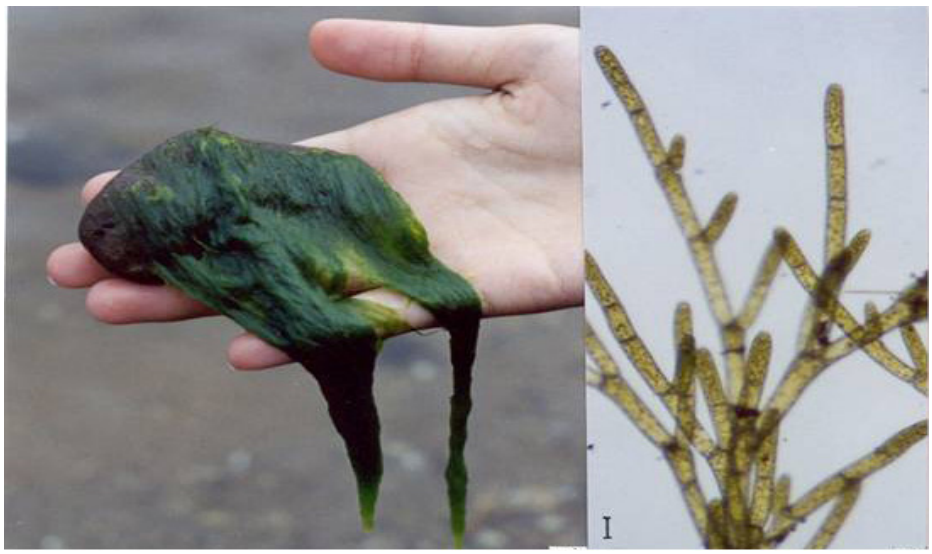

Fig 1. *Cladophora glomerata* Kützing (Chlorophyta) in Nan River, Thailand. Left: in the field, Right: under light microscope.

Fig 2. *Nostochopsis lobatus* Wood em. Geitler (Cyanophyta) in Nan River,Thailand. Left: in the field, Right: under light microscope.

rivers. The shape of the mucilaginous colony varies from spherical when young, changing to an amorphous shape in a mature stage, and it may be hollow, and torn. The colour of the colony varies from dark green, or an orange to rusty colour.

For the microscopic features, the trichome has lateral branching, the main branch containing many cells of 4 -10 µm in length, and 4 – 6 µm in width. The minor branch is made of 1 -3 cells. Heterocysts (diameter 3 - 4 µm) are present in three locations: intercalary, terminal or pedicellate and lateral or sessile. In Thailand, three common names are Lon, Kai Hin (stone egg) and Dok Hin (stone flower).

Restraint Water Immersion Stress-induced Gastric Ulcers in Rats

C. glomerata and *N. lobatus* showed an anti-ulcer effect when tested on the restraint water immersion stress-induced ulcers in rats. An anti-peptic ulcer drug: Cimetidine (a H_2–antagonist) was used as a reference drug. The data obtained are summarized in Table 1.

Ethyl Phenylpropiolate-induced Ear Edema in Rats (EPP)

The effects of *C. glomerata* and *N. lobatus* on EPP-induced ear edema in rats are shown in Table 2. Similar to Phenylbutazone (a nonsterodial anti-inflammatory

Table 1. Effects of the ethanolic extracts of *C.glomerata* and *N. lobatus* on water immersion stress-induced gastric ulcers in rats.

Group	Ulcer index (mm)	Inhibition(%)
Control	16.4 ± 3.3	-
Cimetidine		
100 mg/kg	3.7 ± 1.0***	72.4
C. glomerata		
100 mg/kg	11.9 ± 1.1	27.4
500 mg/kg	6.8 ± 1.5**	58.5
N. lobatus		
100 mg/kg	6.8 ± 2.0**	58.5
500 mg/kg	1.0 ± 0.4***	93.9

Data expressed as mean ± S.E.M. (n = 6-8).
Significantly different from the control group: $^{**}p < 0.01$, $^{***}p < 0.001$.

Table 2. Effects of the ethanolic extracts of *C. glomerata* and *N. lobatus* on ethyl phenylpropiolate-induced ear edema in rats.

Group	Inhibition of ear edema(%)			
	15 min	30 min	1 h	2 h
Control (Acetone)	-	-	-	-
Phenylbutazone				
1 mg/ear	83	72	63	60
C. glomerata				
3 mg/ear	87	79	50	56
N. lobatus				
3 mg/ear	62	47	27	21

drug), both algae exerted an inhibitory effect on edema formation.

Acetic Acid-induced Writhing Response in Mice

Aspirin (a COX-inhibitor), *C. glomerata* and *N. lobatus* extracts showed an analgesic effect by inhibiting the writhing response in mice induced by acetic acid. Data are illustrated in Table 3.

Table 3. Effects of the ethanolic extracts of *C. glomerata* and *N. lobatus* on acetic acid-induced writhing response in mice.

Group	No. of writhes	Inhibition (%)
Control	27.7 ± 2.0	-
Aspirin		
150 mg/kg	7.6 ± 2.1***	72.6
C. glomerata		
100 mg/kg	18.1 ± 2.4**	34.7
500 mg/kg	4.3 ± 1.2***	84.5
N. lobatus		
100 mg/kg	15.2± 1.9***	45.1
500 mg/kg	3.3 ± 1.4***	88.1

Data expressed as mean ± S.E.M. (n = 7-8).
Significantly different from the control group: $^{**}p < 0.01$, $^{***}p < 0.001$.

Blood Pressure of Pentobarbital Anesthetized Rats

An intravenous injection of *C. glomerata* extract elicited a hypotensive response causing a decrease in blood pressure. Fig.3 depicts typical blood pressure responses to various doses of *C. glomerata* in pentobarbital anesthetized rats. The results obtained are summarized in Table 4. *N. lobatus* given at doses up to 100 mg/kg did not produce hypotension.

Table 4. Effect of the ethanolic extract of *C. glomerata* on mean arterial blood pressure (MABP) of pentobarbital anesthetized rats.

Dose(mg/kg)	Decrease of MABP (%)
25	26.15 ± 6.64
50	45.00 ± 5.91
100	63.78 ± 5.15

Data are expressed as mean ± S.E.M. n = 8.

Scavenging Activity Against DPPH Radical

Fig. 4 shows the dose-response curves of (DPPH) radical-scavenging response curves are shown in Table 5.

Table 5. The 50% inhibition concentration (IC50) for DPPH radical-scavenging activity of glutathione, *C. glomerata* and *N. lobatus*.

Extract/anti-oxidant	IC50 (mg/ml)
Glutathione	0.055
C. glomerata	1.876
N. lobatus	13.435

Discussion

The screening for pharmacological activities indicated that both *C. glomerata* and *N. lobatus* exhibit anti-gastric ulcer, anti-inflammatory, analgesic, and anti-oxidant activities, but only *C. glomerata* showed a hypotensive activity.

The experimental models used in the present study are commonly employed to detect pharmacological activities. Gastric ulcers can be induced in experimental animals and humans by physical or psychological stress[6]. The restraint water immersion stress-induced gastric ulcers in rat is a widely used experimental model for induction of acute stress ulcers in rats. The EPP-induced ear edema in rat model is a useful screening model to investigate the anti-inflammatory activity of the test substance on the acute phase of inflammation[7]. The writhing response in mice is a model of visceral pain[8]

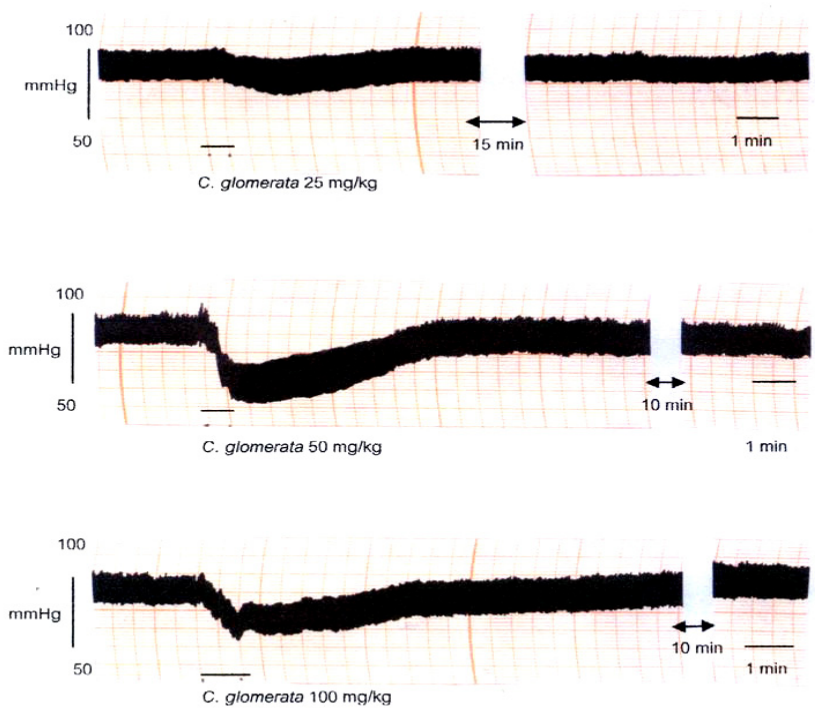

Fig 3. Effect of the ethanolic extract of *C. glomerata* on blood pressure of pentobarbital anesthetized rats.

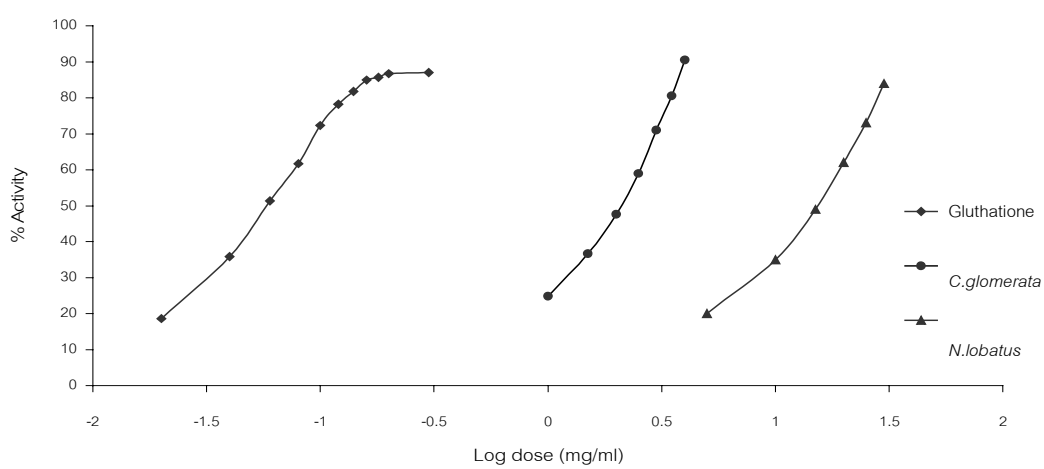

Fig 4. Comparison of dose response curves of glutathione, *C. glomerata* and *N. lobatus*.

and it is used to study both the peripherally and centrally acting analgesic activity[10,11]. Additionally, the scavenging activity against DPPH radical model is among the most widely used models for assessing an anti-oxidant activity.

The roles of free radicals and active oxygen in the pathogenesis of human diseases including cancer, aging and atherosclerosis have been recognized[12,13,14]. Interestingly, anti-oxidant activity has been proposed to play roles in various pharmacological activities such as anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial or antiviral activities.

The anti-gastric ulcer, anti-inflammatory, analgesic, anti-oxidant, and hypotensive activities of the two algae (*C. glomerata* and *N. lobatus*) found in the study suggest their therapeutic values. They may be employed as nutri-pharmaceuticals to relieve peptic ulcers, dyspepsia, rheumatoid arthritis, hypertension, etc. However, further detailed studies of these pharmacological activities are required to validate their therapeutic potentials.

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