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Short Communication

**First results on qualitative characteristics and biological activity
of nematocyst extracts from *Chrysaora plocamia*
(Cnidaria, Scyphozoa)**

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ABSTRACT. We performed qualitative and quantitative characterizations of the biological activity of nematocyst extracts from 30 specimens of *Chrysaora plocamia* collected off Huayquique, Iquique, Chile. After extracting the nematocysts from the jellyfish tentacles, we characterized them with UV and IR sweeps and determined their biological activity through antioxidant and hemolytic analyses. The preliminary results suggest that the extracted molecular could be amino acids originating in proteins, with antiradicalary activity of around 65% as compared with an antioxidant of commercial origins and very low hemolytic activity.

Keywords: jellyfish, nematocyst, bioactivity, antioxidant, hemolysis.

**Primeros resultados sobre las características cualitativas y
actividad biológica de extractos de nematocistos de
Chrysaora plocamia (Cnidaria, Scyphozoa)**

RESUMEN. Se caracteriza cualitativa y cuantitativamente la actividad biológica de extractos de nematocistos de 30 ejemplares de *Chrysaora plocamia* colectados frente a la localidad de Huayquique, Iquique, Chile. Se obtuvo un extracto de nematocistos de los brazos de las medusas y se caracterizó mediante un barrido en el rango ultravioleta e infra-rojo y luego se determinó su actividad biológica mediante análisis antioxidante y hemolítico. Los resultados obtenidos sugieren que las moléculas del extracto podrían ser aminoácidos procedentes de proteínas, con actividad antiradicalaria, de alrededor de 65% en comparación con un antioxidante de origen comercial, y una actividad hemolítica bastante baja.

Palabras clave: medusas, nematocistos, bioactividad, antioxidante, hemólisis.

Chrysaora plocamia (Lesson, 1830) is one of the 14 Scyphozoan jellyfish inhabiting Chilean waters (Sielfeld, 2002). This species is distributed throughout the eastern South Pacific Ocean from Paita off northern Peru to Punta Arenas off southern Chile (Lesson, 1830; Vanhöffen, 1888 *fide* Kramp, 1961). Massive amounts of dead jellyfish or their remains have been found washed ashore off Iquique, especially in summer and part of spring; the nematocysts that the jellyfish use for feeding and defense are one of the most frequent causes of skin irritations in swimmers (Vera *et al.*, 2005). This species is not toxic and causes neither moderate nor severe poisoning in humans, unlike *Physalia physalis*, another species reported in Chilean waters whose toxins produce severe systemic complications (Vera *et al.*, 2005). However, the mildly toxic venom of *C. plocamia* can cause slight cutaneous

and ophthalmologic manifestations within the first 24 h, and delayed long-term reactions in individuals who have been sensitized through previous contacts that result in an immune response (Vera *et al.*, 2005).

Although frequently observed in coastal zones, there is little information on the *C. plocamia* biology and even less all about its nematocysts and the purification, composition, and action mechanisms of its venom, as is available for other congeneric species such as *C. quinquecirrha* or *C. achlyos* (Long-Rowe & Burnett, 1994; Faisal *et al.*, 1999; Takatochi *et al.*, 2004). Therefore, we performed a preliminary qualitative characterization to measure the biological activity of the nematocyst extracts of 30 *C. plocamia* specimens collected off

Huayquique, Iquique, in plastic bags with scuba diving to a maximum depth of 35 m in January and February 2007. Each specimen was identified to the species level according to Kramp (1961) and Fagetti (1973), and the external bell diameter was measured.

Later, based on the modified technique of Burnett *et al.* (1992) and Burnett & Goldner (1970), the jellyfish tentacles were extracted and soaked on a 0.5 mm filter. The resulting extract was refrigerated in labeled 25-mL vials kept in containers until their analysis in the Basic Sciences Laboratory of the Universidad Santo Tomás, Iquique campus. Each sample was then centrifuged at 4,000 rpm for 2 min in a Hettich centrifuge (Boeco-28, rotor 1624) in a 2:3 mL sample:saturated sucrose solution; the resulting decanting was re-centrifuged for 1 min. The sample was then subjected to cold magnetic stirring (Velp Scientifica) for 15-20 min at 1200 rpm until obtaining the nematocyst extract. The nematocyst extract yield and aperture were estimated by counting at 0.2 mL under a light microscope. In order to qualitatively characterize the nematocyst extract in sucrose in terms of amino acids and peptides, a 250-700 nm UV sweep was done in a Shimadzu UV-VIS 1240 spectrophotometer using a blank with distilled water and a control with a saturated sucrose solution and an IR sweep of 700-4000 nm, with a sucrose blank with distilled water. The presence of proteins was confirmed qualitatively in the nematocyst extract by subjecting it to a Biuret test.

Afterwards, the biological activity of the extract was tested, evaluating the free radical-trapping activity through a DPPH assay (diphenylpicrylhydrazyl, Abuin *et al.*, 2002) in triplicate. For this, 1 mL of the extract was added to 2 mL of DPPH solution (10 mg DPPH (99%) in 1 L of methanol, DPPH 10 mg·L⁻¹), that was later incubated in a thermoregulated bath for 30 min. Its absorbency was then read at 517 nm by using a blank with methanol. The extract's antioxidant activity was calculated as follows: $A = 100 \cdot (1 - \text{Abs sample} / \text{Abs reference})$ (Burda *et al.*, 2001). This activity was compared to BHT (Hydroxytoluene Butylated), a commercial antioxidant frequently used in the food industry.

The extract's hemolytic activity was also proven using the hemolysis assay with human erythrocytes according to Zou *et al.* (2001) and Mayasuki *et al.* (1987). For this, 5 mL of blood taken from a young, healthy donor who had been fasting for 12 hours were placed in a recipient with the anti-clotting agent EDTA. The plasma was eliminated in triplicate by centrifuging the sample (3500 rpm for 5 min) and the erythrocyte suspension was washed with physiological saline (0.9%) to eliminate the plasma residue. Finally, 1 mL of the original suspension (5 mL of washed erythrocytes gauged at 250 mL with 0.9% physiological saline) was added to the different volumes of the extract

until reaching 3 mL with the physiological saline. Then, these were incubated at 37°C in a thermoregulated bath for 2 h, centrifuged at 3500 rpm for 5 min, and the supernatant was quantified at 541 nm.

All the specimens sampled were alive and mature. Their bell diameters fluctuated between 130 and 250 mm. The nematocyst extract yield per specimen ranged from 75 to 85%, whereas the nematocyst rupture yield varied from 50 to 70% every 0.2 mL.

With regard to the UV range spectrum, a small peak was observed at 280 nm along with a range of analytes that absorbed between 350 and 700 nm (Fig. 1). The IR sweeps of the extracts revealed signals of the most "concentrated" bands of the extract in group 1 (2800-3000 nm), associate with O-methyl unions or aldehyde-forming O groups; group 2 (1500-1200 nm) related to NO₂, O-NO₂, and C-NO₂ bonds; group 3 (1200-1000 nm) associated with C-O, C-OH, and C-N bonds, in which the most probable is the C-O bond but there are average probabilities for C-N bonds; and group 4 (1000-700 nm) related to C-O, O-O, N-O, and NO₂ bonds, in which the most probable is C-O (Fig. 2).

The percentage of free radical trapping activity (Fig. 3) indicated an antiradicalary activity of the nematocyst extract of approximately 65% as compared to a commercial antioxidant used in the food industry (BHT), whose activity is much more effective (97%). On the other hand, the nematocyst extract showed low hemolytic activity in human erythrocytes (Fig. 4).

The extract presented a C-O bond in its structures that could have been an aldehyde or carbonyl group, and a C-N

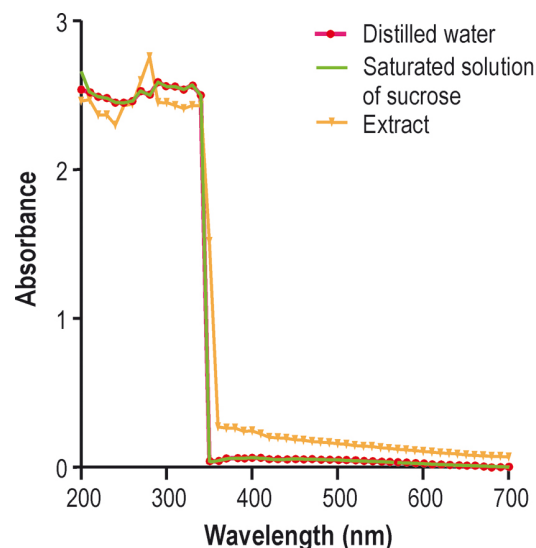


Figure 1. Sweep UV-Vis of nematocyst extracts.

Figura 1. Barrido UV-Vis del extracto de nematocistos.

bond, nitrogen that can be associated with a nitro group. These molecules (oxygenated and/or nitrogenated) give positive results in the Buriet test for proteins, corresponding to the 280-nm peak in the UV sweep. This suggests that molecules of the extract could be amino acids from proteins. The presence of a proteic component is also observed in species with greater toxic potential (Vera *et al.*, 2004, 2005). On the other hand, the range of analytes absorbed between 350 and 700 nm suggests an effect of the sucrose concentration given the greater absorption of the extract.

Given the antioxidant activity of the extract, it can be deduced that this would probably be due to the presence of proteins containing aromatic groups. The presence of a proteic component is also observed in species with greater toxic potential (Vera *et al.*, 2004).

The hemolytic activity of the toxin has been proven in other cogeneric species (*C. quinquecirrha* and *C. achlyos*) and it has been lethal and/or very sensitive in rats. Its potential was found to be different when comparing the toxin in both species, being lower for *C. achlyos* (Long-Rowe & Burnett, 1994; Faisal *et al.*, 1999). This species was also

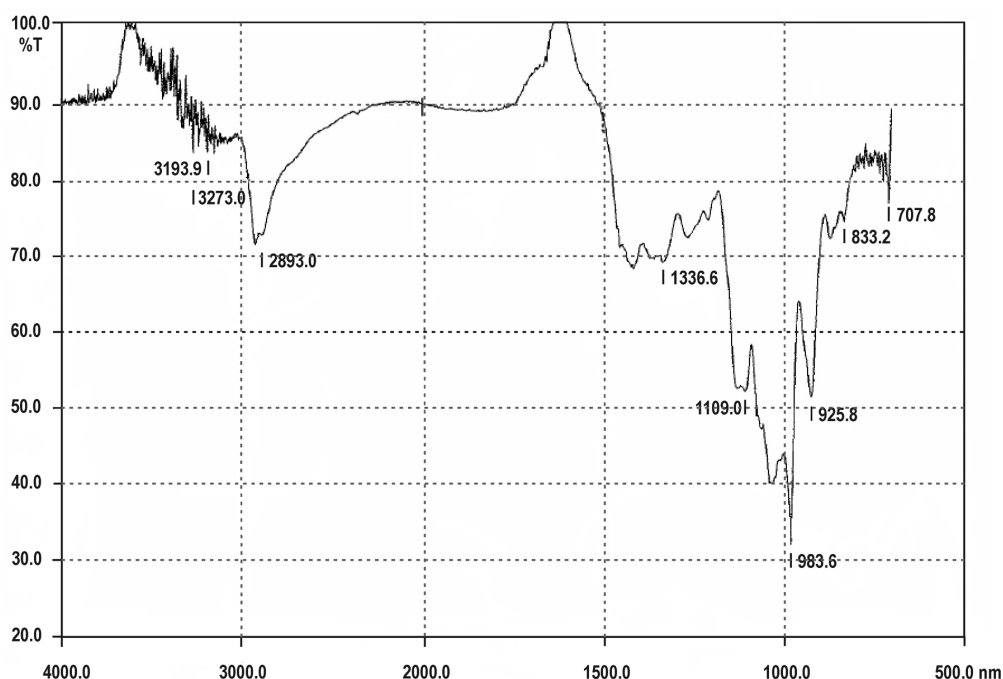


Figure 2. Sweep IR of nematocyst extracts.

Figura 2. Barrido IR del extracto de nematocistos.

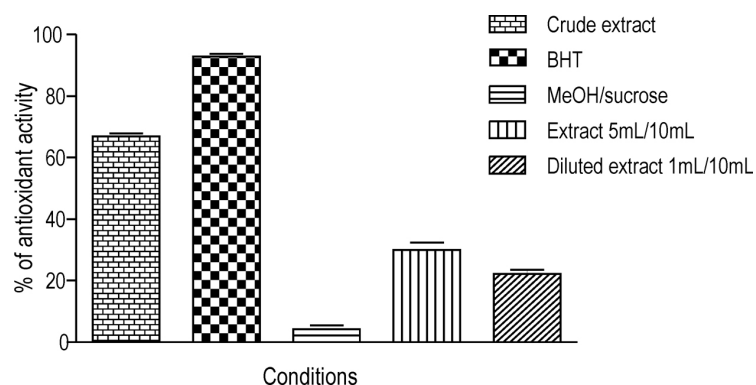


Figure 3. Antioxidant activity of nematocyst extracts.

Figura 3. Actividad antioxidante del extracto de nematocistos.

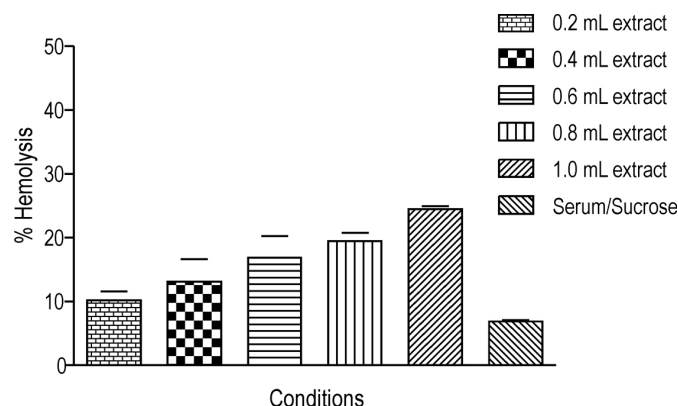


Figure 4. Hemolytic activity of nematocyst extracts.

Figura 4. Actividad hemolítica del extracto de nematocistos.

found to be lethal in other tissues of the diamond killifish (*Adinia xenica*) (Takatochi *et al.*, 2004). However, for *C. plocamia*, the molecular components tested in the nematocyst extract show a low, almost null hemolytic activity. Therefore, we can conclude that they have low toxicity, at least in human erythrocytes. This could generally explain their decreased cutaneous and dermatological effect in comparison with other species (Vera *et al.*, 2005). In spite of these first results, it is necessary to carry out new molecular studies in order to determine the structure of proteins present in the nematocyst extract, to test hemolysis in other species, and to determine future antioxidant applications of the extract.

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