SEASONAL VARIATION OF THE ANTIBACTERIAL ACTIVITY OF *Aplysina gerardogreeni* FROM THE GULF OF CALIFORNIA

Variacion estacional de la actividad antibacteriana de *Aplysina gerardogreeni* del Golfo de California

RESUMEN. Las esponjas del género *Aplysina* producen compuestos bioactivos de importancia farmacológica que presentan actividades antibacterianas y anticancerígenas. A pesar de su importancia se conoce poco en cuanto a la variación temporal de su bioactividad, por lo que el objetivo de este trabajo es describir la variación estacional de la actividad antimicrobiana *de Aplysina gerardogreeni*. Estas se recolectaron estacionalmente en Punta Arena de la Ventana, Baja California Sur, México, durante un ciclo anual (2008) mediante buceo SCUBA. Con los ejemplares, se prepararon extractos orgánicos de cada época del año y se probaron frente a cepas de *Escherichia coli* y *Staphylococcus aureus* utilizando el método de difusión en agar. Los extractos fueron activos frente a *E. coli* y *S. aureus* con una evidente variación en su bioactividad, dependiendo de la época del año. De manera general, los extractos de otoño mostraron la mayor actividad, presentando halos de inhibición entre 22.4 y 22.6 mm, mientras que la menor actividad frente a las dos cepas se presentó con los extractos de primavera. Estos resultados muestran que el estudio químico de estas esponjas tiene gran potencial en la búsqueda de compuestos con actividad farmacológica.

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In the search for bioactive compounds with pharmaceutical potential, sponges play a very important role because most of the marine bioactive compounds are isolated from them (Munro *et al*., 1999). These metabolites play an important ecological role for sponges in their natural environment because they are produced as a defense mechanism against competition, predation, or colonization pressure (Pawlik, 1993). In spite of their importance, these compounds represent an energetic cost to the organism because they are synthesized under stress conditions caused by interactions with other organisms or by the abiotic environment, which determine a seasonal pattern in their bioactivity (Page *et al*., 2005; Ferreti *et al*., 2009; Turon *et al*., 2009). Knowing these variations in the wild is significant to solve one of the main restrictions for the production of these compounds, *i.e.*, the supply of the bioactive compounds. Diverse bromide metabolites bioenergetically related with tyrosine have been obtained from *Aplysina gerardogreeni*, and they have shown interesting antibacterial and antitumoral activity. However, little is known about the seasonal bioactivity variation of these compounds. For this reason, the objective of this work is to assess the antibacterial activity of extracts from *Aplysina gerardogreeni* and their seasonal variation during an annual cycle.

Sponges were collected by hand using SCUBA diving in the rocky reef of Punta Arena, Baja California Sur, México (24º 02´ N and 109º 49´ W) during winter (January), spring (March), summer (end of July) and autumn (November) of 2008. Three specimens were collected in three different zones according to their depth (2, 4, and 6 m). Samples were placed on ice for their immediate transfer to the laboratory where they were frozen at – 20 °C. Prior to the assay they were thawed, weighted and cutted.

Approximately 20 g of each tissue sample were extracted with 150 mL of acetone/methanol mixture (1:1) at room temperature. The extracted solution was filtered and evaporated under reduced pressure in a rotary evaporator. Methanol (150 mL) was added, and the methanol-soluble extract was again evaporated under reduced pressure until a dark brownish and oily extract was obtained.

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The antibacterial activity of the extracts was evaluated against Gram-negative bacteria *Escherichia coli* (ATCC BAA-196) and Gram-positive *Staphylococcus aureus* (ATCC BAA-42) using the agar diffusion test. Each strain was inoculated in plates with Müeller-Hinton medium at 10^8 cells mL^{-1}. Afterwards, 25 L of a solution of each extract (80 mg mL^{-1}) were added to filter paper discs (6 mm diameter). Four discs were placed in each plate; two with the extracts, one as a positive control (30 mg Tetracycline), and one as a negative control (solvent MeOH). Every assay was quadruplicated. The plates were incubated at 35 °C for 24 h, after which the inhibition halos (mm) were measured. The data obtained showed a normal-like distribution according to the Kolmogorov-Smirnov test ($p = 0.020$).

We compared the bioactivities between seasons using one-way ANOVA. A HOBO data recorder was used to obtain temperature data *in situ* records (summer and autumn), and sea surface temperature (winter and spring) data were obtained from the NOAA (http://www.pfeg. noaa.gov/).

The results showed that the extracts were active against the two strains tested throughout the whole year with a significant seasonal variation in activity ($F_{0.95,3\ g.l.}=41.3$, $P=0.05$ for *E. coli* and $F_{0.95,3\ g.l.}=28.6$, $P=0.05$ for *S. aureus*). The extracts during autumn showed a high activity against *E. coli*, exhibiting average inhibition halos of 22.4 mm, in accord with the highest temperature record for the area (26.6 °C); while the lowest activity was observed during spring. The extracts were more active against *S. aureus* during summer and autumn (average inhibition halos of 22.6 mm, Fig. 1b) and the lowest activity values were obtained in spring (16.2 mm) when the temperature was recorded at its lowest (19.5 °C).

A similar pattern was observed in *Aplysina fistularis* at Isla Espíritu Santo, B.C.S., which showed a higher antibacterial activity during the warm months (Betancourt-Lozano *et al*., 1998). Nevertheless, this pattern is not observed in all sponges, *e.g*., *Agelas* and *Petrosia* from the Mediterranean and *Latrunculia* sp. nov. from New Zealand showed a higher cytotoxic activity during the cold season (Ferretti *et al*., 2009; Duckworth & Batershill, 2001). A common factor in these studies is that higher bioactivity is shown as a result of a rise on the number of associated organisms, such as competitors or organisms that use the sponge as a settling or fixing substratum (Betancourt-Lozano *et al*., 1998; Turon *et al*., 2009; Duckworth & Batershill 2001). This sponge has a significant antibacterial activity that inhibits both Gram-positive and Gram-Negative bacteria. Similar results were observed

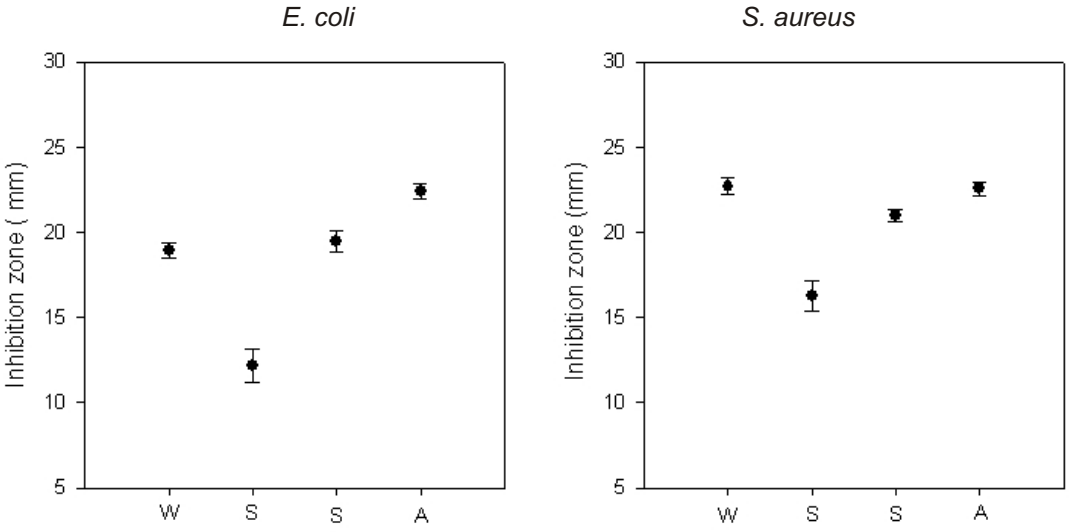

Figure 1. Values of antibacterial activity of *Aplysina gerardogreeni* extracts against strains of *E. coli* and *S. aureus* in different seasons. Bars represent standard error.

with extracts of *Aplysina aerophoba* and *A. lacunosa* (Sepcic *et al*., 1997; Kazanjian & Fariñas 2006). The above shows that the chemical study of *Aplysina gerardogreeni* has a great potential in the search of pharmacologically active compounds.

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