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Toxicity of methanol fraction from marine tunicates *Pyura sp* and *Polycarpa aurata* to the brine shrimp *Artemia salina* Leach

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Abstract. Marine tunicates have been known to produce bioactive compounds with anti-bacterial, anti-fungal, anti-viral and anti-cancer properties. However there have been few studies on marine tunicates from the Sangkarang Archipelago in Indonesia. The purpose of the present study was to determine the toxicity of crude extracts from two marine tunicates against the brine shrimp *Artemia salina* Leach. Toxicity tests of the methanol fractions extracted from the marine tunicates *Polycarpa aurata* and *Pyura sp* were performed on *Artemia salina* Leach. Sample tunicates were collected from Sangkarang waters, then air-dried and freeze dried before proceeding to extraction. Active compounds from the dried tunicate samples were extracted using methanol solvent. The chemical extraction process and Brine Shrimp Lethality Test (BSLT) were conducted according to standard procedures. The LC₅₀ values were 73.2 ug/ml for the *Pyura sp* extract, and 55.0 ug/ml for the *Polycarpa aurata* extract. These results show a level of toxic activity towards *Artemia salina* Leach which warrants further study of the bioactive compounds produced by these two tunicates.

1. Introduction

Tunicates, also known as ascidians, belong to the Phylum Chordata, and are characterized by unique body form called a tunic [1,2,3]. Nowadays, approximately 3000 species of tunicate are recognized worldwide, inhabiting a variety of marine habitats from shallow coastal waters to the deep seas. In tropical coral reef ecosystems, many members of this group tend to be solitary, while others form colonies [4,5]. As sessile organisms, tunicates obtain their food through filtering organic material from the surrounding water. This feeding mode also allows this group to harbour microorganisms as symbionts [6,7,8,9,10].

Previous findings have indicated that some members of this group have potential in the medical field, particularly as sources of anti-microbial, anti-bacterial and anti-fungal compounds [7,11,12]. Other studies shown that bioactive compounds from several tunicates have been used in anti-inflammatory, anti-viral, and anti-cancer treatments [13,14,15,16]. The Sangkarang Archipelago, also known as the Spermonde Archipelago, is located to the South-west of Makassar, capital city of South Sulawesi Province, Indonesia. There are still few studies on tunicates from this region [5,17], even though bio-prospecting of this group has been discussed previously [17]. In order to explore the bio-prospecting potential of marine tunicates, more research is required. The Brine Shrimp Lethality Test (BSLT) is a standard toxicity test against *Artemia salina* Leach which can serve as an initial screening for the bioactivity of potentially bioactive compounds. Therefore, it was considered important to carry

out a study to apply this widely recognised test as an initial evaluation of the bioactive potential of two tunicates found in the Sankarang Archipelago: *Polycarpa aurata* and *Pyura* sp.

2. Methods

2.1. Sample collection.

Tunicates *Polycarpa aurata* and *Pyura* sp. were collected from the waters around Barrang Caddi Island, Sangkarang Archipelago, Indonesia. Samples were kept in a cool box during transporting from the field to the laboratory. In the laboratory, the tunicate samples were cleaned to remove fouling organisms then rinsed with running tap water before being cleaned with distilled water. The samples were then air dried, followed by freeze drying before proceeding to the extraction process.

2.2. Extraction.

Dried tunicates were crushed then soaked in methanol solvent for 24 h. The resulting solution was removed and kept. The maceration process was repeated three times. All three liquid portions were combined and evaporated using rotary evaporator. The crude extract (methanol fraction) from each tunicate species was used for the Brine Shrimp Lethality Test (BSLT).

2.3. Brine Shrimp Lethality Test (BSLT).

The test was conducted according to commonly used standard procedures at the Biochemistry Laboratory, Faculty of Mathematics and Natural Sciences, Hasanuddin University. *Artemia salina* cysts were kept in sterile sea water for 48 h until hatching. The hatched larvae were then used for the BSLT. A series of tunicate extract concentrations was prepared for each of the two species (62.5 ppm; 125 ppm; 250 ppm; 500 ppm), dissolved in dimethyl sulfoxide (DMSO). *Artemia salina* larvae were exposed to different concentrations of the two tunicate methanol fraction and a control. In each test, 10 larvae were placed into 10 ml of control solution or treatment (tunicate extract) solution, with three replicates for the control and each treatment. Larval survival was counted after 24 h incubation time, determined as the ratio (expressed as a percentage) of live larvae in the treated sample compared to the control.

2.4. Data Analysis.

In order to determine the LC₅₀ concentration, data on the percentage of dead *Artemia salina* larvae after the treatments with tunicate methanol fraction solutions was analysed using probit analysis.

3. Results and Discussion

The results of the BSLT toxicity tests of *Pyura* sp and *Polycarpa aurata* methanol fraction extracts on *Artemia salina* Leach (Table 1) show that, for each of these two tunicate extracts, a large percentage of mortality occurred at the lowest extract concentration.

Table 1. BSLT results on the toxicity of *Polycarpa aurata* and *Pyura* sp. methanol fraction crude extracts towards *Artemia salina* Leach

Tunicate	[ppm]	X Axis (log [sample])	% live larvae relative to control	Y Axis (probit value)
<i>Pyura</i> sp	62.5	1.80	30	4.48
	125	2.10	13	3.87
	250	2.40	0	0
	500	2.70	0	0
<i>Polycarpa aurata</i>	62.5	1.80	60	5.25
	125	2.10	30	4.48
	250	2.40	0	0
	500	2.70	0	0

The regression analysis between log [sample] versus probit for *Polycarpa aurata* indicates that extract concentration has strong correlation with probit value as $y = -5.750x + 15.011$ with $R^2 = 0.850$. Furthermore, there is a strong correlation between extract concentration and probit value for *Pyura sp* as indicated by the formula $y = -6.720x + 17.536$ with $R^2 = 0.8539$. LC_{50} values were calculated from the probit values as 73.2 ug/ml for *Pyura sp*. and 55.0 ug/ml for *Polycarpa aurata*.

The Brine Shrimp Lethality Test is widely used to determine the toxicity of chemical compounds that have a toxic effect after a short time of contamination, in this case 24 h. The LC_{50} value indicates the concentration at which the chemical compound can kill 50% of the test organism population, and is thus a measure of the toxicity of the compound. Meyer [18] categorised toxicity levels into three categories as follows: (1) 1-10 ug/ml - highly toxic; (2) 10-100 ug/ml - toxic; (3) 100-1000 ug/ml - low toxicity. The present study on the methanol fraction from marine tunicates *Pyura sp* and *Polycarpa aurata* indicates that both crude extracts contain compounds which are toxic to *Artemia salina*. These results can be used to determine basic concentrations for the use of these fractions in studies to further evaluate the compounds they contain as a natural biochemical resource, in particular as candidates in anti-cancer studies.

4. Conclusion

The methanol fractions from the two marine tunicates *Pyura sp* and *Polycarpa aurata* both showed toxic activity towards *Artemia salina*. This result shows that these compounds warrant further study.

4. Acknowledgement

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