

Toxic Effects of the Methanol Extracts of some Tunicate Species

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ABSTRAK

Toksikita ekstrak metanol rajah tunikat (*Phallusia* sp., *Eudistoma obscuratum*, *Arcidium robustum*, *Didemnum* sp., *D. malle*, *Clavelina picta* and *Aplidium* sp.) ke atas anak udang dan larva bustar keempat nyamuk vektor (*Culex quinquefasciatus*, *Anopheles maculatus*, *Aedes aegypti* dan *Ae. albopictus*) telah dikaji. Ekstrak metanol tersebut adalah sangat toksik ke atas anak udang dengan nilai LC_{50} daripada 0.8 hingga 26.0 $\mu\text{g/ml}$ dan yang paling toksik ialah ekstrak daripada *Aplidium* sp. Ekstrak-ekstrak tersebut juga efektif ke atas larva nyamuk dengan nilai LC_{50} daripada 1.2 hingga 55.6 $\mu\text{g/ml}$. Aktiviti larvisidal ekstrak adalah sangat signifikan ke atas *An. maculatus* dengan nilai LC_{50} daripada 1.2 hingga 6.2 $\mu\text{g/ml}$ dan di antara mereka yang paling toksik ialah ekstrak *E. obscuratum*.

Kata kunci: ekstrak metanol, tunikat, toksisiti, anak udang, larva nyamuk

ABSTRACT

The toxicities of the methanol extracts of seven tunicates (*Phallusia* sp., *Eudistoma obscuratum*, *Arcidium robustum*, *Didemnum* sp., *D. malle*, *Clavelina picta* and *Aplidium* sp.) on brine shrimp and the 4th instar larvae of four vector mosquitoes (*Culex quinquefasciatus*, *Anopheles maculatus*, *Aedes aegypti* and *Ae. albopictus*) were investigated. The extracts were very toxic to the brine shrimp with LC_{50} values ranging from 0.8 – 26.0 $\mu\text{g/ml}$ and the most toxic being the extract from *Aplidium* sp. The extracts were also effective against the mosquito larvae with LC_{50} values ranging from 1.2 – 55.6 $\mu\text{g/ml}$. Larvicidal activity of the extracts was most significant on *An. maculatus* with LC_{50} values ranging from 1.2 – 6.2 $\mu\text{g/ml}$ and of these, the most toxic was the extract of *E. obscuratum*.

Key words: methanol extracts, tunicates, toxicity, brine shrimp, mosquito larvae

INTRODUCTION

Tunicate or generally known as sea squirt provides a rich source of biologically active compounds. A wide spectrum of organic compounds with interesting biological activities such as antibacterial, antiviral, antihelminthic, antiinflammation and anticancer have been isolated from tunicates (Andersson et al. 1983; Davidson 1993; Ireland et al. 1993; Kinetart et al. 1993; Costa et al. 1997). These include cyclic peptides, alkaloids, terpenoids, macrolides, polyethers, prenylated hydroquinones and other compounds with diverse chemical structures (Watters & Van Den Brink 1993; Horton et al. 1994).

The toxicity of extracts of marine organisms against brine shrimp had been investigated by many workers. The methanol extracts of the tunicates, *Styela pigmentata* and *Pyura pallida*, were effective against brine shrimp with LC_{50} values of 7.8 and 3.8 $\mu\text{g/ml}$, respectively (Mary et al. 1991). The extracts of the sponges, *Dysidea etheria* and *D. zvara*, were also toxic against brine shrimp with LC_{50} values of 38.0 and 0.2 $\mu\text{g/ml}$, respectively (Cardellina & Barnekow 1988; Crispino et al. 1989). Brine shrimp was also shown to be susceptible to the extracts of the green algae, *Neomeria australis*, with LC_{50} values ranging from 9.0-16.0 $\mu\text{g/ml}$ (Barnekow et al. 1989). Although there were many reports on the larvicidal effects of plant extracts on mosquitoes (Heller 1940; Amonkar & Reeves 1970; Supavan et al. 1974; Chavan 1983; Zebitz 1986; Ibrahim et al. 1996), no work on the toxicity of tunicate extracts on mosquito larvae has been documented.

In this study, methanol extracts of seven Malaysian tunicates (*Phallusia* sp., *Rudistoma obscuratum*, *Atridium robustum*, *Didemnum* sp., *D. molle*, *Clavelina picta* and *Aplidium* sp.) were screened for their toxic effects on brine shrimp and 4th instar larvae of *Culex quinquefasciatus*, *Anopheles maculatus*, *Aedes aegypti* and *Ae. albopictus*.

MATERIALS AND METHODS

The tunicates were collected from Pulau Perhentian and Pulau Sibu in peninsular Malaysia by scuba diving. Soft samples were collected together with their substrates to prevent injury while branched samples were cut at their bases. The samples were separated from their substrates and washed with distilled water to remove sand, sediments and other impurities. The samples were identified based on shape, morphology, size and colour, according to keys and references of Aw (1997), Allen and Steens (1996), Steyer (1986), Barth and Brosthears (1982) and Barnes (1974).

The samples were homogenized into fine pieces by a Waring blender and soaked in methanol for 48 hr with continuous stirring. The methanol extracts were concentrated *in vacuo* to give gummy yellow to dark brown

viscous mass. The soaking was repeated twice to ensure exhaustive extraction of the samples.

BRINE SHRIMP LETHALITY TEST

Brine shrimp eggs were allowed to hatch and mature as nauplii in two days in a hatching tank filled with artificial sea water. The free-swimming nauplii were attracted by a light to a compartment from which they could be collected for the assay proper. Vials containing 1-1000 $\mu\text{g/ml}$ samples were prepared by dissolving the samples in 2 ml methanol and transferring the solution to each vial. The sea water was added to achieve the correct concentration. Ten shrimps were added to three vials for each dose via a disposable pipette. The number of deaths out of 30 shrimps per dose was recorded after 24 h and LC_{50} values of 95% confidence intervals were determined for each compound by the probit analysis method as described by Raymond (1985). The control solution consisted of 30 nauplii in 2 ml methanol and the artificial sea water. The standard toxicant was prepared by dissolving potassium dichromate in 2 ml methanol and the brine medium to obtain 1-1000 $\mu\text{g/ml}$ concentrations. For acceptable readings, the LC_{50} values for the toxicant should fall within 27-35 $\mu\text{g/ml}$ (Mary et al. 1991).

LARVICIDAL TEST

The 4th instar larvae of four vector mosquitoes, *Culex quinquefasciatus*, *Anopheles maculatus*, *Aedes aegypti* and *Ae. albopictus*, served as the test organisms. The larvae colonies of these mosquitoes were collected from the Medical Entomology Insectary of the Institute for Medical Research, Kuala Lumpur. Each extract in 5 ml methanol was dissolved in distilled water to prepare 10000 $\mu\text{g/ml}$ stock solution from which concentrations of 10-1000 $\mu\text{g/ml}$ were prepared by dilution. Two hundred fifty ml of each extract was placed in a beaker. Twenty-five larvae of each mosquito were transferred into each beaker using a disposable pipette. The bioassay was carried out in two stages. Initially, all tunicate extracts were screened using concentrations of 1000 and 100 $\mu\text{g/ml}$. Extracts producing high mortality rates were further tested at lower concentrations of 1-100 $\mu\text{g/ml}$. The treatment was replicated 3 times on each concentration (WHO 1981). A control was prepared by the addition of 5 ml methanol to the distilled water in each beaker which contained 25 larvae. Solutions of DDT dissolved in water at 1-200 $\mu\text{g/ml}$ concentrations were used as standard toxicant. Mortality counts were made after 24 h. LC_{50} and 95% level of confidence were obtained by using the probit analysis method as described by Raymond (1985), whereas the corrected mortality were obtained from Abbott's formula (Abbott 1925).

RESULTS AND DISCUSSION

The methanol extracts of the seven tunicate extracts showed very high toxic effects on the brine shrimp with their LC_{50} values ranging from 0.8 to 26.0 $\mu\text{g/ml}$ (Table 1). They were more toxic than the standard toxicant, potassium dichromate, towards the test organisms. The LC_{50} values of the extracts of *Aplidium* sp., *Didemnum molle* and *Clavelina picta* reported by us are lower than those reported for the extracts of *Styela pigmentata* and *Pyura pallida* (Mary et al. 1991). Amongst the extracts studied, the extract of *Aplidium* sp. was the most toxic with LC_{50} value of 0.8 $\mu\text{g/ml}$.

TABLE 1. Toxicity values ($\mu\text{g/ml}$) of tunicate extracts against brine shrimp

Extracts	LC_{50} $\mu\text{g/ml}$	95% Confidence Interval
<i>Didemnum</i> sp.	25.97	9.53 - 51.96
<i>Phallusia</i> sp.	17.13	4.99 - 35.81
<i>Eudistoma obscuratum</i>	22.91	5.82 - 51.82
<i>Atrichium robustum</i>	11.55	2.18 - 26.67
<i>Didemnum molle</i>	2.03	0.5 - 8.32
<i>Aplidium</i> sp.C	0.81	0 - 4.80
<i>Clavelina picta</i>	3.90	0.12 - 12.02
Potassium dichromate	27.5	24.4 - 36.9

The high toxicity values of the extracts may suggest that the active compounds in the extracts were inhibiting the osmo-regulatory system in balancing the osmotic pressure in the brine shrimp. Consequently there would be imbalance of ions in the body. Mortality may also be due to inhibition of the respiratory system by interfering with the exchange of gases in the body of the organism.

The larvicidal test of the extracts indicated that they were also highly effective against the four vector mosquitoes with LC_{50} values ranging from 1.2 - 55.6 $\mu\text{g/ml}$ (Table 2). However, the effect of each extract towards *Aedes aegypti*, *Ae. albopictus* and *Culex quinquefasciatus* was non selective as their LC_{50} values showed little variation. The only exception was the relatively strong activity of the extracts against *Anopheles maculatus*, with LC_{50} values ranging from 1.2 - 6.2 $\mu\text{g/ml}$. The extract of *Eudistoma obscuratum* was the most effective, exhibiting LC_{50} value of 1.2 $\mu\text{g/ml}$. The larvae of *An. maculatus* was found to be the most susceptible towards the extracts. The most effective extract against *Ae. aegypti* was from *Aplidium* sp. with a LC_{50} value of 18.8 $\mu\text{g/ml}$. While the most active extracts against

TABLE 2. LC₅₀ values (µg/ml) of tunicate extracts against mosquito larvae

Sample	LC ₅₀ (95% Confidence Interval)			
	<i>Cx. quinquefasciatus</i>	<i>Ae. maculatus</i>	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>
<i>Didemnum</i> sp.	42.80 (37.24-49.28)	3.14 (2.0-4.27)	42.09 (32.69-54.23)	35.81 (25.15-50.98)
<i>Phallusia</i> sp.	44.17 (29.59-65.97)	4.87 (1.29-17.94)	45.18 (29.59-65.97)	37.04 (27.39-50.09)
<i>Eudistoma obscuratum</i>	46.59 (43.75-49.8)	1.18 (0.26-2.37)	22.89 (14.87-35.05)	39.32 (37.43-38.33)
<i>Atridium robustum</i>	55.55 (39.72-77.89)	4.04 (2.64-5.18)	38.69 (39.72-77.89)	40.59 (32.26-51.08)
<i>Didemnum molle</i>	44.29 (41.56-47.35)	6.23 (4.04-8.97)	36.37 (26.51-49.89)	42.0 (39.85-44.29)
<i>Aplidium</i> sp.	46.49 (36.36-59.49)	2.42 (1.66-3.11)	18.83 (12.63-22.71)	40.82 (38.87-42.94)
<i>Clavelina picta</i>	32.44 (21.56-48.78)	5.32 (3.59-7.28)	20.38 (18.27-22.37)	45.13 (39.25-51.96)
DDT	0.0018	0.0059	0.0095	0.0047

Ae. albopictus and *Cx. quinquefasciatus* were *Didemnum* sp. (LC₅₀ of 35.8 µg/ml) and *Clavelina picta* (LC₅₀ of 32.4 µg/ml), respectively.

The results indicated that the extracts contained active principles which may be responsible for the larvicidal activity. These extracts were less toxic than the standard insecticide, DDT. However, the active principles of the extracts, when isolated in pure form, might possess higher larvicidal activity. The active constituents of the crude extracts might be acting by different mode of action such as by paralyzing physiology and osmo-regulation system of the larvae. These results should encourage further studies to isolate and purify the active compounds and study their pathological effects on mosquito larvae.

CONCLUSION

The results indicate that the tunicate extracts were highly toxic against the brine shrimp and moderately toxic against the mosquito larvae. Some of the extracts have the potential to be developed as alternatives to synthetic

insecticides in mosquito control. These extracts are environment friendly, biodegradable and less harmful compared to the synthetic insecticides, which have been reported to cause undesirable side effects to human (Reynolds 1989). Further research should be carried out to determine the residual life-span as well as their performance under field conditions. The active ingredients of the extracts need to be isolated and identified and their potential as commercial insecticidal agents be assessed.

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