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Toxic Effects of the Methanol Extracts of some Tunicate Species

IBRAHIM JANTAN, ZETI AZURA MOHAMED HUSSEIN, ALMA AWALUDDIN & NAZNI WASI AHMAD

ABSTRAK

Toksisiti ekstrak metanol tujuh tunikat (Phallusia sp., Eudistoma obscuratum, Atriolum robustum, Didemnum sp., D. molle, Clavelina picta and Aplidium sp.) ke atas anak udang dan larva instar 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 µg/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 55.6µg/ml. Aktiviti larvisidal ekstrak adalah sangat signifikan ke atas An. maculates dengan nilai LC_{50} daripada 1.2 hingga 6.2 µg/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, Atriolum robustum, Didemnum sp., D. molle, 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 µg/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 µg/ml. Larvicidal activity of the extracts was most significant on An. maculatus with LC_{50} values ranging from 1.2 – 6.2 µg/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, antihelmintic, antiinflammation and anticancer have been isolated from tunicates (Andersson et al. 1983; Davidson 1993; Ireland et al. 1993; Rinehart 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 Brenk 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 µg/ml, respectively (Mary et al. 1991). The extracts of the sponges, *Dysidea etheria* and *D. avara,* were also toxic against brine shrimp with LC_{50} values of 38.0 and 0.2 µg/ml, respectively (Cardellina & Barnekow 1988; Crispino et al. 1989). Brine shrimp was also shown to be susceptible to the extracts of the green algae, *Neomeris annulata,* with LC_{50} values ranging from 9.0-16.0 µg/ml (Barnekow et al. 1989). Although there were many reports on the larvicidal effects of plant extracts on mosquitoes (Haller 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., Eudistoma obscuratum, Atriolum 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 Steene (1996), Sterer (1986), Barth and Broshears (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 µg/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 µg/ml concentrations. For acceptable readings, the LC_{50} values for the toxicant should fall within 27-35 µg/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 µg/ml stock solution from which concentrations of 10-1000 µg/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 µg/ml. Extracts producing high mortality rates were further tested at lower concentrations of 1-100 µg/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 µg/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 µg/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 µg/ml.

TABLE 1. Toxicity values (µg/ml) of tunicate extracts against brine shrimp

Extracts	LC_{50} µg/ml	95% Confidence Interval
Didemnum sp.	25.97	9.52 - 51.96
Phallusia sp.	17.13	4.99 – 35.81
Eudistoma obscuratum	22.91	5.82 – 51.82
Atriolum robustum	11.55	2.18 – 26.67
Didemnum molle	2.03	0.5 – 8.32
Aplidium sp.C	0.81	0 – 4.80
Clavelina picta	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 larvacidal 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 µg/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 µg/ml. The extract of *Eudistoma obscuratum* was the most effective, exhibiting LC_{50} value of 1.2 µg/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 µg/ml. While the most active extracts against

TABLE 2. LC_{50} values (mg/ml)of tunicate extracts against mosquito larvae

Sample	LC_{50} (95% Confidence Interval)			
	Cx. quinquefasciatus	*An. maculatus*	*Ae. aegypti*	*Ae. albopictus*
Didemnum 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)
Phallusia 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)
Eudistoma obscuratum	46.59 (43.75-49.8)	1.18 (0.26-2.27)	22.89 (14.87-35.05)	39.32 (37.43-38.33)
Atriolum robustum	55.55 (39.72-77.89)	4.04 (2.64-5.48)	38.69 (39.72-77.89)	40.59 (32.26-51.08)
Didemnum molle	44.29 (41.56-47.35)	6.23 (4.04-8-.97)	36.37 (26.51-49.89)	42.0 (39.85-44.29)
Aplidium 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)
Clavelina picta	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. (LC_{50} of 35.8 µg/ml) and *Clavelina picta* (LC_{50} 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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Ibrahim Jantan
Zeti Azura Mohamaed Hussein
Alma Awaludin
Department of Pharmacy,
Faculty of Allied Health Sciences,
Universiti Kebangsaan Malaysia,
Jalan Raja Muda Abdul Aziz,
0300 Kuala Lumpur, Malaysia

Nazni Wasi Ahmad
Medical Entomology Department,
Institute for Medical Research Malaysia,
50586 Kuala Lumpur, Malaysia