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RELATIVE HEMOLYTIC POTENCIES OF HOLOTHURINS OF THIRTY PHILIPPINE HOLOTHURIANS

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ABSTRACT

Thirty Philippine holothurians of Families Holothriidas, Stichopodidae, Synaptidae, and Chiridotidae, mostly collected from San Fernando, La Union and Calatagan, Batangas, were investigated for their crude holothurin yield and hemolytic potency. Crude holothurin yield of different parts of the sea cucumbers in ethanolic extracts ranged from 0.17% to 22.6% of dried samples. Hemolytic potency tested in 2% human RBC suspensions ranged from 1,564 HI/g to 666,667 HI/g dry crude holothurin. Statistical tests on the data showed significant variation in content and activity. In crude holothurin content, gut > Cuvierian tubules > body wall. In hemolytic potency, Cuvierian tubules > gut or body wall or gonad.

Among members of the genera Actinopyga, Holothuria, Bohadschia, and Stichopus, body wall crude holothurin content was highest in Stichopus. Hemolytic activity, however, was highest in Actinopya. Crude holothurin content yield of the gut and corresponding hemolytic activity did not differ markedly among the different samples. Between Bohadschia and Holothuria, Holothuria was superior in both crude holothurin yield and hemolytic potency of the Cuvierian organs. The implications of the results such as the possible relation between chemical nature of the holothurins and activity are discussed.

Introduction

The holothurian or sea cucumber is food to the Chinese as an ingredient of soups, noodles and other dishes. Some Filipinos relish it either raw or slightly boiled or broiled and pickled salad style. Previous findings showed that present in the holothurian body well, gut, coelomic fluid, Cuvierian tubules and in practically every other part of its body are biologically active triterpene glycosides. These substances were first known as *holothurins* but are now more specifically designated by chemists as echinosides, bivittosides, stichoposides, thelothurins, etc. due the variations of the glycoside in different holothurian species studied (cited by Burnell and Apsimon, 1983).

Holothurin, discovered and given the name by Nigrelli of the New York Aquarium and Yamanouchi at the Seto Marine Laboratory in Japan, is one of the natural saponins. The early investigations already demonstrated its marked hemolytic activity and toxicity to various animals including fishes and mice (Nigrelli, 1952; Yamanouchi, 1955). Taken orally, holothurin is inactivated presumably by acidic digestive juices; administered intravenously or subcutaneously, it is a toxin.

Experimental results have revealed a wide spectrum of effects of holothurin in living systems (Table 1) (Pocsidio, in press). Promising some boon to mankind is the antifungal and antitumor property. Research on the pharmacological activities as well as the complete structure of the glycosides and their biosynthesis are being undertaken in the laboratories in Osaka, Japan and Vladivostok, Russia.

Table 1. Effects of holothurin on biological systems

Observations	*References*
Toxicity to animals (protozoans, coelenterates, molluscs, worms, crustaceans, fishes, amphibians, mice)	Yamanouchi, 1955; Nigrelli, 1952; Glynn, 1965; Bakus, 1968; Bakus and Green, 1974; Ruggieri and Nigrelli, 1974
Antifungal activity (against cultures of *Candida albicans*, *Saccharomyces cerevisiae*, *S. carlsbergensis* and others; against dermatophytosis but without effect on gram-negative and gram-positive bacteria)	Shimada, 1969; Ruggieri and Nigrelli, 1974; Anisimov *et al.*, 1972a; Baranova *et al.*, 1973
Antitumor activity (against Sarcoma-180, Krebs-2-2ascites, B-16 melanoma tumors; against human epidermal cercinoma KB cells) in mice	Nigrelli, 1952; Nigrelli and Zahl, 1952; Sullivan *et al.*, 195; Sullivan and Nigrelli, 1956; Nigrelli and Jakowska, 1960; Leiter *et al.*, 1962; Nigrelli *et al.*, 1967; Cairns and Olmsted, 1973; Ruggieri and Nigrelli, 1974
Hemolytic action (stronger than digitonin, quillaia and other saponins) using frog, rabbit, and human RBC	Nigrelli, 1952; Yamanouch, 1955; Jakowska *et al.*, 1958; Nigrelli and Jakowski, 1960; Thron, 1964; Lasley and Nigrelli, 1971; Pocsidio 1983
Stimulation of hemopoesis (in frog)	Jakowska *et al.*, 1958
Increased rate of amoeboid migration (human WBC)	Lasley and Nigrelli, 1970 and 1971
Diminished parasite load (*Trypanosoma lewisi* in rats)	
Mutagenicity and clastogenicity upon metabolic activation	Styles, 1970
Effects on Nerves and Muscles using amphibian and mammlian neuro-muscular preparations: neurotoxicity; diministed action currents but conduct velocity not altered, effects irreversible; cholinergic trans-	Pocsidio, 1983a Friess *et al.*, 1959, 1960, 1965, 1968, 1970 and 1972; Friess and Durant, 1963 and 1965; Thron *et al.*, 1963 and 1964

Table 1 (Continued)

Observations	*References*
mission blocked, effects irreversible; direct contractual effect on muscle	
Effects on the Heart (on rabbit sinus node and dog Purkinje fiber preparations): automaticity significantly decreased, duration of action potential reduced, delay in the A-V node, resting membrane potential decreased	Ricciutti and Damato, 1971
Developmental alterations induced:	
1. arrested division of eggs in the sea urchin, *Strongylocentrotus intermedius*	Anisimov *et al.*, 1972, 1973, and 1974
2. cytolysis of blastomeres in S. *intermedius*	
3. extreme animalization in the sea urchin *Arbacia punctulata* (excessive development of the ciliary tuft, thickened apical ectoderm, failure to gastrulate, absence of archenteron and skeletal spicules)	Nigrelli and Jakowska, 1960; Ruggieri and Nigrelli, 1960 and 1974; Colon *et al.*, 1974.
4. inhibition of hatching in A. *punctulata*	Goldsmith *et al.*, 1958
5. retardation of pupation of fruit fly	Quaglio *et al.*, 1957
6. disintegration of whole planarians and failure of posterior segments of cut planarians to regenerate	Nigrelli and Zahl, 1952;
7. growth was suppressed in some protozoans	Nigrelli and Jakowska, 1960
8. necrosis of onion root tips	Nigrelli and Jakowska, 1960

The toxicity of holothurin has been correlated with a geographic pattern with incidence of toxicity increasing towards the tropics (Bakus and Green, 1974). From among our comparatively more variable holothurian fauna may yet be found more information on the toxicities of the tropical sea cucumbers and the excellent source of a most potent saponin.

Studies are now being conducted on Philippine holothurians. Some studies have been done on the isolation of holothurin, the hemolytic assay for its potency, and cytological effects (Pocsidio, 1983a, 1983b, 1986, 1987). Presented in this report are the preliminary results of hemolytic tests on extracts from 30 Philippine littoral holothurian species. The occurrencies of crude holothurins and their activities in different parts of the sea cucumbers and the relative potencies of the common genera are included.

Materials and Methods

Collection of specimens

Sea cucumbers wre collected from several localities, mostly from San Fernando, La Union and Calatagan, Batangas. From the littoral areas were gathered the following species: *Actinopyga echinites*, A. *mauritiana*, A. *miliaris*, Actinopyga sp., *Bohadschia argus*, B. *graeffei*, B. *marmorata*, B. *vitiensis*, *Holothuria atra*, H. *coluber*, H. *fuscocinerea*, H. *hilla*, H. *impatiens*, H. *klunzingeri*, H. *nobilis*, H. *pervicax*, H. *pulla*, H. *rigida*, H. *sanguinolenta*, H. *scabra*, H. *tigris* of Family Holothuriidae, *Stichopus naso*, S. *chloronotus*, S. *variegatus*, S. *variegatus* var. *hermanii*, Stichopus sp., Family Stichopodidae, *Opheodesoma grisea*, *Pendekaplectana nigra*, *Synapta maculata*, Family Synaptidae and *Polychieira rufescens*, Family Chiridotidae. Except for two species which were collected in January 1982, the animals were collected from December 1983 to March 1985. Listed in Tables 2-5 are the samples, dates and places of collection. The animals were transported to the laboratory either fresh or sundried.

Processing of sea cucumbers for extraction of crude holothurin

The animals varied in number and size and the amount of crude holothurin obtained from them was determined on dry weight basis, i.e., in crude holothurin per gram of dried sea cucumber body wall, gut, gonad, or Cuvierian tubules. There was a total of 79 samples of body wall, 32 gut, two gonad, 16 Cuvierian tubules. All the specimens were sundried as were those in previous studies. The dried materials were cut into small pieces and put inside labelled plastic bags and stored in the refrigerator until use for the extraction procedure.

Ethanolic extraction

In the previous studies, a stepwise procedure for extraction in three different absolute alcohols was followed. In the present study, the specimens were refluxed with 95% ethanol, technical grade (RTC Supply House). The residue that were obtained after evaporation were stored in vials inside a dessicator kept inside the refrigerator for the hemolytic potency test.

Hemolytic assay

The hemolytic assay was after the method by Fujita and Nishimoto (1952). The least concentration of crude holothurin that could cause 100% hemolysis in a 1 mL 2% human RBC suspension within three to five hour was tested. With the following formula, the hemolytic potency of a sample would be in units of hemolytic index per gram of dry crude holothurin (HI/g):

$$HI/g = \frac{v}{P/100 \times 3}$$

whereby V = total volume of test solution
P = concentration of holothurin in %
S = volume of holothurin solution causing 100% hemolysis

Per sample, three tests were run. Blood for the tests were drawn from volunteers. Red blood cell suspensions and holothurin solutions were prepared in 0.15M phosphate buffer of pH 7.2-7.3. Standardization of blood samples was against Merck Saponin which has hemolytic potency of 33,333 HI/g.

Statistical analysis

Analysis of variance and the Duncan's test (Steel and Torrie, 1960) were the basis for the interpretation of the data.

Results and Discussion

From the different samples were obtained crude holothurins varying in amounts ranging from 0.17% to 22.6% of dried material (Tables 2-4). Out of 127 samples, 71 had been tested for their hemolytic activities (Table 5). The results of the assay showed a range of hemolytic potency from 1,564 HI/g to 666.667 HI/g. From the results of the statistical analysis were the following inferences.

The percentage of crude holothurin content yield on dry weight basis of the body walls, gut, and Cuvierian tubules differ significantly at $F_{.05}$ and $F_{.01}$ levels in the following order of crude holothurin content: Gut > Cuvierian tubules > Body wall. The hemolytic activities of the different samples were in the following order of potency: Cuvierian tubules > Gut or Body wall or Gonad. Among the four genera Actinopyga, Bohadschia, Holothuria, and Stichopus, highest crude holothurin content of the body wall was from Stichopus. There was no significant differences among the three other genera in their body wall crude holothurin yield. Hemolytic activity of body wall crude holothurin was highest, however, in Actinopyga while the three other genera did not exhibit significant differences. Crude holothurin content yield of the gut and corresponding hemolytic activity did not differ markedly among the different samples although content yield in Bohadschia was shown by the Duncan's test to be greater. Between Bohadschia and Holothuria, Holothuria was superior in both crude holothurin content yield and hemolytic potency of the Cuvierian organs.

Nigrelli *et al.* (1955) analyzed the crude holothurin from the Cuvierian tubules of the Bahamian sea cucumber *Actinopyga agassizi* and found it to contain 60% glycosides and pigments, 1% cholesterol, 5-10% insoluble proteins, salts, polypeptides, and 30% free amino acids.

In the present study, due to lack of necessary equipment no chemical analysis was done. The quantitative determinations of yield and hemolytic activity of the extracts from the different sea cucumbers, however, suggest a variability in the composition of the crude holothurin within different parts of the body of the animal and in general, among different genera. The high activity of crude holothu-

Table 2. Crude holothurin content in the body walls of sea cucumbers of different species

Species	*Date and Place of Collection*[1]		*Extract No.*	*Dried body walls, g*	*Crude Holothurin, g*	*% Crude Holothurin Content, DW basis*[2]
Family Holothuriidae						
Actinopyga echinites	1-26-84	LUSFI	16	31.2	0.0541	0.17
	1-26-84	LUSFI	17	37.2	0.3281	0.88
	1-26-84	LUSFI	18	73.7	0.1318	0.18
	1-26-84	LUSFI	19	74.7	0.2322	0.31
	2-21-84	QLpb	32	25.8	0.1150	0.44
	2-28-84	BSbbs	42	58.1	0.6410	1.10
	3-20-84	LUSFI	9	100.5	2.4791	2.47
	12-28-84	LUSFI	125	62.1	1,5057	2.43
	12-28-84	LUSFI	127	37.1	0.4795	1.29
	3-8-85	BCbbs	110	32.5	0.0885	0.27
	3-8-85	BCbbb	100	21.7	0.3925	1.81
Actinopyga mauritana	3-9-85	BCbbb	131	32.7	0.6391	1.95
Actinopyga miliaris	2-21-84	QLpb	33	20.8	0.0830	0.40
	5-1-84	BCbbb	52	29.4	0.3730	1.27
	3-9-85	BCbbs	133	34.9	0.8540	2.45
Actinopyga sp.	2-1-84	BCbbb	22	45.7	1,1595	2.54
Bohadschia argus	2-28-84	BCbbb	39	112.9	4.1306	3.66
Bohadschia graeffei	12-22-83	LUSFI	3	38.8	0.4227	1.09
Bohadschia marmorta	2-1-84	BCbbb	23	37.7	1,9255	5.11
	2-28-84	BCbba	43	63.8	1.4432	2.26
	28-85	BCbbs	107	97.5	0.4257	0.44
	3-8-85	BCbbb	124	116.3	3.1558	2.71

Table 2 (Continued)

Species	*Date and Place of Collection*[1]		*Extract No.*	*Dried body walls, g*	*Crude Holothurin, g*	*% Crude Holothurin Content, DW basis*[2]
	2-21-84	QLpb	36	20.7	0.5078	2.45
	2-21-84	QLpb	37	17.6	0.8504	4.83
	2-28-84	BCbbb	44	62.9	1.4967	2.38
	4-23-84	CNM	47	41.3	3.0330	7.34
	3-9-85	BCbbb	138	19.5	1.4323	7.35
Stichopus variegatus	12-26-84	LUSFI	4	49.2	0.8418	1.71
var. *hermanii*	12-26-84	LUSFI	6	101.0	0.7217	0.71
Family Synaptidae						
Opheodesoma grisea	3-8-85	BCbbb	64	36.3	2.3528	6.48
Pendekaplectana nigra	3-8-85	BCbbb	60	21.7	1.4914	6.87
Synapta maculata	3-8-85	BCbbb	63	101.8	3.2096	3.15
Family Chiridotidae						
Polycheira rufescens	7-15-83	LUSFp	8	153.6	0.33190	0.22

[1] AT Tiwi, Albay
BCbba Balongbato (Alvarez Farms), Calatagan, Batangas
BCbbb Balongbato (Burot Point), Calatagan, Batangas
BCbbs Balongbato (Sandbar), Calatagan, Batangas
CNM Mercedes, Camarines Norte
IGnv Nueva Valencia, Guimaras, Iloilo
LNK Kauswagan, Lanao del Norte
LUSFI Lingsat, San Fernando, La Union
LUSFp Poro, San Fernando, La Union
PBsi Silaqui Island, Bolinao, Pangasinan
Qlpb Padre Burgos, Lucena, Quezon

[2] DW basis: dry weight basis

rin from the Cuvierian organs furthermore emphasizes their apparent function as defensive mechanisms. The Cuvierian tubules are sticky filaments which are ejected from the anus of the animals whenever they are irritated. When these are absent, holothurin as an antipredator adaptation must become concentrated in other parts of the body such as the body wall which condition is obviously exemplified in this study, by the represented membes of genus Actinopyga. Actually, the noxiousness of the integument was proven in experiments done by DeVore and Brodie (1982).

The high yield of crude extracts from the body walls of the members of the genus Stichopus, probably, may be attributed to the presence of other lipoidal substances such as stanols, sterols, and steroidal glycosides together with the triterpene glycosides. Members of the genus, *Stichopus japonicus* and *Stichopus tremulus* were reported having complex mixtures of the compounds (Nomura *et al.*, 1980; Ballantine, Lavis and Morris, 1981; Kalinovskaya *et al.*, 1983). The sterols, especially, have been implicated to be involved in the development of resistance in the cell membrane and tissues of the sea cucumbers against their own surface active saponins (Popov *et al.*, 1983). In various proportions to the triterpene glycosides and other substances in different sea cucumbers, these may add to the variation of the activity.

There might also be the relation of the chemical nature of the holothurin – particularly, the sugar components and sulfate content, to hemolytic activity. Voogt and Van Rheenan (1982) had observed a correlation between these aspects of starfish saponin structure and hemolysis. These might cause the greater effectivity of the Actinopyga and Holothuria holothurins over those of Bohadschia and Stichopus. The mechanism for hemolysis might then be apart from the cytostatic action displayed markedly, on the other hand, by members of the latter two genera (Shcheglov *et al.*, 1979; Kuznetsova *et al.*, 1982). Noteworthy is the consideration that so far the studies on complete structure of holothurins have revealed similarities of the holothurins from *Actinopyga echinites* and *Holothuria leucospilota. Both species exhibit the* same sugar and sulfate content and differ only in the side chain of their aglycone. *Bohadschia bivittata* and *Stichopus japonicus* holothurins both lack sulfates and may contain more than four sugar units (cited by Burnell and Apsimon, 1983).

Species differences have not been statistically determined in the present work. These would be dealt with when the data will have been completed. Moreover, the sites and month of collection are factors that cannot be entirely disregarded.

Qualitative and quantitative determinations relating hemolytic units to sulfate and sugar groups need to be verified. Imperative for these future studies would be the purified glycosides, hence, the need for adequate equipment.

Table 2 (Continued)

Species	*Date and Place of Collection*[1]		*Extract No.*	*Dried body walls, g*	*Crude Holothurin, g*	*% Crude Holothurin Content, DW basis*[2]
Holothuria pulla	12-18-83	LNK	21	56.2	0.3639	0.65
	1-26-84	LUSF1	11	27.8	1.4125	5.23
	3-30-84	IGnv	56	103.0	1.3864	1.35
	3-30-84	IGnv	112	4.7	0.1203	2.56
	5-1-84	BCbbb	51	82.3	0.8427	1.02
	3-8-85	BCbbb	108	33.3	1.1259	3.38
	3-8-85	BCbbb	104	191.7	6.8776	3.59
	3-8-85	BCbbb	129	24.6	1.5501	6.30
Holothuria rigida	2-28-84	BCbba	45	18.4	0.3277	1.78
Holothuria sanguinolenta	1-10-82	AT	13	60.0	1.4525	2.42
Holothuria scabra	2-1-84	BCbbb	26	203.8	1.6674	0.82
	2-28-84	BCbba	41	60.2	0.7323	1.22
	12-1-84	BCbbs	106	199.1	3.9148	1.97
	3-8-85	BCbbs	130	101.9	3.5392	3.47
	3-8-85	BCbbs	136	196.3	5.5016	2.80
Holothuria tigris	1-10-82	AT	14	120.8	2.2223	1.84
Family Stichopodidae						
Stichopus chloronotus	5-1-84	BCbbb	50	24.7	0.8104	3.28
Stichopus naso	12-26-83	LUSFI	5	46.8	1.1418	2.44
Stichopus sp.	4-23-84	CNM	46	40.6	1.3482	3.32
Stichopus variegatus	2-1-84	BCbbb	25	7.7	0.1400	1.82
	2-20-84	QLpb	34	39.9	2.3800	5.97
	2-21-84	QLpb	35	27.4	0.9853	3.60

Table 3. Crude holothurin content in the gut of sea cucumbers of different species

Species	*Date and Place of Collection*[1]		*Extract No.*	*Dried gut, g*	*Crude Holothurin, g*	*% Crude Holothurin Content, DW basis*[2]
Family Holothuriidae						
Actinopyga echinites	126-84	LUSFI	77(18)	4.4	0.2906	6.60
	1-26-84	BCbbs	80(42)	6.8	0.4216	6.20
Actinopyga miliaris	2-21-84	QLpb	152(33)	7.3*	0.2003	2.74
	5-1-84	BCbbb	96(52)	0.1	0.0155	15.50
Actinopyga sp.	2-1-84	BCbbb	99(22)	3.1	0.1275	4.11
Bohadschia argus	2-28-84	BCbbb	65(39)	3.8	0.3420	9.00
	2-28-84	BCbbb	75(39)	5.0	0.2114	4.23
Bohadschia graeffei	12-22-83	LUSFI	72(3)	6.1	0.5919	9.70
Bohadschia marmorata	2-1-84	BCbbb	97(23)	2.4	0.0638	2.66
	2-28-84	BCbba	81(43)	21.8	1.7080	7.83
	3-8-85	BCbbb	118(124)	11.7	0.6072	5.19
	3-8-85	BBbbs	141(107)	17.4	1.9027	10.94
Bohadschia vitiensis	12-20-83	LUSF1	85(1)	1.3	0.1763	13.56
	2-28-84	BCbbb	82(38)	10.6	1.3248	12.50
	3-8-85	BCbbb	122(126)	5.1	0.7056	13.84
Holothuria atra	12-18-83	LNK	92(20)	12.4	0.4853	3.91
	2-28-84	BCbba	73(40)	4.7	0.1831	3.90
	3-8-85	BCbbb	120(109)	2.6	0.0743	2.86
Holothuria pervicax	12-22-83	LUSFp	86(2)	8.6	0.2634	3.06
Holothuria pulla	12-18-83	LNK	90(21)	16.1	0.5384	3.34
	1-26-84	LUSF1	79(11)	4.8	0.3519	7.33
	5-1-84	BCbbb	70(51)	7.5	0.3573	4.76

Table 3 (Continued)

Species	*Date and Place of Collection*[1]		*Extract No.*	*Dried body walls, g*	*Crude Holothurin, g*	*% Crude Holothurin Content, DW basis*[2]
Holothuria rigida	2-28-84	BCbba	84(45)	0.4	0.0203	5.51
Holothuria scabra	2-1-84	BCbbb	101(26)	1.7	0.0741	4.36
	2-1-84	BCbbb	151(26)	12.1*	0.5676	4.69
	2-28-84	BCbba	74(41)	2.8	0.1497	5.35
	12-1-84	BCbbs	121(106)	2.2	0.0636	2.89
Family Stichopodidae						
Stichopus naso	12-26-83	LUSFI	87(5)	0.8	0.1808	22.60
Stichopus sp.	4-23-84	CNM	94(46)	1.1	0.1186	10.78
Stichopus variegatus	2-1-84	BCbbb	95(25)	1.0	0.0181	1.81
	2-21-84	QLpb	98(36)	1.1	0.0460	4.18
	2-28-84	BCbbb	83(44)	1.5	0.0534	3.56
Stichopus variegatus	12-26-84	LUSF1	88(4)	3.9	0.1765	4.53
var. *hermanii*	12-26-84	LUSF1	89(6)	2.5	0.0931	3.72

[1] See Table 2
[2] Dry weight basis
*gonads

Table 4. Crude holothurin content in the Cuvierian tubules of sea cucumbers of different species

Species	*Date and Place of Collection*[1]		*Extract No.*	*Dried gut, g*	*Crude Holothurin, g*	*% Crude Holothurin Content, DW basis*[2]
Family Holothuriidae						
Bohadschia argus	2-28-84	BCbbb	143(39)	7.4	0.0370	0.50
Bohadschia graeffei	12-22-83	LUSF1	145(3)	9.4	0.0162	0.17
Bohadschia marmorata	2-1-84	BCbbb	155(23)	9.3	0.2326	2.50
	2-28-84	BCbba	146(43)	37.4	0.9200	2.46
Bohadschia vitiensis	12-20-83	LUSFI	147(1)	17.0	0.0756	0.45
	2-28-84	BCbbb	144(38)	21.3	0.3155	1.48
	5-13-84	PBsi	154(59)	6.3	0.3727	5.92
	3-8-85	BCbbb	153(126)	12.9	0.5050	3.92
	3-8-85	BCbbs	161(105)	8.2	0.4739	5.78
Holothuria fuscocinerea	2-21-84	QLpb	150(31)	6.9	0.7386	10.70
	5-1-84	BCbbb	162(53)	9.9	0.8567	8.67
Holothuria pervicax	12-22-83	LUSFp	157(2)	11.2	0.1506	1.34
Holothuria pulla	12-18-83	LNK	165(21)	106.4	6.0971	5.73
	1-26-84	LUSF1	156(11)	6.9	1.0387	15.05
	5-1-84	BCbbb	142(51)	12.2	0.1590	1.30
	3-8-85	BCbbb	164(108)	6.6	0.1639	2.48

[1]See Table 2.
[2]Dry weight basis.

Table 5. Hemolytic activities of crude holothurins from different parts of sea cucumber of different species

Species	*Date and Place of Collection*[1]		*Extract Nos.*[2]	*HI/g (Hemolytic index per g dry crude holothurin)*			
				Body wall	*Gut*	*Cuvierian tubules*	*Gonads*
Family Holothuriidae							
Actinopyga echinites	2-28-84	BCbbs	42-80	85,714	93,333	–	–
A. mauritiana	3-9-85	BCbbb	131	302,020	–	–	–
A. miliaris	5-1-84	BCbbb	52-96	50,159	111,111	–	–
A. miliaris	2-21-84	QLpb	33-152	33,333	–	–	143,834
Actinopyga sp.	2-1-84	BCbbb	22-99	28,191	101,058	–	–
Bohadschia argus	2-28-84	BCbbb	39-75-143	63,810	324,768	666,667	–
B. graeffei	12-22-83	LUSF1	3-72-145	181,714	17,801	523,809	–
B. marmorata	2-28-84	BCbba	43-81-146	40,000	342,857	666,667	–
B. marmorata	3-8-85	BCbbs	107-141	124,444	444,444	–	–
B. marmorata	2-1-84	BCbbb	23-96-155	11,259	77,576	304,762	–
B. vitiensis	3-8-85	BCbbb	126-122-153	76,623	57,143	457,142	–
B. vitiensis	12-20-83	LUSF1	1-85-147	65,057	61,838	592,593	–
Holothuria atra	3-9-85	BCbbs	139	122,843	–	–	–
H. atra	2-28-84	BCbba	40-73	36,565	20,571	–	–
H. atra	3-8-85	BCbbb	109-120	41,481	123,810	–	–
H. coluber	5-13-84	PBsi	55	7,000	–	–	–
H. fuscocinerea	5-1-84	BCbbb	53-162	46,603	–	101,587	–

Table 5 (Continued)

Species	*Date and Place of Collection*[1]		*Extract Nos.*[2]	*HI/g (Hemolytic index per g dry crude holothurin)*			
				Body wall	*Gut*	*Cuvierian tubules*	*Gonads*
H. fuscocinerea	2-21-84	QLpb	31-150	24,713	–	16,212	–
H. hilla	3-9-85	BCbbs	132	50,217	–	–	–
H. impatiens	7-15-83	LUSFp	7	9,439	–	–	–
H. klunzingeri	5-1-84	BCbbb	49	20,571	–	–	–
H. nobilis	5-13-84	PBsi	54	8,169	–	–	–
H. pervicax	12-22-83	LUSF1	2-86-157	32,064	37,714	91,967	–
H. pulla	5-1-84	BCbbb	51-70-142	24,762	26,032	114,286	–
H. rigida	2-28-84	BCbba	45-84	24,550	309,524	–	–
H. sanguinolenta	1-10-82	AT	13	78,307	–	–	–
H. scabra	2-1-84	BCbbb	26-101-151	76,191	8,667	–	53,333
H. scabra	12-1-84	BCbbs	106-121	4,027	91,429	–	–
H. tigris	1-10-82	AT	14	5,926	–	–	–
Family Stichopodidae							
Stichopus naso	12-26-83	LUSF1	5-87	6,753	3,302	–	–
S. chlornotus	5-1-84	BCbbb	50	12,445	–	–	–
S. variegatus	2-28-84	BCbbb	44-83	1,852	29,815	–	–
S. v. hermanii	12-26-84	LUSF1	6-88	34,285	44,502	–	–
Stichopus sp.	4-23-84	CNM	46-94	14,730	5,714	–	–

Table 5 (Continued)

Species	*Date and Place of Collection*[1]		*Extract Nos.*[2]	*HI/g (Hemolytic index per g dry crude holothurin)*			
				Body wall	*Gut*	*Cuvierian tubules*	*Gonads*
Family Synaptidae							
Opheodesoma grisea	3-8-85	BCbbb	64	6,624	–	–	–
Pendekaplectana nigra	3-8-85	BCbbb	60	5,238	–	–	–
Synapta maculata	3-8-85	BCbbb	63	1,564	–	–	–
Family Chiridotidae							
Polycheira rufescens	7-15-83	LUSFp	8	39,682	–	–	–

[1]See Table 2.

[2]The numbers correspond to extracts from the same sample or animal. Some animals were without guts most probably because of previous eviscerations. As species characteristic, some species are without Cuvierian tubules. Gonads had been collected only from *A. miliaris* and *H. scabra*.

Summary and Conclusions

In 30 Philippine holothurian species that have been investigated, variation in crude holothurian yield as ethanolic extracts and hemolytic activity in 2% human RBC suspensions have been observed. Differences in the composition of the crude extracts was suggested by the results obtained in the assay. The data, upon statistical analysis, showed significant differences in the crude holothurin content of the different parts of the sea cucumber in the order Gut > Cuvierian tubules > Body wall and hemolytic activity in the order Cuvierian tubules > Gut or Body wall or Gonad. Actinopyga and Holothuria holothurins were more effective than Bohadschia and Stichopus holothurins in causing hemolysis. Possible relation between sulfate and sugar contents of triterpene glycosides and the hemolytic mechanism has been indicated.

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