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Antileishmanial, Antimalarial and Antimicrobial activity of the Jamaican 'Touch-me-not' sponge *Neofibularia nolitangere*

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ABSTRACT

The crude extracts and fractions of the Jamaican sponge *Neofibularia nolitangere* were examined for biological activity. The dried animal was extracted successively in three organic solvents (hexane, methylene chloride and methanol) and tested for their potential as antileishmanial, antimalarial and antimicrobial agents. Fractions of the crude methylene chloride extract demonstrated notable antimalarial properties giving percentage inhibitions of 87% and 78% respectively for fractions Y4 and Y5 at 20 µg/mL. Fractions Y4 and Z4 showed remarkable antileishmanial activity inhibiting the growth of the pathogen by 93.31% and 91.77% respectively at 20 µg/mL. No significant activity was observed in the antimicrobial assays.

INTRODUCTION

Over the years, marine organisms including sponges, soft corals, bryozoans, molluscs and tunicates have been the source of several secondary metabolites that display unusual structures and possess biological activities (Bhakuni and Rawat, 2005). It is reported that marine sponges are among the richest sources of interesting chemicals produced by marine organisms and are considered to be true "chemical factories"(Laport *et al.,* 2009). Although a range of compounds have been isolated over the years, the biological activities and roles which many of them play are still unknown but some sponge metabolites are thought to serve a number of functions including protection from predation, fouling or infections, growth regulation, induction of metamorphosis and communication (Sepčić *et al.,* 2010; Cardellina II, 1986).

Research has shown that the chemical (lipophilic or hydrophilic extracts) properties of some sponges provide a refuge for mesofauna from fish predation (Huang *et al.,* 2006). Researchers have extended the feeding deterrent properties of sponge extracts to the terrestrial biosphere by observing the responses of insects or pests to marine natural products for application in pest control (Cardellina II, 1986). Our present study focuses on the highly toxic large reddish-brown sponge *Neofibularia nolitangere* the colonies of which are composed of several connected, thick walled cylinders or mounds, each with large exhalent openings (Sheppard, 2010). More commonly referred to as the 'touch-me-not' sponge, handling this sponge with the bare hands may cause severe burning and blistering which may take several days to subside (Voss, 1976). The *Neofibularia* genus belongs to the Desmacellidae family (formerly Biemnadae) and are widely distributed in the world's oceans, from shallow waters to depths of at least 2165 m (Yong and Chung, 2008).

A study of the butanol and methanol extracts of the *N. nolitangere* from Curacao demonstrated protein phosphatase inhibition (PP1) activity at concentrations of 54 and 167 µg/mL respectively while moderate antibacterial activity against *B. subtilis* was observed in the butanol extract with a minimum inhibitory concentration of 2 mg/mL (Fagerholm, 2010). With the continuous emergence of drug resistance as well as high manufacturing costs, the discovery of novel, effective, safe and affordable drugs for the treatment of different diseases remains relevant (Atta-ur-Rahman *et al.,* 2008).

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MATERIALS AND METHODS

Sponge Material

Neofibularia nolitangere was collected by SCUBA at Columbus Park, Discovery Bay in July 2009. The animal was cleaned of debris, cut into small pieces and lyophilized. A specimen of the sponge is housed in the Marine Natural Products Laboratory, Department of Chemistry, UWI, Mona (Accession number: 0117).

Extraction and Fractionation

The dried animals (428 g) were extracted at room temperature using three organic solvents: *n*-hexane (2 x 4L), methylene chloride (2 x 4L), and then finally methanol (2 x 4L). The extracted solvents were filtered and each was concentrated using a rotary evaporator to yield 13 g, 4 g and 80 g of crude residues respectively. A portion of the hexane extract (12 g) was subjected to flash column chromatography using silica gel. Eluents with increasing polarity starting from *n*-hexane through to methylene chloride to methanol were used to elute the fractions of the crude to obtain a total of five fractions (Z_1 – Z_5). Approximately 3.5 g of the methylene chloride extract was applied on a silica gel column and eluted with increasing portions of ethyl acetate in hexane to 100 % ethyl acetate to methanol. This yielded seven fractions (Y_1 – Y_7).

Antileishmanial Assay

The antileishmanial activity of the compounds was tested *in vitro* on a culture of *Leishmania donovani* promastigotes. In a 96-well microplate assay compounds with appropriate dilution were added to the *Leishmania* promastigotes culture (2 x 106 cell/mL). The plates were incubated at 26 °C for 72 hours and growth of *Leishmania* promastigotes was determined by Alamar blue assay (Mikus and Steverding, 2000). Pentamidine and amphotericin B were used as the standard antileishmanial agents.

Antimalarial Assay

The assay for antimalarial activity was determined by analysing the plasmodial LDH activity. In this method, a suspension of red blood cells infected with D6 or W2 strain of *P. falciparum* was added to the wells of a 96-well plate containing 10 µL of serially diluted test samples. The plate was flushed with a gas mixture of 90% N_2, 5% O_2, and 5% CO_2 and incubated at 37 °C for 72 hours in a modular incubation chamber. In this method, 20 µL of the incubation mixture was mixed with 100 µL of the Malstat™ reagent and incubated at room temperature for 30 minutes. Twenty microliters of a 1:1 mixture of nitro blue tetrazolium/phenazine ethosulfate (NBT/PES) was then added and the plate was further incubated in the dark for 1 hour. The reaction was then stopped by the addition of 100 µL of a 5% acetic acid solution. The plate was read at 650 nm. Artemisinin and chloroquine were included in each assay as antimalarial drug controls. IC_{50} values were computed from the dose response curves (Makler and Hinrichs, 1993).

Antimicrobial Assay

Antimicrobial activity of the samples was determined using modified versions of the Clinical and Laboratories Standards Institute (formerly NCCLS) methods (Pfaller *et al.,* 2002; Wikler *et al.,* 2006; Woods *et al.,* 2003). Samples (dissolved in DMSO) were serially diluted in 20% DMSO/saline and transferred (10µL) in duplicate to 96 well flat bottom microplates. Final sample test concentrations were $1/100^{th}$ the DMSO stock concentration. Drug controls ciprofloxacin (for bacteria) and amphotericin B (for fungi) were included in each assay. All organisms were read at either 530 nm using the Biotek Powerwave XS plate reader (Bio-Tek Instruments, Vermont) or 544 ex/ 590 em, (*M. intracellulare*, *A. fumigatus*) using the Polarstar Galaxy Plate Reader (BMG LabTechnologies, Germany) prior to and after incubation: *Candida spp.* at 35 °C for 46 – 50 hours, *Staphylococcus spp.*, *E. coli*, and *P. aeruginosa* at 35 °C for 16 – 20 hours, *C. neoformans* at 35 °C for 70 – 74 hours, *A. fumigatus* at 35 °C for 46 – 50 hours, and *M. intracellulare* at 37 °C and 10% CO_2 for 70 – 74 hours. For all organisms excluding *M. intracellulare* and *A. fumigatus*, optical density was used to monitor growth (Pfaller *et al.,* 2002; Wikler *et al.,* 2006). Media supplemented with 5% Alamar Blue™ was utilized for growth detection of *M. intracellulare* (Woods *et al.,* 2003; Franzblau *et al.,* 1998) and *A. fumigatus* (Pfaller *et al.,* 2002).

All samples were tested in duplicate at one test concentration (50 µg/mL) and the percent inhibitions were calculated relative to blank and growth controls. All organisms were obtained from the American Type Culture Collection and include the fungi *Candida albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 204305 and the bacteria *Staphylococcus aureus* ATCC 29213, methicillin-resistant *S. aureus* ATCC 33591 (MRSA), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068.

RESULTS AND DISCUSSION

The antimalarial, antileishmanial and antimicrobial activity of the crude organic extracts (hexane, methylene chloride and methanol) and subfractions of the Jamaican *N. nolitangere* were examined. The hexane and methylene chloride extracts were subjected to flash column chromatography on silica gel with elution in solvents of progressively increasing polarity. The hexane extract was fractionated to afford four main fractions denoted Z1-Z4, while chromatography of the methylene chloride extract yielded seven main fractions denoted Y1-Y7.

Screening of the samples for antimalarial activity against *Plasmodium falciparum* led to the discovery of two bioactive sub-fractions from the methylene chloride extract, Y5 and Y6, which inhibited the growth of the organism by 87% and 78% respectively when administered at a concentration of 20 µg/mL. However, none of the crude extracts of the sponge showed any activity against the pathogen. The results are shown in Table 1.

Table. 1: Antimalarial activities of the crude extracts and fractions of *N. nolitangere* against *P. falciparum* (D6 clone) at 20 µg/mL.

Extracts	Fractions	% Inhibition
Hexane	Crude Z	0
	Z1	0
	Z2	0
	Z3	41
	Z4	16
Methylene chloride	Crude Y	0
	Y1	28
	Y2	30
	Y3	0
	Y4	0
	Y5	87
	Y6	78
	Y7	8
Methanol	Crude X	0
Control - Chloroquine (IC_{50} /ngmL^{-1})		<26.4
Control - Artemisinin (IC_{50} /ngmL^{-1})		<26.4

Extracts showing % Inhibition < 50 are considered inactive at 15.9 µg/ml.

While the crude extracts showed very minimal antileishmanial activity, selected subfractions of extracts showed promising results against the pathogen. As shown in Table 2, two fractions of the hexane extract, Z4 and Z3, inhibited the growth of the parasite by 91.77% and 79.71% respectively at 20 µg/mL. Fractions of the methylene chloride extract also demonstrated antileishmanial activity with the most bioactive fraction being Y4, which inhibited growth by 93.31% at 20 µg/mL, followed by Y3 (54.13 %) then Y2 (35.88 %). These results showed that the activity exhibited by the sub-fractions of the extracts of *N. nolitangere* was comparable with that exhibited by the standard antibiotic amphotericin B which is used against the parasites. Currently, pentavalent antimonials such as sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime), and amphotericin B are the drugs recommended to treat leishmaniasis (Monzote, 2009). These drugs have been in use for over 50 years and treatment requires long courses of administration. Additionally, the drugs also exhibit therapeutic failure, long duration for healing of lesions and the risk of diabetes, among other side effects (Sabina *et al.*, 2005; Croft, 1988; Ribeiro *et al.*, 1999). The metabolites in the extracts responsible for the activity have great potential for drug development and subsequent commercial medications.

Table. 2: Antileishmanial activities of the crude extracts and sub-fractions of *N. nolitangere* at 20 µg/mL.

Extracts	Fractions	% Inhibition
Hexane	Crude Z	10.96
	Z1	0
	Z2	0
	Z3	79.71
	Z4	91.77
Methylene chloride	Crude Y	11.26
	Y1	5.83
	Y2	35.88
	Y3	54.13
	Y4	93.31
	Y5	5.73
	Y6	5.94
	Y7	2.53
Methanol	Crude X	0
Control - Amphotericin B		100.0

The antibacterial activity of the extracts, expressed as percent inhibition, against five strains with values ranging from 0 % inhibition to 36 % inhibition are shown in Table 3. Low antibacterial activity was demonstrated by the crude hexane and methylene chloride extracts against the growth of the *Escherichia coli* bacteria with 21 % and 34 % inhibition respectively. The most significant antibacterial activity was demonstrated by fraction Y3 that inhibited the growth of Methicillin-resistant *Staphylococcus aureus* (MRSA) by 36 %. Based on the results, it can be concluded that the extracts of *N. nolitangere* have limited antibacterial potential against the five strains tested.

Table. 3: Antibacterial activities of the crude extracts and sub-fractions of *N. nolitangere* at 50 µg/mL.

Extracts	Fractions	% Inhibition				
		SA	***MRSA***	***EC***	***PA***	***MI***
Hexane	Crude Z	0	1	21	10	0
	Z1	0	0	20	3	0
	Z2	0	0	33	0	0
	Z3	11	29	15	0	0
	Z4	0	3	33	1	0
Methylene chloride	Crude Y	0	0	34	0	0
	Y1	0	0	9	2	0
	Y2	0	7	18	0	0
	Y3	14	36	31	18	0
	Y4	3	11	32	0	0
	Y5	9	8	30	2	0
	Y6	0	1	31	0	0
	Y7	0	1	26	0	0
Methanol	Crude X	0	2	0	3	0
Control - Ciprofloxacin (IC_{50})		0.10	0.09	0.007	0.18	0.38
Control - Ciprofloxacin (MIC)		0.25	0.25	0.31	1.00	1.00

SA-Staphylococcus aureus, MRS-Methicillin-resistant Staphylococcus , EC-Escherichia coli , PA-Pseudomonas aeruginosa , MI- Mycobacterium intracellulare,

Table. 4: Antifungal activities of the crude extracts and fractions of *N. nolitangere* at 50 µg/mL.

Extracts	Fractions	% Inhibition				
		CA	*CG*	*CK*	*CN*	*AF*
Hexane	Crude Z	7	4	6	28	0
	Z1	33	8	2	35	0
	Z2	26	11	0	45	1
	Z3	37	8	0	39	3
	Z4	33	13	34	36	0
Methylene chloride	Crude Y	0	0	6	25	0
	Y1	0	0	0	25	1
	Y2	40	8	0	30	0
	Y3	25	4	0	0	0
	Y4	25	9	0	34	0
	Y5	30	14	0	33	3
	Y6	0	10	0	28	3
	Y7	0	12	0	22	2
Methanol	Crude X	0	0	7	7	0
Control - Amphotericin B (IC_{50})		0.19	0.33	0.56	0.55	1.01
Control - Amphotericin B (MIC)		1.25	0.63	1.25	1.25	1.25

CA-Candida albicans, CG-Candida glabrata, CK- Candida krusei, CN-Cryptococcus neoformans, AF-Aspergillus fumigatus

The results for the antifungal studies are summarized in Table 4. The crude extracts of *N. nolitangere* showed minimal antifungal activity against the pathogens screened at 50 µg/mL. As shown in the table, the percent growth inhibition values ranged from 0 to

45%. Though considered inactive, the fractions of both the hexane and methylene chloride extracts exhibited minimal percentage growth inhibition against *C. albicans* and *C. neoformans*. The highest percentage growth inhibition was displayed by fraction Z2 that inhibited the growth of *C. neoformans* by 45% at 50 µg/mL followed by fraction Y2 that inhibited the growth of *C. albicans* by 40%. The fraction that inhibited the growth of *C. krusei* to the greatest extent was Z4 (34%). None of the samples showed notable results when screened against *C. glabrata* and *A. fumigatus.*

CONCLUSION

The crude methylene chloride and methanol extracts of *N. nolitangere* exhibited antimalarial and anti-insecticidal activity respectively suggesting potential applications in the health sector. Potent antileishmanial activity comparable to standard antibiotics in current use was recorded in relatively polar fractions from the hexane and methylene chloride extracts. This study has therefore demonstrated the potential for the use of extracted specimens of *N. nolitangere* for a range of applications.

REFERENCES

Atta-ur-Rahman S, Atia-tul-Wahab C and Choudhary M. Discovery of leishmanacidal agents from medicinal plants, Pure Appl. Chem. 2008; 80: 1783 – 1790.

Bhakuni D and Rawat D. 2005. Bioactive Marine Natural Products, Anamaya Publishers, New Delhi, India.

Cardellina II J. Marine natural products as leads to new pharmaceutical and agrochemical agents, Pure & Appl. Chem. 1986*;* 58: 365 - 374.

Croft S. Recent developments in the chemotherapy of Leishmaniasis, Trends Pharmacol. Sci., 1988, 9: 376 – 381.

Epifanio R, Gabriel R, Martins D, and Muricy G. The sesterterpene variabilin as a fish predation deterrent in the western Atlantic sponge *Ircinia strobilina*, J. Chem. Ecol., 1999; 25: 2247 – 2254.

Fagerholm A, Habrant D and Koskinen A. Calyculins and related marine natural products as serine-threonine protein phosphatase PP1 and PP2A inhibitors and total syntheses of Calyculin A, B and C, Mar. Drugs, 2010*;* 8:122–172.

Franzblau S, Witzig R, McLaughlin J, Torres P, Madico G, Hernandez A, Degnan M, Cook M, Quenzer V, Ferguson R. and Gilman R. Rapid low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar blue assay, J. Clin. Microbiol.,1998*; 36*, 362 – 366.

Huang X, Guo Y, and Song G. Fortisterol, a novel sterol with an unusual seven-membered lactone ring B from the Chinese marine sponge *Biemna fortis* Topsent, J. Asian Nat. Prod. Res., 2006; 8, 485-489.

Laport M, Santos O and Muricy G. Marine Sponges: Potential Sources of New Antimicrobial Drugs, Curr. Pharm. Biotech., 2009; 10: 86 -105.

Makler M. and Hinrichs D. Measurement of the lactase dehydrogenase activity of *Plasmodium falciparum* as an assessment of parasitemia, Am. J. Trop. Med. Hyg., 1993;48, 205-210.

Mikus J. and Steverding D. A simple colorimetric method to screen drug cytotoxicity against Leishmania using the dye Alamar Blue, Parasitol. Int., 2000; 48, 265-269.

Monzote L. Current treatment of Leishmaniasis: A Review, The Open Antimicrobial Agents Journal, 2009; 1, 9 – 19.

Pfaller M, Chaturvedi V, Espinel-Ingroff A, Ghannoum M, Gosey L, Odds F, Rex J, Rinaldi M, Sheehan D, Walsh T and Warnock D. National Committee on Clinical Laboratory Standards (NCCLS) *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard* , 2002; *M27-A2*, Second edition, *22*.

Pfaller M, Chaturvedi V, Espinel-Ingroff A, Ghannoum M, Gosey L, Odds F, Rex J, Rinaldi M, Sheehan D, Walsh T and Warnock D. National Committee on Clinical Laboratory Standards (NCCLS), *Reference method for broth dilution antifungal susceptibility testing of filamentous fungi, Approved Standard,* 2002; *M38-A*, 22.

Ribeiro A, Drummond J, Volpini A, Andrade A and Passos V. Electrocardiographic changes during low-dose short-term therapy of cutaneous leishmaniasis with the pentavalent antimonial meglumine, Braz. J. Med. Biol. Res., 1999; 32, 297 – 301.

Sabina H, Tasneem S, Samreen K, Choudhary M and Aliya R. Antileishmanial activity in the crude extract of various seaweeds from the coast of Karachi, Pakistan, Pak. J. Bot., 2005; 37: 163 – 168.

Sepčić K, Kauferstein S, Mebs D and Turk T. Biological activities of aqueous and organic extracts from Tropical marine sponges, *Mar. Drugs*, 2010; 8: 1550-1566.

Sheppard C. Coralpedia Volume 1.0, A Guide to Caribbean Corals, Octocorals and Sponges, Available at: http://coralpedia.bio. warwick.ac.uk/en/sponges/neofibularia_nolitangere.html [Accessed January 18, 2010]

Voss G. 1976. Seashore life of Florida and the Caribbean, Banyan Books, Miami, Fl., 26.

Wikler M, Cockerill F, Craig W., Dudley M, Eliopoulos G, Hecht D, Hindler J, Low D, Sheehan D, Tenover F, Turnidge J, Weinstein M, Zimmer B, Ferraro M and Swenson J. National Committee on Clinical Laboratory Standards (NCCLS) *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, Seventh Edition,* 2006; *M7-A7*, *26*.

Woods G, Brown-Elliott B, Desmond E, Hall G, Heifets L, Pfyffer G, Ridderhof J, Wallace R, Warren N and Witebsky F. National Committee on Clinical Laboratory Standards (NCCLS), *Susceptibility Testing of Mycobacteria, Nocardia, and Other Aerobic Actinomycetes, Approved Standard,* 2003; *M24-A*, 23.

Yong, J. and Chung, J. A new species of the genus *Biemna* (Demospongia: Poecilosclerida: Desmacellidae) from Korea, Anim. Cells and Syst., 2008; 12,241 – 243.

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