Assessing Pressurized Liquid Extraction for the High-Throughput Extraction of Marine-Sponge Derived Natural Products[⊥]

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[Supporting Information]

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General Experimental Procedures.

Initial Standard Solvent Partitioning (SSP) workup of the first specimen (02600) began with three successive extractions using 500 mL / 24 hr in methanol, which were then evaporated to generate 1,371.0 mg of the total polar extract (TPE). The TPE was subsequently extracted using 100 mLs of water (sample coded W, 1,289.0 mg) and dichloromethane (sample coded F, 80.1 mg). The W fraction was then partitioned using 100 mL of sec-butanol (sample coded WB, 75.0 mg) and the remaining water (sample coded WW, 1,213.0 mg) which retained mostly inorganic salts. Additionally the organic F layer was evaporated then re-extracted three times (100 mL X 3) with hexanes (sample coded FH = 21.0 mg) to remove unwanted lipid components and partitioned between 100 mL of 90% aqueous methanol. The 90% aqueous methanol layer was then adjusted to a 50:50 (180 mL) mixture of H_2O and methanol (sample coded FM = 38.0 mg) and partitioned between 180 mL of dichloromethane (sample coded FD = 20.7 mg). The total organic extract yield was approximately 155.0 mg and involved 2,180 mL of solvent, primarily methanol.

ASE 100 Parameters Used During Extraction(s).

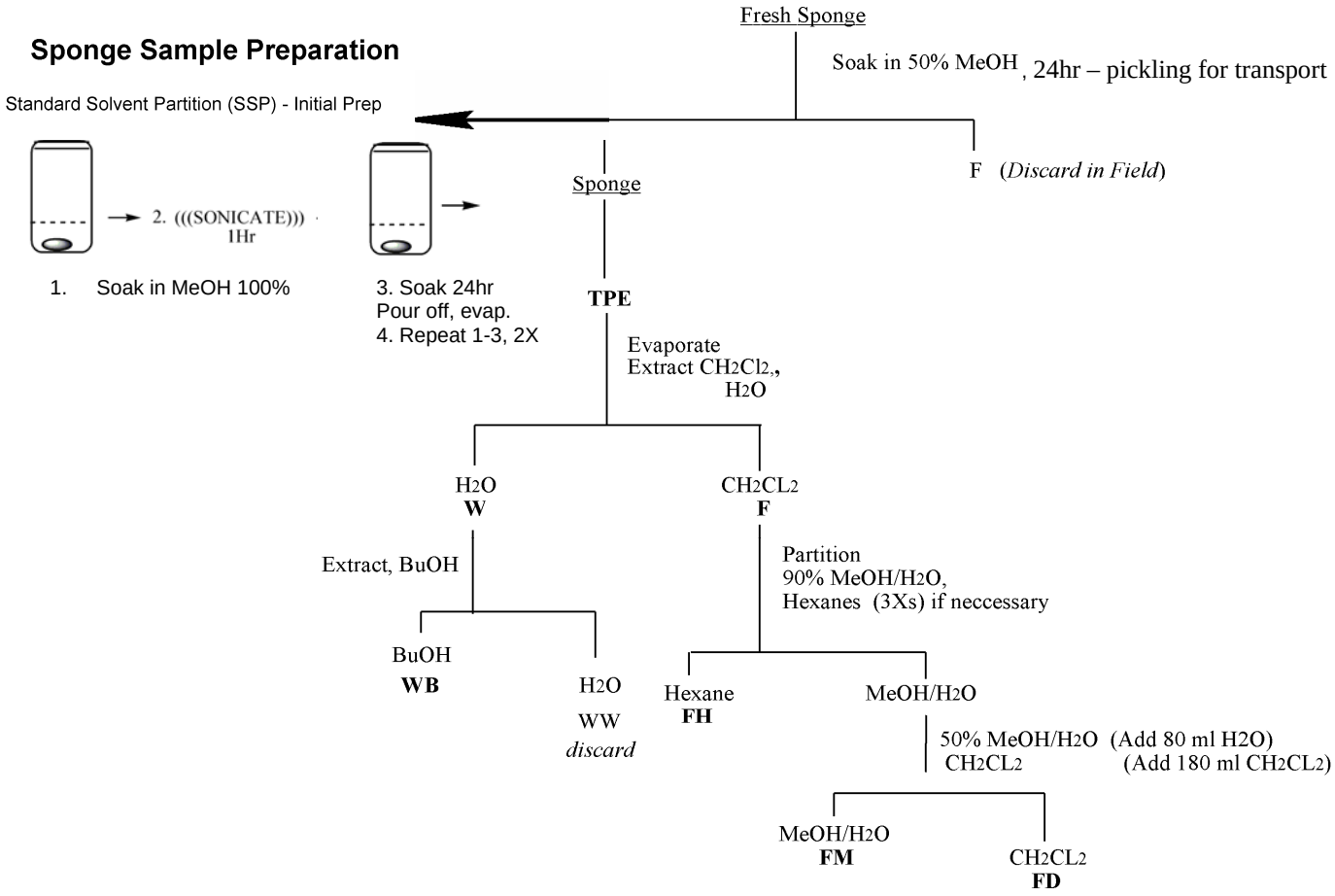
SETUP

Temperature: off (room temperature ~ 22-27 ˚C) and 100˚C
Static time: 5 minutes
Flush volume: 50%
Purge time: 100 seconds
Static cycle: 3

METHOD EDITOR

Cell size: 100 mL
Units: p.s.i (using nitrogen)
Reduce relief: off
Preheat time: 0 minutes
Preheat purge: off
Bypass heat up: off
Key sound: on
Error sound: on

Chart S1. UCSC experimental procedure for Standard Solvent Partitioning (SSP) of marine extracts.



NOTES: All aliquots of solvent are 100 mL unless otherwise specified.

TPE: Total Polar Extract
W: Water
F: Fat

Chart S2. UCSC experimental procedure for ASE processing of marine extracts.

Fresh Sponge

Soak in 50% MeOH

24 hr – pickling for transport
Store @ 4˚C until further extraction

F (*Discard in Field*)

Sponge Sample Preparation

100g Sponge -

ASE (50g)

Aqueous/Organic ASE Extraction

50g Sponge/cell

	Sample Code: (X = ASE)
1. Extract: (1700 PSI, 100* C) w/ 200ml Water	XWW I, II, III
2. Extract: (1700 PSI, 100* C) w/ 200ml Hexanes	XFH I, II, III
3. Extract: (1700 PSI, 100* C) w/ 200ml Dichloromethane	XFD I, II, III
4. Extract: (1700 PSI, 100* C) w/ 200ml MeOH	XFM I, II, III

NOTES:
X = ASE
30 minute extraction time/ solvent @ 1700 PSI and 100˚C
(e.g. 200 mL of Water, Hexanes, DCM and MeOH ~ 120 minutes for ASE I).

Table S1. Comparative Extraction Yields of *A. constricta* (coll. no. 03505) Using SSP versus ASE

Standard Solvent Partitioning (SSP) of an 50.2 g weight sample.

Fraction Codes (Volume)	MeOH Extractions[a]	Solvent Partition/ Evaporate	**TOTAL**
TPE	ND	5,212.3 mg	5,212.3 mg
W	ND	4,675.0 mg	4,675.0 mg
F	ND	525.0 mg	525.0 mg
WW (100 mL) "salts"	ND	4,439.1 mg	4,439.1 mg
WB (100 mL)	ND	235.9 mg	235.9 mg
FH (300 mL)	ND	195.6 mg	195.6 mg
FD (180 mL)	ND	283.1 mg	283.1 mg
FM (100 mL)	ND	46.3 mg	46.3 mg
Total Organic Extract	ND	760.9 mg	760.9 mg
Organic Solvent Use	1.5 L	0.68 L	2.18 L
Process Time	72 hr	8 hr	80 hr
Extraction Efficiency (EE)[b]	ND	ND	349.0 mg/L
Total Extraction Yield (TEY)[c]	ND	ND	1.5 %

[a]Three successive extractions using MeOH (500 mL each) were performed and decanted after 24 hrs. [b]Total organic extract (mg) / Solvent Use (L). [c]Total organic extract (mg) / specimen weight (mg) • 100%. Codes: ND = Not Determined; TPE = Total Polar Extract; W = Water soluble; F = Fat soluble; WW = Water soluble/ Water; WB = Water soluble/Butanol; FH = Fat soluble/Hexanes; FD = Fat soluble/ Dichloromethane; FM = Fat soluble/Methanol.

Accelerated Solvent Extraction (ASE) of a 50.14 g weight sample with % yield in parenthesis.

Fraction Codes (Volume)	ASE Run I	ASE Run II	ASE Run III	**TOTAL**
XWW (200 mL) "salts"	3,421.2 mg (89%)	230.6 mg (6%)	192.1 mg (5%)	3,844.8 mg
XFH (200 mL)	130.7 mg (60%)	56.1 mg (25%)	33.2 mg (15%)	220.0 mg
XFD (200 mL)	171.5 mg (74%)	41.2 mg (18%)	17.4 mg (8%)	230.1 mg
XFM (200 mL)	536.7 mg (87%)	51.9 mg (9%)	22.2 mg (4%)	610.8 mg
Total Organic Extract	838.9 mg (79%)	149.2 mg (14%)	72.8 mg (7%)	1,060.9 mg
Organic Solvent Use	0.6 L	0.6 L	0.6 L	1.8 L
Process Time	2 hr	2 hr	2 hr	6 hr
Extraction Efficiency (EE)[a]	1,398.2 mg/ L	248.6 mg/L	121.3 mg/L	589.4 mg/L
Total Extraction Yield (TEY)[b]	1.7%	0.3 %	0.1 %	2.1%

[a]Total organic extract (mg) / solvent use (L). [b]Total organic extract (mg) / specimen weight (mg) • 100%. X = ASE; XWW = Water soluble/Water; XFH = Fat soluble/Hexanes; XFD = Fat soluble/Dichloromethane; XFM = Fat soluble/Methanol. ND = Not Determined. Note: Processing time using ASE/ solvent: ~ 30 minutes not including rotatory evaporation.

Table S2. Comparative Extraction Yields of *Z. fuliginosa* (coll. no. 03501) Using SSP versus ASE

Standard Solvent Partitioning (SSP) of an 50.2 g weight sample.

Fraction Codes (Volume)	MeOH Extractions[a]	Solvent Partition/ Evaporate	**TOTAL**
TPE	ND	2,948.0 mg	2,948.0 mg
W	ND	2,528.4 mg	2,528.4 mg
F	ND	419.6 mg	419.6 mg
WW (100 mL) "salts"	ND	2160.2 mg	2160.2 mg
WB (100 mL)	ND	398.2 mg	398.2 mg
FH (300 mL)	ND	242.9 mg	242.9 mg
FD (180 mL)	ND	57.1 mg	57.1 mg
FM (100 mL)	ND	119.6 mg	119.6 mg
Total Organic Extract	ND	817.8 mg	817.8 mg
Organic Solvent Use	1.5 L	0.68 L	2.18 L
Process Time	72 hr	8 hr	80 hr
Extraction Efficiency (EE)[b]	ND	ND	375.1 mg/L
Total Extraction Yield (TEY)[c]	ND	ND	1.6 %

[a]Three successive extractions using MeOH (500 mL each) were performed and decanted after 24 hrs. [b]Total organic extract (mg) / Solvent Use (L). [c]Total organic extract (mg) / specimen weight (mg) • 100%. Codes: ND = Not Determined; TPE = Total Polar Extract; W = Water soluble; F = Fat soluble; WW = Water soluble/ Water; WB = Water soluble/Butanol; FH = Fat soluble/Hexanes; FD = Fat soluble/ Dichloromethane; FM = Fat soluble/Methanol.

Accelerated Solvent Extraction (ASE) of a 50.0 g weight sample with % yield in parenthesis.

Fraction Codes (Volume)	ASE Run I	ASE Run II	ASE Run III	**TOTAL**
XWW (200 mL) "salts"	2,768.2mg (86%)	418.6 mg (9%)	161.2 mg (5%)	3,220 mg
XFH (200 mL)	162.1 mg (68%)	41.1 mg (17%)	35.8 mg (15%)	238.3 mg
XFD (200 mL)	180.9 mg (62%)	73.8 mg (25%)	36.8 mg (13%)	289.8 mg
XFM (200 mL)	621.2 mg (87%)	66.7 mg (9%)	22.2 mg (3%)	710.0 mg
Total Organic Extract	964.2 mg (78%)	181.6 mg (15%)	94.8 mg (8%)	1,238.1 mg
Organic Solvent Use	0.6 L	0.6 L	0.6 L	1.8 L
Process Time	2 hr	2 hr	2 hr	6 hr
Extraction Efficiency (EE)[a]	1,607.0 mg/L	302.6 mg/L	158.0 mg/L	687.8 mg/L
Total Extraction Yield (TEY)[b]	1.9 %	0.4 %	0.2 %	2.5 %

[a]Total organic extract (mg) / solvent use (L). [b]Total organic extract (mg) / specimen weight (mg) • 100%. X = ASE; XWW = Water soluble/Water; XFH = Fat soluble/Hexanes; XFD = Fat soluble/Dichloromethane; XFM = Fat soluble/Methanol. ND = Not Determined. Note: Processing time using ASE/ solvent: ~ 30 minutes not including rotatory evaporation.

Table S3. Comparative Extraction Yields of *F. reticulata* (coll. no. 05417) Using SSP versus ASE

Standard Solvent Partitioning (SSP) of an 50.4 g weight sample.

Fraction Codes (Volume)	MeOH Extractions[a]	Solvent Partition/ Evaporate	**TOTAL**
TPE	ND	2,289.9 mg	2,289.9 mg
W	ND	1,764.9 mg	1,764.9 mg
F	ND	525.0 mg	525.0 mg
WW (100 mL) "salts"	ND	1,651.4 mg	1,651.4 mg
WB (100 mL)	ND	113.5 mg	113.5 mg
FH (300 mL)	ND	200.8 mg	200.8 mg
FD (180 mL)	ND	526.1 mg	526.1 mg
FM (100 mL)	ND	83.1 mg	83.1 mg
Total Organic Extract	ND	922.5 mg	922.5 mg
Organic Solvent Use	1.5 L	0.68 L	2.18 L
Process Time	72 hr	8 hr	80 hr
Extraction Efficiency (EE)[b]	ND	ND	423.2 mg/L
Total Extraction Yield (TEY)[c]	ND	ND	1.8 %

[a]Three successive extractions using MeOH (500 mL each) were performed and decanted after 24 hrs. [b]Total organic extract (mg) / Solvent Use (L). [c]Total organic extract (mg) / specimen weight (mg) • 100%. Codes: ND = Not Determined; TPE = Total Polar Extract; W = Water soluble; F = Fat soluble; WW = Water soluble/ Water; WB = Water soluble/Butanol; FH = Fat soluble/Hexanes; FD = Fat soluble/ Dichloromethane; FM = Fat soluble/Methanol.

Accelerated Solvent Extraction (ASE) of a 50.4 g weight sample with % yield in parenthesis.

Fraction Codes (Volume)	ASE Run I	ASE Run II	ASE Run III	**TOTAL**
XWW (200 mL) "salts"	1,822.5 (92%)	89.1 (5%)	59.4 (4%)	1,981.8 mg
XFH (200 mL)	30.1 mg (30%)	14.3 mg (42%)	9.8 mg (28%)	54.2 mg
XFD (200 mL)	146.6 mg (60%)	61.2 mg (25%)	38.8 mg (15%)	246.6 mg
XFM (200 mL)	180.7 mg (70%)	50.3 mg (19%)	27.6 mg (11%)	258.6 mg
Total Organic Extract	357.4 mg (64%)	125.8 mg (23%)	76.2 mg (14%)	559.4 mg
Organic Solvent Use	0.6 L	0.6 L	0.6 L	1.8 L
Process Time	2 hr	2 hr	2 hr	6 hr
Extraction Efficiency (EE)[a]	595.6 mg/L	209.6 mg/L	127.0 mg/L	310.8 mg/L
Total Extraction Yield (TEY)[b]	0.7 %	0.2 %	0.2 %	1.1 %

[a]Total organic extract (mg) / solvent use (L). [b]Total organic extract (mg) / specimen weight (mg) • 100%. X = ASE; XWW = Water soluble/Water; XFH = Fat soluble/Hexanes; XFD = Fat soluble/Dichloromethane; XFM = Fat soluble/Methanol. ND = Not Determined. Note: Processing time using ASE/ solvent: ~ 30 minutes not including rotatory evaporation.

Table S4. Comparative Extraction Yields of *J. coriacea* (coll. no. 00102) Using SSP versus ASE

Standard Solvent Partitioning (SSP) of an 50.4 g weight sample.

Fraction Codes (Volume)	MeOH Extractions[a]	Solvent Partition/ Evaporate	**TOTAL**
TPE	ND	2,391.5 mg	2,391.5 mg
W	ND	2,093.8 mg	2,093.8 mg
F	ND	297.7 mg	297.7 mg
WW (100 mL) "salts"	ND	1,921.3 mg	1,921.3 mg
WB (100 mL)	ND	172.5 mg	172.5 mg
FH (300 mL)	ND	132.4 mg	132.4 mg
FD (180 mL)	ND	74.1 mg	74.1 mg
FM (100 mL)	ND	91.2 mg	91.2 mg
Total Organic Extract	ND	470.2 mg	470.2 mg
Organic Solvent Use	1.5 L	0.68 L	2.18 L
Process Time	72 hr	8 hr	80 hr
Extraction Efficiency (EE)[b]	ND	ND	215.7 mg/L
Total Extraction Yield (TEY)[c]	ND	ND	0.9 %

[a]Three successive extractions using MeOH (500 mL each) were performed and decanted after 24 hrs. [b]Total organic extract (mg) / Solvent Use (L). [c]Total organic extract (mg) / specimen weight (mg) • 100%. Codes: ND = Not Determined; TPE = Total Polar Extract; W = Water soluble; F = Fat soluble; WW = Water soluble/ Water; WB = Water soluble/Butanol; FH = Fat soluble/Hexanes; FD = Fat soluble/ Dichloromethane; FM = Fat soluble/Methanol.

Accelerated Solvent Extraction (ASE) of a 50.2 g weight sample with % yield in parenthesis.

Fraction Codes (Volume)	ASE Run I	ASE Run II	ASE Run III	**TOTAL**
XWW (200 mL) "salts"	1,723.5 (87%)	158.5 (8%)	99.1 (5%)	1,981.8 mg
XFH (200 mL)	73.8 mg (53%)	43.2 mg (31%)	22.1 mg (16%)	139.1 mg
XFD (200 mL)	93.4 mg (65%)	33.1 mg (23%)	16.5 mg (12%)	143.0 mg
XFM (200 mL)	121.4 mg (52%)	82.3 mg (35%)	31.4 mg (13%)	235.1 mg
Total Organic Extract	288.6 mg (56%)	158.6 mg (31%)	70.0 mg (14%)	517.2 mg
Organic Solvent Use	0.6 L	0.6 L	0.6 L	1.8 L
Process Time	2 hr	2 hr	2 hr	6 hr
Extraction Efficiency (EE)[a]	481.0 mg/L	264.0 mg/L	116.7 mg/L	287.3 mg/L
Total Extraction Yield (TEY)[b]	0.6 %	0.3 %	0.1 %	1.0 %

[a]Total organic extract (mg) / solvent use (L). [b]Total organic extract (mg) / specimen weight (mg) • 100%. X = ASE; XWW = Water soluble/Water; XFH = Fat soluble/Hexanes; XFD = Fat soluble/Dichloromethane; XFM = Fat soluble/Methanol. ND = Not Determined. Note: Processing time using ASE/ solvent: ~ 30 minutes not including rotatory evaporation.

Table S5. Comparative Extraction Yields of *A. constricta* (coll. no. 03505) Using ASE at 22 ˚C and 100 ˚C accelerated solvent extraction (ASE)*

fraction codes (volume)	22 ˚C	100 ˚C
XWW (200 mL) “salts”	886.2 mg	3,101.3 mg
XFH (200 mL)	66.1 mg	119.2 mg
XFD (200 mL)	43.5 mg	152.3 mg
XFM (200 mL)	208.7 mg	501.2 mg
total organic extract	318.3 mg	772.7 mg
organic solvent use	0.6 L	0.6 L
process time	2.0 h	2.0 h
extraction efficiency (EE)[a]	530.5 mg/L	1287.8 mg/L
total extraction yield (TEY)[b]	0.6 %	1.5 %

*Sample processed using approximately 50.0 g wet weight specimens. [a]Total organic extract (mg) / solvent use (L). [b]Total organic extract (mg)/specimen weight (mg) • 100%. Codes: X = ASE; XWW = Water soluble/Water; XFH = Fat soluble/Hexanes; XFD = Fat soluble/Dichloromethane; XFM = Fat soluble/Methanol. Note: Processing time using ASE/solvent: ~30 min not including rotatory evaporation.

Table S6. Comparative Extraction Yields of *Z. fuliginosa* (coll. no. 03501) Using ASE at 22 ˚C and 100 ˚C accelerated solvent extraction (ASE)*

fraction codes (volume)	22 ˚C	100 ˚C
XWW (200 mL) “salts”	622.4 mg	2,801.5 mg
XFH (200 mL)	56.0 mg	157.3 mg
XFD (200 mL)	19.3 mg	169.7 mg
XFM (200 mL)	194.1 mg	598.8 mg
total organic extract	269.4 mg	925.8 mg
organic solvent use	0.6 L	0.6 L
process time	2.0 h	2.0 h
extraction efficiency (EE)[a]	449.0 mg/L	1543.3 mg/L
total extraction yield (TEY)[b]	0.5 %	1.9 %

*Sample processed using approximately 50.0 g wet weight specimens. [a]Total organic extract (mg) / solvent use (L). [b]Total organic extract (mg)/specimen weight (mg) • 100%. Codes: X = ASE; XWW = Water soluble/Water; XFH = Fat soluble/Hexanes; XFD = Fat soluble/Dichloromethane; XFM = Fat soluble/Methanol. Note: Processing time using ASE/solvent: ~30 min not including rotatory evaporation.

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Table S7. Comparative Extraction Yields of *F. reticulata* (coll. no. 05417) Using ASE at 22 ˚C and 100 ˚C accelerated solvent extraction (ASE)*

fraction codes (volume)	22 ˚C	100 ˚C
XWW (200 mL) "salts"	529.7 mg	1801.6 mg
XFH (200 mL)	19.5 mg	78.2 mg
XFD (200 mL)	99.3 mg	149.3 mg
XFM (200 mL)	104.7 mg	151.6 mg
total organic extract	223.5 mg	379.1 mg
organic solvent use	0.6 L	0.6 L
process time	2.0 h	2.0 h
extraction efficiency (EE)[a]	372.5 mg/L	631.8 mg/L
total extraction yield (TEY)[b]	0.4 %	0.8 %

*Sample processed using approximately 50.0 g wet weight specimens. [a]Total organic extract (mg) / solvent use (L). [b]Total organic extract (mg)/specimen weight (mg) • 100%. Codes: X = ASE; XWW = Water soluble/Water; XFH = Fat soluble/Hexanes; XFD = Fat soluble/Dichloromethane; XFM = Fat soluble/Methanol. Note: Processing time using ASE/solvent: ~30 min not including rotatory evaporation.

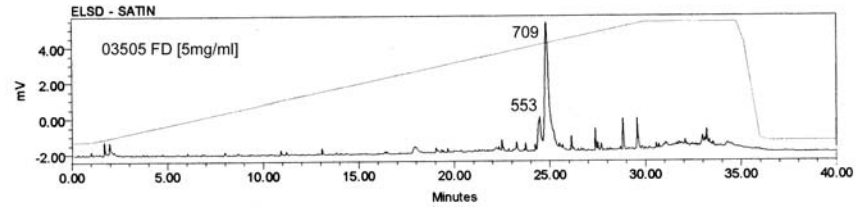
Table S8. Comparative Extraction Yields of *J. coriacea* (coll. no. 00102) Using ASE at 22 ˚C and 100 ˚C accelerated solvent extraction (ASE)*

fraction codes (volume)	22 ˚C	100 ˚C
XWW (200 mL) “salts”	376.8 mg	1,533.4 mg
XFH (200 mL)	17.4 mg	62.5 mg
XFD (200 mL)	105.7 mg	136.9 mg
XFM (200 mL)	86.2 mg	103.5 mg
total organic extract	209.3 mg	302.9 mg
organic solvent use	0.6 L	0.6 L
process time	2.0 h	2.0 h
extraction efficiency (EE)[a]	348.8 mg/L	504.8 mg/L
total extraction yield (TEY)[b]	0.4 %	0.6 %

*Sample processed using approximately 50.0 g wet weight specimens. [a]Total organic extract (mg) / solvent use (L). [b]Total organic extract (mg)/specimen weight (mg) • 100%. Codes: X = ASE; XWW = Water soluble/Water; XFH = Fat soluble/Hexanes; XFD = Fat soluble/Dichloromethane; XFM = Fat soluble/Methanol. Note: Processing time using ASE/solvent: ~30 min not including rotatory evaporation.

Figure S1. ELSD analysis of coll. no. 03505 extracts processed using: (**a**) SSP (FD) vs. (**b**) ASE (100 ˚C, XFD) and (**c**) ASE (22 ˚C, XFD) vs. (**d**) ASE (100 ˚C, XFD) with annotations including *m/z* ions. (**e**) Underwater photo of *A. constricta* and, (**f**) associated chemistry.

(**a**)



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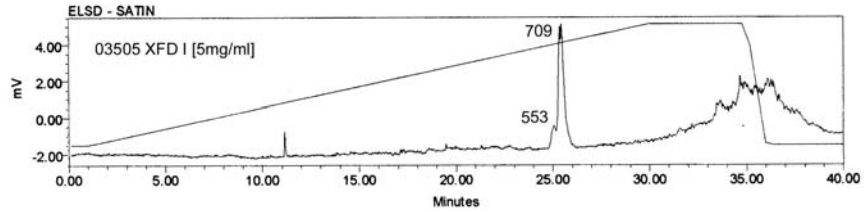

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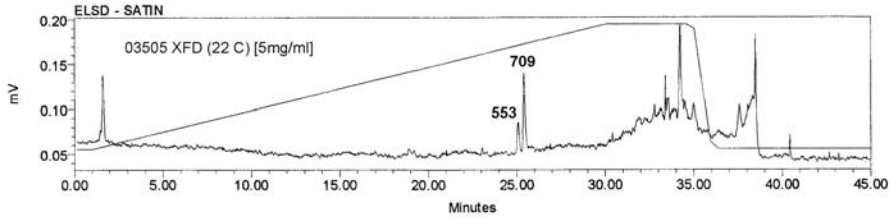

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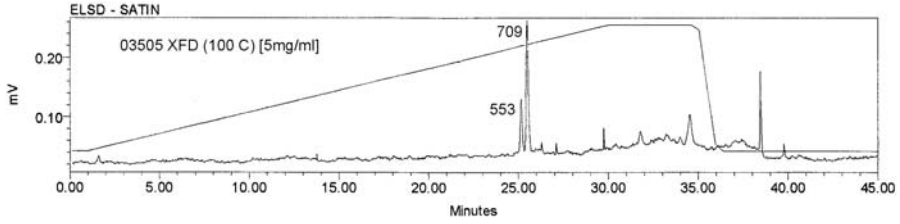
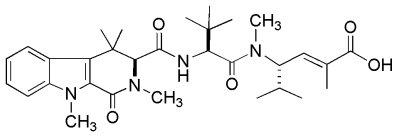
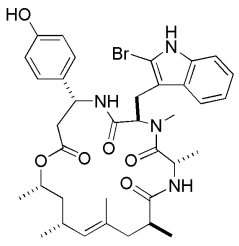


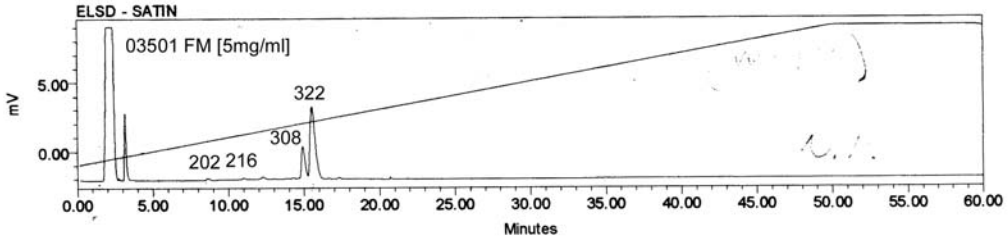




(**e**) 03505 *Auletta* cf. *constricta* (PNG); (**f**) milnamide C, 553 amu $[M+H]^+$, jasplakinolide, 709 $[M+H]^+$

Figure S2. ELSD analysis of coll. no. 03501 extracts processed using: (**a**) SSP (FM) vs. (**b**) ASE (100 ˚C, XFM) and (**c**) ASE (22 ˚C, XFM) vs. (**d**) ASE (100 ˚C, XFM) with annotations including *m/z* ions. (**e**) Underwater photo of *Z. fuliginosa* and, (**f**) associated chemistry.

(**a**)



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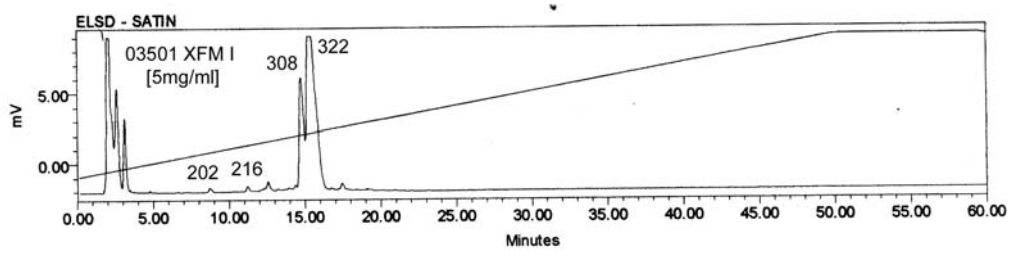

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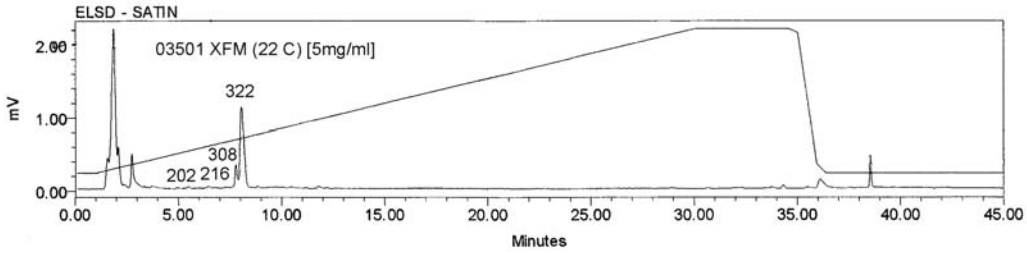

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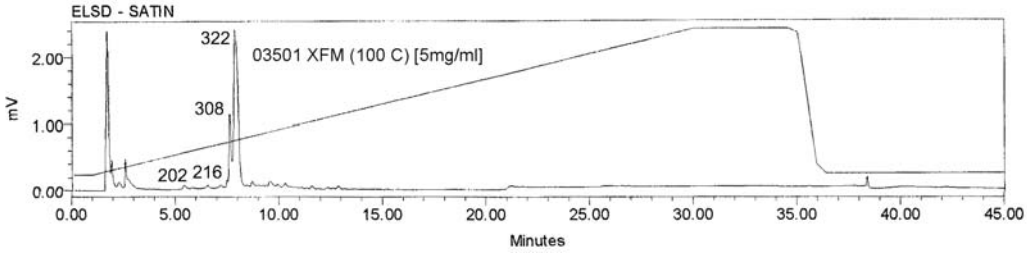

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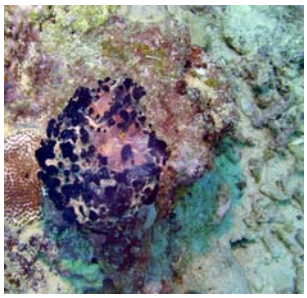
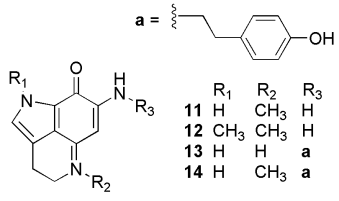


03501 *Zyzzya fuliginosa* (PNG)

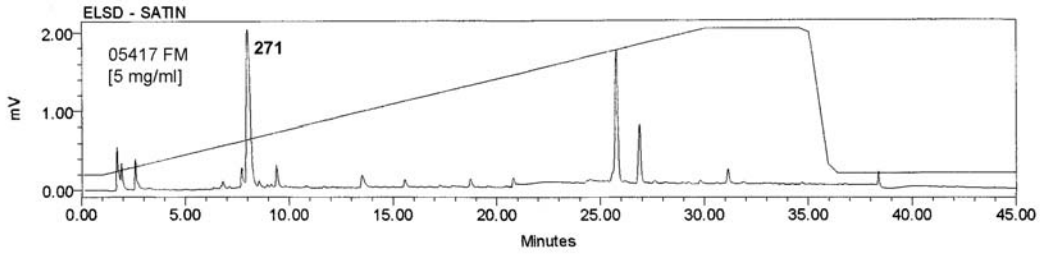
(**f**)



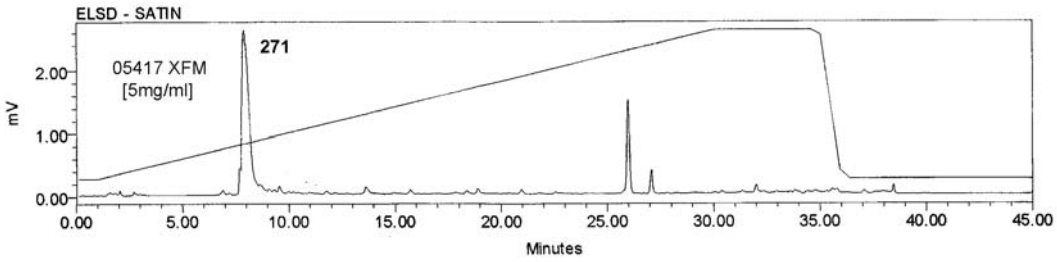
makaluvamine C (**11**), 202 amu $[M+H]^+$
makaluvamine H (**12**), 215 amu $[M+H]^+$
makaluvamine D (**13**), 308 amu $[M+H]^+$
makaluvamine J (**14**), 322 amu $[M+H]^+$

Figure S3. ELSD analysis of coll. no. 05417 extracts processed using: (**a**) SSP (FM) vs. (**b**) ASE (100 ˚C, XFM) and (**c**) ASE (22 ˚C, XFM) vs. (**d**) ASE (100 ˚C, XFM) with annotations including *m/z* ions. (**e**) Underwater photo of *F. reticulata* and, (**f**) associated chemistry.

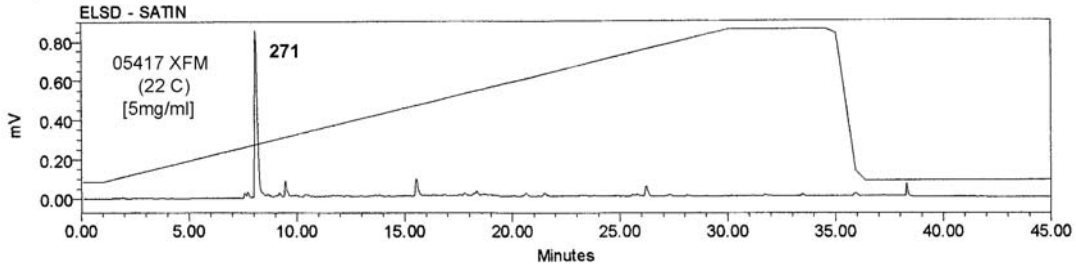
(**a**)



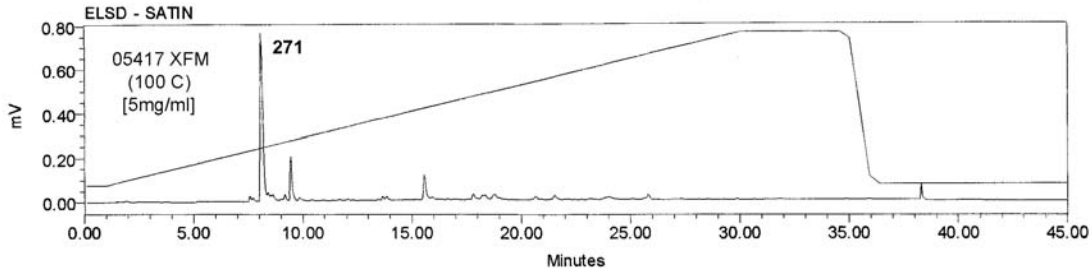
(**b**)



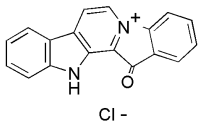
(**c**)



(**d**)



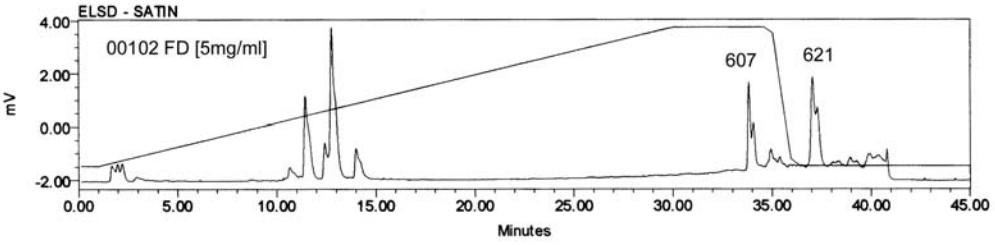
(**e**) 05417 *Fascaplysinopsis reticulata* (PNG)



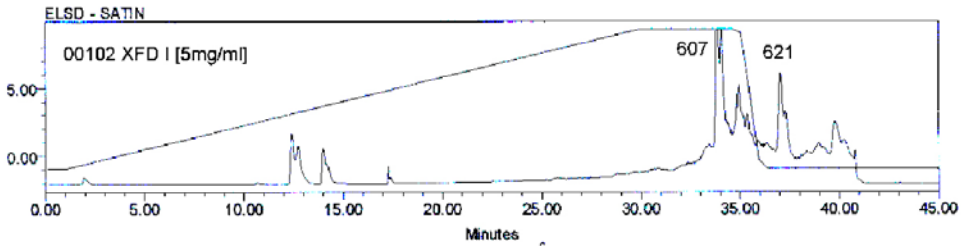
(**f**) fascaplysin, 271 amu $[M]^+$

Figure S4. ELSD analysis of coll. no. 00102 extracts processed using: (**a**) SSP (FD) vs. (**b**) ASE (100 ˚C, XFD) and (**c**) ASE (22 ˚C, XFD) vs. (**d**) ASE (100 ˚C, XFD) with annotations including *m/z* ions. (**e**) Underwater photo of *J. coriacea* and, (**f**) associated chemistry.

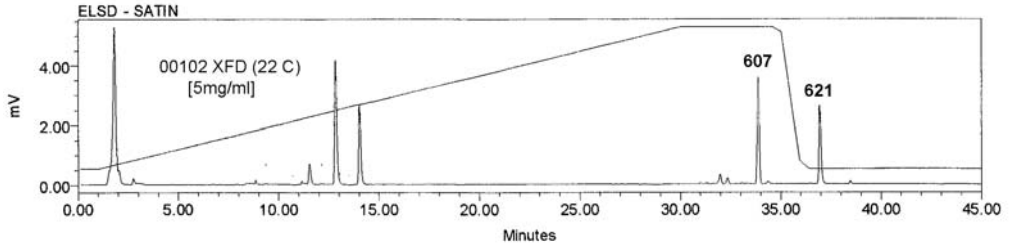
(**a**)



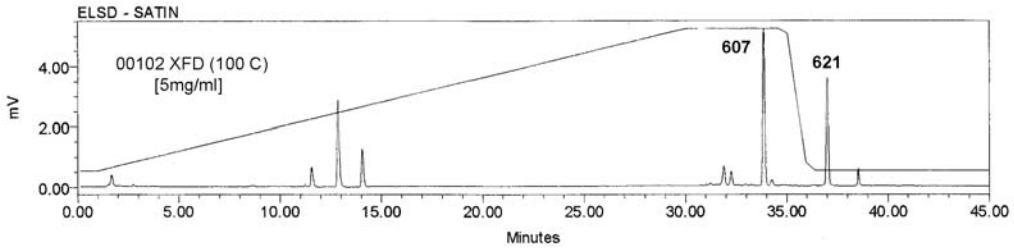
(**b**)

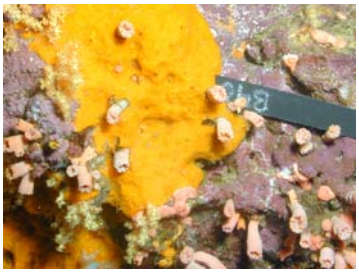


(**c**)

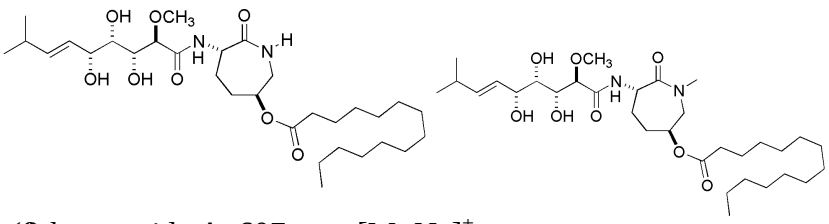


(**d**)





(**e**) 00102 *Jaspis coriacea* (Fiji)



(**f**) bengamide A, 607 amu $[M+Na]^+$,
bengamide B, 621 amu $[M+Na]^+$