SUPPLEMENTARY MATERIAL

Isolation of Stigmast-5,24-dien-3-ol from marine brown algae *Sargassum tenerrimum* and its Antipredatory effect

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1. Experimental

General Remarks. The ^{1}H and ^{13}C NMR were recorded in the deuterated solvents ($CDCl_3$) on Bruker 300 MHz spectrometer. The mass spectra were recorded on electrospray ionization-quadrupole time of flight Mass spectrometry (ESI- QTOF MS) QSTAR XL, Applied Biosystem, Canada. Column chromatography was performed with Sephadex LH20 and silica gel (60-120 mesh) as packing material. Thin-layer chromatography was performed with Kieselgel 60 F254 (Merck aluminum support plates).

Collection, extraction, and isolation. The marine algae *Sargassum tenerrimum* was collected from the intertidal zone at Anjuna Goa, central western coast of India. Sample was frozen as soon as possible and transferred to the laboratory. Identification was carried out by emeritus scientist Dr. P. A. Thomas of the Vizhingam Research Center of the Central Marine Fisheries Research Institute, Kerala, India. The sample is deposited at the CSIR-National Institute of Oceanography, Dona-Paula, Goa, India (Vocher specimen no. NIO1001). The freshly collected brown alga was initially freeze dried. The sample was homogenized in a blender with little water and exhaustively extracted at room temperature with MeOH (1 Lit. x 3). The combined extracts were filtered and concentrated under vacuum on a rotary evaporator at low temperature to give crude methanolic extracts. Methanolic extract (~5 g) of the algae *S. tenerrimum* was fractionated with hexane (100 ml x 3), ethyl acetate (100 ml x 3) followed by butanol (100 ml x 3). The hexane fraction (200 mg) was chromatographed initially on series of sephadex column eluting with chloroform in methanol. Subsequently, purification was carried out on silica gel column (60-120 mesh) using different proportion of hexanes and ethyl acetate mixture as eluting solvents. These resulted in the purification of Fucosterol **(1)** as white solid.

Feeding experiment

Laboratory palatability assays. Pawlik protocol (Pawlik et al. 1995) was used to perform aquarium feeding assays using rock pool fishes, which were captured on the same site from which brown algae were collected. The artificial food (treated) pellets were prepared by adding agar (1.5 g) to 60 mL of distilled water and 3.0 g of commercially available artificial fish food followed by heating in microwave oven until boiling. Test solution (crude extracts or pure

compound) was added to 15 ml above melt. The concentration used for test compounds was calculated on the basis of the natural concentration found in fresh seaweed tissues. The control food pellets were treated with the same amount of solvent used in treated pellets. After hardening gel solution by cooling at 5 ^{o}C, the pellets were cut and offered in control-treated pairs, to fish at the top of the aquarium (1 pellet at a time). Five replicates were made of each test solution, using 5 different aquaria with 3 fishes each. A significant difference between the number of treated and control pellets eaten was determined for an individual 5 replicate assay (1-tailed test, Pawlik et al. 1995). The consumption of treated food and control food was assumed to be dependent on each other, since consumption of one food item means less consumption of the other in same container (Peterson et al. 1989). Differences were considered significant only when $p < 0.05$ i.e. 5%.

Observation table:

Tank	Crude	Hexane fraction	Ethyl acetate fraction	Butanol fraction	Fucosterol
1	3	2	2	3	3
2	2	2	2	4	3
3	3	2	1	4	3
4	3	2	3	4	3
5	3	3	2	5	3
Mean	**2.8**	**2.2**	**2.0**	**4.0**	**3.0**
Std. dev.	**0.447**	**0.447**	**0.707**	**0.707**	**0.000**
Std. error	**0.199**	**0.199**	**0.316**	**0.316**	**0.000**
Error bar	**2.8±0.199**	**2.2±0.199**	**2.0±0.316**	**4.0±0.316**	**3.0±0.000**

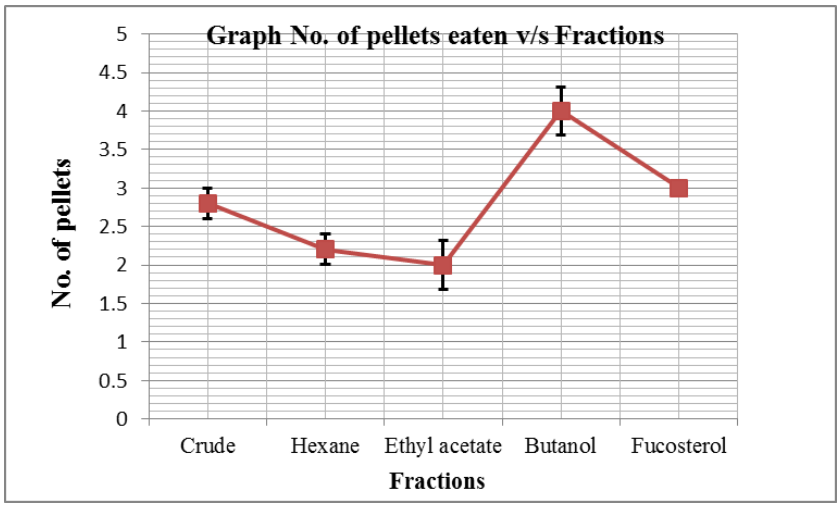

Figure 1. Error bar graph for laboratory palatability assays.

Field palatability assays. Field assay was performed at Anjuna Goa, Central western coast of India in the month of November 2013. Field strip were prepared by using previously reported protocol by (Pawlik and Fenical 1992). For each experiment, 5 treated and 5 control strips were arranged in pairs and attached to 2 ropes. The ropes were anchored slightly above the bottom near to the same site where seaweeds were collected. After 3 h, the ropes were recovered and the numbers of strips eaten were measured. During the experiments several fishes were observed feeding on the test strips.

Observation table:

Pond	Crude	Hexane fraction	Ethyl acetate fraction	Butanol fraction	Fucosterol
1	3	3	1	5	3
2	3	3	1	5	2
3	3	3	1	5	3
4	3	2	0	5	3
5	3	4	2	5	3
Mean	**3.0**	**3.0**	**1.0**	**5.0**	**2.8**
Std. dev.	**0.000**	**0.707**	**0.707**	**0.000**	**0.447**
Std. error	**0.000**	**0.316**	**0.316**	**0.000**	**0.199**

Error bar	**3.0±0.000**	**3.0±0.316**	**1.0±0.316**	**5.0±0.000**	**2.8±0.199**

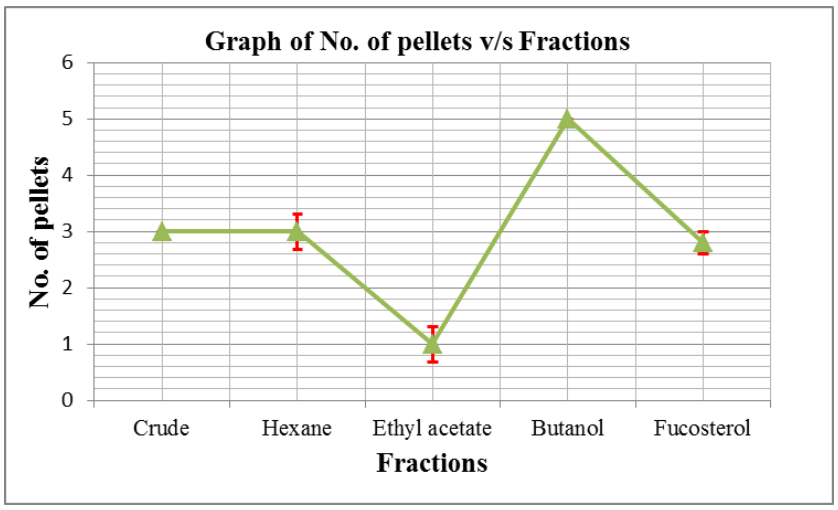

Figure 2. Error bar graph for field palatability assays.

2. Characterization data of Fucosetrol

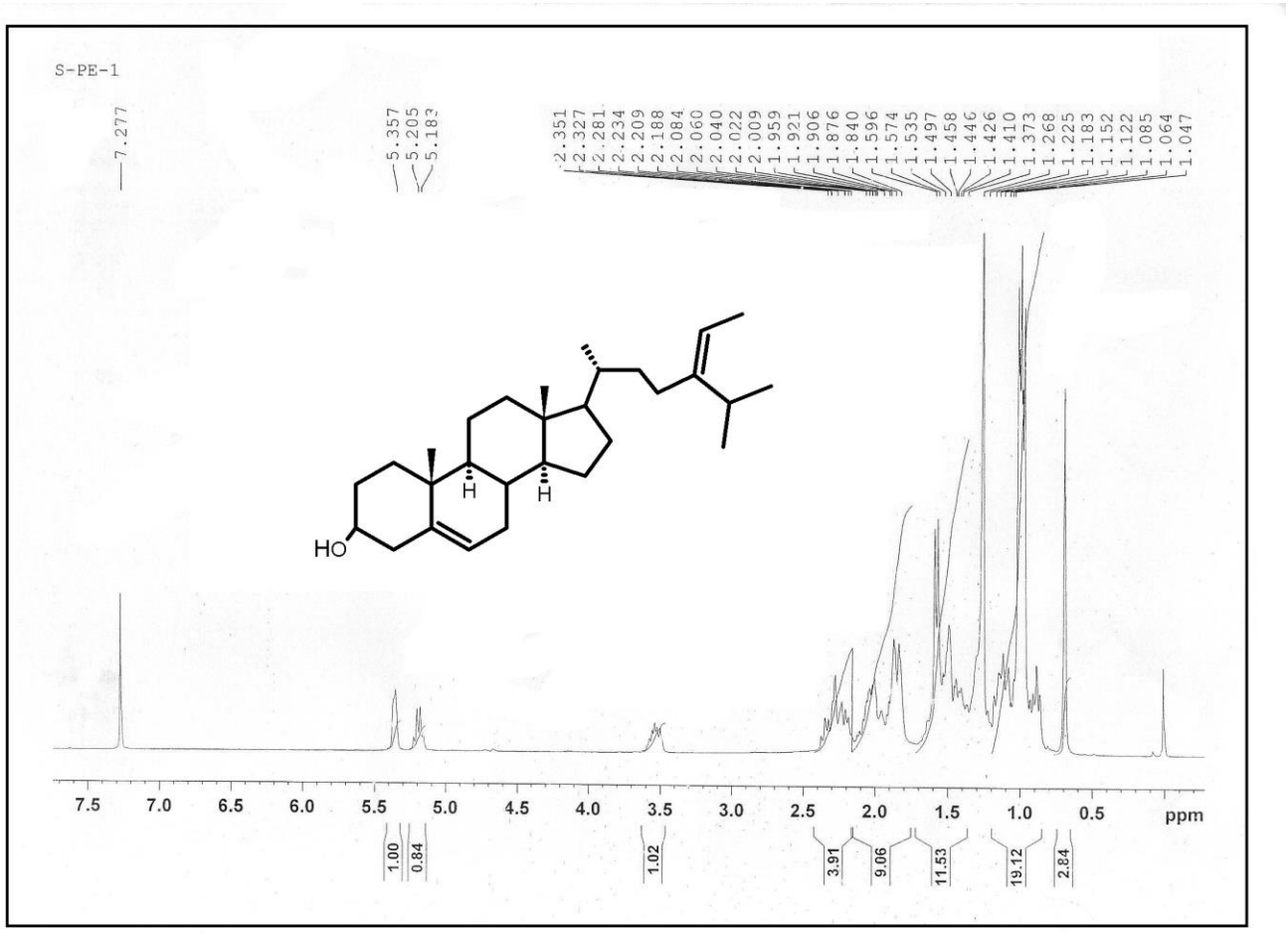

Figure S1. ^{1}H NMR ($CDCl_3$) spectrum of fucosterol 1

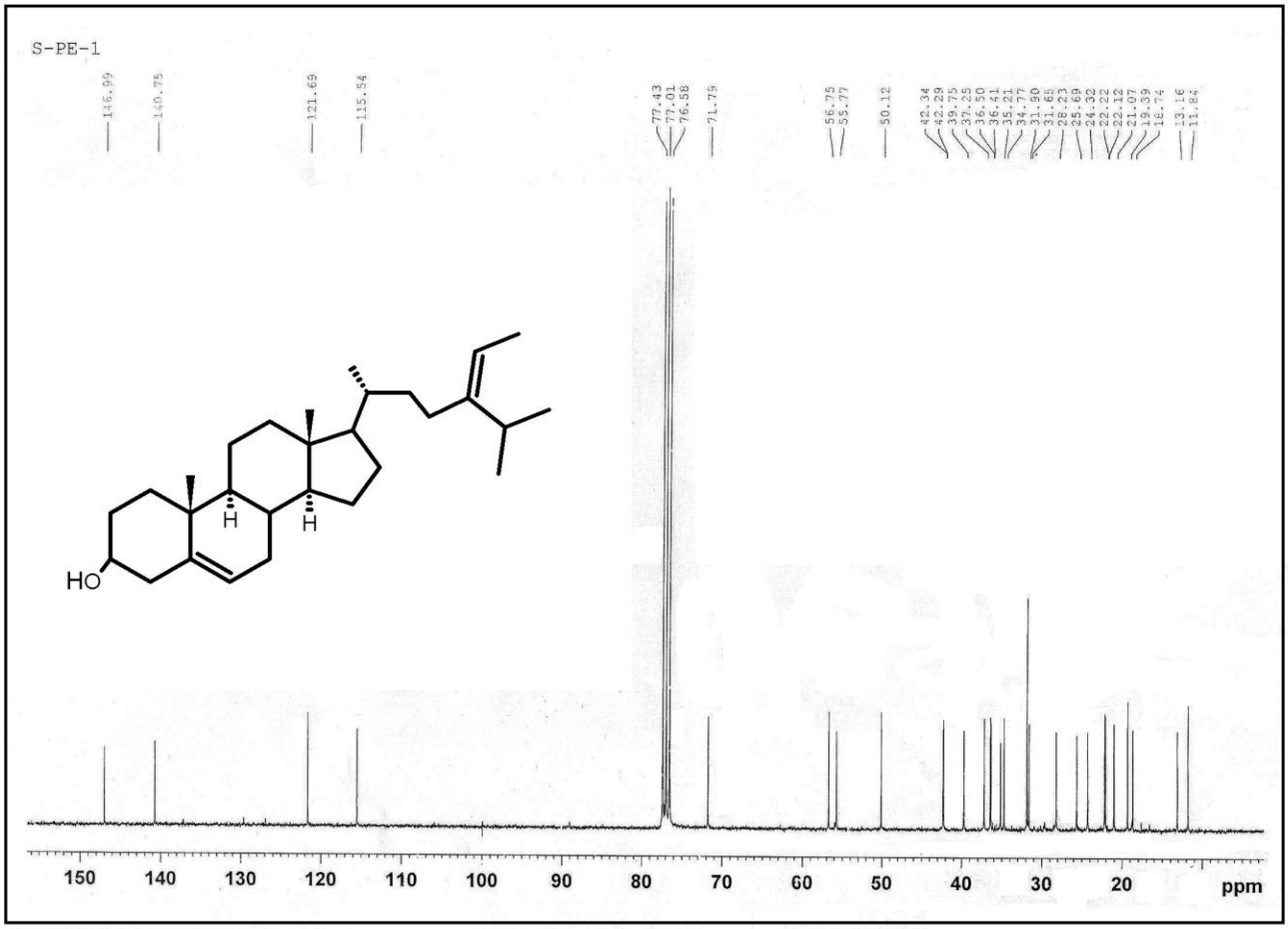

Figure S2. ^{13}C NMR ($CDCl_3$) spectrum of fucosterol 1

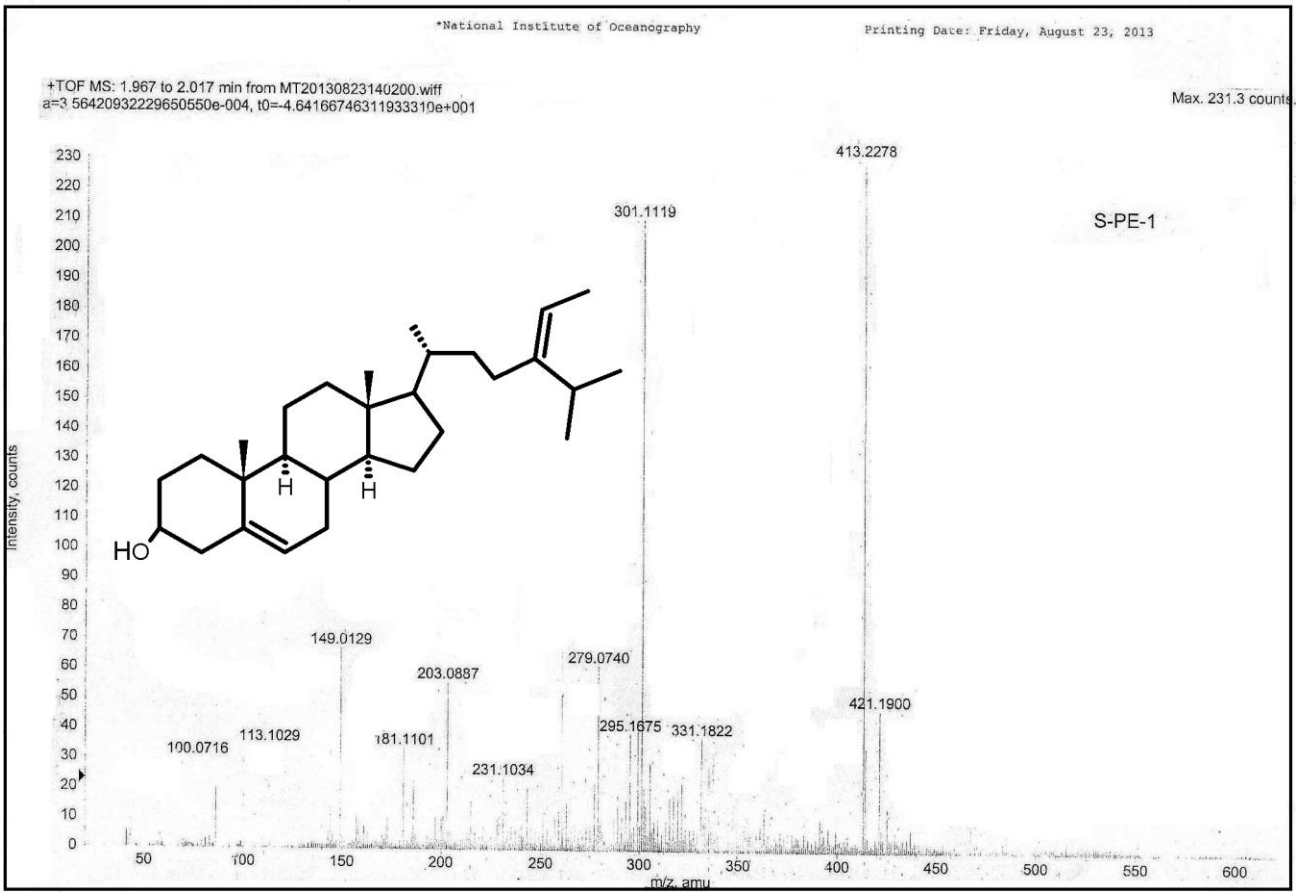

Figure S3. ESI-MS of fucosterol 1

References:

1 J. Pawlik, B. Channas, R. Toonen and W. Fenical, *Mar. Ecol. Prog. Ser.*, 1995, **127**, 183.

2 C. Peterson, and P. Renaud, *Oecologia.*,1989, **80**, 82.

3 J. Pawlik and W. Fenical, *Mar Ecol Prog Ser.*,1992, **87**, 183.