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# Comparative analysis of protein profiles of aqueous extracts from marine sponges and assessment of cytotoxicity on different mammalian cell types

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ABSTRACT

Marine natural products extracted from sponges represent a new source for drug discovery. Here we describe a simple method for preparing aqueous extracts from 7 Mediterranean demosponges, which allowed the extraction of water-soluble compounds, such as proteins by homogenization of sponge tissue in phosphate buffered saline (PBS).

The comparative analysis by SDS-PAGE showed differences in number of bands, bandwidth and intensity among the sponges analyzed. The PAS/silver staining revealed a substantial and different glycoprotein assortment among the demosponges studied.

To further study the biological activities present in the sponge extracts, we determined the non-cytotoxic doses on four different mammalian cell types demonstrating that the optimal non-cytotoxic doses were cell type- and extract-dependent.

In conclusion, the extraction method described in this paper represents a fast and efficient procedure for the extraction of water-soluble proteins from marine sponges. Furthermore, the cell viability data suggest the feasibility of this method for the direct *in vitro* cell-based assays.



## 1. Introduction

Several studies regarding the chemistry of molecules extracted from marine organisms have fully demonstrated that sponges provide the largest number of biologically active natural compounds (Leal et al., 2012). Over 60% of the potentially useful bioactive compounds discovered so far from living organisms have been obtained from marine fauna, 70% of

*Abbreviations:* BHK-21/C13, baby hamster kidney cells; BSA, bovine serum albumine; CaCo-2, human colon adenocarcinoma cells; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; GFAP, glial fibrillary acidic protein; MTT, 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide; PBS, phosphate buffered saline; PAS, periodic acid Schiff; PLL, poly-L-lysine; PMA, phorbol 12-myristate 13-acetate; RPMI, Roswell Park Memorial Institute; RT, room temperature; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; THP-1, human monocytic leukemia cell line.

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which comes from sponges (Faulkner, 2002). Indeed, Porifera, due to their typically sessile condition, have evolved the ability to produce potent toxins as defensive tools against predators or competitors (Loh and Pawlik, 2014). There is a worldwide interest in marine natural products since they represent one of the few *de novo* sources for drug discovery (Cragg and Newman, 2013). Moreover, it has been demonstrated that the biologically active molecules from marine organisms, possess unique pharmacological properties that are proving to be useful against cancer, AIDS, autoimmune and neurodegenerative diseases (Schmitz et al., 1993; Vinothkumar and Parameswaran, 2013).

Purification procedures of these compounds usually include initial extraction with methanol, partitioning of the extract with organic solvents and chromatographic steps (Blunt et al., 2014).

However, the majority of the organic solvents are toxic for cells or not well tolerated by some bioassays. Furthermore, these solvents tend to exclude water-soluble natural compounds.

In this regard, it has been recently reported that sponges represent a promising resource of water-soluble bioactive compounds, suggesting that the therapeutic potential of molecules isolated from sponges cannot be attributed only to the secondary metabolites extracted by organic solvents, but also to water-soluble molecules. In this respect, bioactive peptides with anticancer potential (Suarez-Jimenez et al., 2012) and lectines with antimicrobial activities (Schröder et al., 2003) have been recently isolated from marine sponges. In addition, other water-soluble molecules such as the chondropsins A and B, two macrolides with anticancer properties, and the 3-alkylpyridinium polymers (poly-APS), which present hemolytic and cytotoxic activities, have been extracted from the sponges *Chondropsis* sp. and *Haliclona sarai*, respectively (Cantrell et al., 2000; Sepčić et al., 1997a,b). Despite the fact that the class Demospongiae is known for producing the largest number and diversity of biologically active molecules among marine invertebrates (Leal et al., 2012), the bioactive potential of water-soluble compounds, in particular bioactive proteins, has been little studied (Wilkesman and Schröder, 2007).

On these grounds, in the present study we used a phosphate buffer in order to prepare aqueous extracts from 7 different Mediterranean demosponges. Some of the species chosen had been previously subjected to *ex situ* experimental rearing by this research group (Di Bari et al, in press).

The method described in this paper has proved to be effective for the extraction of water-soluble proteins. By using a panel of mammalian cultured adherent cells, we assessed the cytotoxicity of the sponge extracts, which was demonstrated to be cell type-dependent. Taken together our results indicated the feasibility of the proposed method for direct *in vitro* cell-based assays aimed at the screening of potential bioactivity of water-soluble compounds present in the aqueous extracts.

## 2. Materials and methods

### 2.1. Chemicals

Dulbecco's modified Eagle's medium (DMEM), RPMI 1640, fetal bovine serum (FBS), penicillin and streptomycin, L-glutamine were obtained from GIBCO (Paisley, Scotland). DNase 1, poly-L-lysine (PLL), trypsin, trypan blue, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), phorbol 12-myristate 13-acetate (PMA), Schiff's reagent and bovine serum albumine (BSA) were provided by Sigma (St. Louis, MO, USA). Glial fibrillary acidic protein (GFAP) antibodies were purchased from Serotec (Oxford, UK).

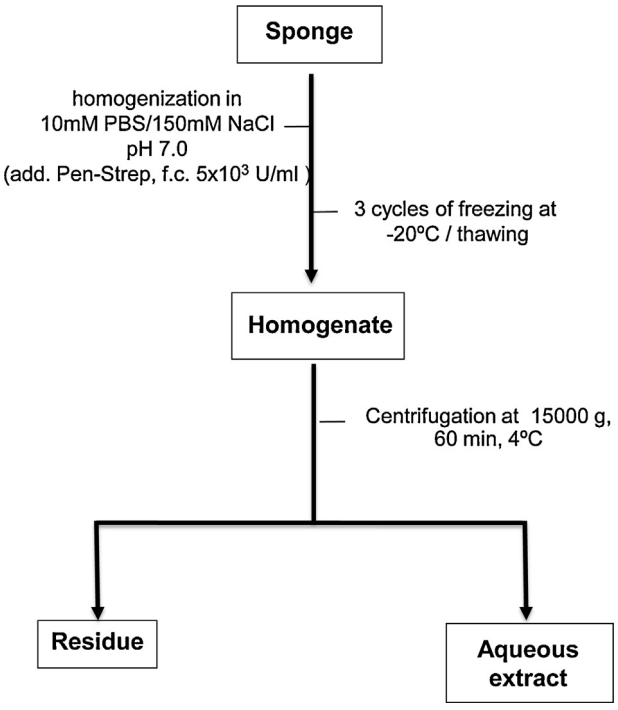


**Fig. 1 – Flowchart of the procedure used for the preparation of the aqueous extracts.**

### 2.2. Sponge collection

For our experiments seven different demosponges, commonly found in the Adriatic Sea, were collected. In particular, specimens from *Tethya aurantium*, *Tethya citrina*, *Hymeniacidon perlevis*, *Ircinia variabilis*, *Chondrilla nucula*, *Aplysina aerophoba* and *Sarcotragus spinosulus* were chosen.

Sponge species were collected by scuba diving in Southern Adriatic Sea, Italy, at depths between 1 and 3 m. Sponges were individually transferred to laboratory in bags filled with seawater and labeled for recognition. During the transport, the samples were protected against contact with air as well as other injuries and temperature was maintained around 18 °C. Once in laboratory, all sponges were numbered and listed with information like date of sampling and location, weighed and then frozen at −80 °C as soon as possible until the extraction.

### 2.3. Preparation of aqueous extracts

Fig. 1 shows a simplified flow-sheet of the procedure used for the preparation of aqueous extracts. Each sponge was homogenized in 10 mM PBS, 150 mM NaCl, pH 7.0 (1:4 w/v). Specimens from *T. aurantium* and *T. citrina* were grounded in PBS with