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DNA affinity screening of extracts tunicate *Cynthia squamulata* from the Atlantic coast

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In Memory of Professor M'hamed Charrouf (1949- 2014)

ABSTRACT

Four extracts of tunicate Cynthia squamulata from the atlantic coast were subject of DNA affinity screening, to identify potential antitumor activity. The assay uses a HPLC method for detecting the presence or absence of interactions between molecules of low molecular weight DNA. Also, this method based on the ability of antitumor agents to reduce DNA peak size. The results obtained in this work show that tow extracts from tunicate Cynthia squamulata (ether and methanol) were positive activities, contrariwise, the petroleum ether and chloroform extract showed negative peak reduction.

Keywords: DNA, extracts, *Cynthia squamulata*, antitumor activity.

INTRODUCTION

Tumor diseases are the second cause of human mortality by disease. All things being equal, this statistic is also true for the whole planet. [1] The causes of cancer are numerous but also increasingly well-known risk factors. [2] For a product to be a good archetype of antitumor agent, it must induce a destruction of tumor cells without affecting healthy cells. Researches on the etiology of cancer have not yet resulted in the development of effective models for the selection of biomolecules may specifically reach tumor cells. [3-5] Analysis of the known mechanisms of the cytotoxic action shows that in 70% of cases, the antitumor activity products act at the DNA or indirectly by inhibition of its biosynthesis, either by physical or chemical change in its structure. Generally, anti-tumor agents are classified into four main groups: alkylating agents, intercalating agents, antimetabolites and spindle poisons. [6-9]

One mechanism of action of different active substances involves interaction with DNA, several antitumor agents use this mechanism. Petuzzo et al. (1991) [10] developed a rapid and efficient method for preliminary determination of DNA interaction for pure compounds and crude extracts, the method uses a HPLC as an affinity probe, using the ability of binding compounds to reduce the size of the DNA peak. Optimization of this method was created by Ainane et al. (2011) [11] from which the test was performed on extracts of seaweeds.

In our work to implement a fast and cheap preliminary assay to identify potential antitumor of tunicate *Cynthia squamulata* from the atlantic cost of Morocco, four extracts obtained from this tunicate were screened.

MATERIALS AND METHODS

Extract Preparation

After harvesting the tunicate *Cynthia squamulata* in Oualidia (South of Eljadida -Morocco) in the period of low tide, It is washed with water. The identity of the tunicate was confirmed in the ecology laboratory of the Faculty of Sciences, Ben M'Sik, Casablanca (Morocco). A voucher specimen is deposited in the Biology Department, Faculty of Sciences, Ben M'Sik. The specimens, cut into small pieces, were placed in $CHCl_3$/EtOH (V/V) for extraction.

The $CHCl_3$/EtOH extract of freshly collected tunicate was filtered and the resulting filtrates were concentrated by evaporation under reduced pressure. The residual solution was then extracted successively with petroleum ether, ether, chloroform and methanol.

DNA affinity test

Calf Thymus DNA and Adriamycine were purchased from Acros organics. HPLC, The HPLC instrument used was a Agilent/HP 1100 Series, equipped with an absorbance UV detector (G1314A UV Detector), the column used was a ZORBAX RP-18 (5µm) 4.6 x 200 mm. DNA solution (0.1 mg/ml) were prepared in H_2O and divided in sterilized vials containing 1 ml of solution which were stored at -20 °C until use.

All extracts were dissolved in MeOH (1.0 mg/ml), solutions were centrifuged and filtrated to avoid injection of solids to the column. The mobile phase system used was H_2O – MeOH (8:2) during 5 minutes and gradient to pure MeOH in 5 minutes, rinse of the column for 20 minutes with MeOH before the next injection, equilibration time was 10 minutes, flow rate 1 ml/min and injection volume was 20 µl. Premixing of the sample with the DNA solution (v:v), (1:1), and incubation of the mixture at room temperature for 5-30 min. were carried on before injection. In these conditions free DNA eluted in approximately 1.8 min. In all experiments an injection of DNA alone coming from the same vial used to incubate the sample was made and the size of the eluted peak was used to compare with the one from the injection of the incubed mixture. All the samples were tested using the methodology described above, to increase the confiability of the procedure, extracts were tested three times. To confirm the efficiency of the method used, Adriamycine was used as positive control of the binding with DNA.

RESULTS AND DISCUSSION

After harvesting and extraction of extracts from *Cynthia squamulata*, four extracts are obtained with solvents of increasing polarity: petroleum ether, ether, chloroform and methanol. Once the extracts were obtained, it was determined their colors and returns relative to the initial amount of freshly tunicate. Data for samples obtained are given in the table 1.

Table 1: The different extracts of *Cynthia squamulata* with yield and color

Extract	Color	Yield (%)
Petroleum ether	Red	7.03
ether	Brown	1.57
chloroform	Green	0.90
methanol	Brown - yellow	4.31
Marc (*)	Brown	84

A solution of DNA (Calf thymus) in the water to 0.1 mg/mL was injected on a grafted silica C18 HPLC column packed with a mixture solvent of H_2O/MeOH (8:2). The peak corresponding to the DNA is detected by UV (254 nm) (Figure 1). The extracts tested and the standard (Adriamycin), are diluted in methanol (1 mg/mL) and co-injected with DNA (20 µL) were eluted for 10 minutes with eluent. By comparison of the areas of DNA peaks (before and after injection of the test product). We consider whether there is an interaction between the product and DNA. A decrease in the peak area reflects an interaction with DNA.

Petroleum ether and chloroform extracts show negative responses (Figure 2 and 4), ether extract produces 6% inhibition of DNA peak (Figure 3). Methanolic extract elicits a positive response, this extract is found to reduce 81% the DNA peak. (Figure 5)

Methanol extract produces a strong interaction with DNA, more than adriamycin (76%) and dichloromethane extract of the tunicate *Lissoclinum fragile* [12], but less than doxorubicin, which produces 100% inhibition of the DNA peak. [10] This result suggests that *Cynthia squamulata* is able to produce compounds that bind DNA.

On the other hand, we compared the results obtained in this study with the latter achieved with Abourriche et al. (1999) [13] on the tunicate *Cynthia savignyi*, hence, we have assembled the results in Table 2. It can be concluded

that the extracts have the same activities for both species, so the species can contain molecules in common who is responsible for the antitumor activity.

Table 2: Results of screening for DNA interaction of extracts

Cynthia squamulata (Present work)		*Cynthia savignyi* [13]	
Extract	DNA peak reduced (%)	Extract	DNA peak reduced (%)
Petroleum ether	0	Hexane	0
Ether	6	Ether	8
Chloroform	0	Methylene chloride	0
Methanol	81	Methanol	90

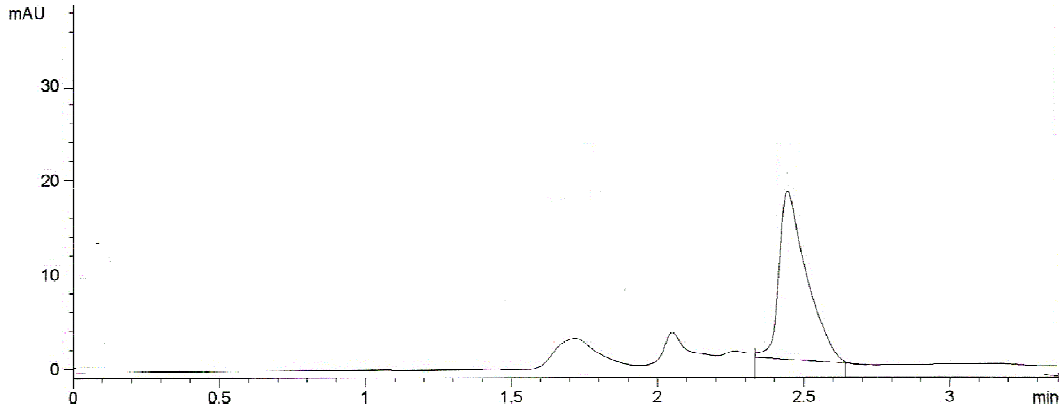

Figure 1: Chromatogram of DNA alone

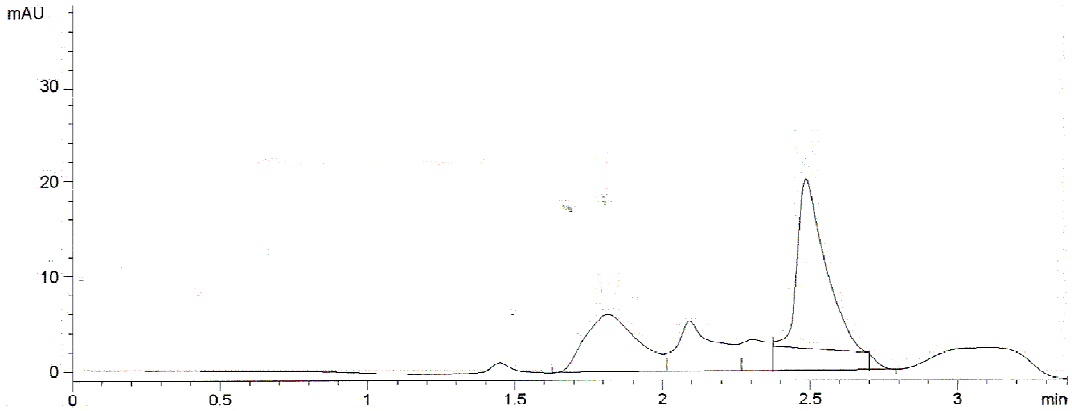

Figure 2: Chromatogram of DNA + Petroleum ether extract

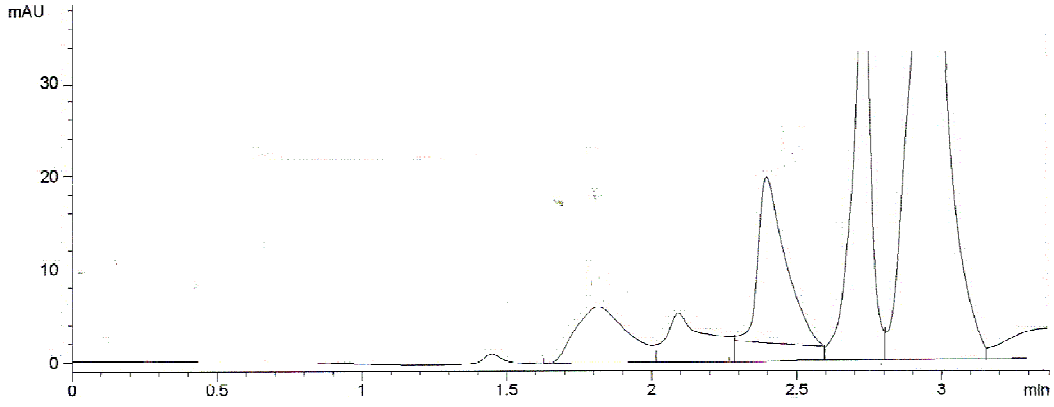

Figure 3: Chromatogram of DNA + Ether extract

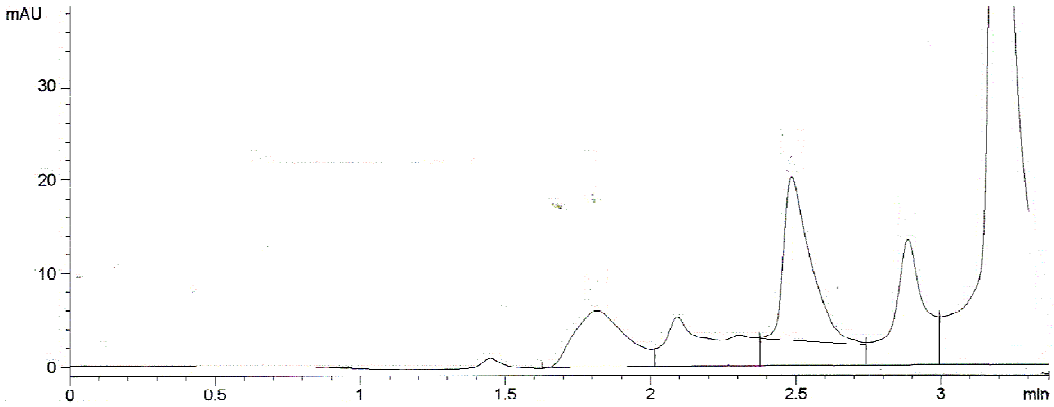

Figure 4: Chromatogram of DNA + chloroform extract

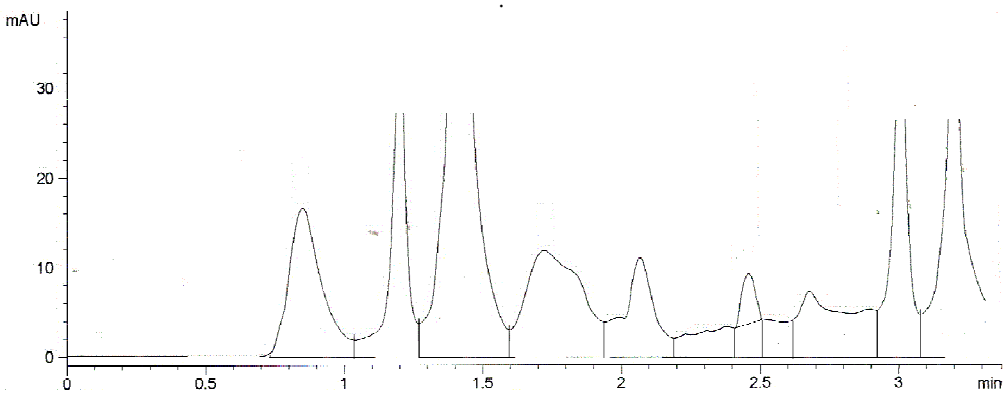

Figure 5: Chromatogram of DNA + Methanol extract.

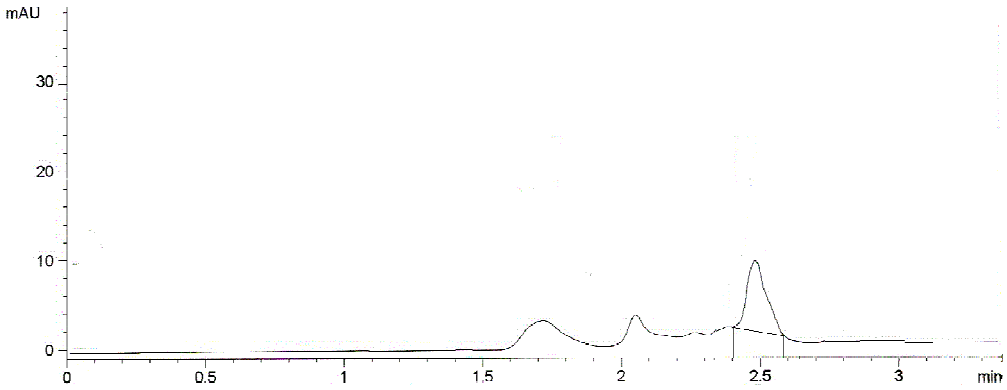

Figure 6: Chromatogram of DNA + Adriamycin

CONCLUSION

In this work we have made a simple and effective method using an HPLC system for measuring the antitumor activities of the extracts obtained from *Cynthia squamulata*. The results of this work show that the ether and methanol extracts of *Cynthia squamulata* provide a reduction of the area of the DNA, against the petroleum ether and chloroform extracts do not provide a reduction in DNA area. On the other hand, Adriamycin which is used as reference substance provides a reduction of the area of the DNA of the order of 76%.

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