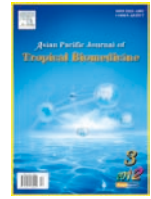




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## Anticoagulant activity of marine bivalve *Donax incarnates* Lin, 1758 Collected from Thazhanguda, Southeast coast of India

V. Karthikeyan A. Gopalakrishnan R. Vijayakumar, P. Bharathirajan

C A S in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai – 608 502 Tamil Nadu – India

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## ABSTRACT

**Objective:** Molluscs are highly delicious seafood and they are also very good source for biomedically imported products. Among the molluscs some have pronounced pharmacological activities or other properties which are useful in biomedical area. **Methods:** In the present study GAGs was isolated from the bivalve such as *Donax incarnates*. **Results:** The isolated GAGs were quantified in crude samples and they were estimated as 6.84 gm/kg crude GAGs in *Donax incarnates*. The bivalve showed the anticoagulant activity of the crude samples 124.53 USP units/mg in *Donax incarnates*. FTIR analysis reveals the presence of anticoagulant substance signals at different ranges. **Conclusions:** The determined in this research show that gastropod *Donax incarnates* tissue is value medicinal due to high quality of anticoagulant compounds.

### 1. Introduction

Anticoagulants have been widely used both clinically and in vitro medical treatments. In clinical practice, they are the drugs of choice for the prevention and treatment of thromboembolic disorders, and prophylaxis of thrombotic events both pre- and post-surgery. It is estimated that 2–4 patients out of 1,000 receive anticoagulant therapy [1, 2]. In the materials field, anticoagulants are used to improve the hemo-compatibility of medical devices and tissue engineering materials [3]. Recently, the concept of “vascular beautifying” has been promoted by the cosmetics industry [4]. Substances with anticoagulant activity are among the first choices as functional components which are being used to open up new areas of application for anticoagulants.

The Phylum Mollusca shows extensive species diversity and their bi-products have received much attention from the beginning of 20th century. Glycosaminoglycans (GAGs) have been isolated from tissues of a large number of vertebrate and invertebrate organisms. Invertebrates were first shown to contain a heparin or heparan sulfate

[5]. An exhaustive assessment showed that molluscs are particularly rich source of the sulfated polysaccharides [6] and it amounts to 90% of the total GAGs content of the molluscs. But the heparins isolated from molluscs are structurally different from human heparin and pharmaceutical heparins [7]. Among the molluscs, some have pronounced bio activities and useful in the biomedical arena. It is surprising that some of the bioactivities are attributed to the presence of polysaccharides, particularly those that are sulfated. Molluscan heparin contains antithrombin-dependent anticoagulant activity associated with the presence of unique 3-O-sulfated glycosamine residues in all anticoagulant heparin [8]. Although molluscan heparin is as significant as that obtained from commercial mammalian sources, much work has not been carried out on the isolation of glycosaminoglycans from marine mollusks. The aim of the present study was, to analyze extraction of crude GAGs, characterization and the anticoagulant activity from the tissue extracts of bivalve *Donax incarnates*.

### 2. Materials and method

#### 2.1. Isolation of the crude glycosaminoglycans

\*Corresponding author: V. Karthikeyan, Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai – 608 502 Tamil Nadu – India

Tel: 04144 – 243070–243071; Fax: 04144 = 243555

E-mail: karthikeyancansee@gmail.com

To dry defatted *Donax incarnates* bivalve sample, 0.4 M sodium sulfate (3.5 L kg<sup>-1</sup> of tissue) was added. The whole content was incubated in a water bath at 55°C for 1 h 30 min and was maintained at pH 11.5 using 10% NaOH solution. Then the pH of the solution was reduced to 7.7 using aluminium sulfate and was heated to 95°C for one hour. After the above process the solution was allowed to cool over night. Cetyl pyridinium chloride (CPC– 3% in 0.8 M NaCl) was added to the supernatant until a complete white precipitation of the complex appeared after incubation at 40°C for a period of 24 h. the sample was subjected to centrifuge at 3000 rpm for 90 min and thus the crude heparin complex was obtained. The precipitate was dissolved in 2 M NaCl at 40°C to dissociate CPC salt from heparin and 2 volumes of 95% methanol was used to precipitate the crude heparin.

## 2.2. Biological Anticoagulant activity assay

The anticoagulant activities of crude heparin samples were determined by comparing with the concentration necessary to prevent the clotting of sheep plasma using USP (United State Pharmacopoeia) method.

## 2.3. FTIR spectro photometry (Fourier Transform– Infra Red spectrum analysis)

The crude samples of *Donax incarnates* (10mg) was mixed with 100mg of dried potassium bromide (Kbr) and compressed to prepare as a salt disc. The disc was then read spectro photometrically (Bio– Rad FTIR–40– model, USA). The frequencies of different components present in each sample were analyzed.

## 3. Results

The amount of heparin–like glycosaminoglycans (Heparin complex) crude was estimated as 6.84 gm per kg of dry tissue in *Donax incarnates* (Table 1).

**Table 1.**

The yield of crude heparin and anticoagulant activity of (glycosaminoglycans) from *Donax incarnates*

S.No	Source	Net yield (gm/kg)	Anticoagulant activity USP units/mg
1	<i>Donax incarnates</i>	6.84	124.53

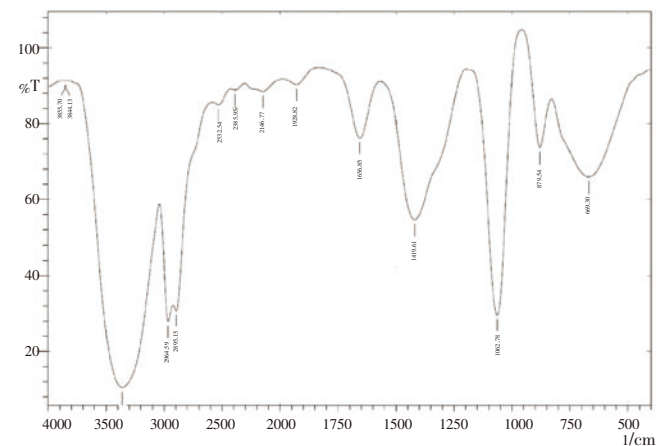
### 3.1. Anticoagulant activity

In the United States pharmacopoeia method, the anticoagulant activity of the *Donax incarnates* crude sample were reported to be 124.53 USP units/mg unites per mg (Table 1.).

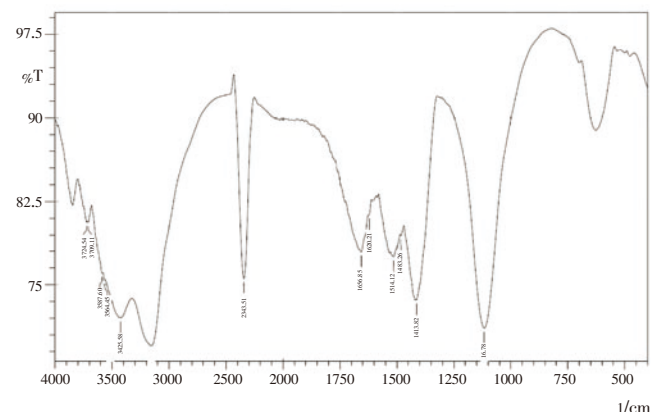
### 3.2. Fourier Transform – Infra Red (FTIR) spectral analysis

FT–IR spectrum of crude anticoagulant GAGs of *Donax incarnates* was obtained and compared with that of standard heparan sulfate (Fig. 1.2).

The IR spectrum of standard heparan sulfate contains 5 peaks at 3360, 2964.59, 2895.15, 1419.61 and 1062.78 cm<sup>-1</sup> (Fig. 1); whereas the IR spectrum of crude sample of GAG from *Donax incarnates* also recorded 5 major peaks 3425.58, 3157.47, 2343.51, 1413.82 and 1116.78 cm<sup>-1</sup>. In the IR spectrum of crude sample of *Donax incarnates* the following peaks each were obtained 1483.26, 1514.12, 1620.21, 1656.85, 3564.45, 3587.6, 3709.11, 3724.54 cm<sup>-1</sup> (Fig.2).



**Figure 1.** Showing the FTIR spectrum of standard heparin sulfate



**Figure 2.** Showing the FTIR spectrum of crude sample in *Donax incarnates*

## 4. Discussion

In the present investigation, the *Donax incarnates* recorded a crude heparin like substances yield of 6.84 g/kg. Previously [9] isolated heparin with an yield of 2.8g/kg and 3.8g/kg from *A. brasiliensis* and *T. mactroides* respectively. The total yield of the molluscan heparin product was almost similar to that of GAG obtained from giant African snails [8]. [10] Obtained 7.02 g/kg of heparin like substances in mollusk *K. opima*. [11] Reported the cephalopod animals as *Sepia aculeate* & *S. brevimana* showed higher net yield of the crude heparin–

like sulfated polysaccharides 21.79g /kg & 24.0 g / kg. [12] Reported that the isolated crude heparin like polysaccharide from cephalopods *Loligo duvauceli* & *Doriteuthis sibogae* were estimated as 16.5g/kg and 8.4g/kg respectively. [13] had quantified the heparin yield as 2.72 g/kg and 2.2 g/kg from *Tridacna maxima* and *Perna viridis* respectively. [14] Reported that the isolated glycosaminoglycans (GAG) from bivalves were estimated as 5.4g/kg wet tissue in *K. opima* and 4.1g/kg wet tissue in *D. cuneatus*. [24] reported that the amount of crude GAG extracted was estimated as 17.2g/kg of tissue in *A. pleuronectus*. [17] reported that the amount of crude anticoagulant GAGs was estimated as 9.85 gm/kg of *M. casta*. The results suggest that *Donax incarnates* are promising source of GAG.

The heparin isolated from marine clams and mussels has identical structural features and anticoagulant activity to the mammalian polysaccharide [15]. Heparin with high anticoagulant activity was isolated from the marine mollusks *A. brasiliensis*, *D. striatus* and *T. mactroides* [9, 15] which showed similar activity like mammalian heparin but differs in molecular weight. In the present investigation, the *Donax incarnates* reported an anticoagulant activity of the crude sample was 124.53 USP units / mg. The marine molluscs, *Spisula solidissima* and *Cyprina islandica* showed anticoagulant activity ranging from 70 to 120 USP units / mg [16]. [5] Demonstrated activity ranging from 130 to 150 USP units / mg of the extracted products of the species *Spisula solidissima* and *Cyprina islandica*. [9] Recorded 320 USP units / mg of anticoagulant activity in *A. brasiliensis* whereas, 220 USP units / mg in *T. mactroides* and 180 USP units / mg in *D. stioiatus*. [11] Reported the anticoagulant activity of the crude sample of *S. aculeata* 376.98 USP units / mg and *S. brevimana* 421.72 USP units / mg. [12] Reported the activity of the crude sample of *L. duvauceli* was 376.98 USP units / mg and *D. sibogae* was 376.98 USP units / mg. [13] Recorded the anticoagulant activity of the crude sample of *T. maxima* 7.4 USP units / mg and *P. viridis* 4.3 USP units / mg. [25] reported that the anticoagulant activity of crude sample of GAG from *Amussium pleuronectus* –15.38USP units/mg (sheep blood). [26] reported that the crude sample of *Euprymna berryi* –415 USP units/mg. [14] Showed the anticoagulant activity was 160 USP units / mg and 154 USP units / mg in *Ketalysia opima* and *D. cuneatus*. [17] Reported the anticoagulant activity of the crude sample of *Meretrix casta* (sheep blood 22.52 USP units / mg) (chicken blood 20.00 USP units / mg) and (human blood 18.60 USP units / mg).

In the present study, FT-IR spectrum of crude anticoagulant GAGs of *Donax incarnates* was obtained and compared with that of standard heparan sulfate and the anticoagulant GAGs from body tissue of *Donax incarnates* crude sample showed a major peaks at 1656.85, cm<sup>-1</sup> which is said to be responsible for the same GAGs group. Similar result was reported previously, assignment of IR absorption bands and 1240 cm<sup>-1</sup> [18] and 1430 cm<sup>-1</sup> in the spectrum of fully o-sulphonated HA were based on the reports by [19] and

bands in the 820–850 cm<sup>-1</sup> spectral region were attributed to C–O–S stretching based on the results of [20], as also observed by [21] in these species of molluscs. The most striking characteristic feature of the spectra of GAGs is a band at 1258.56 cm<sup>-1</sup>, which represents the sulfate groups as also reported at 1257.08 cm<sup>-1</sup> which determined the activity of heparin. Similarity in the band pattern between the standard heparin and the sample was the presence of one definite band at 1656.85 cm<sup>-1</sup> in crude sample and at 1656.85 cm<sup>-1</sup> is standard heparin indicating the presence of GAGs group in the samples analyzed. The determined in this research show that bivalve *Donax incarnates* muscle is medicinal due to high quality of anticoagulant compounds.

The classes of crustacean and mollusca do not possess any blood coagulation system similar to that of mammals and other vertebrates and thus the presence of compounds that act specifically upon the proteins of the blood coagulation system is indeed remarkable. It has been previously speculated that mast cells and their heparin may serve as modulators of the immune reactions or other body defense mechanisms and thus the action of heparin upon the coagulation cascade, due to its strong anionic character, may be a pharmacological rather than a biological activity [23]. The determined in this research show that bivalve *Donax incarnates* body muscle of crude sample is value drug due to high quantity GAGs, well-balanced anticoagulant activity. The evidence presented in the preceding sections reveals that molluscan glycosaminoglycan possesses diver's pharmacological properties like anticoagulant activities. Hence, based on the above, it could be concluded that the heparin like substance of *Donax incarnates* may be used as an alternative to the mammalian heparin and *Donax incarnates* be a potential source for the heparin-like substances.

The present study was revealed that species *Donax incarnates* showed more anticoagulant activity. They represent potential pharmacological leads perhaps possessing novel and uncharacterized mechanisms of action that might ultimately benefit the ongoing global search for clinically useful anticoagulant compounds.

### Conflict of interest statement

We declare that we have no conflict of interest

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