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Screening of antiangiogenic potential of twenty two marine invertebrate extracts of phylum Mollusca from South East Coast of India

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PEER REVIEW

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Comments

This is a valuable research work in which authors have demonstrated the anti-angiogenic property of marine invertebrates *in ovo* angiogenesis (CAM assay) model and ocular angiogenesis experimental model in rats. The activity was accessed based on the extent of inhibiting new vessel formation induced by VEGF, chemical injury and hyperoxia. Out of 22 extracts studied, *Telescopium telescopium* was found to be a potential candidate for further development for ocular angiogenesis.
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ABSTRACT

Objective: To evaluate the antiangiogenic potential of twenty two marine invertebrate species of Phylum Mollusca from south east coast of India.
Methods: Live specimens of molluscan species were collected and their methanolic extracts were evaluated for preliminary antiangiogenic activity using the *in ovo* chick chorio-allantoic membrane assay. The extracts were further evaluated for *in vivo* antiangiogenic activity using chemical cautery induced corneal neovascularization assay in rats and oxygen induced retinopathy assay in rat pups.
Results: In the chick chorio-allantoic membrane assay, four methanolic extracts of marine molluscan species *viz. Meretrix meretrix, Meretrix casta, Telescopium telescopium* and *Bursa crumena* methanolic extracts exhibited noticeable antiangiogenic activity at the tested concentration of 200 μg whereby they significantly inhibited the VEGF induced proliferation of new blood vessels. Among these four extracts, the methanolic extract of *Meretrix casta* exhibited relatively higher degree of antiangiogenic activity with an inhibitiory percentage (64.63%) of the VEGF induced neovascularization followed by the methanolic extracts of *Telescopium telescopium* (62.02%), *Bursa crumena* (60.48%) and *Meretrix meretrix* (47.01%). These four methanolic extracts were further evaluated for *in vivo* antiangiogenic activity whereby the methanolic extract of *Telescopium telescopium* exhibited most noticeable inhibition (42.58%) of the corneal neovascularization in rats in comparison to the sham treated group, and also exhibited most noticeable inhibition (31.31%) of the oxygen induced retinal neovascularization in rat pups in comparison to the hyperoxia group that was observed for considerable retinal neovascularization.
Conclusions: The significant antiangiogenic activity evinced by the extract of *Telescopium telescopium* merits further investigation for ocular neovascular diseases.

KEYWORDS
Marine invertebrate, Mollusca, Antiangiogenic, CAM, Cautery, Retinopathy

1. Introduction

Among the 34 fundamental phyla of life, 17 have been reported on land while 32 of them are found in the sea (with some overlaps) and among these, the marine organisms represent a majorly unexplored domain, having highest

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chances for the identification of compounds with higher potency and novel biological activities[1]. This has been shown in the recent past as much attention has been given to the approach of isolating novel chemical structures and compounds from marine natural products (MNPs) whereby MNPs bioprospecting has yielded a considerable number of drug candidates with potent anticancer properties with some of these possessing novel mechanisms of action[2]. Other than anticancer agents, many therapeutically successful drugs like lipid lowering agents, immuno-suppressants, antifungals, antivirals, anti-inflammatory, analgesic, anti-malarial, anti-HIV, *etc.* have been obtained from marine sources.

Marine invertebrates constitute one of the major groups of marine organisms from which a wide range of medicinal benefits have been devised in addition to the large numbers of MNPs that have been discovered till date[3]. Seafood diet from edible marine invertebrates such as molluscs has been linked with various medicinal benefits to improve human health[4]. Throughout history, molluscs have provided a wide range of human resources, including food, shells, dyes and medicines. In many cultures, shelled gastropods and bivalves are regarded as a delicacy or healthy food and they also feature in a range of traditional natural remedies[5]. In most cases, there has been no scientific research undertaken to substantiate the health benefits of molluscs. However, there is increasing interest in the bioactivity of mollusc extracts and secondary metabolites. Currently, natural products isolated from molluscs are particularly well represented in the anticancer compounds in clinical trials *e.g.* elisidepsin, a novel marine-derived cyclic peptide belonging to the Kahalalide family of compounds currently under phase II development with preliminary evidence of antitumor activity[6].

The formation of new blood vessels out of pre-existing capillaries or angiogenesis is a sequence of events that is of key importance in a broad array of physiologic and pathologic processes. In several diseases, excessive angiogenesis is a part of the pathology. These diseases include cancer (both solid and hematologic tumors[7], cardiovascular disease (atherosclerosis)[8], chronic inflammation (rheumatoid arthritis, Crohn's disease), diabetes (proliferative diabetic retinopathy)[9], neovascular wet age related macular degeneration[10], retinopathy of prematurity[11], psoriasis[12] and AIDS complications[13]. These diseases may benefit from the therapeutic inhibition of angiogenesis. A growing tumor needs an extensive network of capillaries to provide nutrients and oxygen to the body tissues. In addition, the new intratumoral blood vessels provide a way for tumor cells to enter the circulation and to metastasize to distant organs. Thus, every organ system may involve diseases in which angiogenesis is an important component. Several compounds from marine sources are under clinical trials and have been shown to possess potent angiostatic effect in the pre-clinical phases *e.g.* squalamine[14]. In recent years, many bioactive compounds have been isolated from cone snails, soft corals, sponges, sea squirts, marine worms, bryozoans, sea slug, sharks and other marine organisms and among these, shark cartilage have been recognized as an important source of bioactive compounds having antiangiogenic potential.

As marine organisms from Indian seas are considered as potential sources of bioactive molecules, this study was undertaken for the first time to explore the antiangiogenic properties of the methanolic extracts of various species of marine invertebrates from phylum Mollusca using *in ovo* chick chorio-allantoic membrane (CAM) assay. The methanolic extracts that showed noticeable *in ovo* antiangiogenic activity were further evaluated for *in vivo* antiangiogenic activity using chemical cautery induced corneal neovascularization assay in rats and oxygen induced retinopathy in rat pups.

2. Materials and methods

2.1. Drugs and chemicals

VEGF, bryostatin 1 and dolastatin 15 were purchased from Sigma-Aldrich, USA. Squalamax (natural shark squalamine extract) was purchased from Nu Gen, USA. Thalidomide was purchased from Natco pharma, Hyderabad. Silver nitrate and potassium nitrate were purchased from Qualigens, Mumbai. Bevacizumab was obtained from Genentech, USA. All the other reagents were of analytical grade and were used without further purification.

All study protocols were approved by standing Institutional Animal Ethics Committee (IAEC) of All India Institute of Medical Sciences (AIIMS), New Delhi, India. All animal experiments were done in accordance with guidelines of Association for Research in Vision and Ophthalmology.

2.2. Collection and identification of marine invertebrates

Live specimens of twenty two marine invertebrates of phylum Mollusca were collected from Cuddalore coastal