



Received on 21 February 2023; received in revised form, 27 April 2023; accepted, 31 May 2023; published 01 October 2023

ANTIBACTERIAL AND ANTIMYCOBACTERIAL PROPERTY OF CRUDE EXTRACT OF BOX JELLY FISH *CHIROPSOIDES BUITENDIJKI* (HORST R, 1907) FROM WEST COAST OF MUMBAI

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Keywords:

Jelly fish, Edwan village, Crude extract, Antibacterial, Antimycobacterial property

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ABSTRACT: The Box jelly fish *Chiropsoides buitendijki* (Horst R, 1907) were collected during low tides from Edwan village, of West Coast of Mumbai. Identification was done at the Central Marine Fisheries Research Institute (CMFRI), Mumbai. Crude extract of *Chiropsoides buitendijki* (Horst R, 1907) was obtained following the 80% methanol and 1% acetic acid, absolute methanol and n-butanol (1:1), and n-butanol. The antibacterial activity was carried out on bacteria *E. coli*, *K. pneumonia*, *S. aureus*, *S. flexneri*, *V. Cholerae*, *C. sporogenes*, *S. enterica*, *E. faecalis*, and fungal strain *Candida albicans* and *Aspergillus niger* whereas the antimycobacterial study was carried out on *Mycobacterium tuberculosis* (MTB) strain, H37 Rv: ATCC No- 27294. From the above study it is confirms that, the crude extract of *Chiropsoides buitendijki* have antibacterial and anti- tuberculosis property.

INTRODUCTION: Many studies investigated bioactive compounds from marine animals for various purposes including anti-bacterial, anti-tumor, anti-inflammatory, nutritional supplements, discovery of novel drugs and its applications for industrial biotechnology¹. Several marine bioactive compounds are of great benefit to pharmaceutical industries to prepare human medicines including cancer therapy, vascular diseases and infectious viral diseases such as AIDS². Jelly fish belongs to a group of free-swimming animals that belongs to the phylum Cnidaria. Cnidarians have special cells called cnidocytes which gives the name to the phylum Cnidarians.

The world fishery for edible jellyfish, which is mostly based in Southeast Asia, produces an annual catch of more than 750,000 tones, with rising demand reaching outside of Asian markets. Jellyfish appear to have qualities of a nutritious diet and are known for their nutritional and medicinal significance in the Chinese pharmacopoeia. Certain Cnidaria jellyfish species from the Mediterranean can serve as an affordable source of raw materials for cutting-edge pharmaceutical, nutraceutical, or cosmetic goods.

Because of their high collagen content and protein content, jellyfish have just been recognized in Europe as novel foods. Jellyfish proteins and other isolated substances have significant antioxidant and other biological properties. Many studies are found on cnidocytes for several purposes, but very scanty study is available on their toxic potential³. However, neurotoxin is found in the ectodermal gland cells⁴.

	<p style="text-align: center;">DOI: 10.13040/IJPSR.0975-8232.14(10).4918-23</p>
	<p style="text-align: center;">This article can be accessed online on www.ijpsr.com</p>
<p>DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(10).4918-23</p>	

Therefore, extensive study is required not only on the nematocyst but also the whole body of jelly fish⁵. Jellyfish stings can cause a burning sensation, excruciating pain, swelling, red streaks, nausea, cramps in the stomach, excessive perspiration, respiratory trouble, heart failure, and other symptoms⁶. In line with this, jellyfish venoms exhibit a diverse range of biological effects, including dermonecrotic, neurotoxic, hemolytic, and cardiovascular effects^{7,8}. Acute heart failure is acknowledged as the primary cause of mortality brought on by jellyfish venoms, and it is thought that the effects of jellyfish venoms are brought on by the combination of numerous poisonous components^{9,10}. Countless number of people is found to be poisoned by jelly fish every year throughout the world, but statistical data is rarely available, even though the fatal cases are not available in this regard. Many research showed the regime for the treatment against cnidarian stings, but confusion still exists as to what is the most effective first aid and clinical management against cnidarians toxin. Significant research is available for the treatment on cnidarian stings, but only a few toxic components have been identified so far, it is one of the most neglected areas in toxicology research. This is most likely due to the difficulty in obtaining jellyfish, which, in contrast to other venomous invertebrates, are widely dispersed in the open ocean. Second, unlike the milking of snake venom, it is very challenging to gather high-purity jellyfish venom without contaminating it with other tissue detritus. Lastly, because all of the poisons discovered from jellyfish venoms up to this point are proteins, many of which are innately vulnerable to harsh environmental conditions and readily denatured, it is necessary to identify the dangerous components found in jellyfish.

MATERIALS AND METHODS:

Collection of Samples: The Box jelly fish *Chiropsoides buitendijki* (Horst, R 1907) were collected during low tides from Edwan village, of West Coast of Mumbai. Animals were taken alive to the laboratory in sea water washed under sea water and then with distilled water.

Identification of Box Jelly Fish: Preliminary identification was done by studying the shape and no. of tentacles and by referring the relevant literature and final confirmation of identification

was done by Dr. Ramkumar, scientist, at the Central Marine Fisheries Research Institute (CMFRI), Mumbai.

Preparation of Box Jelly Fish Crude Extracts:

Crude extract of *Chiropsoides buitendijki* (Horst, R 1907) was obtained following the 80% methanol and 1% acetic acid, absolute methanol and n-butanol (1:1), and n-butanol by applying method of¹¹ with some modifications. 10 grams of Box jelly fish samples; 10 ml mixture of equal volume of 80 % methanol and 1% acetic acid, 10 ml of absolute methanol and n-butanol (1:1) and 10 ml of n-butanol was added and kept standing for 24 hrs. Solvent were then removed, by squeezing Box jelly fish samples, and filtered through Whatman filter paper No.1. The homogenate centrifuged at 5000 rpm for 15 minutes in cold centrifuge at -8°C and the supernatant was collected. The remaining solvent was evaporated at low pressure using Rotary Vacuum Evaporator at 45°C. The resultant compound was subjected to Millipore filter system and finally dried in vacuum desiccators and stored at 4°C in a refrigerator till further use for antibacterial and antimycobacterial study.

Ethical Approval: Ethical approval is sought from Maharashtra State Biodiversity Board, Nagpur, Maharashtra for collection of Box jelly fish samples for research purpose (No.:MSBB/Desk-5/ /Research/ 841/2022-23).

Procurement of Bacterial Cultures: The pure culture of bacteria *E. coli*, *K. pneumoniae*, *S. aureus*, *S. flexneri*, *V. cholerae*, *C. sporogenes*, *S. enterica*, *E. faecalis* and fungal strain *Candida albicans* and *Aspergillus niger* were collected from APX laboratories, Plot No. B7/1, Kothari Warehouse No.3, S. V. Road, Chitalsar, Manpada, Thane west-607, Mumbai, India and *Mycobacterium tuberculosis* (MTB) strain, H37 Rv: ATCC No- 27294 was procured from Maratha Mandal's Central Research Laboratory, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, R.S.No. 47A/2, Bauxite Road, Belgaum-590010, India.

Antibacterial and Antimycobacterial Study: The antibacterial study was carried out by using bacteria seeded plate –Well method as proposed by¹² and the anti-Mycobacterial activity of crude

extracts of *Chiropsoides buitendijki* (Horst, R 1907) were assessed against *M. tuberculosis* strain H37 Rv: ATCC No- 27294 using microplate alamar blue assay (MABA) as proposed by¹³.

RESULTS AND DISCUSSION:

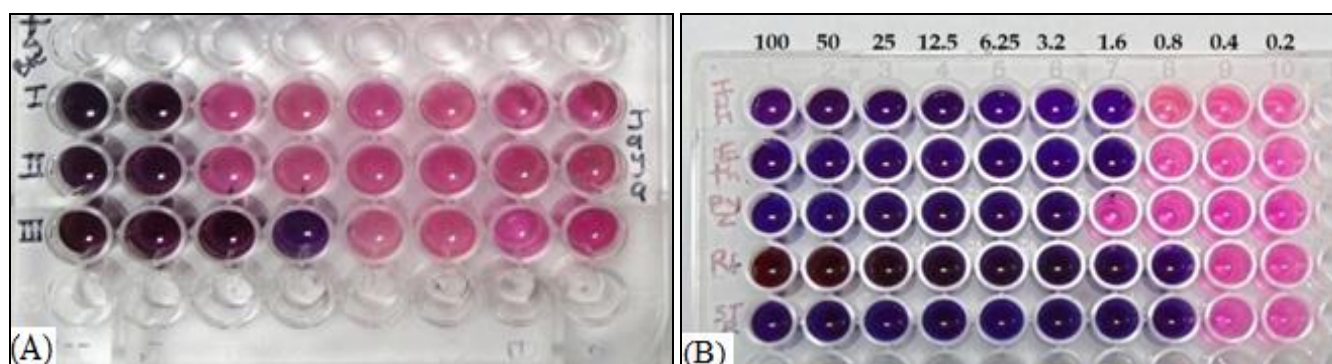
TABLE 1: SHOWING EFFECT OF CRUDE EXTRACT OF *CHIROPSONIDES BUITENDIJKI* (HORST, R 1907) IN DIFFERENT ORGANIC MIXTURES ON DIFFERENT BACTERIAL AND FUNGAL STRAINS IS IN THE FORM OF ZONE OF INHIBITION

Sr. no.	Microbes	80 % methanol and 1%acetic acid	absolute methanol and n-butanol (1:1)	n-butanol
1	<i>E. coli</i>	---	---	8.3mm
2	<i>K. pneumoniae</i>	---	---	9.0mm
3	<i>S. aureus</i>	---	---	9.83mm
4	<i>S. flexneri</i>	---	8.16mm	9.16mm
5	<i>V. cholerae</i>	---	19.44mm	19.34mm
6	<i>C. sporogenes</i>	---	20.17mm	20.26mm
7	<i>S. enterica</i>	---	36.19mm	40.03mm
8	<i>E. faecalis</i>	---	14.31mm	25.23mm
9	<i>Candida albicans</i>	---	---	---
10	<i>Aspergillus niger</i>	---	---	---

TABLE 2: SHOWING EFFECT OF CRUDE EXTRACT OF *CHIROPSONIDES BUITENDIJKI* (HORST, R1907) IN DIFFERENT ORGANIC MIXTURES AND STANDARD DRUGS ON *M. TUBERCULOSIS* STRAIN H37 RV: ATCC NO- 27294 USING MICROPLATE ALAMAR BLUE ASSAY (MABA)

Sr. no.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
1	n-Butanol crude extract	S	S	R	R	R	R	R	R	R	R
2	Absolute Methanol + n-Butanol (1:1)	S	S	R	R	R	R	R	R	R	R
3	8% Methanol + 1% acetic acid	S	S	S	S	R	R	R	R	R	R
4	Isoniazid	S	S	S	S	S	S	S	R	R	R
5	Ethambutol	S	S	S	S	S	S	S	R	R	R
6	Pyrazinamide	S	S	S	S	S	S	R	R	R	R
7	Rifampicin	S	S	S	S	S	S	S	S	R	R
8	Streptomycin	S	S	S	S	S	S	S	S	R	R

* S= Sensitive *R=Resistant



PHOTOGRAPH 1: PHOTOGRAPH SHOWING, A) EFFECT OF CRUDE EXTRACT OF *CHIROPSONIDES BUITENDIJKI* IN DIFFERENT ORGANIC MIXTURES B) STANDARD DRUG CONCENTRATIONS ON *M. TUBERCULOSIS* STRAIN H37 RV: ATCC

DISCUSSION: Box jelly fish *Chiropsoides buitendijki* (Horst, R1907) belonging to the phylum cnidaria and class cubozoa have been reported for its multitudinous medicinal values. The study carried out by¹⁴ showed antibacterial activity against toxin of *Anemonia sulcata*. In another study

¹⁵ have isolated the neurotoxin Ueq 12-1 from *Anemonia sulcata* and *Urticina eques* showed *Corynebacterium glutamicum*, and *Staphylococcus aureus* bacterial inhibition of human pathogens¹⁶. studied on cytolytic actinoprin, cardio stimulatory proteins and cytolysins isolated from sea

anemones; *Heteractis magnifica* and *Stichodactyla mertensii* showed higher antibacterial potentials against *Staphylococcus aureus* and *Salmonella typhi*. According to¹⁷ sea anemone neurotoxin was found effective against *Bacillus subtilis* at minimal effective concentration and showed moderate antibacterial potency against *E. coli* and *Salmonella enteric*¹⁸. studied the methanolic extract of nematocysts of *Stichodactyla mertensii* and *Stichodactyla gigantean* showed antibacterial property against *Staphylococcus aureus*, *Salmonella typhi* and *Vibrio cholera*¹⁹. Isolated the bioactive compounds of *Leptogorgia virgulata* of the family Gorgoniidae showed antibacterial property. According to²⁰ the active component homarine and a homarine analog showed inhibited growth of *Vibrio harveyi* and *Micrococcus luteus*, indicating antibacterial activity²¹. Isolated mesoglea of jellyfish *Aurelia aurita* exhibited higher activity against Gram- negative and Gram-positive bacteria further²². Isolated the venom from jelly fish *Chrysaora quinquecirrha* found moderately effective on human pathogens (*Escherichia coli*, *Vibrio cholerae*, *Salmonella paratyphi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Proteus vulgaris* and *Klebsiella oxytoca*), whereas *Salmonella paratyphi* is found more sensitive against venom of *Chrysaora quinquecirrha*.

The present study was undertaken to examine the effect of crude extract of *Chiropsoides buitendijki* prepared in different organic solvents, 80% methanol and 1% acetic acid, absolute methanol and n-butanol (1:1), and n-butanol to find antibacterial property. The crude extract of *Chiropsoides buitendijki* was also tested on standard drugs Isoniazid, Ethambutol, Pyrazinamide, Rifampicin, and Streptomycin against *Mycobacterium tuberculosis* (MTB) strain, H37 Rv: ATCC No- 27294 to find the antimycobacterial property. From **Table 1** Showing effect of crude extract of *Chiropsoides buitendijki* (Horst, R 1907) in different organic mixtures on different bacterial and fungal strains is in the form of zone of inhibition. It was observed that in 80 % methanol and 1% acetic acid against all the bacterial and fungal strains no zone of inhibition was observed. In case of absolute methanol and n-butanol (1:1), the zones of inhibitions were observed against *S. flexneri* (8.16mm), *V. cholerae* (19.44mm), *C. sporogenes*

(20.17mm), *S. enterica* (36.19mm), and *E. faecalis* (14.31mm) respectively. In n-butanol the zone of inhibition was observed in *E. coli* (8.3mm), *K. pneumoniae* (9.0mm), *S. aureus* (9.83mm), *S. flexneri* (9.16mm), *V. cholerae* (19.34mm), *C. sporogenes* (20.26mm), *S. enterica* (40.03mm), and *E. faecalis* (25.23mm) respectively. In case of fungal strains *Candida albicans* and *Aspergillus niger* no zone of inhibitions was noted in different organic mixtures. **Table 2** Showing effect of crude extract of *Chiropsoides buitendijki* in different organic mixtures and standard drugs on *M. tuberculosis* strain H37 Rv: ATCC No- 27294 using microplate alamar blue assay (MABA) and Photograph: 1, Photograph Showing, a) Effect of crude extract of *Chiropsoides buitendijki* in different organic mixtures b) standard drugs against *Mycobacterium tuberculosis* (MTB) strain H37 Rv: ATCC No- 27294.

The sensitivity of the crude extract and standard drugs against *Mycobacterium tuberculosis* (MTB) strain, H37 Rv: ATCC No- 27294 and it was found that in Isoniazid (1.6 µg/ml), Ethambutol (1.6 µg/ml), Pyrazinamide (3.125µg/ml), Rifampicin (0.8µg/ml), and Streptomycin (0.8µg/ml) respectively. The sensitivity of crude extract prepared in different organic solvents was also checked against *Mycobacterium tuberculosis* (MTB) strain, H37 Rv: ATCC No- 27294 and it was found that in 80 % methanol and 1% acetic acid (12.5µg/ml), in absolute methanol and n-butanol (1:1) (50µg/ml), and in n-butanol (50µg/ml) respectively.

From the above results it is confirmed that Box jelly fish *Chiropsoides buitendijki* (Horst, R1907) contains bioactive compounds which showed antibacterial and antimycobacterial property.

CONCLUSION: Marine animals produce varieties of natural toxins in order to utilize them for their defense mechanism and to protect them from other predators. Recently, many marine toxins have risen in the field of novel drug discovery and proven its wide spectrum of pharmaceutical and biomedical potential. However, with respect to the Cnidarian toxins the efforts have reveals on its neurotoxins potentials that are being used for various therapeutic applications in nervous system and very scanty reports are available on its bioactive

compounds. The screening of Cnidarian bioactive compounds and evaluating its activity on different animal model is highly recommended and there is a need to be a major focus in drug discovery and therapy. Therefore, we have screened the effect of crude extract of Box jelly fish *Chiropsoides buitendijki* (Horst, R1907) and it confirms that the Box jelly fish contains bioactive compounds which showed antibacterial and antimycobacterial property.

ACKNOWLEDGEMENT: Authors are thankful to Dr. Ramkumar, scientist, at the Central Marine Fisheries Research Institute (CMFRI), Mumbai for final identification and confirmation of species. Authors are also thankful to the Director, Maharashtra State Biodiversity Board, Nagpur, Maharashtra for giving permission for collection of species. Thanks are also due to APX laboratories, Thane, Maharashtra for providing bacterial strains and, Maratha Mandal's Central Research Laboratory, Belgaum-590010, India, for *Mycobacterium tuberculosis* (MTB) strain H37 Rv: ATCC No- 27294.

CONFLICT OF INTEREST: Authors have no conflict of interest

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How to cite this article:

Dolnar JL and Zodape GV: Antibacterial and antimycobacterial property of crude extract of box jelly fish *Chiropsoides buitendijki* (horst r, 1907) from west coast of Mumbai. Int J Pharm Sci & Res 2023; 14(10): 4918-23. doi: 10.13040/IJPSR.0975-8232.14(10).4918-23.

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