The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(3): S.I (3), 537-552, 2025 www.thebioscan.com

BIOACTIVE COMPOUNDS DERIVED FROM THE BOX JELLYFISH-CHIROPSOIDES BUITENDIJKIFROM THE WEST COAST OF MUMBAI.

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Web of Science Researcher Profile ID: JFS-3657-2023

DOI: 10.63001/tbs.2025.v20.i03.S.I(3).pp537-552

KEYWORDS
Bioactive, Edwan village, Jellyfish, Novelmedications
Received on:

16-06-2025

Accepted on:

12-07-2025

Published on:

20-08-2025

ABSTRACT

During low tide, box jellyfish (Chiropsoides buitendijki, Horst R, 1907) were gathered from Edwan village, located on Mumbai's western coast. The species was identified by experts at the Central Marine Fisheries Research Institute (CMFRI) in Mumbai. To prepare a crude extract, 80% methanol mixed with 1% acetic acid was used.The crude extract was processed for the separation of bioactive compounds by using TLC. The separated bioactive compounds were further processed for their structural determination by using GC-MS and FTIR techniques. From the analysis, we confirmed eighteen compounds as Sulfuric acid dimethyl ester: Trans-(2 Chlorovinyl) methyldiethoxysilane,Ethanone, 1-(1a, trimethyloxireno[g]benzofuran-5-yl), 2, 3, 5,6a, 6b-hexahydro-3, 3, 6a 4-[(1R)-1-Aminoethyl]-N-4-pyridinyl-Trans cyclohexane carboxamide, Terbutaline, N-trifluoro acetyl-O, O, o-tris (trimethylsilyl) derive, Cyclopentasiloxane, Deca methyl, Phloroglucinaldehyde, tris(trimethylsilyl) ether, 5- [2- [4 (Ethoxy carbonyl) phenyl] diazenyl]-2-hydroxybenzeneacetic acid, Benzaldehyde, 4-methoxy , Benzaldehyde, 3-methoxy, o-Anisic acid, 3,4-dichlorophenyl ester, Decanoic acid, methyl ester, 8-Methylnonanoic acid, methyl ester, Pentadecanoic acid, 14-methyl-, methyl ester, Tetra decanoic acid, 10,13-dimethyl-, methyl ester, Tridecanoic acid, methyl ester, Methyl stearate, and Heptadecanoic acid, 16-methyl-, methyl ester respectively.These isolated compounds revealed therapeutic applications that might help the pharmaceutical industry design novel medications in the future.

INTRODUCTION

Cnidarians display an impressive range of body structures and are divided into five primary groups. These include Anthozoa, which consists of true corals, sea anemones, and sea pens; Cubozoa, recognized for box jellyfish with potent venom; Hydrozoa, the most diverse group that features siphonophores, hydroids, fire corals, and various medusae;Staurozoa, which are stalked jellyfishes, and Scyphozoa, also known to be actual jellyfish [1]. Despite their diversity, all cnidarians share a common feature: stinging cells known as nematocysts. These organisms are categorized together based on the belief that their nematocysts were inherited from a shared ancestor [2]. Cnidarians occupy a high position in the marine food web and are capable of competing with fish for available food. As a result, the large-scale removal of top predatory fish due to commercial fishing may increase food availability for jellyfish [3]. Moreover, studies indicate that salinity has a notable impact on jellyfish distribution and behaviour [4]. Furthermore, it was noted that the predicted decline in pH levels in oceans as CO2 concentrations rise could lead to an increase in the long-term frequency of jellyfish [5].In the last ten years, researchers have isolated over 3,000 compounds from cnidarians, with terpenoids being the most common. Sea anemones, in particular, are known to produce fluorescent pigments called zoanthoxantins, along with polypeptide toxins that affect voltage-gated sodium (Na^+) and potassium (K^+) channels. A major breakthrough in marine natural products research came in the late 1960s with the discovery of prostaglandins in corals [6].Among the most powerful toxins identified is palytoxin, which originates from *Palythoa* species belonging to the Zoanthidae family. The toxicity of jellyfish poses a serious health concern in several regions, leading to increased toxicological research on these organisms, particularly because the venoms of jellyfish and sea anemones have relatively low toxicity.

Jellyfish are recognized as free-swimming organisms belonging to the phylum Cnidaria, which is named after specialized cells called cnidocytes that are characteristic of its members.

Several species of jellyfish are considered edible, including *Lobonema smithii*, *Rhopilema esculentum*, *Nemopilema nomurai*, and *Lobonemoides gracilis*[7].Primarily found in Southeast Asia, this species contributes to an annual harvest of over 750,000 tons, with growing interest extending beyond Asian markets. Jellyfish are valued as a nutritious food option [8] and have long been used in traditional Chinese medicine for their health benefits. In addition, their high collagen and protein content [9, 10] along with antioxidant properties, make jellyfish an affordable source of raw materials for the pharmaceutical, nutraceutical, and cosmetic industries. They have also been recognized as novel foods in Europe.Previous studies have shown that polysaccharides extracted from *Nemopilema nomurai* and *Rhopilema esculentum* exhibit anti-inflammatory, antioxidant, and immune-modulating properties. Moreover, proteins and other substances obtained from jellyfish demonstrate strong antioxidant activity and a range of other biological functions. Although many studies have explored the applications of cnidocytes, research on their toxic effects is still scarce [11].However, neurotoxins have been found in the ectodermal gland cells.

Jellyfish are invertebrate animals found in marine environments, with approximately 200 recognized species belonging to the class Scyphozoa within the phylum Cnidaria [7].Several species of jellyfish, such as *Lobonema smithii*, *Rhopilema hispidum*, *Rhopilema esculentum*, *Nemopilema nomurai*, and *Lobonemoides gracilis*, are considered edible [8]. These jellyfish are valued for their high nutritional content, including proteins, amino acids, carbohydrates, essential vitamins, and minerals, making them important from both economic and dietary perspectives [10, 12]. In many Asian countries, dried jellyfish are considered a tasty and special food. Jellyfish are also thought to have health benefits and have long been used in traditional medicine to help treat issues like arthritis, high blood pressure, and back pain [13]. In China, jellyfish have been harvested for commercial use along the coast for more than 1,700 years [9]. Only species from the Order *Rhizostomeae* are chosen for food, as they are larger and have firmer bodies than other types of scyphozoans. When processed, these jellyfish develop a pleasing, almost crunchy texture that is highly valued in cooking.

Many cubozoan species possess highly potent toxins within their stinging cells, known as nematocysts. This significant toxicity often results in intense stings that can lead to heart problems or even cardiac arrest in humans [14]. Certain species within the *Carybdeida* order can cause Irukandji syndrome, a condition that leads to symptoms like lower back pain, nausea, anxiety, and severe high blood pressure [15, 16]. Additionally, species from the *Chirodropida* order have been linked to fatal cases in humans [17]. Reports indicate that the Gulf of Thailand has a greater number of deadly incidents compared to the Andaman Sea [18]. Consequently, cubozoans have garnered a lot of interest from researchers and regulatory bodies, as they pose a public health risk and contribute to economic losses in the tourism sector [19].

Box jellyfish, part of the class *Cubozoa*, are marine invertebrates known for their unique cube-shaped bodies. Some species in this group carry extremely potent venom, which they inject through their tentacles upon contact. Stings from jellyfish like *Chironex fleckeri*, *Carukia barnesi*, and *Malo kingi* can cause intense pain and may be deadly to humans.*Chironex fleckeri* is actually considered one of the most venomous animals in the world[20]. When its tentacles touch a person or prey, it quickly releases nematocysts-specialized cells that inject fast-acting venom. Although stings from *C. fleckeri* can be life-threatening, most victims suffer from extreme pain and severe damage to the surrounding tissue [21, 22]. Box jellyfish venom contains a mix of biologically active proteins that can cause severe effects, including the destruction of red blood cells (hemolysis), damage to cells (cytotoxicity), the creation of pores in cell membranes, inflammation, cardiovascular collapse in living organisms, and even death in laboratory animals [23,24].

India boasts a rich biodiversity that remains largely untapped in terms of discovering new drug entities.India has a coastline of about 8,014 kilometers, which includes two major island groups-the Andaman and Nicobar Islands and the Lakshadweep Islands. The country's Exclusive Economic Zone covers a large area of 2,013,410 square kilometers, along with territorial waters spanning 155,889 square kilometers. Along the coast, India has several delicate ecosystems such as coral reefs, mangroves, lagoons, sand dunes, seagrass beds, and wetlands [25]. Mumbai, a coastal city on India's west coast (located between 18°51' to 19°33' N and 72°43' to 73°01' E), is surrounded by the Arabian Sea and has a 100-kilometer-long coastline. The coastal zones in and around Mumbai are known for their rich biodiversity and support a wide variety of marine life.

MATERIALS AND METHODS:

a) Sample Collection:

Box jellyfish (*Chiropsoides buitendijki*, Horst, 1907) were gathered during low tide from Edwan village, located on the western coast of Mumbai. The live specimens were transported to the laboratory in seawater. Once there, each jellyfish was rinsed twice with seawater, followed by a final rinse in distilled water. The cleaned samples were initially stored on ice and later transferred to a deep freezer set at -8°C in the Department of Zoology, at S.S. & L.S. Patkar College of Arts & Science and V.P. Varde College of Commerce & Economics, Goregaon West, Mumbai.

b) Identification of box jellyfish:

Initial identification was carried out by examining the jellyfish's body shape and the number of tentacles, along with consulting relevant scientific literature. The species was then confirmed by Dr. Ramkumar, a scientist at the Central Marine Fisheries Research Institute (CMFRI), Mumbai.

c) **Preparation of crude extract from box jellyfish:**

The crude extract of *Chiropsoides buitendijki* (Horst, 1907) was prepared using a modified version of a method involving 80% methanol and 1% acetic acid. Ten grams of jellyfish tissue were blended, after which 10 ml of a solution made from equal parts of 80% methanol and 1% acetic acid was added. This mixture was left to stand in a water bath at 45°C for 24 hours. The resulting solution was then filtered using Whatman No.1 filter paper. The filtered homogenate was centrifuged at 5000 rpm for 15 minutes at -8°C using a cold centrifuge (Remi, Serial No. VCDX-5983). The supernatant was collected into a conical flask and concentrated under low pressure using a rotary vacuum evaporator at 45°C. The concentrated extract was further purified using a Millipore filtration system, dried in a vacuum desiccator, and stored at -20°C in a refrigerator until further use.

d) Ethical Approval:

Permission for collecting box jellyfish samples for research was obtained from the Maharashtra State Biodiversity Board, Nagpur, Maharashtra, under approval numbers MSBB/Desk-5/Research/841/2022-23 and MSBB/Desk-5/Research/397/2023-24. A voucher specimen of *Chiropsoides buitendijki* has been deposited in the repository of the Zoological Survey of India, Western Regional Centre, Pune, under the reference ZSI-WRC Misc/19.

TLC ANALYSIS:

The sample analysis was carried out using the Chem. Tech. TLC model of HPTLC at Anchrom Test Lab, located in Mulund, Mumbai. In this setup, the stationary phase consisted of an aluminium plate precoated with silica gel ($^{60}F_{254}$). The mobile phase was a solvent mixture of chloroform, toluene, and ethanol in a 4:4:1 ratio (v/v/v). Sample development was performed in a twin trough chamber. For densitometric scanning, a deuterium lamp set at 254 nm wavelength was used.

GC-MS: (GAS CHROMATOGRAPHY- MASS SPECTROMETRY):

The samples were examined using GC-MS at the Sophisticated Analytical Instruments Facility (SAIF), IIT Madras. An Agilent Model 8890 Gas Chromatography System paired with a Single Quadrupole Mass Spectrometer (5977B MSD) was employed to separate and identify thermally stable volatile compounds. The GC system is equipped with Split/Splitless (SSL) injectors and capillary columns suited for various types of analyses. Compound identification was carried out using the NIST spectral library.

FTIR -SPECTROPHOTOMETER:

FTIR spectrophotometers at RSIC – IIT Powai, Mumbai, were used to analyze and identify the bioactive compounds. Instruments used included the USA-made Mercury Plus (300 MHz, H1 NMR) and the Magna 550 FTIR spectrophotometer. For FTIR analysis, samples were blended with potassium bromide (KBr) to create pellets, which were then scanned across a wavelength range of 4000 cm^{-1} to 400 cm^{-1}.

All chemicals and reagents used in the IR analysis were of analytical grade, sourced from M/S S.D. Fine Chemicals, Thane, India.

RESULTS:

CHARACTERIZATION OF CRUDE EXTRACT BY TLC

Extracts obtained from the box jellyfish *Chiropsoides buitendijki*, collected from Edwan village in Palghar District, were applied to TLC plates for analysis. The plates were developed in a twin trough chamber using a solvent mixture of chloroform, toluene, and ethanol in a 4:4:1 (v/v/v) ratio. After drying, the plates were treated with Anisaldehyde reagent to visualize the spots. Densitometric scanning was carried out using a deuterium lamp at 254 nm. (Refer to Fig. No. 1 for the photograph.) Preparative TLC was then conducted, and the spots corresponding to Rf values of R1–0.17, R2–0.22, R3–0.4, and R4–0.9 were collected by scraping and dissolving them in methanol. The solvent was evaporated to obtain purified compounds, which were subsequently analyzed using FTIR spectroscopy.

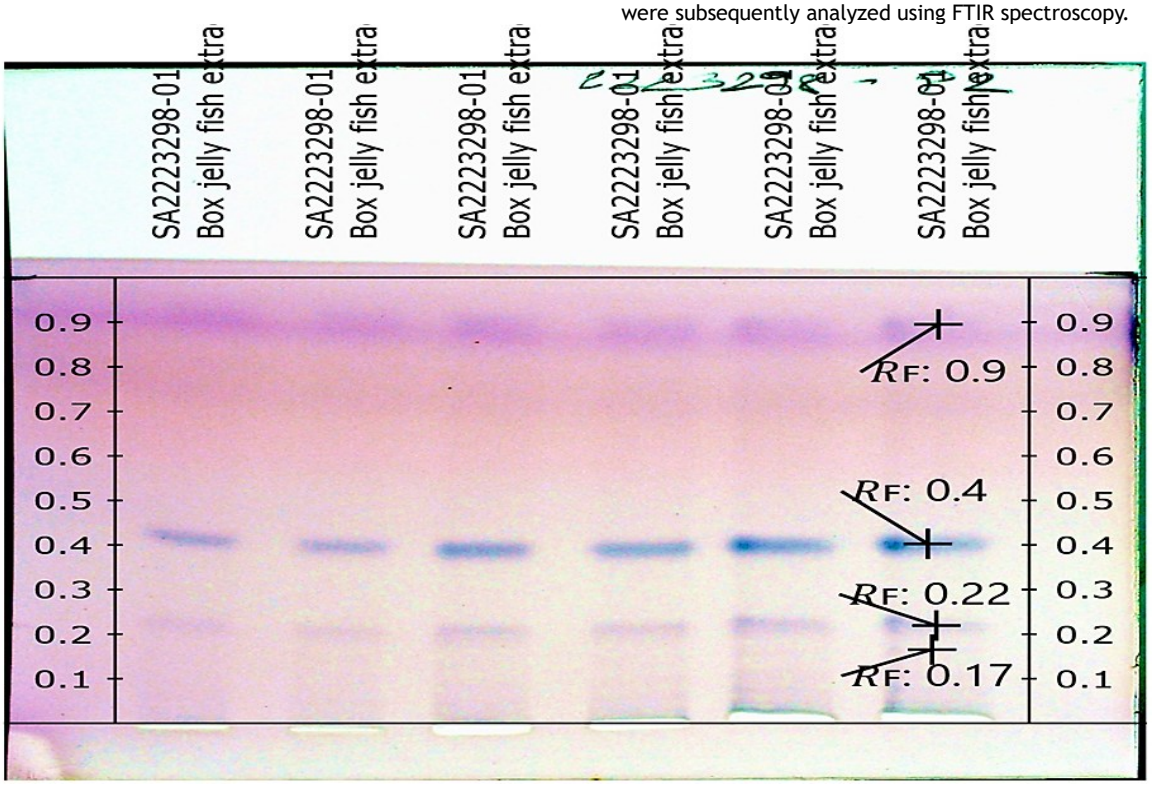


Fig.No.1.Showing TLCanalysisofcrudeextractsofboxjellyfish-*Chiropsoides buitendijki*

CHARACTERIZATION OF ISOLATED EXTRACTS OF BOX JELLYFISH- *CHIROPSOIDES BUITENDIJKI* ON GC-MS

GC-MS of the extracts isolated from box jellyfish- *Chiropsoides buitendijki* have been performed and the results are presented in Fig. No. 2 to 2.16, 3 to 3.10, and 4 to 4.4.

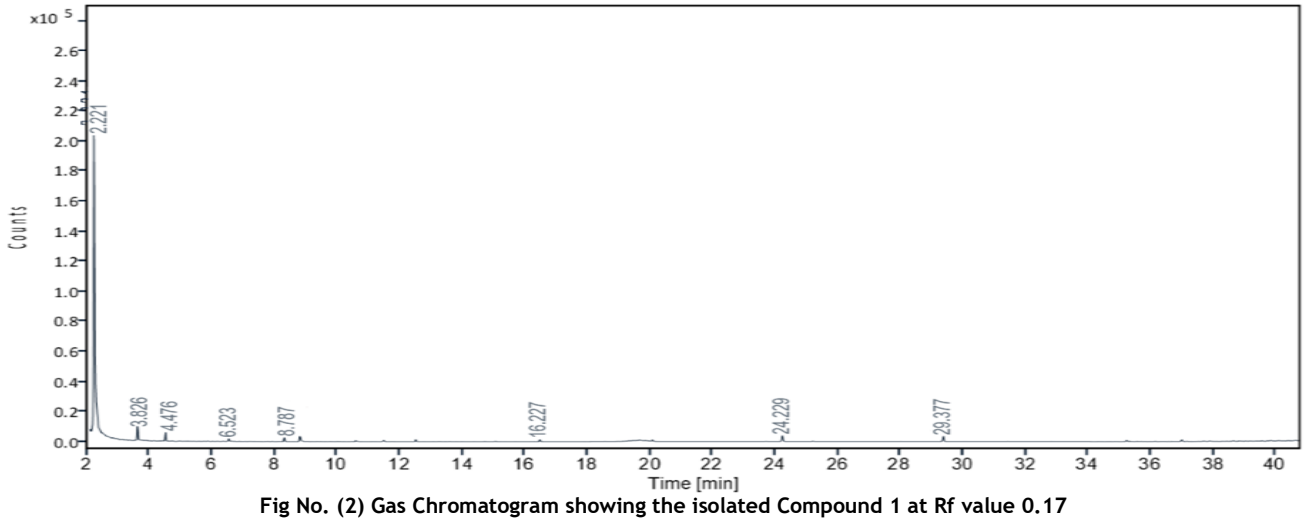


Fig No. (2) Gas Chromatogram showing the isolated Compound 1 at Rf value 0.17

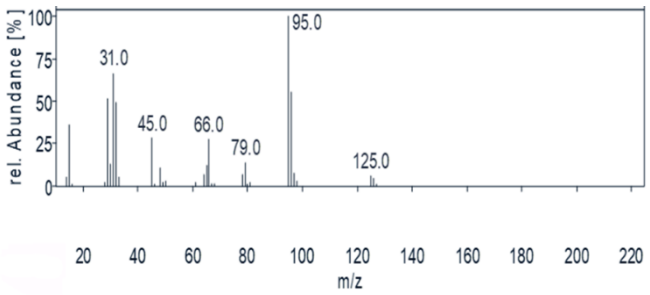


Fig. No. (2.1) Compound 1 (a) at Rt value 2.221

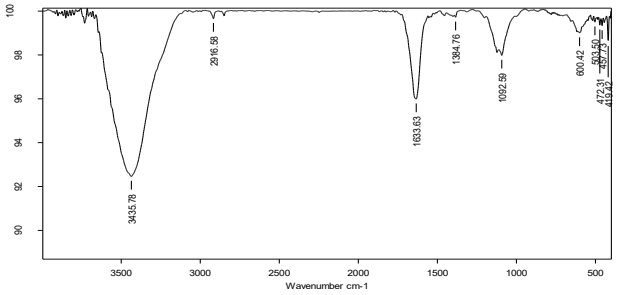


Fig. No. (2.2) FTIR spectra for compound 1 (a)

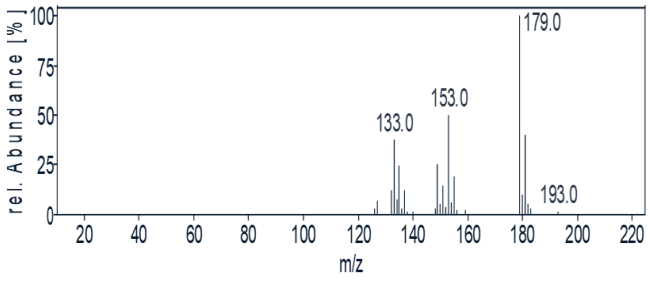
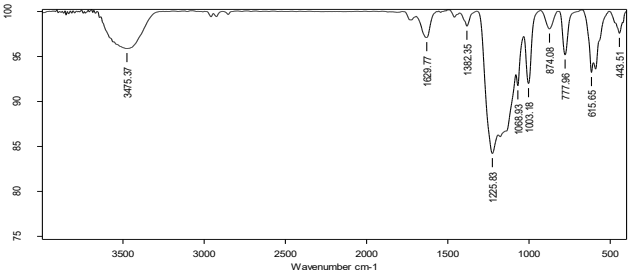


Fig. No. (2.3) Compound 1 (b) at Rt value3.826



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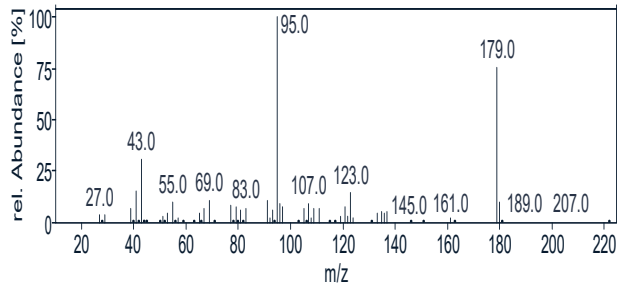


Fig. No. (2.5) Compound 1 (c) at Rt value 4.476

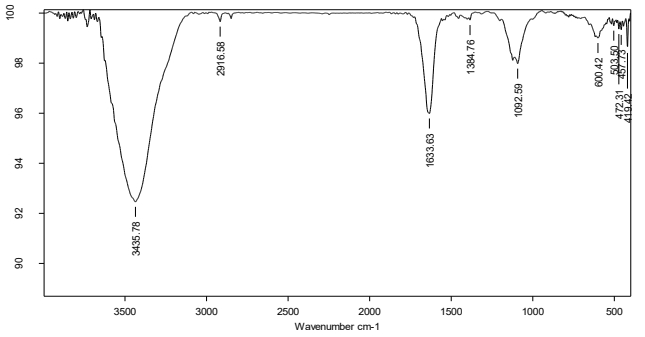


Fig. No. (2.6) FTIR spectra for compound 1 (c)

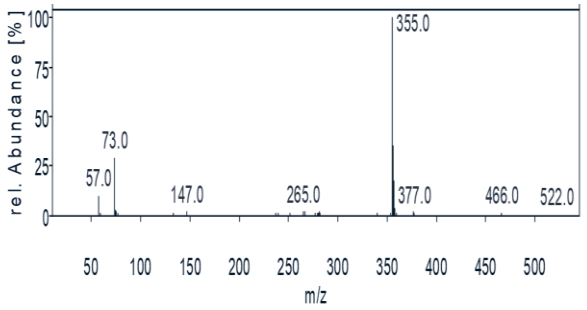


Fig. No. (2.7) Compound 1 (d) at Rt value 6.523

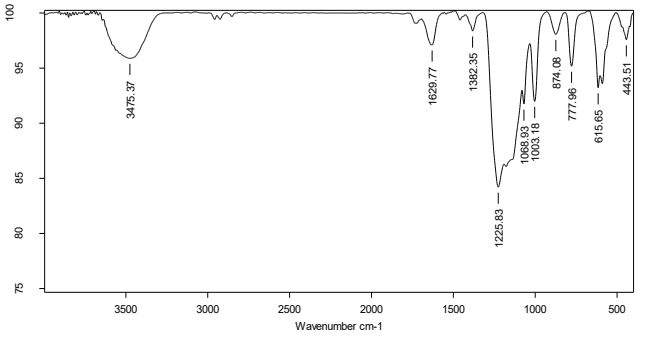


Fig. No. (2.8) FTIR spectra for compound 1 (d)

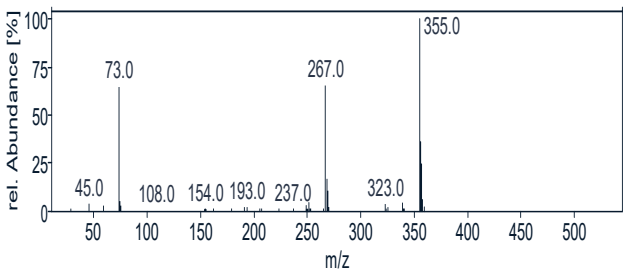
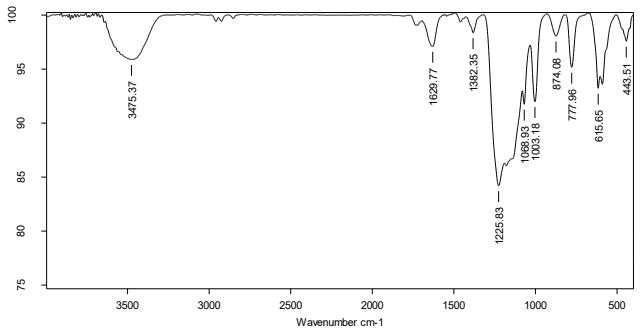


Fig. No. (2.9) Compound 1 (e) at Rt value 8.787



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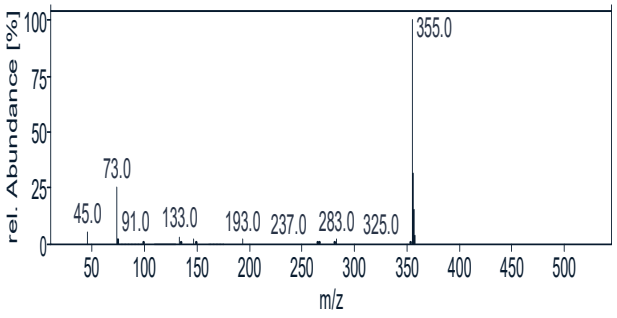
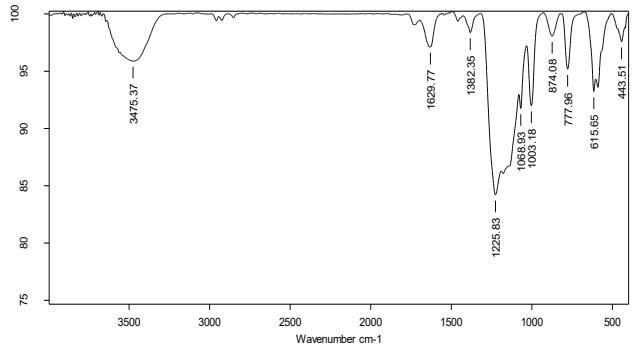


Fig. No. (2.11) Compound 1(f) at Rt value16.227



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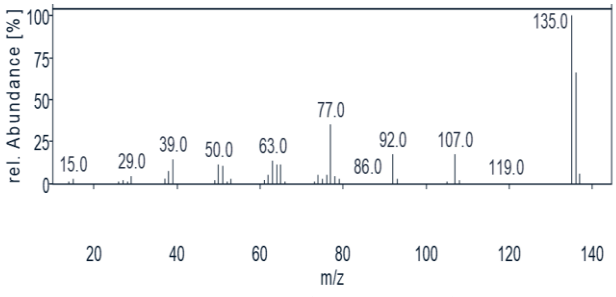
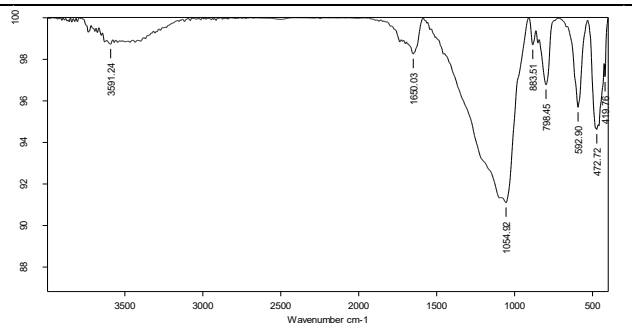


Fig. No. (2.13) Compound 1 (g) at Rt value24.229



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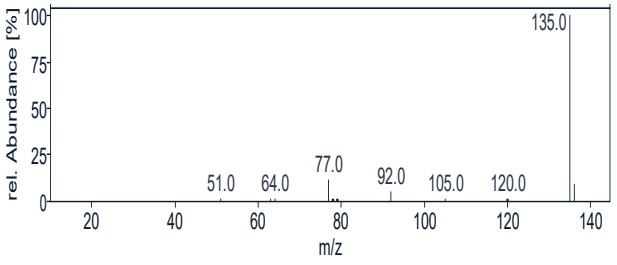
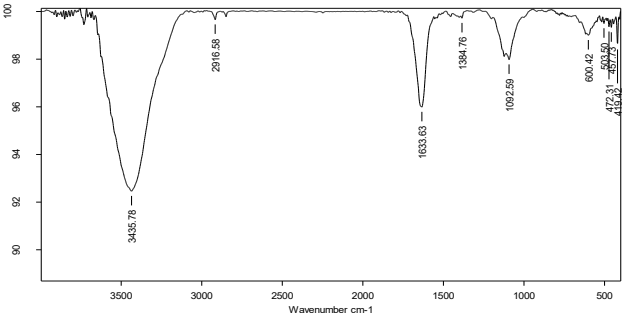


Fig. No. (2.15) Compound 1(h)Rt value29.377



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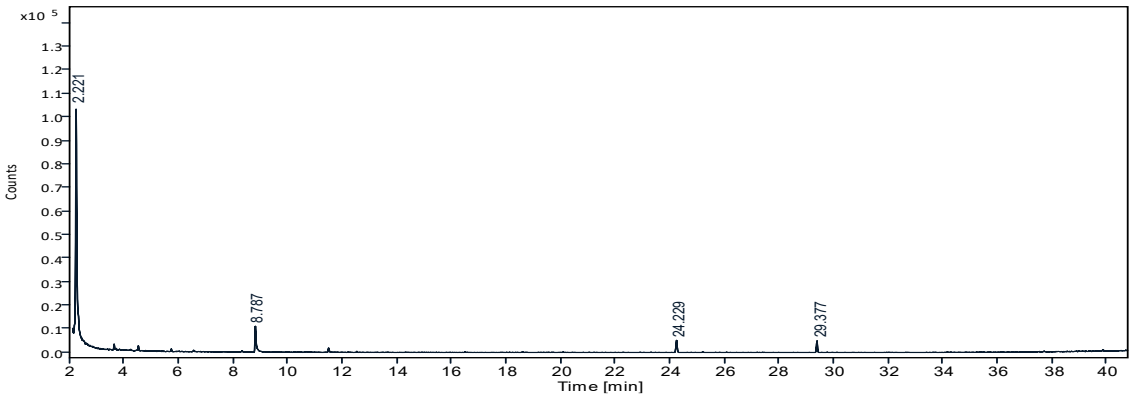


Fig No. (3) Gas Chromatogramshowing the isolated Compound 2 at Rf value 0.4

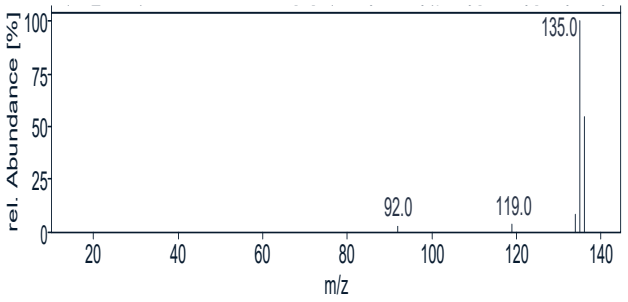
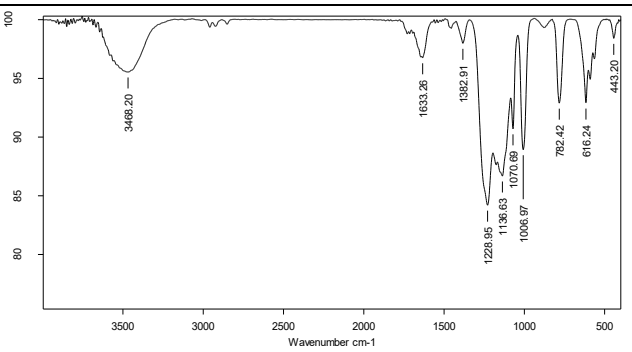


Fig. No. (3.1) Compound 2(a)at Rt value 8.787



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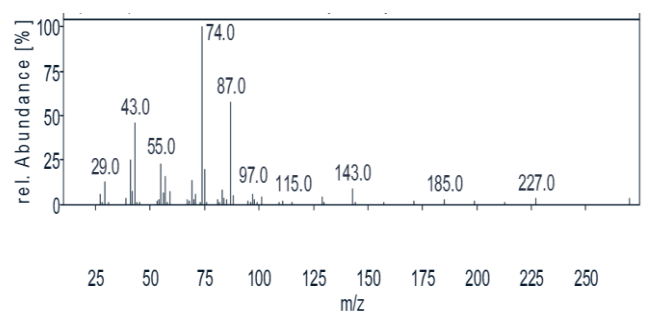

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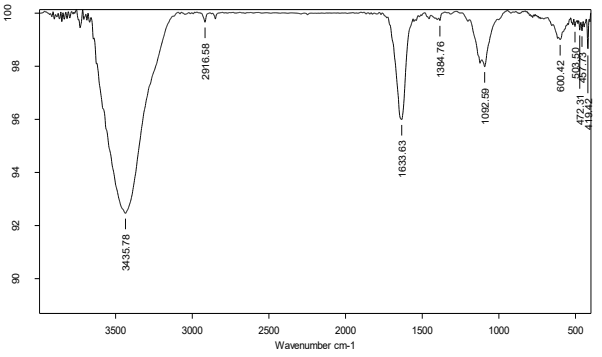

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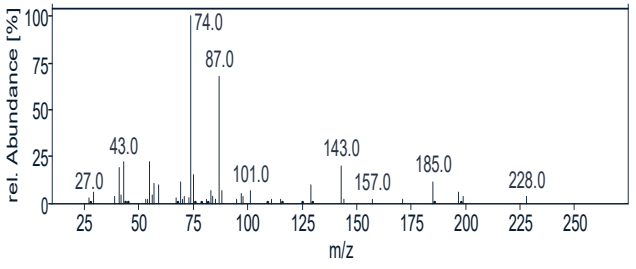
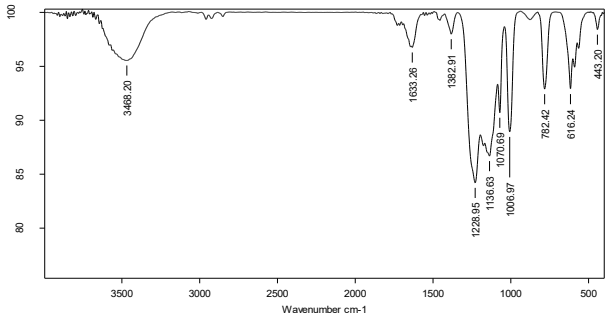


Fig. No. (3.5) Compound 2(c) at Rt value 24.229



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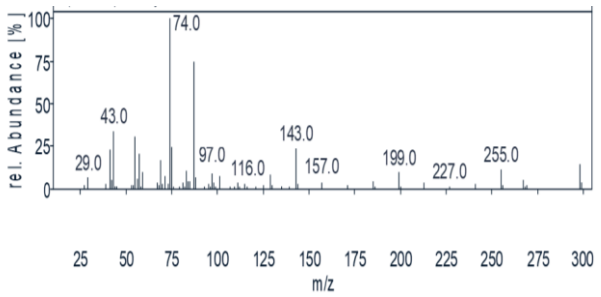

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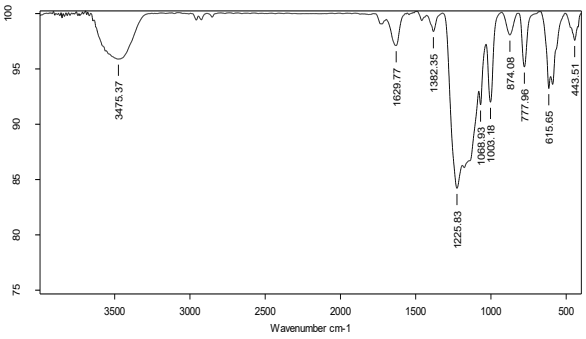

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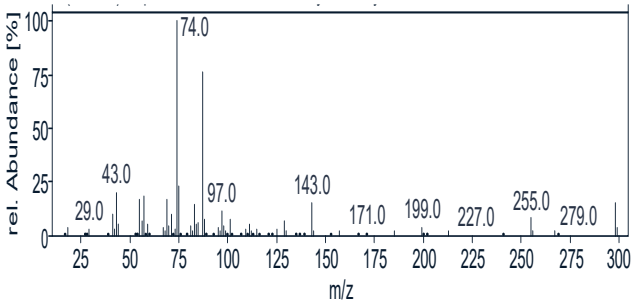


Fig. No. (3.9) Compound 2(e) at Rt value 29.377

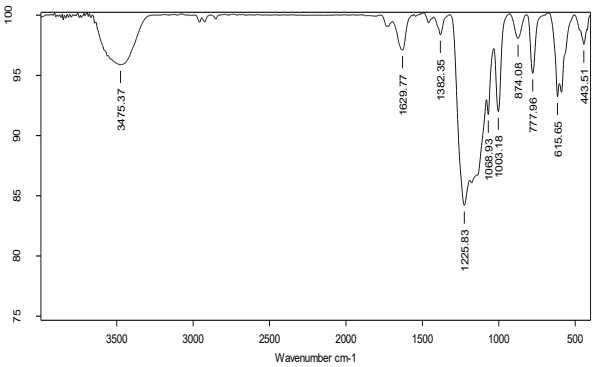


Fig. No. (3.10) FTIR spectra for compound 2 (e)

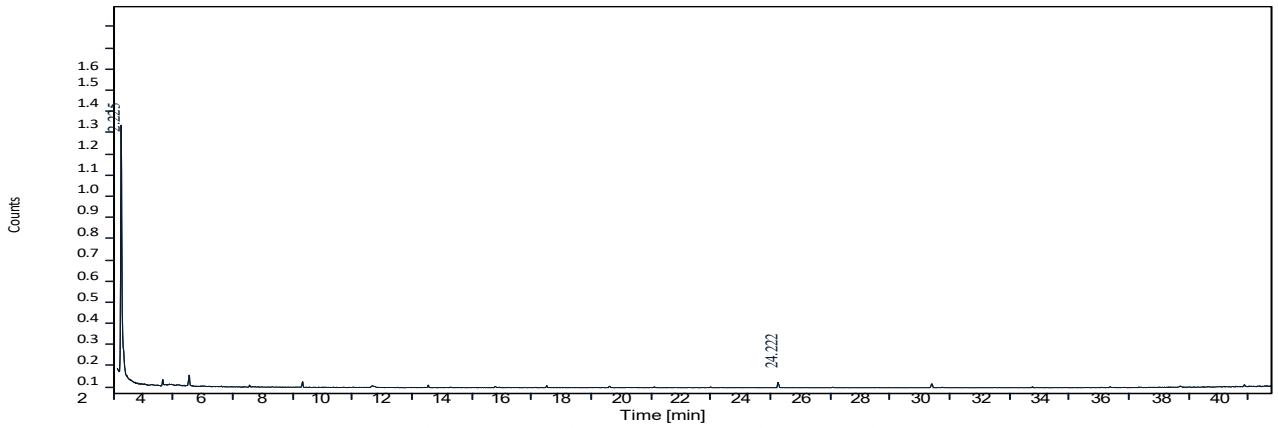


Fig. No. (4) Gas Chromatogramshowing the isolated Compound 3 at Rf value 0.9

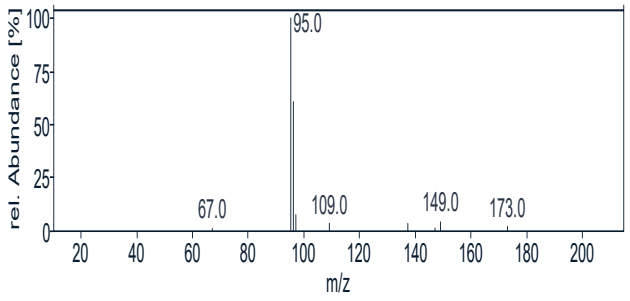


Fig. No. (4.1) Compound 3 (a) at Rt value 2.225

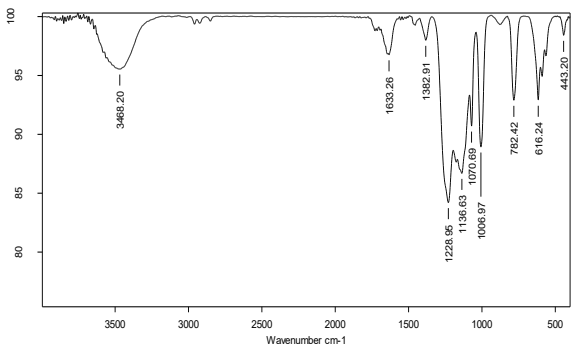


Fig. No. (4.2) FTIR spectra for compound 3 (a)

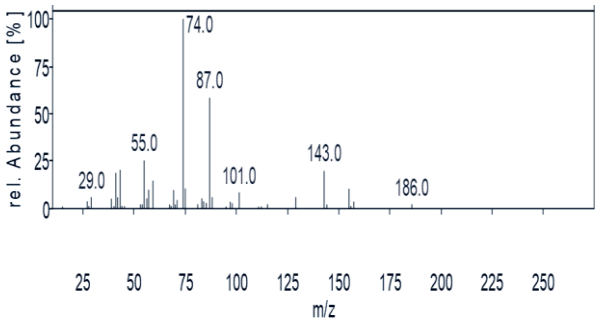


Fig. No. (4.3) Compound 3 (b) at Rt value 24.222

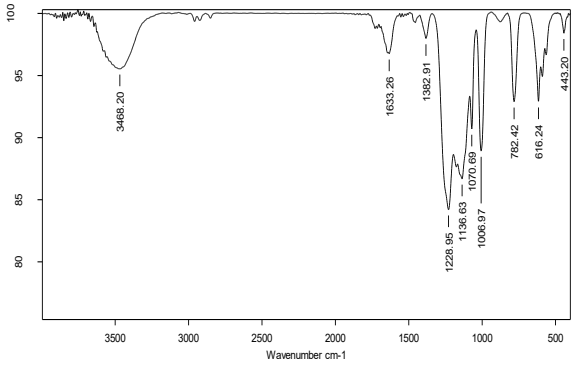


Fig. No. (4.4) FTIR spectra for compound 3 (b)

DISCUSSION:

The gas chromatograms of the extracts from the box jellyfish *Chiropsoides buitendijki*, presented in Figs No2 to 4 reveal numerous peaks throughout the analysis. The gas chromatography (GC) run lasted for a total of 53 minutes, with the system temperature being increased up to 350°C. Although some selected peaks with specific retention times (RT) were successfully identified, many others could not be distinguished. This limitation was due to overlapping peaks, a lack of matching data in the existing spectral library, and the absence of previously reported references related to *Chiropsoides buitendijki* extracts**.**The peaks detected in the GC analysis at specific Rt valuesof 2.221, 2.225, 6.523, 8.787, 8.796, 24.222, 24.229, and 29.377.The peaks at specific retention times (Rt) were used solely for capturing the mass spectra by subjecting the eluted compounds to electron impact (EI+) ionization in the mass spectrometer. (Fig. No. 2.1 to 2.16, 3.1 to 3.10, 4.1 to 4.4) display the mass spectra of the compounds eluted at these respective Rt valuesof 2.221, 2.225, 6.523, 8.787, 8.796, 24.222, 24.229, and 29.377,from the box jellyfish *Chiropsoides buitendijki*.The mass spectra revealed M/Z (mass-to-charge)peaks recorded at retention times 2.221, 2.225, 6.523, 8.787, 8.796, 24.222, 24.229, and 29.377, correspond to compounds with specific molecular mass of 126.13g/mol, 194.73g/mol, 222.28 g/mol, 247.34 g/mol, 537.8 g/mol, 370.76 g/mol, 378.63 g/mol, 328.32 g/mol,136.14g/mol, 136.147g/mol, 297.133 g/mol, 186.29g/mol, 186.291g/mol, 270.45g/mol, 270.45g/mol, 228.37g/mol, 298.51 g/mol, 298.50 g/mol, of isolated compounds of the extracts of box jellyfish- *Chiropsoides buitendijki*.

Fig. No. (2.1) The fragmentation peaks identified in the mass spectra at the retention time (Rt) of 2.221 min for Compound 1 (a) at R1- 0.17 are as follows: 126.13g/mol.M/Z at 126 (3%), M/Z at 125 (5%), M/Z at 95 (100%), M/Z at 79 (22%), M/Z at 66 (38%), M/Z at 45 (40%), M/Z at 31 (66%). Fig. No. (2.3) The fragmentation peaks obtained from the mass spectrum at the retention time (Rt)of3.826 min for Compound 1 (b) at R1- 0.17 are as follows: 194.73g/mol.M/Z at 193 (2%), M/Z at 179 (100%), M/Z at 153 (51%), M/Z at 133(37%), M/Z at 135 (24%). Fig. No. (2.5)The fragmentation peaksidentified in the mass spectra at the retention time (Rt)of4.476 min for Compound 1 (c) at R1- 0.17 are as follows: 222.28 g/mol.M/Z at 222 (1%), M/Z at 197 (75%), M/Z at 123 (14%), M/Z at 95(100%), M/Z at 91(10%), M/Z at 43(30%), M/Z at 27(2%).Fig. No. (2.7) The fragmentation peaks obtained from the mass spectrum at the retention time (Rt)of 6.523min forCompound 1 (d) at R1- 0.17 are as follows: 537.8 g/mol.M/Z at 538 (2%), M/Z at 466 (1%), M/Z at 377 (1%), M/Z at 357(17%), M/Z at 356(35%), M/Z at 355(100%), M/Z at 73(32%), M/Z at 57(1%). Fig. No. (2.9) The fragmentation peaks obtained from the mass spectrum at the retention time (Rt)of 8.787 min for Compound 1 (e) at R1- 0.17 are as follows: 370.76 g/mol.M/Z at 372 (2%), M/Z at 357 (27%), M/Z at 356 (35%), M/Z at 355(100%), M/Z at 267(65%), M/Z at 73(65%), M/Z at 43(1%). Fig. No. (2.11) The fragmentation peaksidentified in the mass spectra at the retention time(Rt)of 16.277 min for Compound 1 (f) at R1- 0.17 are as follows: 378.63 g/mol.M/Z at 379 (2%), M/Z at 355 (100%), M/Z at 283 (1%), M/Z at 91(2%), M/Z at 73(25%), M/Z at 45(5%). Fig. No. (2.13)The fragmentation peaks obtained from the mass spectrum at the retention time(Rt) of24.229 min forCompound 1 (g) at R1-0.17 are as follows: 136.14g/mol.M/Z at 136 (66%), M/Z at 135 (100%), M/Z at 107(17%), M/Z at 92(17%), M/Z at 77(35%), M/Z at 39(15%), M/Z at 15(2%). Fig No. (2.15) The fragmentation peaks identified in the mass spectra at the retention time (Rt) of 29.377min for Compound 1 (h) at R1- 0.17 are as follows: 297.133 g/mol.M/Z at 136 (9%), M/Z at135 (100%), M/Z at 92 (4%), M/Z at 77 (11%), M/Z at 64 (1%), M/Z at 51 (1%).

In the case of Rf value 0.22 showed similar compounds to R3-0.4 therefore cannot be repeated.

Fig. No. (3.1) The fragmentation peaks obtained from the mass spectrum at the retention time(Rt)of 2.221 min forCompound 2 (a) at R3-0.4 are as follows: 328.32 g/mol.M/Z at 329 (2%), M/Z at 136 (54%), M/Z at 135 (100%), M/Z at 134(7%), M/Z at 119(3%), M/Z at 92(2%). Fig. No. (3.3) The fragmentation peaks identified in the mass spectra atthe retention time(Rt)of 8.787min for Compound 2 (b)at R3-0.4 are as follows: 270.45g/mol.M/Z at 270(2%), M/Z at 87 (57%), M/Z at 75(100%), M/Z at 55(22%), M/Z at 43(46%), M/Z at 41(25%), M/Z at 29(18%).Fig. No. (3.5) The

fragmentation peaks obtained from the mass spectrum atthe retention time(Rt) of 24.229 min for Compound 2 (c) at R3-0.4 are as follows: 228.37g/mol.M/Z at 228(2%), M/Z at 185 (10%), M/Z at 143(19%), M/Z at 87(67%), M/Z at 74(100%), M/Z at 43(19%), M/Z at 27(3%).Fig. No. (3.7)The fragmentation peaks identified in the mass the retention time(Rt)of 29.377 min Compound 2 (d) at R3-0.4 are as follows: 298.51 g/mol. M/Z at 298(18%), M/Z at 255 (10%), M/Z at 143(23%), M/Z at 87(74%), M/Z at 74(100%), M/Z at 43(33%), M/Z at 29(4%).Fig. No. (3.9)The fragmentation peaks obtained from the mass spectrum atthe retention time(Rt)of 29.377 min for Compound 2 (e) at R3-0.4 are as follows: 298.51 g/mol.M/Z at 298(18%), M/Z at 243 (16%), M/Z at 87(76%), M/Z at 75(23%), M/Z at 74(100%), M/Z at 43(20%), M/Z at 29(2%).

Fig. No. (4.1) The fragmentation peaks obtained from the mass spectrum atthe retention time(Rt) of 2.225 min for Compound 3 (a) at R4- 0.9 are as follows: 247.34 g/mol.M/Z at 275 (1%), M/Z at 173 (1%), M/Z at 109 (3%), M/Z at 96(60%), M/Z at 95(100%). Fig. No. (4.3)The fragmentation peaks obtained from the mass spectrum atthe retention time(Rt) of 24.222min for Compound 3 (b) at R4-0.9 are as follows: 186.29g/mol.M/Z at 186 (1%), M/Z at 143 (19%), M/Z at 136(3%), M/Z at 101(5%), M/Z at 87(58%), M/Z at 74(100%), M/Z at 55(24%).

FTIR STUDIES

The FTIR analysis was performed using KBr pellets. Compounds isolated at Rf values of R1 - 0.17, R2 - 0.22, R3 - 0.4, and R4 - 0.9 from each extract were subjected to TLC, and their corresponding IR spectra are presented in Figures 2.2 to 2.16, 3.1 to 3.10, and 4.2 to 4.4. The IR spectra obtained for each compound at the respective Rf values were found to be distinct from one another. The wave numbers of significant IR peaks, along with their intensity, vibration type, and the likely functional groups present in the compounds isolated at Rf values R1- 0.17, R3- 0.4, and R4- 0.9, are summarized in Table No. (1) to (3). The identification of potential functional groups was made by analyzing the IR band characteristics as wave number, intensity, and type of vibration comparing them with standard references [26, 27].

Table No. (1) Correlation of IR spectra of the Compound 1 isolated from *Chiropsoides buitendijki (*box jellyfish) at Rf value 0.17 on TLC

Sr.No.	Wave number: cm-1 intensity	Type of IR vibrations	Probable Functional Group
1	3468.20	O-H or N-H stretching	-OH or -NH2 or -NHR or -COOH
2	1633.26	C=C, C=O or C=N Stretching	-C=C or -CO- or -COO- or -CHOor Ar
3	1382.91	C-H Bending or CH3 Bending or S=O Bending	-C-H or -CH=CH2or -CH3
4	1228.95	C-O stretching	-C-O-C- or -OH or -COO-
5	1136.63	C-O stretching	-C-O-C- or -OH or -COO-
6	1070.69	C-O stretching	-C-O-C- or -OH or -COO-
7	1006.97	C-C Stretching	-C-C- or -C6H5- or -C-C-
8	782.42	C-H out of plane bending C-C Stretching	C=C or -C-C- or -C6H5- or -C-C-
9	616.24	C-O bending vibration	-C-OH
10	443.20	C-O-C- bending	-C-O-C group

Table No. (2) Correlation of IR spectra of the Compound 2 isolated *Chiropsoides buitendijki (*box jellyfish)at Rf value 0.4 on TLC

Sr.No.	Wave number: cm-1 intensity	Type of IR vibrations	Probable Functional Group
1	3435.78	-OH Stretching (Hydrogen bonded)	-COOH or -OH or Ar-OH
2	2916.58	-CH Stretching (asymmetric)	-CH2 or -CH3 or Cycloalkanes, Aliphatic compounds
3	1633.63	C=C Stretching	-CH=CH- or Aromatics or Conjugated dienes, Heterocyclic aromatic compounds
4	1384.76	C-H Bending (symmetric) or CH3 Stretching or S=O Stretching	-CH2 or -CH3 or Cycloalkanes, Aliphatic compounds, sulfate
5	1092.59	C-O Stretching S-O Stretching	-OH or -O- or -COO- or Ar-OH, sulfate
6	600.42	C-Cl Stretching, C-C Bending	-Cl or Cycloalkanes, Aliphatic compounds with branched or cyclic structures
7	503.50	C-Cl Stretching, C-C Bending	-Cl or -Ar-Cl or Cycloalkanes, Aliphatic compounds
8	472.31	C-Cl Stretching, C-C Bending	-Cl or -Ar-Cl or Cycloalkanes, Aliphatic compounds
9	457.73	C-Cl Stretching, C-C Bending	-Cl or -Ar-Cl or Chlorocycloalkanes,Chloroalkanes
10	419.42	C-Cl Stretching, C-C Bending	-Cl or Cycloalkanes, Aromatic compounds

Table No. (3) Correlation of IR spectra of the Compound 3 isolated from *Chiropsoides buitendijki (*box jellyfish) at Rf value 0.9 on TLC

Sr.No.	Wave number: cm-1 intensity	Type of IR vibrations	Probable Functional Group
1	3591.24	-OH Stretching (Hydrogen bonded)	-COOH or -OH or Ar-OH
2	1650.03	C=C Stretching	-CH=CH- or Aromatics or Conjugated dienes, Heterocyclic aromatic compounds
3	1054.92	C-O Stretching	-O- or -COO- or -OH or Ar-OH
4	883.51	C-H Bending, C-C Stretching	-CH=CH- or Aromatics, Cycloalkanes
5	798.45	C-H Bending, C-C Stretching	-CH=CH- or Aromatics, Cycloalkanes
6	592.90	C-Cl Stretching, C-C Bending	-Cl or -Ar-Cl or Cycloalkanes, Aliphatic compounds
7	472.72	C-Cl Stretching, C-C Bending	-Cl or -Ar-Cl or Cycloalkanes, Aliphatic compounds
8	419.76	C-Cl Stretching, C-C Bending	-Cl or -Ar-Cl or Cycloalkanes, Aliphatic compounds, Chloroalkanes

The FTIR technique requires very pure samples. Therefore, preparative TLC was carried out to isolate the pure compounds against the box jellyfish- *Chiropsoides buitendijki* at Rf values atR1- 0.17, R3- 0.4, and R4- 0.9 respectively. The IR spectra of these compounds were recorded and are shown in Table No. (1-3) the wave number, intensity of IR peaks and the types of vibration of the IR bands, the possible functional groups in the compounds were identified using information from standard textbooks [26, 27].

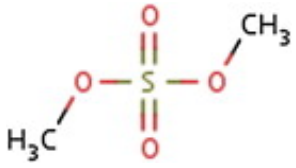
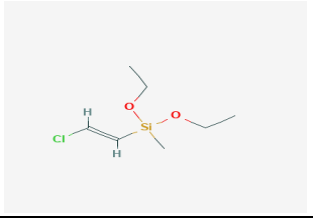
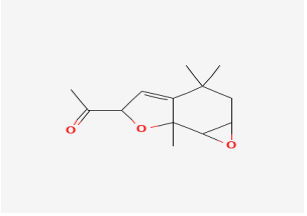
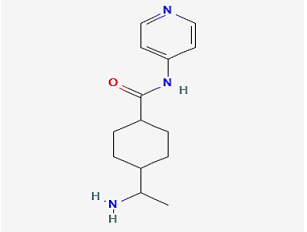
Many researchers have discovered bioactive compounds in different types of marine organisms, such as saxitoxin (STX), domoic acid (DA), ciguatoxin (CTX), brevetoxin (BTX), tetrodotoxin (TTX), okadaic acid (OA), azaspiracid (AZA), and palytoxin (PLTX), these compounds can pose health risks to humans if consumed through seafood, inhaled from polluted water, or absorbed through the skin [28].Researchers studied cnidarians extensively and them considered the oldest existing group of venomous organisms and the biggest category of toxic animals, highlighting their ecological and economic significance [29]. The Cnidarians hold the top position in their hierarchy, with approximately 10,000 species found globally [30]. Most of these compounds are recognized as hazardous to human health. Cnidarians serve as a source of bioactive peptides that hold potential for the development of new pharmaceutical drugs.The venoms primarily consist of enzymes, powerful pore-forming toxins, and neurotoxins [31]. Cnidarians have been found to produce a diverse array of peptides, including toxins such as Phospholipase A2 (PLA2), neurotoxins that target sodium and potassium channels, cytolysins, peptide toxins that affect acid-sensing ion channels (ASICs), and several other types of toxic compounds.[1]Neurotoxin 2 (ATX-II), a toxin found in *Anemonia sulcata* that blocks Na + channels, has been shown to have both toxic and antibacterial properties [32, 33]. The neurotoxin Ueq 12-1, derived from *Anemonia sulcata* and *Urticina eques*, has been shown to inhibit the growth of several human pathogens, such as *Corynebacterium glutamicum* and *Staphylococcus aureus*. Cytolytic actinoporinsproteins with heart-stimulating properties, cytolysins extracted from sea anemones *Heteractis magnifica* and *Stichodactyla mertensii* have demonstrated enhanced antibacterial activity against pathogens like *Staphylococcus aureus* and *Salmonella typhi* [34]. Crassicorin-I, a newly identified neurotoxin derived from a sea anemone, was effective against *Bacillus subtilis* and showed moderate antimicrobial activity against *E. coli* and *Salmonella enterica* [35]. Additionally, methanol extracts obtained from the nematocysts of *Stichodactyla mertensii* and *Stichodactyla gigantea* exhibited moderate antibacterial effects against human pathogens such as *Staphylococcus aureus*, *Salmonella typhi*, and *Vibrio cholera* [36].The sea whip *Leptogorgia virgulata*, belonging to the Gorgoniidae family, produces homarine or a similar compound, both of which are considered key elements of the innate immune system, as reported in studies by [37] and [38]. Aurelin, a compound extracted from the mesoglea of the jellyfish *Aurelia aurita*, has demonstrated enhanced antibacterial activity against both Gram-negative and Gram-positive bacteria [39].The jellyfish *Gonionemus vertens* produces neurotoxins that can alter the adhesion of macrophages. The raw venom of *Pelagia noctiluca* and its substances have cytotoxic and antiproliferative effects mainly on cancerous cell lines. Venom extracted from the tentacles of *Chironex fleckeri*, known for causing intense pain and damage to nearby tissues, was found to swiftly destroy human cells. Comparable outcomes were seen through tests measuring LDH release or ATP depletion [40]. Lau et al.[41] suggest that the interaction between jellyfish venom components and human factors could be a potential avenue for using jellyfish venom components in drug discovery to speed up the development of new medicines. The structural characteristics of a cytotoxic protein (CcTX-1) extracted from *Cyanea capillata's* venom, known for its cytotoxicological effect [42]. The venom of *Nemopilema nomurai* jellyfish exhibits potent anticancer properties causing cytotoxicity against HepG2 cells through apoptotic cell death [43].Daiz-Garcia et al. [44] discovered two small toxins, PpV9.4 and PpV19.4, in the venom of *Physalia physalis*, which have been shown to suppress insulin secretion. Additionally, Lazcano-Pérez et al.[45] examined the venom of *Palythoa caribaeorum* and identified it as a potential anticancer agent due to its strong inhibitory impact on glioblastoma and lung cancer cells. The venom of *Cassiopea andromeda* caused targeted cytotoxicity in breast adenocarcinoma patients' cancerous tissue by acting on mitochondria through ROS generation [46]. A peptide isolated from the venom of *Chrysaora quinquecirrha* exhibited strong cytotoxic effects on alveolar epithelial carcinoma and cervical cancer cells, while having no effect on healthy human lymphocytes [47]. *Ehrlich ascites* have been reported to exhibit both anticancer and antioxidant activities [48]. A recently identified toxic peptide, PpVα, from *Protopalythoa variabilis* was shown to alleviate the negative effects of 6-hydroxydopamine (6-OHDA) on zebrafish movement and to inhibit the excessive production of reactive oxygen species (ROS) triggered by 6-OHDA [35].

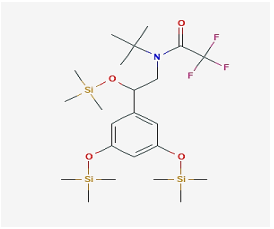
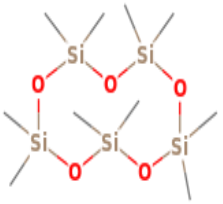
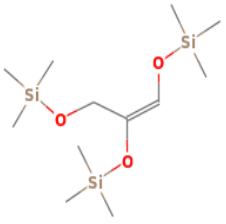
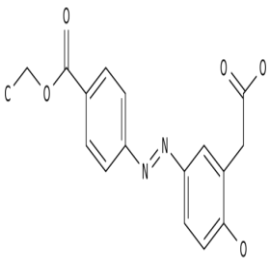
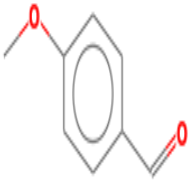
Moreover, researchers have discovered a new Kunitz-like peptide (PcKuz3) in *Palythoa caribaeorum*, which shows potential as a neuro-protective compound for treating neurodegenerative

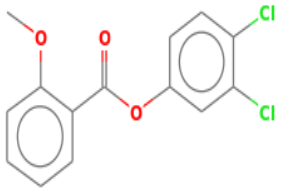
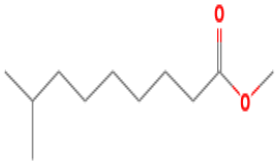
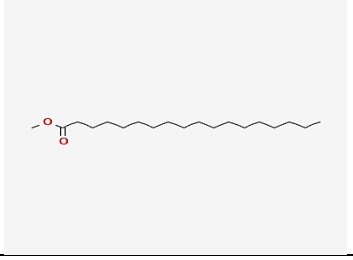
illnesses [35]. Several sesquiterpenes obtained from *Capnella imbricate* and *Dendronephthya rubeola* displayed anti-inflammatory and antiproliferative effects, as well as cytotoxicity against certain cancer cell lines. Capnell-9(12)-ene-8β,10α-diol has been found to effectively disrupt the interaction between the cancer-related transcription factor Myc and its partner protein Max, indicating its potential as a therapeutic agent in cancer treatment. Meanwhile, chabranol—a nor-sesquiterpene derived from *Nephthea chabroli*—has shown moderate cytotoxicity against P-388 mouse lymphocytic leukemia cells, with an ED50 value of 1.81 µg/mL, as noted in the study by Cheng et al. [49]*Nephthea erecta* produces two proteins, oxygenated ergostanoids 1 and 3 that play a role in inflammatory responses. Hwang et al. [50] Crassocolide H was found to inhibit the growth of KB cells with an IC50 of 5.3 µg/mL, while Crassocolide L demonstrated activity against HeLa cells, showing an IC50 of 8.0 µg/mL. Additionally, the aqueous extract of *Lobophytum* species contained lobohedleolide, (7Z)-lobohedleolide, and 17-dimethylaminolobohedleolide, all of which exhibited moderate anti-HIV effects, with IC50 values ranging from 7 to 10 µg/mL in an in vitro assay by Rashid et al [51] Prostanoids, specifically claviridic acid, extracted from *Clavularia viridis*, exhibited significant inhibition of phytohemagglutinin-stimulated proliferation in peripheral blood mononuclear cells (PBMC) at a concentration of 5 µg/mL. Additionally, they showed notable cytotoxic effects against human gastric cancer cells (AGS), with IC50 values ranging from 1.73 to 7.78 µg/mL [52]. According to Duh et al [53]extracts of *Claviri denone* showed strong cytotoxic activity against mouse lymphocytic leukemia (P-388) and human colon adenocarcinoma (HT-29) cells, and exhibited highly potent cytotoxic effects on human lung adenocarcinoma (A549) cells, with ED50 values ranging from 0.52 pg/mL to 1.22 µg/mL. Additionally, halogenated prostanoids were found to be cytotoxic to MOLT-4 human T lymphocyte leukemia cells (IC50 0.52 µg/mL), DLD-1 human colorectal adenocarcinoma cells (IC50 0.6 µg/mL), and IMR-90 human diploid lung fibroblast cells (IC50 4.5 µg/mL), as reported in a study [54].

The three distinct and well-separated compoundswere isolated from crude extracts of box jellyfish-*Chiropsoides buitendijki*. The preparative HPTLC was performed to isolate the pure compounds from the extracts. Analysis of the extracts was performed to determine the nature of the compounds using GC-MS and FTIR techniques, and the compounds were identified, Table No. (4)as, Sulfuric acid dimethyl ester: Trans-(2 Chlorovinyl) methyl diethoxy silane, Ethanone, 1-(1a, 2, 3, 5,6a, 6b-hexahydro-3, 3, 6a-trimethyloxireno[g]benzofuran-5-yl), 4-[(1R)-1-Aminoethyl]-N-4-pyridinyl-Trans-cyclohexanecarboxamide, Terbutaline, N-trifluoro acetyl-O, O, o-tris(trimethylsilyl) derive, Cyclopentasiloxane, decamethyl, Phloroglucinaldehyde, tris(trimethylsilyl) ether, 5- [2- [4-(Ethoxy carbonyl) phenyl] diazenyl]-2-hydroxybenzeneacetic acid, Benzaldehyde, 4-methoxy , Benzaldehyde, 3-methoxy, o-Anisic acid, 3,4-dichlorophenyl ester, Decanoic acid, methyl ester, 8-Methylnonanoic acid, methyl ester, Pentadecanoic acid, 14-methyl-, methyl ester, Tetra decanoic acid, 10,13-dimethyl-, methyl ester, Tridecanoic acid, methyl ester, Methyl stearate, and Heptadecanoic acid, 16-methyl-, methyl ester,respectively.

Table No. (4) Showing names of the compounds,molecular weights, molecular formula, and structures of the compounds isolated from box jellyfish-*Chiropsoides buitendijki*.

Sr.No.	Nameof theCompound	Molecular Weight	Molecular Formula	Structure
1	Sulfuricacid dimethyl ester	126.13g/mol	$(CH_3O)_2SO_2$	
2	Trans-(2 Chlorovinyl) methyldiethoxy silane	194.73g/mol	$C_7H_{15}ClO_2Si$	
3	Ethanone, 1-(1a,2,3,5,6a,6b-hexahydro-3,3,6a-trimethyloxireno[g]benzofuran-5-yl)	222.28 g/mol	$C_{13}H_{18}O_3$	
4	4-[(1R)-1-Aminoethyl]-N-4-pyridinyl-trans-cyclohexanecarboxamide	247.34 g/mol	$C_{14}H_{21}N_3O$	

5	Terbutaline, N-trifluoro acetyl-O, O, o-tris(trimethylsilyl)deriv.	537.8 g/mol	$C_{23}H_{42}F_3NO_4Si_3$	
6	Cyclopentasiloxane, decamethyl	370.76 g/mol	$C_{10}H_{30}O_5Si_5$	
7	Phloroglucinaldehyde, tris(trimethylsilyl) ether	378.63 g/mol	$C_{15}H_{30}O_3Si_3$	
8	5- [2- [4-(Ethoxy carbonyl) phenyl] diazenyl]-2-hydroxybenzeneacetic acid	328.32 g/mol	$C_{17}H_{16}N_2O_5$	
9	Benzaldehyde, 4-methoxy	136.14g/mol	$C_8H_8O_2$	
10	Benzaldehyde, 3-methoxy	136.147g/mol	$C_8H_8O_2$	

11	o-Anisic acid, 3,4-dichlorophenyl ester	297.133 g/mol	$C_{14}H_{10}Cl_2O_3$	
12	Decanoic acid, methyl ester	186.29 g/mol	$C_{11}H_{22}O_2$	
13	8-Methylnonanoic acid, methyl ester	186.291 g/mol	$C_{11}H_{22}O_2$	
14	Pentadecanoic acid, 14-methyl-, methyl ester	270.45 g/mol	$C_{17}H_{34}O_2$	
15	Tetra decanoic acid, 10,13-dimethyl-, methyl ester	270.45g/mol	$C_{17}H_{34}O_2$	
16	Tridecanoic acid, methyl ester	228.37 g/mol	$C_{14}H_{28}O_2$	
17	Methyl stearate	298.51 g/mol	$C_{19}H_{38}O_2$	
18	Heptadecanoic acid, 16-methyl-, methyl ester	298.50g/mol	$C_{19}H_{38}O_2$	

CONCLUSION

We conducted a screening of the organic extract to isolate and purify individual compounds for structural elucidation. The chemical structures of these isolated compounds were further analyzed to assess their biological activities. The findings indicate that the crude extract from the box jellyfish *Chiropsoides buitendijki* exhibits promising biomedical potential. These results highlight the significance of our study in contributing to the development of novel pharmaceutical drugs. Consequently, further investigations at the molecular level are recommended to better understand the physiological effects and mechanisms of action of these bioactive compounds.A clinical study is also essential, as it can aid the pharmaceutical industry in developing new drugs by evaluating their safety and effectiveness. This research is crucial for ensuring safe usage and assessing safety parameters, ultimately contributing to the future elimination of diseases affecting humanity.

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 the collection of species. Dr. Kishori Apte, National Toxicological Centre, APT Testing & Research Pvt. Ltd., Pune, Maharashtra, for supervising and assisting during the toxicological studies.

Funding sources:

The authors are thankful to Mahatma Jyotiba Phule Research & Training Institute (MJPRF), Nagpur, Govt. of Maharashtra, for their
financial support and cooperation for the research.

Conflict of interest:

The authors have no conflict of interest.

Data Availability Statement:

This statement does not apply to this article.

Ethics Statement:

Maharashtra State Biodiversity Board, Nagpur, under approval numbers MSBB/Desk-5/Research/841/2022-23 and MSBB/Desk-5/Research/397/2023-24 for the collection of box jellyfish specimens for research. National Toxicology Centre, Pune. The study was approved by the APT Foundation Ethical Committee (CPCSEA RP. No APTRF/RP-02/2223).

Informed Consent Statement:

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration:

This research does not involve any clinical trials.

Author Contributions:

Jaya Dolnar: Data collection, analysis & writing; Prof. Dr. Gautam V. Zodape: Conceptualization and supervision

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