IJPSR (2020), Volume 11, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 December 2019; received in revised form, 10 October 2020; accepted, 20 November 2020; published 01 December 2020

GC-MS ANALYSIS AND ANTI-INFLAMMATORY ACTIVITY OF *MUREX TRIBULUS*

J. Esther Mereen * and Jemma Hermelin Jesy Diaz

Department of Zoology, St. Mary's College (Autonomous), Thoothukudi, Affiliated to Manonmaniam Sundaranar University, Abhishekapatti, Tirunelveli - 627012, Tamil Nadu, India.

Keywords:

Murex tribulus, Gastropod, Bioactive compounds, Anti-inflammatory activity

Correspondence to Author:
J. Esther Mereen

Research Scholar, Department of Zoology, St. Mary's College (Autonomous), Thoothukudi, Affiliated to Manonmaniam Sundaranar University, Abhishekapatti, Tirunelveli - 627012, Tamil Nadu, India.

E-mail: esthermereen@gmail.com

ABSTRACT: The aim of this study was to screen the presence of bioactive compounds through GC-MS analysis and carry out the ant-inflammatory test of the methanolic tissue extract of *Murex tribulus*, a spiny predatory gastropod that is widespread from the Central Indian Ocean to Western Pacific Ocean. The GC-MS analysis of the methanolic flesh extract revealed the presence of four bioactive compounds, 1, 2 dimethyl hydrazine, urea, cis-9 hexadecenoic acid, and 1, 1, 1, 3, 5, 5, 5 hepta-methyltrisiloxane. Among the compounds identified, 1, 1, 1, 3, 5, 5, 5-Heptamethyltrisiloxane was the most abundant antimicrobial compound (92.87%) present in the methanolic tissue extract of *M. tribulus*. The results of the GC-MS analysis showed that the bioactive compounds present in the tissue extract showed anti-inflammatory responses. Hence *in-vitro* anti-inflammatory activity in the tissue extract of *M. tribulus* was determined by proteinase inhibitory activity and 5-lipooxygenase inhibitory assay. The anti-inflammatory test showed promising results with high percentage of inhibition such as 53.50% at a concentration of 1000 µg/ml in proteinase inhibitory activity and 75.42% at the concentration of 1000 µg/ml in %-lipoxygenase inhibitory assay.

INTRODUCTION: Among the marine invertebrates, the molluscs are the potential source of bioactive substances. The bioactive compounds isolated from the gastropods are considered to have a role in the chemical defence of the animals against their predators. Muricidae, commonly known as murex or rock whelks, have a long history of pharmacological use, being listed in the Materia medica by Discorides in 1st century AD, reported by Arabic scholars in 9th Century, and sold in medieval Jewish pharmacies from 11th -14th Century AD [1, 2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(12).6184-88

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(12).6184-88

Over the years, numerous bioactive compounds have been reported in the family of Muricidae among which some have the property to reduce the oxidation reactions. Many promising lead compounds have been reported from marine sources having anti-inflammatory activity.

In-vitro and *in-vivo* anti-inflammatory activity of tissue extracts and associated indole compounds from the marine Muricidae *Dicathais orbita* were tested for their ability to inhibit the production of the recognised pro-inflammatory modulator nitric oxide (NO) and cytokines, such as tumour necrosis factor alpha (TNFα) and prostaglandin E2 (PGE2) [3]. Muricidae extracts have demonstrated wound healing properties and anti-inflammatory activity in addition to their antimicrobial properties. Muricidae produce a suite of brominated indoles with anti-inflammatory, anti-cancer, and steroid-ogenic activity as well as choline esters with

muscle-relaxing and pain relieving properties. These compounds are used in wound healing, stomach pain and menstrual problems traditionally. Hence, the present study was taken to elucidate the anti-inflammatory activity of the tissue extract of *M. tribulus* and to characterize the bioactive compounds in the methanolic tissue extract of *M. tribulus* by GC-MS analysis.

MATERIALS AND METHODS:

Specimen Collection and Identification: In the present study, the gastropod, *Murex tribulus* was collected from Gulf of Mannar, Thoothukudi coastal region (Long.$78^0$8" to 79^0 30" E and Lat. 8^0 35" to 9^0 25" N) by trawl catch used for crabs. These animals are found entangled in the nets used for crabs.

The collected fresh molluscs were preserved with ice and transported to the laboratory and identified by standard literature [4]. The shells were broken and the whole body of the organism was removed, cut into small pieces, air dried, powdered and it was used for further studies.

FIG. 1: DORSAL VIEW OF *M. TRIBULUS*

FIG. 2: VENTRAL VIEW OF *M. TRIBULUS*

Collection and Preparation of Extract: About 50 g of tissue powder was immersed into 150 ml of methanol in a conical flask and was cold steeped at 18 °C. The extracts were filtered using Whatman No.1 filter paper and stored at 4 °C for further experimental work.

GC-MS Analysis: GC-MS analysis was performed using an Agilent 7820A gas chromatograph coupled to an Agilent5977E mass selective detector in the positive ion-electron impact (EI) mode. The separation was achieved using a DB-5 MS fused silica capillary column, 30m × 0.25 mm *i.e.*, 0.25 µm film thickness. GC oven temperature was programmed from 100 °C to 270 °C at a rate of 10 °C/min. Helium was used as the carrier gas; inlet pressure was 25kPa; linear velocity: 1 mL/min at 210 °C. Injector temperature: 250 °C and injection mode: split 1:50. MS scan conditions: source temperature, 200 °C; interface temperature, 250 °C; E energy, 70eV; mass scan range, 40-350 amu.

Identification of Compounds: Interpretation on the spectrum was conducted using the database of National Institute Standard and Technology (NIST), WILEY 8, and FAME having more than 62,000 patterns.

The unknown compounds found in the methanol fraction of the tissue were matched with the spectrum of the known components stored in NIST, WILEY 8, FAME, and MS library and predicted from Dukes ethnobotanical database.

***In-vitro* Anti-Inflammatory Activity:**

Preparation of Extract: 1 gm of the sample was dissolved in 20 ml of the methanol solvent and incubated at 40 °C, 60-70-RPM in an orbital shaker for 24 h. The extract was filtered through Whatman no.1 filter paper and used for further study.

Proteinase Inhibitory Activity: To the different concentration of the extract (250 µg/ml, 500 µg/ml, 750 µg/ml, and 1000 µg/ml) 0.06 mg of trypsin and 1 ml of 20 mM Tris-HCl solution (pH-7 to7.4) was added, and the mixture was incubated at 370C for 5-10 min. To this extract 1 ml of 0.8% casein solution was added, and the mixture was incubated for 20 min at room temperature, and 1 ml of 70% perchloric acid solution was added to arrest the reaction. To avoid the cloudy suspension, the reaction mixture was centrifuged at 5000 rpm for 5 min, and the measurement was taken at 210 nm. The percentage of inhibition was calculated by using the following formula [5].

% of inhibition = (Absorbance of Control-Absorbance of the sample) / (Absorbance of control) × 100

5-lipooxygenase Inhibitory Assay: 70 mg of linoleic acid and an equal weight of tween 20 was dissolved in 4 ml of oxygen-free water and mixed back and forth with a pipette to avoid air bubbles. A sufficient amount of 0.5N sodium hydroxide was added to yield a clear solution and then made up to 25 ml using oxygen-free water. This was divided into 0.5 ml portions and flushed with nitrogen gas before closing, and kept frozen until needed. The reaction was carried out in a quartz cuvette at 25 °C with 1 cm light path. The assay mixture contained 2.75 ml Tris buffer of pH 7.4, 0.2 ml of sodium linoleate, and 50 ml of enzyme, and the OD was measured in 234 nm [6].

% of inhibition = (Absorbance of Control-Absorbance of the sample) / (Absorbance of control) × 100

RESULTS:

GC-MS Analysis: GC-MS analysis of body tissue of *M. tribulus* exhibited 4 peaks, with the retention times ranging from 5.992 to 17.679 min. All the four compounds were characterized as 1, 2 dimethylhydrazine, Urea, cis-9 Hexadecenoic acid and 1, 1, 1, 3, 5, 5, 5-Heptamethyltrisiloxane. Among the compounds identified, 1, 1, 1, 3, 5, 5, 5-Heptamethyltrisiloxane was the most abundant antimicrobial compound (92.87%) present in the methanolic tissue extract of *M. tribulus*. The identified compounds, 1, 2 dimethylhydrazine, Urea, cis-9 Hexadecenoic acid and 1, 1, 1, 3, 5, 5, 5-Heptamethyltrisiloxane have the role in anti-depressant, antineoplastic, antibacterial, anti-viral, antibiotic, antiparasitic, analgesics for osteo-porosis, anti-asthmatics, anti-acne, anti-psoriatics, medicaments and the inclusion of biologically active materials for therapeutic substances. These compounds constitute a promising novel class of pharmaceuticals for the treatment of diseases.

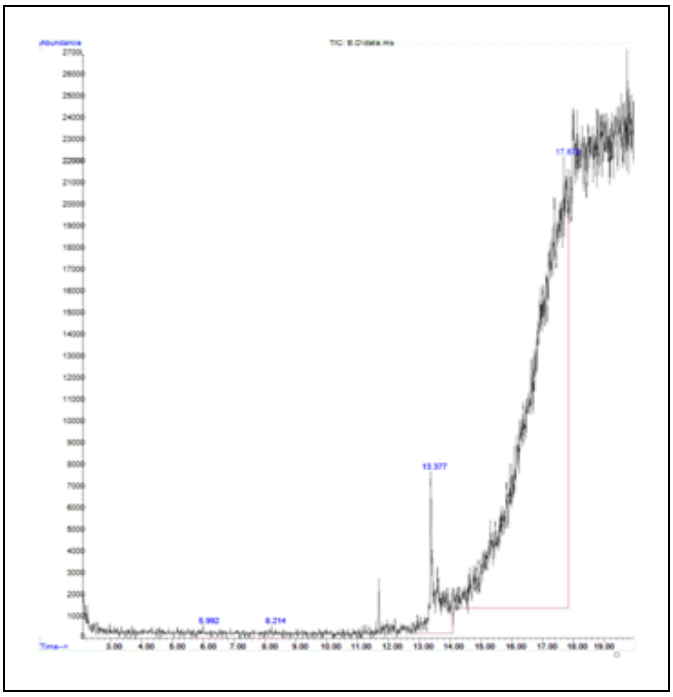

FIG. 3: CHROMATOGRAM

TABLE 1: BIOACTIVE COMPOUNDS FROM THE METHANOLIC EXTRACT OF *M. TRIBULUS*

S. no.	CAS No.	Compound name	Rt Time (in min)	Molecular formula	Molecular weight (g/ml)
1.	000540-73-8	1,2-dimethyl-hydrazine	5.992	$C_2H_8N_2$	60.01
2.	000057-13-6	Urea	8.214	CH_4N_2O	60.06
3.	1000333-19-5	cis-9-Hexadecenoic acid	13.377	$C_{16}H_{30}O_2$	254.41
4.	001873-88-7	1,1,1,3,5,5,5-Heptamethyl-trisiloxane	17.679	$C_7H_{21}O_2Si_3$	221.49

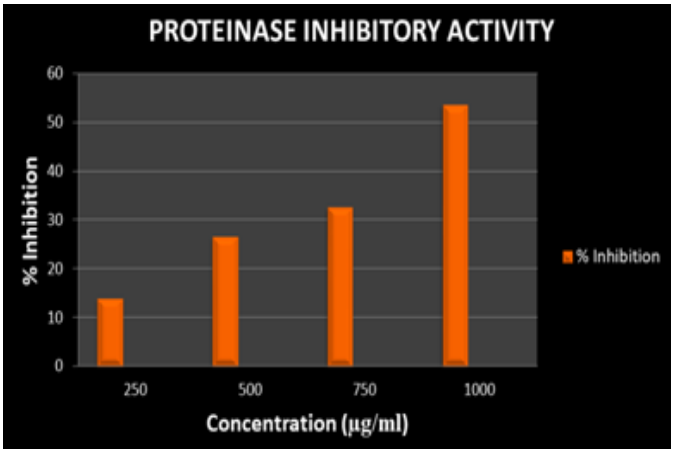

FIG. 4: PERCENTAGE OF INHIBITION IN THE TISSUE EXTRACT OF *M. TRIBULUS* (PIA)

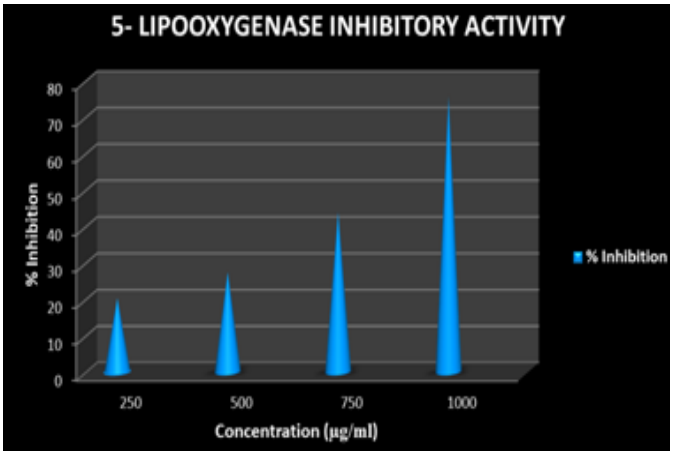

FIG. 5: PERCENTAGE OF INHIBITION IN THE TISSUE EXTRACT OF *M. TRIBULUS* (5- LIA)

***In-vitro* Anti-inflammatory Activity:**

Proteinase Inhibitory Activity (PIA): Aceclofenac drug was used as a standard to compare with the sample value. The percentage of inhibition showed variation from 13% to 54%. The percentage of inhibition was increased with increase in concentration (250 µg/ml-13.87%, 500 µg/ml-26.54%, 750 µg/ml- 32.57%, 1000 µg/ml-53.50%) of the extract.

5-lipooxygenase Inhibitory Assay (5- LIA): The methanolic tissue extract of *M. tribulus* showed inhibition activity ranging from 20% to 75%. Maximum inhibition activity of 75.42% was observed at 1000 µg/ml, and minimum activity of 20.34% was recorded at 250 µg/ml concentration. The anti-inflammatory activity at 500 µg/ml of concentration was 24.47%, and at 750 µg/ml, it was 43.96%. The lipooxygenase inhibitory activity was increased with an increase in the concentration of the extract.

DISCUSSION: GC-MS analysis of methanolic tissue extract showed the presence of bioactive compounds that were further confirmed with the library data. GC-MS analysis showed 1, 1, 1, 3, 5, 5, 5- Heptamethyltrisiloxane as the major constituent with peak area (92.87%). The other minor constituents with low area normalization were also identified by the molecular mass from the library database. Similarly, antimicrobial compounds such as Hexanal–2 methyl, 1, 2 Benzenadicarboxylic acid, diisooctyl ester, 2, 2–Dimethyl propionic acid, hexadecyl ester and pseudoephedrine were identified in column fractionated extract of *P. glaucum* [7].

The presence of antibacterial compounds in the oyster *Pteria chinersis* and bivalve *Perna viridis* have been reported using various solvent extracts [8, 9, 10]. In the present study, anti-inflammatory activity in the methanolic tissue of *M. tribulus* was investigated by proteinase inhibitory activity and 5-Lipooxygenase inhibitory assay. In both the assays the activity was found to increase with the increase in the concentration of the sample. At 1000 µg/ml concentration the proteinase inhibitory activity showed 53.50% inhibition, and at 250 µg/ml concentration, it was 13.87%. Similarly, in 5-Lipooxygenase inhibitory assay, the percentage of inhibition was 20.34% at 250 µg/ml concentration and 75.42% at 1000 µg/ml concentration. Muricidae feature in a number of records of traditional medicines and are also well known for their bioactive secondary metabolites [11], which include the brominated indole precursors to the dye Tyrian purple [12, 13]. This dye is dominated by 6, 6 dibromoindigo, although the purple secretion from some muricids has been shown to contain a mixture of both brominated and non-brominated indigo and indirubin [13].

There was a report that the methanolic extracts of *Cypraea errones* and *C. arabica* exerted a moderate anti-inflammatory effect against Carrageenan-induced inflammation at a dose of 10 mg/kg [14]. The active components were identified as sesterterpenes, which caused *in-vivo* rat paw edema inhibition. The anti-inflammatory effect of the 100% acetone column purified extracts of *D. margariticola* experimented on albino rats. The extract at the concentration of 50 and 100 mg/kg, paw edema showed a significant decrease in the paw thickness in a dose-dependent manner compared to that of control, at the 5th h of experiment [15]. Indole anti-cancer drug leads have regularly shown promise for the treatment of a range of diseases, including inflammation [16].

However, these indole compounds are only found in the hypobranchial glands and reproductive material of Muricidae and are present in trace amounts in the operculum and flesh, and their presence in the shells is uncertain, which are the parts mostly used in traditional anti-inflammatory applications. Consequently, Muricidae natural medicines could be optimized to include standard concentrations of these bioactive compounds along with other well-characterized components in the shell of flesh that may improve the bioavailability and activity.

CONCLUSION: *Murex tribulus* is a commonly available trash organism caught in the cast nets used for crabs on the Thoothukudi coast. Due to its easy availability and non-economical status, it can be widely used as a source of the drug to treat various diseases. Further studies and research may help in the proper designing of drugs using this species. The identification of compounds responsible for numerous pharmacological activities through GC-MS analysis can be utilized to prepare such

compounds artificially. Very little research has been undertaken to visualize the potency of this organism. Hence further research can be done to recognize its yet undiscovered values.

ACKNOWLEDGEMENT: Authors are grateful to the Principal and Head of the Department of Zoology, St. Mary's College (Autonomous), Thoothukudi, Tamil Nadu, India, for the facilities provided to carry out the study.

CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest.

REFERENCES:

1. Meyerhof M and Sobhy GP: The Abridged Version of "The Book of Simple Drugs", of Ahmad Ibn Muhammad Al-Ghafiqi by Gregorius Abul-Farag. Al Ettemad Printing Press and Publising House 1932.
2. Voultsiadou E: Therapeutic properties and uses of marine invertebrates in the ancient greek world and early byzantium. J Ethnopharm 2009; 130: 237247.
3. Ahmad TB: *In-vitro* and *in-vivo* anti-inflammatory activity of extracts from a marine mollusc. PhD thesis, Southern Cross University, Lismore, NSW, 2017.
4. Subbarao NV: Indian sea shells (Part-1): Polyplacophora and Gastropoda. Rec Zool Sur India 2003.
5. Sakat S, Juvekar AR and Gambhire MN: *In-vitro* antioxidant and anti- Inflammatory activity of methanol extract of *Oxalis corniculata* Linn. International journal of Pharma and Pharmacological Sciences 2010; 2(1): 146-55.
6. Viji V and Helen A: Inhibition of lipoxygenases and cyclooxygenase-2enzymes by extracts isolated from *Bacopa monniera* (L.). Wettst. Journal of Ethnopharmacology 2008; 118: 305-11.
7. Thilaga RD, Vimala S and Subavathy P: Isolation and characterization of bioactive Compounds and antibacterial activity of marine Gastropod *Phalium glaucum*. Int Journal of Pure and Applied Zoology 2014; 2 (3): 218-23.
8. Chellaram C, Gnanambal KM and Edward JKP: Anti-bacterial activity of the winged oyster, *Pteria chinensis* (pteroida: pteridae). Indian J Mar Sci 2004; 33: 369-72.
9. Li C, Song I and Zhao J: A review of advances in research on marine molluscan antimicrobial peptides and their potential application in aquaculture. Molluscan Res 2009; 29: 17-26.
10. Ding GF, Huang FF, Yang ZS, Yu D and Yang YF: Anticancer activity of an oligopeptide isolated from hydrolysates of Sepia ink. Chi J Nat Med 2011; 9: 151-55.
11. Benkendorff K, Rudd D, Nongmaithem BD, Liu L, Young F, Edwards V, Avila C and Abbott CA: Are the traditional medical uses of *Muricidae molluscs* substantiated by their pharmacological properties and bioactive compounds? Mar. Drugs 2015; 13: 5237-75.
12. Benkendorff K: Natural product research in the Australian marine invertebrate Dicathais orbita. Mar Drugs 2013; 11: 1370-98.
13. Cooksey CJ:Tyrian purple: 6,6′-dibromoindigo and related compounds. Molecules 2001; 6 (9): 736-69.
14. Kumar SS: Studies on the cowries (Mollusca: Gastropoda: Cypraeidea) of Gulf of Mannar, Southeast coast of India. Ph. D Thesis submitted to Manonmaniam Sundaranar University, Tirunelveli, 2003; 1-86.
15. Chellaram C and Edward JKP: Anti-inflammatory potential of coral reef-associated gastropod. Drupa Margariticola. Indian J Sci Technol 2009; 2: 75-77.
16. Gul W and Hamann MT: Indole alkaloid marine natural products: an established source of cancer drug leads with considerable promise for the control of parasitic, neurological and other diseases. Life Sci 2005; 78: 442-53.

How to cite this article:
Mereen JE and Diaz JHJ: GC-MS analysis and anti-inflammatory activity of *Murex tribulus*. Int J Pharm Sci & Res 2020; 11(12): 6184-88. doi: 10.13040/IJPSR.0975-8232.11(12).6184-88.