

“GC-MS Profiling and Pharmacological activities of *Telescopium telescopium* from Punnakayal Mangrove Region”

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DOI: <https://doi.org/10.63001/tbs.2026.v21.i01.pp2246-2321>

KEYWORDS

*Antioxidant,
Anti-inflammatory,
and GC-MS analysis.*

Received on: 28-02-2026

Accepted on: 15-03-2026

Published on: 26-03-2026

Abstract

The present study evaluates the pharmacological activities of *Telescopium telescopium* collected from the mangrove region. The research focuses on assessing antioxidant, anti-inflammatory, and GC-MS analysis properties of the species. Specimens were collected from the Punnakayal mangrove region, and tissue extracts were prepared using standard extraction methods. The biological activities were analyzed through established in vitro assays. The results demonstrated significant antioxidant activity, indicating the presence of bioactive compounds capable of scavenging free radicals. The extracts also exhibited notable anti-inflammatory effects. These findings suggest that *Telescopium telescopium* possesses potential therapeutic properties and can be considered a promising source of bioactive compounds for pharmaceutical applications. The study highlights the importance of mangrove gastropods in drug discovery and provides baseline data for further pharmacological investigations.

1 Introduction

With more than 70% of the earth's surface, the oceans represent the largest habitat of the earth and a prolific resource of organisms with high biological and chemical diversity. Although most drugs are still derived from terrestrial sources, a considerable

number of drugs, drug candidates and other metabolites from marine organisms have been identified in recent years (Ulrike Lindequist, 2016). Marine invertebrates have to date provided a substantial diversity of natural products (Leal

et al., 2012). These natural products have an extensive array of therapeutic properties, including antimicrobial, antioxidant, anti-inflammatory, anticancer and other medicinal properties (Perdicalis *et al.*, 2013; Senthil kumar and Kim, 2013). A number of marine natural products have provided important leads for drug development and many are now used in formulation of novel drugs (Tarek Ahmad *et al.*, 2017).

Marine organisms are a rich source of structurally novel and biologically active metabolites. Primary and secondary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry molluscs are the slow moving organisms with high biological and chemical diversity (Thilaga, 2005). A number of molluscs are also used in

traditional Chinese, Indian, South African and Middle Eastern medicines as in homeopathic remedies (Benkendorff, 2014). Bioactive compounds derived from these soft bodied molluscs exhibit potential biological activity with unique structural features and can be considered safe to some existing synthetic drugs (Suleria *et al.*, 2016).

Inflammation is a pathophysiological response of living tissue to injury that leads to local accumulation of plasmatic fluid and blood cells (Vittalrao *et al.*, 2011). Inflammation results in the liberation of endogenous mediators like histamine, serotonin, bradykinin and prostaglandins etc. Prostaglandins and hyperalgesic, potent vasodialators and also contribute to erythema, edema and pain (Anilkumar, 2010). Pain due to inflammation is an unpleasant sensation that is a consequence of

complex neurochemical processes in the central and peripheral nervous system (Musa *et al.*, 2009). Drug that are currently used for management of mild to moderate, severe pains and for inflammatory conditions are opioids, non – steroidal anti-inflammation drugs (NSAIDS) and corticosteroids. Studies reveal that the risk of gastrointestinal irritation (Wallace, 2000), liver damage (Bower *et al.*, 2007) and renal damage (Howland *et al.*, 2005) were significantly associated with acute use of non- steroidal anti-inflammatory drugs (Kohila Subathra Christy *et al.*, 2014).

One of the most common therapeutic applications for molluscs in traditional ethnomedicine appears to be for the treatment of inflammatory condition. Inflammation is a complex mechanism involving the activation and deactivation of immune cells in response to stimuli and tightly

regulated signaling pathways. If left unchecked, inflammation will result in cellular and tissue damage that can lead to chronic disease (Alessandri *et al.*, 2013). Macrophages play a key role in initiating and maintaining the inflammatory response, and are activated by pathogen associated molecules and cytokines, which stimulate pro-inflammatory, signaling pathway (Fujiwara and Kobayashi, 2005). Current non-steroidal anti-inflammatory drugs have been linked to increase blood pressure, greatly increased risk of congestive heart failure, occurrence of thrombosis and they can also predispose patients to serious gastrointestinal erosion (Aisen *et al.*, 2003). These side effects are common to almost all non-steroidal anti-inflammatory drugs (Vane and Botting, 1998). Because anti-inflammatory drugs are among the most consumed pharmaceuticals, with

over 70 million prescriptions and 30 billion tablets of non – steroidal anti-inflammatory drugs sold over the counter each year (Maroon *et al.*, 2006). Hence there is an urgent need to search for safer and cheaper sources of anti-inflammatory drugs.

Oxidative stress is considered an imbalance between the prooxidants and antioxidants in the body, and usually eliminated by antioxidant defense system (Rahal *et al.*, 2014), including many antioxidant enzymes and non – enzymatic antioxidants. Unfortunately when there are more free radicals present that can be quenched by the body's antioxidant defense system. Hence, damage to tissues, and even diseases began to appear. The most effective and widely used strategy to reduce oxidative stress is supplement exogenous antioxidants (Poljsak *et al.*, 2013). Antioxidants

from natural sources play a paramount role in helping endogenous antioxidants to neutralize oxidative stress (Sasikumar *et al.*, 2009). In recent years, there have been concerns over the safety of synthetic antioxidants, therefore antioxidants derived naturally are attracting more attention. Natural products show strong antioxidant activity in scavenging free radicals and receiving cellular damage caused by oxidation and have been added in health supplements, food additives, and pharmaceuticals (Nimse and Pal, 2015; Massini *et al.*, 2016; Xu *et al.*, 2017).

Many anti-inflammatory and antioxidant activities have been explored by various researchers. Anti-inflammatory activity of tissue extracts of *H. pugilines* and *Natica didyma* was done by Ravi *et al.* (2012), on Borquayl *et al.*, 2017 investigated anti-inflammatory activity of the extracts

from *Oliva. sp.*, *Patella rustica* and *Littorina littorea*. Ahmad *et al.*, 2018 reviewed the anti-inflammatory, immune – modulatory and wound healing properties of molluscs. Esther meerin *et al.*, 2019 analyzed the anti-inflammatory activity of *Murex Tribulus* and Queensley *et al.*, 2021 on *Pachymelania aurila* and *Tympanotonons fuscatus radula*.

Antioxidant activities of varies mollusc have been carried out various authors Prem Anand *et al.* (2010) on *Pleuroplaca trapezicm*, Nagash *et al.*, 2010 on *Loligo duvaucelli* and *Donax striatus*, on *Amusium pleuronectes* by Shankar Kanchana *et al.*, 2013, on *Bursa spinosa* by Subhapradha *et al.*, 2013. Invitro antioxidant activity of different gastropod mollusc and Echinoderm was tested by Abrami Pachiappan *et al.*, 2014. Lawrence Sheringham *et al.*, 2016 examined the antioxidant activity

of ethylactitate and methanol extracts of *Littorina littorea* and *Galatea paradoxa*. Ponnusamy *et al.*, 2016 investigated the antioxidant properties of tissue extract of Cephalopodes. Antioxidant capacity of the extracts from *Perna viridis* was explored by (Krishnamoorthy *et al.*, 2019). Bavani Saraswathy *et al.* (2019) tested the antioxidant potential of *P. glauctum*. Subavathy and Mary Bapista Janet, (2020) examined the antioxidant property of *T. brunneus*, *C. annulus* and *B. spiriata*. Queensley *et al.*, 2021 on *Pachymelania aurita* and *Tympanotonons fuscatus radula*. Though many antioxidant and anti-inflammatory studies have been carried out on marine molluscs, the pharmacological works on mangrove gastropods are scanty. Hence the present study aimed at to test antioxidant and anti-inflammatory

potentials of the mangrove gastropods
T. telescopium.

2 Materials and Methods

Collection and extraction of samples

Live specimens of *T. telescopium* were collected from Punnakayal mangrove (lat 8 38'19.2" N Long 78 07' 09.8" E). They were immediately brought to the laboratory and their soft tissues were removed by breaking the shells. The whole body muscle of the sample (50 g) was cut into small pieces and the tissue sample was used for extraction using the solvent methanol and water. The extracts were cold stored overnight at -18°C and filtered with Whatman No. 1 filter paper. The filtrate was poured in previously weighed petriplates and evaporated to dryness in rotary evaporator. (Becerro *et al.*, 1994;

Wright, 1998). The dried extracts were used for anti-inflammatory and anti oxidant activities.

1 Anti-inflammatory activity

i) Test Animals

The adult Wistar albino rats of either sex weighing between 25-50 g and 100-180 g were used respectively. The animals were housed under standard environmental conditions (temperature of $22 \pm 1^{\circ}\text{C}$ with an alternating 12 hrs light-dark cycle and relative humidity of $60 \pm 5\%$) in the Department of Pharmacology, Sankaralingam Bhuvaneswari college of Pharmacy, Sivakasi, fed with standard pellet – diet and water *adlibitum*. Prior approval of Institutional Animal Ethics Committee (IAEC) (SBCP/ 2015 -16 CPCSEA/IAE/I/I/P) was obtained.

ii) Carrageenan – Induced Paw Edema

Rats were divided into 4 groups of 6 animals each. The control group (Group 1) was injected with saline (1 ml/kg) into the plantar region of the right hind paw. Anti-inflammatory activity was evaluated by injecting carrageenan (Sigma 0.05 ml of 1% W/V) subcutaneously into the plantar region of the right hind paw. Group 2 (standard) was given the standard drug Diclofenac sodium (10 mg/kg) an hour prior to carrageenan injection, Group 3 and 4 were treated with test extracts (Methanol extract of *T. telescopium*) at the dose levels of 200 and 400 mg/kg respectively. All the doses were administered orally. The thickness of the right paw was measured before and after Carrageenan injection at time intervals 1, 2, 3, and 4 hours respectively.

The degree of edema formation at the hind paw was measured by Plethysno graphically and the

percentage increase of paw edema thickness was calculated using the method of Duwiejua *et al.* (1994).

$$\% \text{ inhibition} = 100 (1 - V_t/V_c)$$

Where V_c represents edema volume in control

V_t represents edema volume in the test organism

2 Antioxidant activity of

T. telescopium (Harborne and Baxter, 1995)

iii) DPPH Radical Scavenging assay

The antioxidant activity of the Methanolic extract of *T. telescopium* was measured on the basis of scavenging activity of the stable 2, 2-diphenyl

1- Picrylhydrazyl (DPPH) free radical according to the method described by Harborne and Baxter, (1995) with slight modification. 1 ml of 0.1 mm DPPH solution in methanol was mixed with 1ml of animal extract solution of

varying concentrations (50, 100, 150 and 200 µg/ml). Corresponding blank samples were prepared and L-ascorbic acid was used as standard. Mixture of 1 ml of methanol and 1 ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-Vis spectrophotometer (UV-Vis Shimadzu). The percentage of antioxidant activity was calculated using the following formula.

Percentage of antioxidant activity =

$$\frac{AC-AS}{AC} \times 100.$$

Where

AC is the absorbance of the control

AS is the absorbance of the test

3 GC-MS-Analysis

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer

System comprising a AOC 20 i auto sampler and gas chromatography interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions such as Column Elite – 1 fused silica capillary column (30 × 0.25 mm ID × 1 mm df, composed of 100% poly siloxane), operating in electron impact mode at 70 eV; Helium (99.999%) was used as a carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 µl (split ratio of 10:1) injector temperature 250°C; and ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5s and fragments from 40 to 550 Da.

4 Identification of bioactive compounds

Interpretation on mass spectrum was conducted using the database of National Institute of Standard Technology (NIST Ver. 21), WILEY and FAME having more than 62,000 patterns. The unknown components found in the body tissues of

T. telescopium were matched with the spectrum of the known components stored in NIST, WILEY and FAME, the MS library and predicted from Duke's Ethno Botanical Database.

Statistical analysis

All the data were assessed statistically by the method of one way ANOVA followed by Dunnett's t-test. P value < 0.1, P < 0.05 and P < 0.001 were considered as statistically significant.

Mean

The average ($\bar{x}$) is calculated as follows.

$$\bar{X} = \frac{(\sum x)}{N}$$

Where,

X = data obtained

$\sum x$ = sum of all the values

n = total number of sample

Standard Error $S.E_{\bar{X}}$

$$S.E_{\bar{X}} = \frac{s}{\sqrt{n}}$$

Analysis of variance (ANOVA)

Sum of X for all the values was squared and a correction factor 'C' was obtained.

$$C = \frac{(\sum X)^2}{N}$$

Total sum of squares = $(\sum X)^2 - C$ where X^2 represents the sum of squared values.

$$\text{Group sum squares} = (\sum x)^2 - C$$

Where N - number of observation in each group

Error Sum of square = Total SS - Group SS

Considering the degrees of freedom for each source of variance, mean square was calculated.

Total SS = Number of values in the Table - 1

Group SS = Number of groups - 1

Error SS = df of total SS - df group SS

3 Results

3.1 Anti-inflammatory activity

T. telescopium methanol extract was tested for its anti-inflammatory properties, and the findings are shown in (Table 8.1). Over the course of the four hours of observation, group 1 (control) paw volume increased. After 4 hours, the dose of 10 mg/kg of diclofenac sodium (Group 2)

(Standard) was statistically significant (56.78±0.02). After 3 and 4 hours of experimental exposure to *T. telescopium* methanolic extract (200 mg/kg bodyweight), the mean volume of paws in the third group (which received the highest dose) decreased by 55.21% and 64.52%, respectively. Group 4 received 400 mg/kg of the extract, and their Paw volume significantly decreased after 3 and 4 hours (56.74% and 69.28%, respectively). After 4 hours, the percentage of inhibition was still quite high (69.28%) despite the greater concentration of the test extract. Groups 3 and 4 treated with methanolic extract of *T. telescopium* showed dose-dependent anti-inflammatory efficacy. After 4 hours, there was a significant difference in the percentage decrease in Paw volume between the groups treated with the extract and those

treated with the conventional treatment, Diclofenac sodium (56.78%). *T. telescopium* methanol extract showed substantial anti-inflammatory action (<0.05) (Table 1).

2 Antioxidant activity

DPPH radical scavenging activity of methanol extract of *T. telescopium* at various doses, compared to that of conventional ascorbic acid, is shown in (Fig. 8.1). The DPPH solution lightens when an antioxidant removes free radicals by donating hydrogen to the reaction. Samples with concentration range 50-200 $\mu\text{g/ml}$ were analyzed. The antioxidant activity of the methanol extract of *T. telescopium* increased with the increasing concentration of the extract that is at 50 $\mu\text{g/ml}$, 56.01%, 100 $\mu\text{g/ml}$ 63.03%, 150 $\mu\text{g/ml}$, 74.06% and at 200 $\mu\text{g/ml}$ 79.04% respectively. At higher dosage the antioxidant activity was also higher. When

compared to standard ascorbic acid. A maximum antioxidant activity in higher dose (200 $\mu\text{g/ml}$) was observed. Inhibitors or scavengers of free radicals, them ethanolic extracts proton-donating capacity suggested they may be a major antioxidant, according to the study findings (Table 2) (Fig. 1).

3 GC-MS analysis

Through GC-MS analysis 25 compounds were structurally elucidated. They are 1,2 Benzenedicarboxylic acid, diethyl ester-, Tetradecanoic acid, Octane,1,1'-oxybis, Pentadecanoic acid, 9-Eicosene, (E)-, Hexadecanoic acid, Methyl ester, cis-9-Hexadecanoic acid, n Hexadecanoic acid, Heptadecenoic acid, cis Vaccenic acid, Heptadecenoic acid,1-Octadecanol, 11-Octadecenoic acid, Methyl ester(11E), Methyl Stearate, oleic acid, Octadecenoic acid, 5,8,11,14,17 Eicosapentanoic acid, methyl ester,

Octanoic acid, 2-dimethylaminoethyl ester, cis-11Eicosenoicacid, methylester, Ethanol,2(octadecyloxy),1,4Pentadlyn 3ol,3(Diethoxymethyl)1,5bis(Trimethy lsilyl)Decane,1,1diethoxy,Nonane,1,1 diethoxy,Bis(2ethylhexyl)phthalate,2,2 Dimethylpropanoic acid, heptadecyl ester. Of these 25 compounds, 11 compounds were biologically active.

Oleic acid could be responsible for anti-inflammatory activity in the present study. Tetradecanoic acid, n-hexadecanoic acid, hexadecanoic acid methyl ester 5,8,11,14,17 In this case, it is possible that eicosapentanoic acid methyl ester is the molecule responsible for the antioxidant effects observed (Table 3), (Fig. 2).

Table 1 : Anti-inflammatory activity of methanol extract of *T. telescopium* against Carragenan induced paw edema in albino rats

Group	Dose	Paw edema (mm)			
		1 hr	2 hrs	3 hrs	4 hrs
1	Control (water 1 ml/kg)	0.22± 0.010	0.44± 0.062	0.44± 0.061	0.45± 0.012
2	Standard (Diclotenac sodium 10 mg/kg)	0.12± 0.014	0.04± 0.005	0.05± 0.006	0.03± 0.02
		48.70%	51.86%	54.62%	56.78%
3	Methanol extract of <i>T. telescopium</i> (200 mg/kg)	0.25± 0.038	0.42± 0.046	0.44± 0.046	0.45± 0.036
		50.06%	52.82%	55.21%	64.52%
4	Methanol extract of <i>T. telescopium</i> (400 mg/kg)	0.28± 0.06	0.28± 0.06	0.29 ±0.06	0.32 ±0.06
		47.02%	49.26%	56.74%	69.28%

One way ANOVA	F	0.078	0.086	4.65	13.31
	Df	3, 12	3, 12	3, 12	3, 12
	P	<0.05	<0.05	<0.05	<0.05

Table 2 : Antioxidant activity of methanol extract of *T. telescopium*

S. No	Concentration (µg/ml)	Solution (ml)		
		Control 1 ml of methoanol and 1 ml of DPPH solution	Standard ascorbic acid	Percentage of scavenging activity of <i>T. telescopium</i>
1	50	40	53.08	56.01
2	100	52	58.23	63.03
3	150	64	70.11	74.06
4	200	70	72.03	79.04

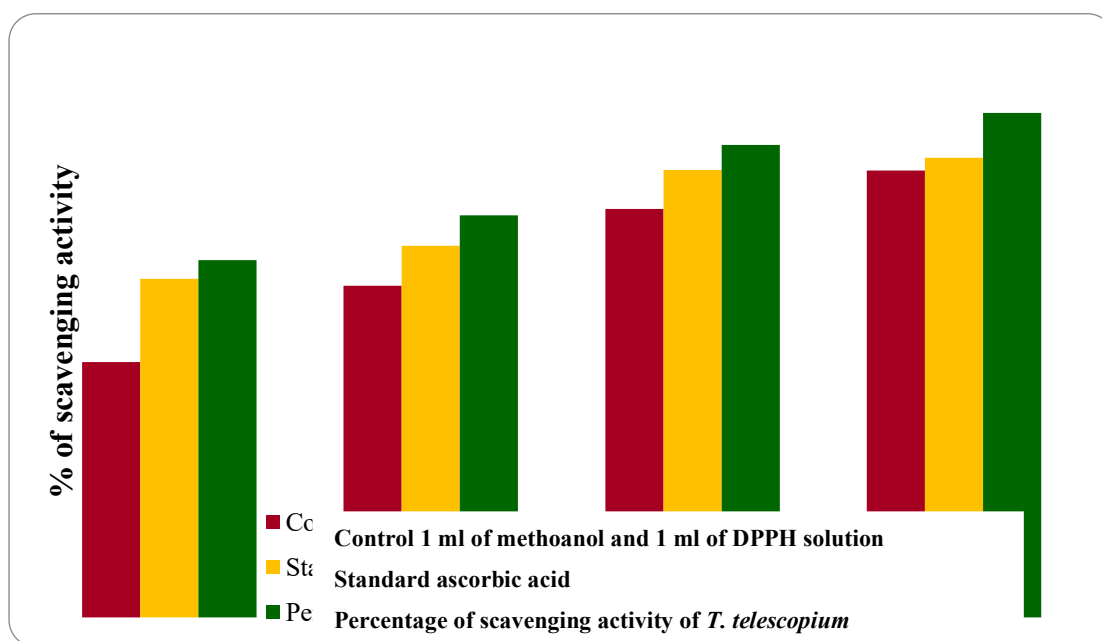


Fig. .1 : Antioxidant activity of methanol extract of *T. telescopium*

Table 3 : Activity of compounds identified in GC-MS analysis

No	RT	Name of the Compound	Formula	M. W	Peak Area %	Compound Nature	**Activity
1	15.565	1,2 Benzenedicarboxylic acid, diethyl ester-	C ₁₂ H ₁₄ O ₄	222	0.67	Plasticizer compound	Antimicrobial Antifouling
2	17.388	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	4.18	Myristic acid	Antioxidant, Cancer preventive, Nematicide, Lubricant Hypocholesterolemic
3	18.013	Octane, 1,1'-oxybis-	C ₁₆ H ₃₄ O	242	1.25	Alkane compound	No activity reported
4	18.503	Pentadecanoic acid	C ₁₅ H ₃₀ O ₂	242	3.26	Saturated fatty acid	No activity reported
5	18.718	9-Eicosene, (E)-	C ₂₀ H ₄₀	280	0.99	Alkene compound	No activity reported
6	19.170	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	1.82	Palmitic acid methyl ester	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
7	19.403	cis-9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	254	8.91	Palmitic acid	No activity reported
8	19.563	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	46.16	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
9	20.221	Heptadecenoic acid	C ₁₇ H ₃₂ O ₂	268	1.09	Unsaturated fatty acid	No activity reported
10	20.442	cis-Vaccenic acid	C ₁₈ H ₃₄ O ₂	282	1.07	11-Octadecenoic acid. (Z)	No activity reported
11	20.617	Heptadecanoic acid	C ₁₇ H ₃₂ O ₂	268	2.53	Saturated fatty acid	No activity reported
12	20.823	1-Octadecanol	C ₁₈ H ₃₈ O	270	1.21	Saturated	Antimicrobial

						Alcoholic compound	
13	21.008	11-Octadecenoic acid, methyl ester(11E)	C19H36O2	296	1.13	Unsaturated fatty acid, methyl ester	No activity reported
14	21.245	Methyl Stearate	C19H38O2	298	0.64	Stearic acid ester	No activity reported
15	21.524	Oleic acid	C18H34O2	282	8.39	Oleic acid	Anti-inflammatory, Antiandrogenic Cancer preventive, Dermatitogenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor
16	21.600	Octadecanoic acid	C18H36O2	256	4.98	Stearic acid	No activity reported
17	23.164	5,8,11,14,17-Eicosapentaenoic acid, methyl ester	C21H32O2	316	1.01	Unsaturated fatty acid ester compound	Cardio protective Antioxidant
18	23.261	Octanoic acid, 2-dimethylaminoethyl ester	C12H25NO2	215	0.90	Saturated fatty acid amino ester	Antimicrobial
19	23.506	cis-11-Eicosenoic acid, methyl ester	C21H40O2	324	1.29	Unsaturated fatty acid ester	No activity reported
20	25.252	Ethanol, 2-(octadecyloxy)-	C20H42O2	314	1.76	Alcoholic ether compound	Antimicrobial
21	25.325	1,4-Pentadlyn-3-ol, 3-(Diethoxymethyl)-1,5-bis(Trimethylsilyl)-	C16H30O3Si2	326	1.20	Unsaturated alcoholic compound with silica	No activity reported
22	25.732	Decane, 1,1-diethoxy-	C14H30O2	230	1.23	Ether compound	No activity reported
23	28.476	Nonane, 1,1-diethoxy-	C13H28O2	216	1.49	Ether compound	No activity reported
24	28.717	Bis(2-ethylhexyl) phthalate	C24H38O4	390	0.75	Plasticizer compound	Antimicrobial Antifouling
25	29.094	2,2-Dimethylpropanoic acid, heptadecyl ester	C22H44O2	340	2.09	Ester compound	Antimicrobial

**Source: Dr. Duke's Phytochemical and Ethnobotanical Databases

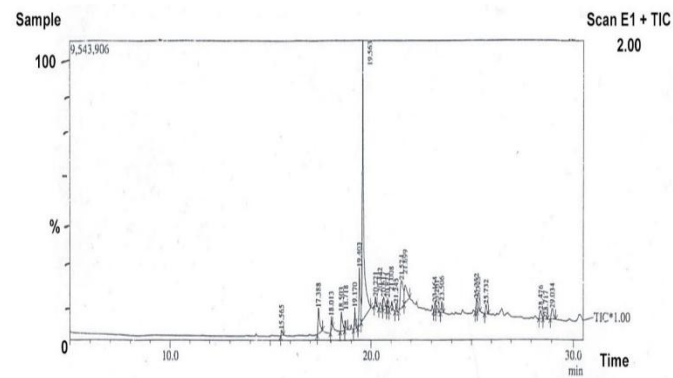
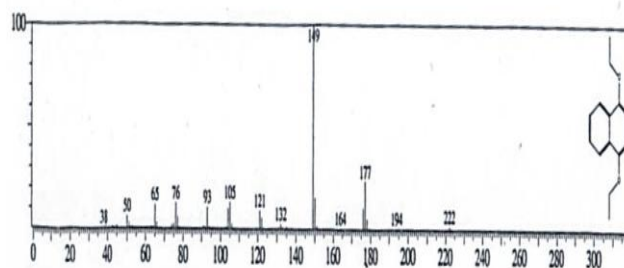
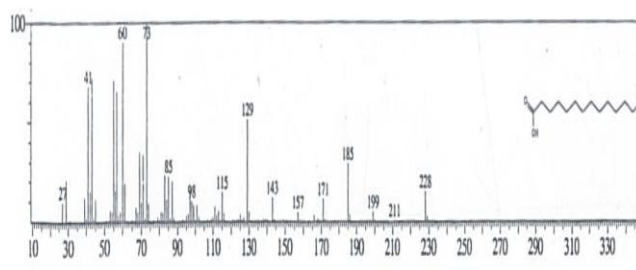


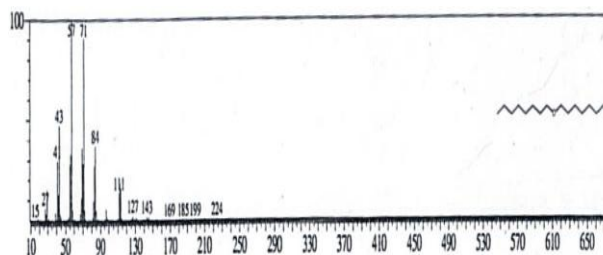
Fig. 8.2 : GC-MS mass spectrum



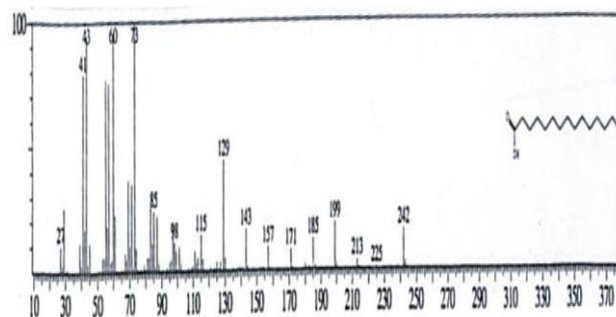
a. 1,2 Benzenedicarboxylic acid, diethyl ester-



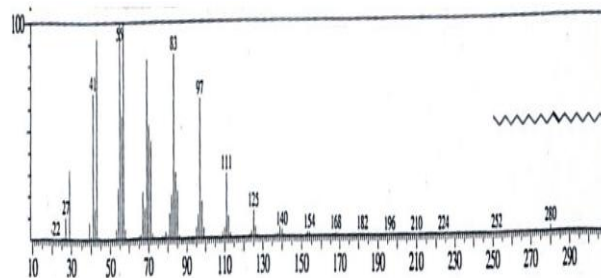
b. Tetradecanoic acid



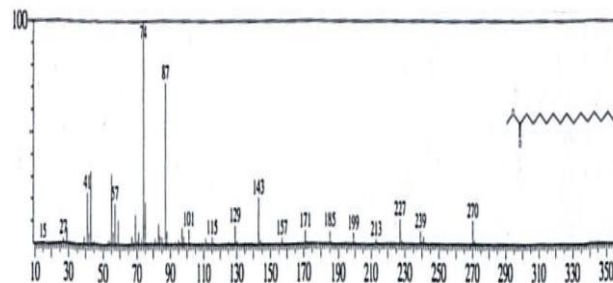
c. Octane, 1,1'-oxybis-



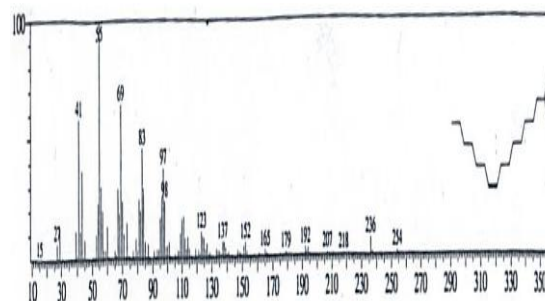
d. Pentadecanoic acid



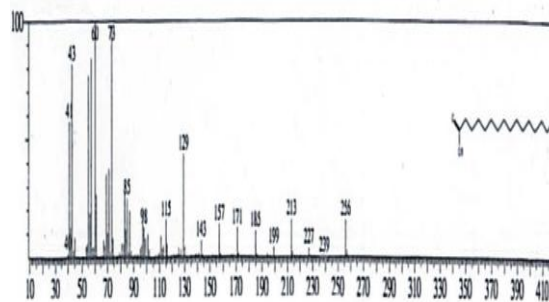
e. 9-Eicosene, (E)-



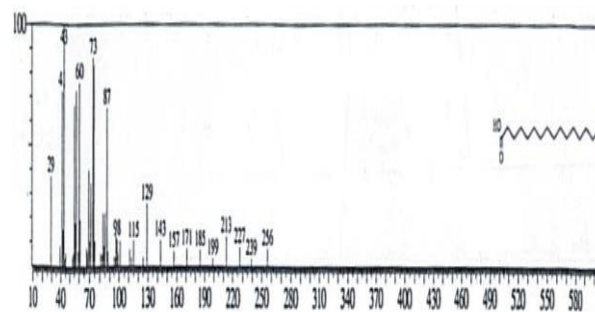
f. Hexadecanoic acid, methyl ester



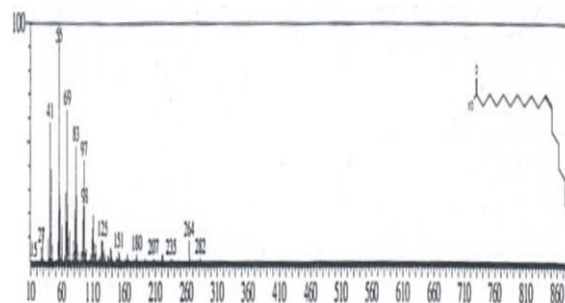
g. cis-9-Hexadecenoic acid



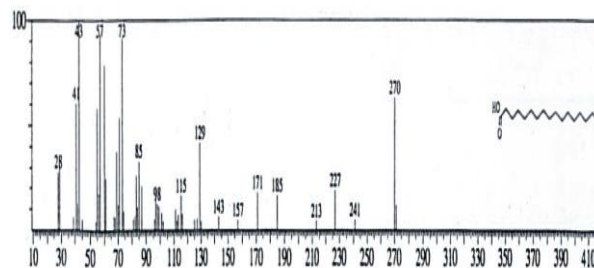
h. n-Hexadecanoic acid



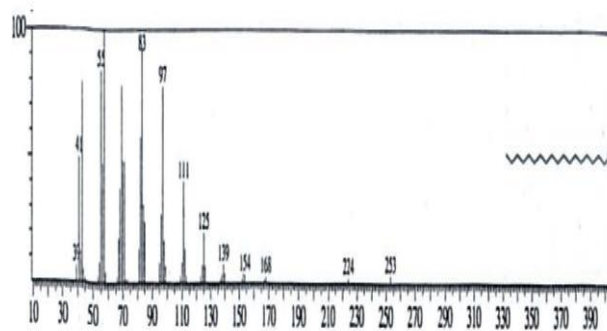
i. Heptadecenoic acid



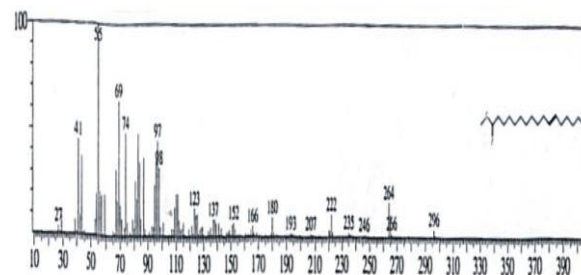
j. cis-Vaccenic acid



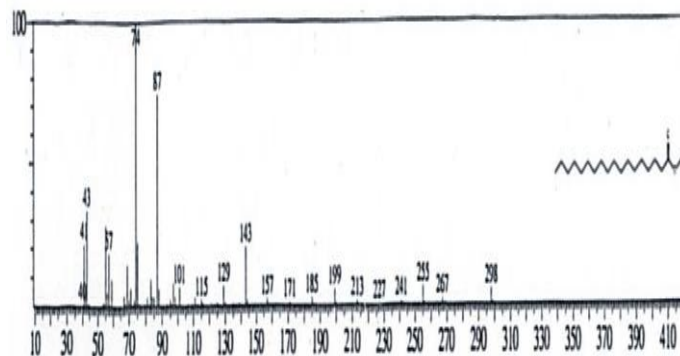
k. Heptadecanoic acid



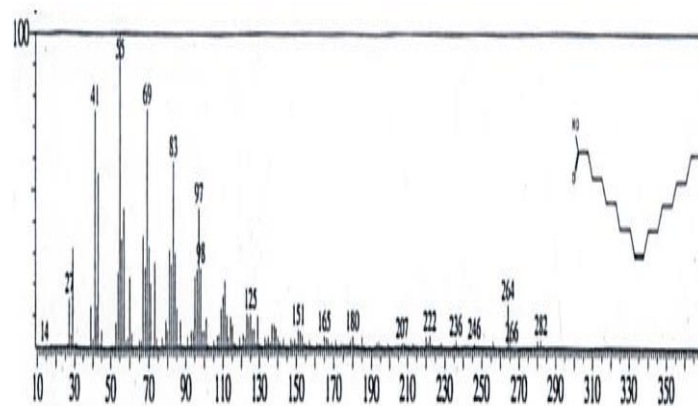
1. 1-Octadecanol



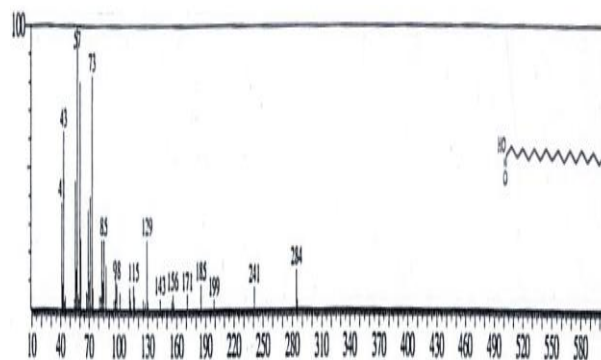
m. 11-Octadecenoic acid, methyl ester(11E)



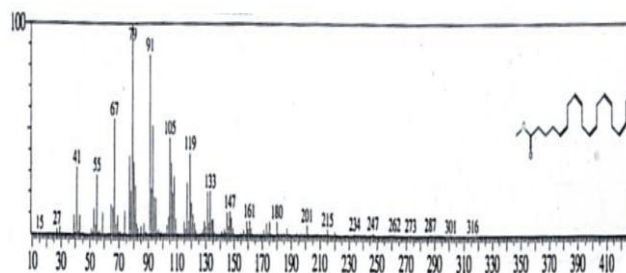
n. Methyl Stearate



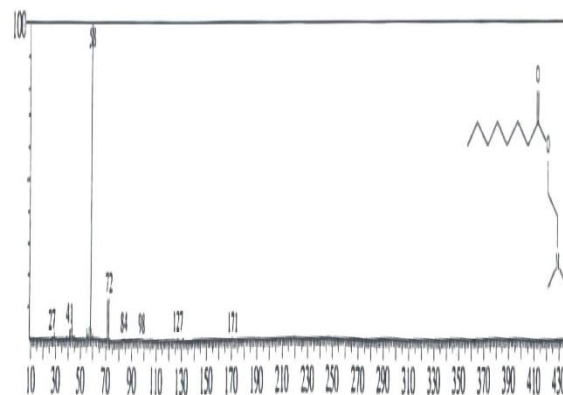
o. Oleic acid



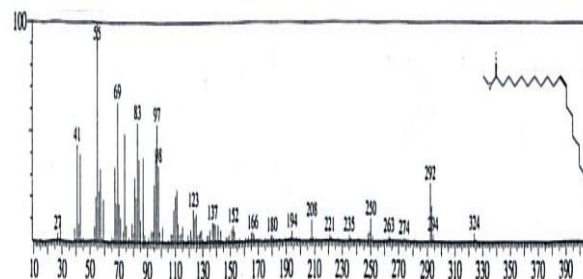
p. Octadecanoic acid



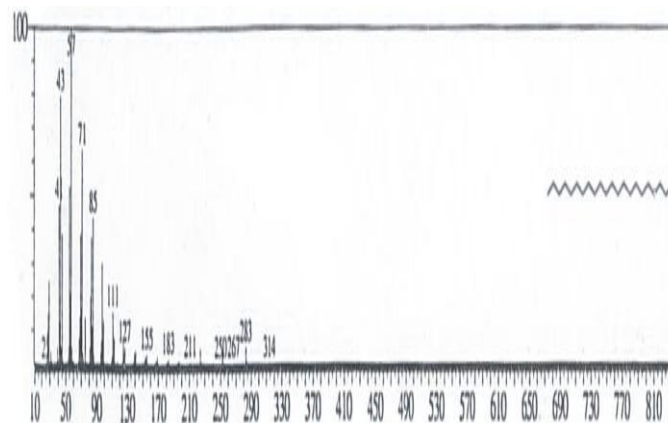
q. 5,8,11,14,17-Eicosapentaenoic acid, methyl ester



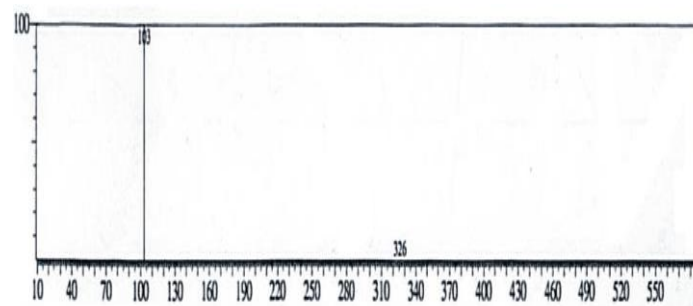
r. Octanoic acid, 2-dimethylaminoethyl ester



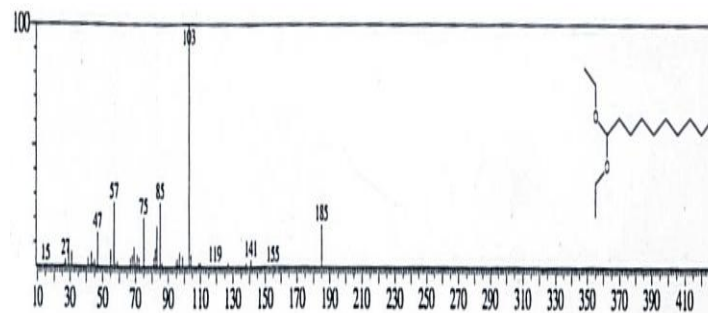
s. cis-11-Eicosenoic acid, methyl ester



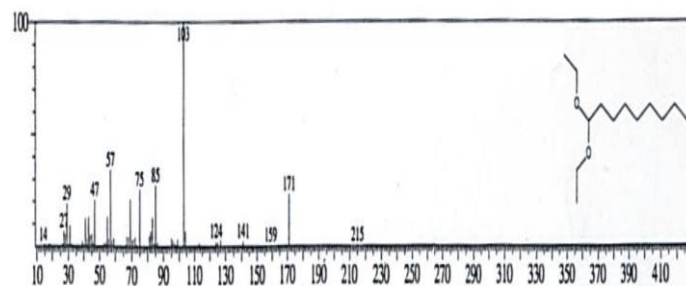
t. Ethanol, 2-(octadecyloxy)-



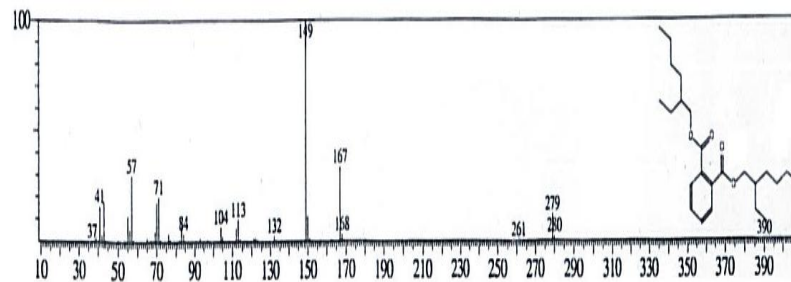
u. 1,4-Pentadlyn-3-ol, 3-(Diethoxymethyl)- 1,5-bis(Trimethylsilyl)-



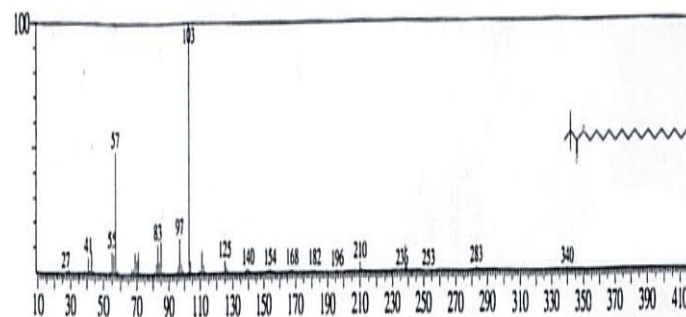
v. Decane, 1,1-diethoxy-



w. Nonane, 1,1-diethoxy-



x. Bis(2-ethylhexyl) phthalate



y. 2,2-Dimethylpropanoic acid, heptadecyl ester

Fig. 8.3 : Chromatogram

Molluscs have been used in ethno medicine by many traditional cultures to treat different aspects of inflammatory conditions (Ahmad *et al.*, 2018). Pain and inflammation are associated with many pathophysiology of various clinical conditions like arthritis, cancer and vascular diseases (Ansari *et al.*, 2012). A number of natural products are used in various traditional medical systems to treat symptoms of pain and inflammation. It is well known that Carrageenan – induced paw edema is characterized by a biphasic event, with involvement of different inflammatory mediators. In the first phase (during the first 2 hrs after carrageenan injection), chemical mediators such as histamine and serotonin play a role, while in second phase (3-4 hrs) after Carrageenan infection Kinin and prostaglandin are also involved (Hernandez – Perez and Rabanal Galago, 2002). The result of the present study revealed that administration of methanol

extract of *T. telescopium* inhibited the edema starting from the first hour and during all phases of inflammation, which is probably due to the presence of oleic acid in the methanol extract of *T. telescopium* which are structurally elucidated through GC-MS. The result of the present study corroborates well with the findings of Ansari *et al.*, 2012 on *Eudistoma viride* who also isolated similar compounds.

Kumar, (2003) had reported that the methanolic extracts of *Cypraea errones* and *C. arabica* exerted moderate anti – inflammatory effect against carrageenan induced inflammation at a dose of 10 mg/kg. The active components were identified as sesterterpenes which prevented paw edema in rat. Subavathy and Thilaga, 2018 also investigated the anti-inflammatory effect of methanolic extract of *Cypraea arabica* and found out the percentage of paw edema was

decreased to 56.33% and 58.01% at 200

mg/kg and 400 mg/kg respectively. In the present study also the anti-inflammatory compound oleic acid was structurally elucidated through GC-MS. It was observed that in paw thickness was inhibited to about 69.28% by the methanol extract of *T. telescopium* at the concentration of 400 mg/kg, where as standard anti-inflammatory drug, Dichlofenac sodium (10mg/kg) inhibited the paw thickness to 56.78%. The present study shows that the gastropods compounds (oleic acid) is effective against carrageenan induced edema. So it can be inferred that upon further purification these gastropod extract may be used to get potent anti inflammatory drugs after clinical trials in the near future.

Similar to the present findings Ravi *et al.* (2012) investigated the potent anti-inflammatory activity of acetone and methanol extracts of the gastropods *Hemifuscs pugilinus* and *Natica didyma*. The maximum activity was exerted by the

acetone extract both in the hyposaline medium ($72.58 \pm 1.66\%$) and *N. didyma* maximum activity was exerted by the acetone extract both in the hyposaline medium ($66.21 \pm 1.66\%$) and in the distilled water medium it was ($49.32 \pm 9.09\%$). The in vivo anti-inflammatory activity was studied in acetone column purified extracts of *Trochus tentorium* and *Drupa margariticola* in the Swiss mice and the extracts exhibited potent anti-inflammatory activity similar to the present findings. The bio-active compounds manocide, pseudopterosins, topsentins and scytonemia could be responsible for the anti-inflammatory activity which were isolated from *T. tentbrium* and *D. margariticola* (Chellaram and Edward, 2009). Similar to the above findings in the present study also compounds like Oleic acid which could be responsible for anti-inflammatory activities have been isolated and characterized through GC-MS.

observed significant anti inflammatory activity of ethyl acetate extracts of *Oliva spp.*, *Patella rustica* and *Littorina littorea* paw edema. The maximum perecentage inhibition of edema (83.90%) was recorded at a dose of 150 mg/kg where as the least % inhibition of 33.42% was found at 50 mg/kg dose. Bavani Saraswathy, (2019) analyzed the anti inflammatory activity of ethanol extract of *P. glaucum* and found 18.18% and 63.63% of paw thickness reduction at the concentration of 200 mg/kg and 400 mg/kg (p.o) respectively. Srinandhini Devi, (2021) studied the in-vitro anti-inflammatory activity of fresh water snail *Pila globosa* that showed potential anti-inflammatory activity in adose dependent manner. The anti-inflammatory activity in higher doses (500 mg/kg) of snail homogenate was similar to the standard drug diclofenac sodium. Similar to the above findings, in the present study also the methanolic extract of *T. telescopium*

showed dose dependent anti inflammatory activity.

The methanol extract of gastropod and bivalve species could be better potential source as a natural antioxidant. Earlier reports evidence that the antioxidant peptide isolated from *Conus betulinus* showed 20-25% of scavenging effect. Similarly, the gastropod *P. trapezium* meat possessed natural antioxidant potential as its methanolic extract was found to exhibit good scavenger of DPPH radical (Yuan *et al.*, 2008). In the present study also the methanolic extract of *T. telescopium* exhibited better scavenging activity (79.04%) at 200 µg/ml concentrations. Highest scavenging ability was observed at 100 µg/ml concentration in the methanolic extract of *Sepeia pharonis* (81.25%) (Ponnusamy *et al.*, 2016) and also observed strong antioxidant activity (84.46%) the methanolic extract of *M. meretrix*. The strong antioxidant activities in methanol extract of *T. telescopium* in

the present study could be due to the

Arumuga Samy and Cyril, (2017)

ability of methanol to dissolve all polar compounds.

Kumar *et al.* (2014) studied antioxidant activity in methanolic extract of

B. zeylanica with maximum percentage inhibition of 65.4% at highest concentration 3.2 mg/ml and minimum inhibition of 4.2% at 0.1 mg/ml. Madhu *et al.* (2014) observed moderate antioxidant activity of methanolic extract of *P. viridis* which showed (76.9%) total reducing power (27.8 µg), total antioxidant activity (174 µg), hydrogen peroxide scavenging activity of 88.12% and nitrous oxide scavenging activity 62.5% at 100 µg/ml, Pachaiappan *et al.*, 2014 observed higher scavenging activity in bivalves. The effect was high in *M. meretrix* (74.52%)

followed by *P. viridis* (73.64%) and lower for *T. attenuata* (23.54%). The above study agree well with the present findings.

examined antioxidant activities of the tissue extracts of marine gastropod *H. pugilinus* and found more efficient radical scavenging activity with IC 50 concentration of 74.73 µg/ml. Bavani Saraiswathy, 2019 was studied the antioxidant activity of ethanol extract of *P. glaucum* and observed the highest percentage of scavenging activity of 75.01% at 150 µg/ml concentration followed by 50.25% at 100 µg/ml and 39.05% at 50 µg/ml concentrations. Subavathy and Mary Baptista Janet, (2020) studied the molecular weight of protein and In-vitro antioxidant activity of marine gastropods *Turbo brunneus*, *Cypraea annulus*, and *Babylonia spirata*. The result of the present study also corroborates well with the above findings.

In the present study, through GC-MS analysis 25 bioactive compounds have been isolated and characterized out of these 25 compounds Oleic acid could be responsible for anti-inflammatory activity

and Tetradecanoic acid, Hexadecanoic

acid methyl ester, n-Hexadecanoic acid and 5,8,11,14,17-Eicosapentaenac acid, methyl ester are the compounds which could be responsible for antioxidant activities. Several studies indicated that molluscs are a very good source of biomedically important products and have developed effective mechanisms that are part of their innate immunity (Chakrabort and Joy, 2020). The methanolic extract of *T. telescopium* also proved to be a source of reservoir of bioactive elements like Oleic acid, Tetradecanoic acid, Hexadecanoic acid methyl ester, n-Hexadecanoic acid and 5,8,11,14,17-Eicosapentaenac acid, methyl ester and may be recommended as a pharmaceutical.

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