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Screening of Novel Bioactive Natural Compounds and their Antibacterial Property from Resilient Marine Gastropod *Planaxis sulcatus* (Born, 1778) Collected from Karwar coast, West coast of India

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Abstract

The study aimed at identifying novel bioactive natural compounds from intertidal marine gastropod Planaxis sulcatus (Born, 1778) Collected from Karwar coast, West coast of India. Crude methanolic extract of intertidal marine gastropod Planaxis sulcatus was tested for preliminary zoochemical screening using standard methods to determine the presence of different chemical compounds: The crude extract was analysed using GC-MS to identify the bioactive components. The disc diffusion method was used to conduct an antibacterial experiment. Accordingly, the inhibition zone around the disc impregnated with gastropod extract was used to test the antibacterial activity. By using the micro broth dilution technique, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were determined. Standard zoochemical testing of the Planaxis sulcatus whole body extract revealed the presence of steroids, alkaloids, flavonoids, glycosides, tannins, and saponins. A total of 130 chemicals were identified as the bioactive components in the extract using GC-MS. Seventeen of these compounds have antimicrobial properties while eleven of these compounds have antioxidant properties, six of these have anticancer properties based on published literature. Some of the other compounds also suggest biological activities other than antimicrobial, antioxidant and anticancer which indicates potential biomedical applications. The gastropod extract showed noteworthy antibacterial activity with minimum inhibitory zone of 12 mm against Pseudomonas aeruginosa and maximum 30 mm against Staphylococcus aureus at 40 µg/ml concentration. The Minimum Inhibitory Concentration (MIC) was found to be 1.72 µg/ml, 1.87µg/ml, 1.99 µg/ml, and 1.87 µg/ml against Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, and Escherichia coli respectively. The MIC value decrease with higher concentration of methanol extracts than that of the pure antibiotic. As a result, Klebsiella pneumonia exhibits higher MIC values and MIC-CFU (214). The tissue extract of Planaxis sulcatus possess bioactive compounds that have potent antibacterial effect.

Keywords: *Planaxis sulcatus, Intertidal molluscs, Bioactive compounds, Antibacterial, GC-MS*

1. Introduction

The marine environment is a great source of naturally occurring bioactive chemicals (Chia et al., 2015; Kailas & Nair, 2015) with unique structural characteristics not seen on land (Eahamban & & Antonisamy, 2012). The marine environment has attracted the attention of researchers in recent years because it is a rich source of plants, animals, and microorganisms that produce a wide range of primary and secondary metabolites as a result of their adaptation to this particular habitat. These metabolites have demonstrated significant biological activities against, for example, cancer and inflammation, as well as in analgesia, immunomodulation, allergy, and antiviral assays (Kiuru et al., 2014). Numerous new metabolites with strong pharmacological effects have been found in marine creatures in recent years. About 300 patents on natural compounds obtained from marine species were

granted between 1969 and 1999 (Proksch et al., 2002). The bulk of these studies focused only on sponges and other marine organisms with respect to bioactive compounds. A few researches have been conducted exclusively on natural compounds extracted from marine molluscs (Benkendorff,, 2010; Li et al., 2009). Molluscs are a very good source of crucial biological products among invertebrates. Due to the fact that the bulk of these creatures spend their lives in hostile intertidal rocky coastlines, where they are subjected to a variety of pressures, they are more prone to injuries and damages brought on by intra- and inter-species competition and microbial infections. As a result, they are capable of adapting to various forms of stress and overcoming them by synthesising secondary metabolites with immunological and antibacterial action (Lumeran, 2019). In India, there hasn't been much research done on the potential of marine molluscs as a source of biologically active compounds (Anbuselvi et al., 2009). Therefore, a thorough screening of marine molluscs for bioactive substances is required. Thus, the current study's objective is to assess the bioactive compounds present and their antibacterial efficacy of gastropod *Planaxis sulcatus* (Born, 1778) entire body tissue extracts against clinically isolated pathogenic bacteria.

2. Materials And Methods

Sampling and Identification

In this study live marine gastropod *Planaxis sulcatus* (Born, 1778), (Family: Planaxidae) were randomly collected by hand picking during low tide from the intertidal zone of rocky shore, Majali, Karwar, Uttar Kannada district of Karnataka, West coast of India (14.88°N,74.11°E). The live specimens were brought to the laboratory in zip lock bag and identified up to species level by the standard literature of Subbarao and scientific names, classification of *Planaxis sulcatus* was adopted from the World Registrar of Marine Species (WRoM) website (http://www.marinespecies.org).The gastropod samples were authenticated as Planaxis sulcatus (Born, 1778), (Family: Planaxidae) by Marine Biology Regional Centre, Zoological Survey of India (ZSI), Chennai.

Figure 1. Sampling site

Figure 2. Planaxis sulcatus (Born, 1778)

Extraction of Sample

The Chellaram *et al*., 2004 (Chellaram et al., 2004) method was used to create the methanolic extract of fleshy tissue. The gathered samples were shattered with a hammer to remove the soft body tissue and cleaned with distilled water to remove sand from the shells before extraction. A hot air oven was used to dry the full body sample at 45 °C for three days before it was powdered. To extract the most polar chemicals, the powdered material was steeped in 100% A.R grade methanol for three days at room temperature. Using Whatman No. 1 filter paper, the solvent's extract was filtered. To obtain the crude methanol extract, the resultant extract was subsequently concentrated under a vacuum on a rotary evaporator at low temperature and reduced pressure.

Screening for preliminary zoochemicals.

For the identification of secondary metabolite present in the extract, a zoochemical analysis was carried out using Standard operating procedures (Raman, 2006; Harborne, 2005).

Identification of alkaloids: Iodine test

A few drops of iodine solution was added to extract. A blue color which disappears on boiling and reappears on cooling indicated the presence of alkaloids.

Identification of Flavonoid: Alkaline reagent test
To 2 ml of the extract, two drops of sodium hydroxide were added. When a few drops of diluted HCL were added, the initial intense yellow colour gradually faded away, proving the presence of flavonoids.

Identification of Glycosides: Keller-kiliani test
Ferric chloride, concentrated sulphuric acid, and glacial acetic acid were used to dissolve the extract in water. The junction's brown ring provided evidence of glycoside presence.

Identification of Tannin: Ferric chloride test
The presence of tannins was determined by adding 2 ml of a 5% neutral ferric chloride solution to 1 ml of extract.

Identification of Terpenoids: Salkowski test
Concentrated Sulphuric acid is carefully added to the extract after it has been combined with 2 ml of chloroform to create a layer. The interface develops a reddish-brown color when terpenoids are detected positively.

Identification of Phenols: Ferric chloride test
Three to four drops of a ferric chloride solution were added to the extracts. Phenols were present because a bluish-black color developed.

Identification of Saponin: Froth test
In a test tube, 10ml of distilled water was combined with about 3ml of the extract's aqueous solution. The test tube was stoppered, agitated forcefully for about five minutes, and then let to stand for thirty minutes while the appearance of honeycomb foam, a sign of saponins, was looked for.

Identification of Steroid: Salkowski test
Chloroform and concentrated H_2SO_4 were added along the test tube walls, and the sample extract was shaken. Steroids were present, as evidenced by the appearance of a red color.

Determination of compounds by Gas Chromatography-Mass Spectroscopy (GC-MS)
GC-MS was used to do a component analysis on the chemicals in the crude sample extract. The number of peaks on the chromatogram showed how many compounds were present in the extract, and the names of the compounds were interpreted based on the spectrum data from each of these peaks and mass spectra data using the library approach method in the GC-MS database. The GC-MS TQ Xevo (Waters USA) instrument with BP capillary column (Length: 30.0 m, Diameter: 0.25 mm, Film thickness: 0.25 m) was filled with about 10µg of methanol extract. Oven temperature controlled from 280° to 360° at 1ºC/min and sustained for 5 minutes at 360° with split ratio 1:100. Injector temperature 250ºC. Carrier gas Helium at 1 ml/min. Ionisation technique, electronic impact at 70 eV, an acquisition range of 30-500 m/z, and a scan rate of 1 amu/s were the MS scan settings that were employed. Based on a comparison of the retention times and computer matching to the NIST MS Data library, the constituents were identified. The test materials' names, molecular weights, and chemical compositions were determined.

Antibacterial Assay
Determination of the Minimum Inhibitory Concentration (MIC)
Microdilution assay (Kelman et al., 2001) was used to assess the MIC of *Planaxis sulcatus* extract against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. For each bacterial species under consideration, a different microplate was used. At 37°C, the microplates were incubated for 24 hours. Using a spectrophotometer, the OD of each well was measured at 600 nm both before and after plate incubation. As the indication, MTT dye is utilized. There were three duplicates of each test run.

Determination of the Minimum Bactericidal Concentration (MBC)
By sub-culturing the wells on a sterile agar plate that showed no discernible growth, the MBC was assessed. On tryptone soya agar plates, 100 µl of the bacterial solutions that are thought to be the MIC and greater concentrations were produced. There were six agar plates for each concentration. At 37 °C, these plates were incubated for 24 hours. After the incubation period, each plate was checked

for growth using both the naked eye and colony-forming units (CFUs), which were estimated on a grid. The MBC value was determined to be the lowest concentration in an agar subculture that showed no discernible growth (Mishra et al., 2015).

Disc Diffusion Method

In order to test the antibacterial activity of the gastropod (*Planaxis sulcatus*) extracts by Disc diffusion method (Clinical, 2019), four species of pathogenic bacteria were used: *Staphylococcus aureus* (MTCC3160), *Escherichia coli* (MTCC1586), *Klebsiella pneumonia* (MTCC432), and *Pseudomonas aeruginosa* (MTCC741). Virulent nutrient broth was used as the inoculum for pathogenic bacterial strains, which were then cultured there for 24 hours at 37 °C. On the Luriabertani Agar plates, pathogens were swabbed, then discs impregnated with various amounts of gastropod extracts were laid on top. To evaluate the impact of antibiotics on infections, control discs were combined with antibiotics. The antibacterial activity was assessed after 24 hours of incubation at 37^0 C using the inhibitory zone surrounding the disc.

3. Results and Discussion

Extraction of Sample

Planaxis sulcatus extract was prepared. Further extract was used for zoochemical analysis and antimicrobial assays and stored at 4°C.

Quantitative analysis of zoochemicals of extract

Zoochemical screening of *P.sulcatus* methanolic extract revealed the presence of alkaloids, flavonoids, glycosides, tannin, saponin and steroids as shown in Table 1. These detected zoochemicals may explain the biological and pharmacological activities of the extract.

Table 1: Preliminary zoochemical screening of *Planaxis sulcatus* (Born, 1778).

Zoochemicals	Method	Methanol extract
Alkaloids	Iodine test	+
Flavonoids	Alkaline reagent test	+
Glycosides	Keller-kiliani test	+
Tannins	Ferric chloride test	+
Terpenoids	Salkowski test	-
Phenols	Ferric chloride test	-
Saponins	Froth test	+
Steroids	Salkowski test	+

GC-MS analysis

14 peaks were found in the gastropod extract according to the GC-MS analysis (Figure 3). Hexadecanoic acid-methyl ester, pentadecanoic acid-14-methyl-methyl ester, tert-Hexadecanethiol, ethanol 2-(octadecyloxy), and octadecanal 2-bromo were the extract's main constituents. Table 2 lists the names of the remaining compounds along with their retention times, molecular weights, and molecular formulas. In the present study of methanolic extract of marine gastropod *P.sulcatus* has shown the major peak for tert-Hexadecanethiol, Ethanol 2-(octadecyloxy)-, Pentadecanoic acid 14-methyl- methyl ester, which has been previously reported for antibacterial, antifungal, antioxidant, anti-inflammatory, Nematicide. Hence the recorded activities of antibacterial activity of the gastropod extract might be due the presence of these bioactive compounds.

Table 2: Chemical compounds identified in the methanolic extract of *Planaxis sulcatus* by GC-MS

Sl. No	Retention Time (min)	Compound name	Formula	Mol. Wt.	Bioactivity
1	4.68	Acetate, [3-(acetyloxy)-4,5-dihydro-5-isoxazolyl]methyl	$C_8H_{11}NO_5$	201	No Activity Reported
2	4.68	Deoxyspergualin	$C_{17}H_{37}N_7O_3$	387	Immunosuppressive Agent (Lorenz et al., 2011) Antiangiogenic action (Hussein, 2016)
3	4.68	HEPES	$C_8H_{18}N_2O_4S$	238	No Activity Reported
4	4.68	Acetic acid, 1,4-dioxa-spiro[4.6]undec-6-yl ester	$C_{11}H_{18}O_4$	214	No Activity Reported
5	4.68	Dec-9-en-6-oxo-1-ylamide	$C_{10}H_{17}NO_2$	183	No Activity Reported
6	4.68	Hexanoic acid, 3-tetradecyl ester	$C_20H_{40}O_2$	312	No Activity Reported
7	4.68	Thiophene, 3-nitro-2-(2-thienylsulfonyl)-	$C_8H_5NO_4S_3$	275	No Activity Reported
8	4.68	Hexanoic acid, 4-hexadecyl ester	$C_{22}H_{44}O_2$	340	No Activity Reported
9	4.68	trans-2-undecenoic acid	$C_{11}H_{20}O_2$	184	No Activity Reported
10	6.53	9H-Imidazo[1,2-a]benzimidazole, 2-(4-chlorophenyl)-9-methyl-	$C_{16}H_{12}ClN_3$	281	No Activity Reported
11	6.53	Nickel, (η-4-diallyl ether)-(2,4-dimethyl-3-pentylisonitrile)	$C_{14}H_{25}NNiO$	281	No Activity Reported
12	6.53	2,5-cyclohexadien-1-one, 2,6-dichloro-4-[(4-methoxyphenyl)imino]-	$C_{13}H_9Cl_2NO_2$	281	No Activity Reported
13	6.53	Benzoic acid, 2-[(trichloroacetyl)amino]-	$C_9H_6Cl_3NO_3$	281	No Activity Reported
14	6.53	Benzoic acid, 2-(2,5-dichlorophenylamino)-	$C_{13}H_9Cl_2NO_2$	281	No Activity Reported
15	6.53	3',4'-Dichloro-2-hydroxybenzanilide	$C_{13}H_9Cl_2NO_2$	281	No Activity Reported
16	6.53	5-Chloro-3-(2-chloro-6-fluoro-phenyl)-[1,2,4]triazolo[4,3-a]pyridine	$C_{12}H_6Cl_2FN_3$	281	No Activity Reported
17	6.53	6,8-Dichloro-2-trifluoromethyl-4-quinolinol	$C_{10}H_4Cl_2F_3NO$	281	No Activity Reported
18	6.53	Anthranilic acid, 3-chloro-N-(o-chlorophenyl)-	$C_{13}H_9Cl_2NO_2$	281	No Activity Reported
19	6.53	Pyridine-3-carbohydrazide, 5,6-dichloro-N2-phenyl-	$C_{12}H_9Cl_2N_3O$	281	No Activity Reported
20	7.52	1-Dimethylamino-2-tozylaminocyclohexane (trans)	$C_{15}H_{24}N_2O_2S$	296	No Activity Reported
21	7.52	9-Oximino-2,7-diethoxyfluorene	$C_{17}H_{17}NO_3$	283	Bacteriostatic, Anti-pathogenic (Al-Rubaye et a., 2017)
22	7.52	Curan-17-oic acid, 19,20-dihydroxy-, methyl ester, (19S)-	$C_{20}H_{26}N_2O_4$	358	No Activity Reported

23	7.52	Acetamide, N-cyclohexyl-2-[(2-furanylmethyl)thio]-	$C_{13}H_{19}NO_2S$	253	No Activity Reported
24	7.52	Nonanoyl chloride	$C_9H_{17}ClO$	176	Anti-cancer (Al-Marzoqi et al., 2016)
25	7.52	5-(Prop-2-enoyloxy)pentadecane	$C_{18}H_{34}O_2$	282	No Activity Reported
26	7.52	2-Pentyl-cyclohexane-1,4-diol	$C_{11}H_{22}O_2$	186	No Activity Reported
27	7.52	2-Propenoic acid, tetradecyl ester	$C_{17}H_{32}O_2$	268	No Activity Reported
28	7.52	1-(3-Methyl-2-butenyl)-3,6-diazahomoadamantan-9-ol	$C_{14}H_{24}N_2O$	236	No Activity Reported
29	7.52	3,5-Dimethyl-cyclohexanone oxime	$C_8H_{15}NO$	141	No Activity Reported
30	10.03	Tetraacetyl-d-xylonic nitrile	$C_{14}H_{17}NO_9$	343	Anti-oxidative, Anti-inflammatory, Anti-viral effects, Anti-asthmatic (Tulika, 2017)
31	10.03	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	$C_{24}H_{46}O_2$	366	Anti-oxidants, Anti-microbial (Tulika, 2017)
32	10.03	13,16-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	No Activity Reported
33	10.03	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	$C_{19}H_{36}O_3$	312	Anti-oxidant (Elaiyaraja, 2018)
34	10.03	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-	$C_{19}H_{36}O_3$	312	No Activity Reported
35	10.03	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-	$C_{20}H_{38}O_2$	310	No Activity Reported
36	10.03	D-Fructose, diethyl mercaptal, pentaacetate	$C_{20}H_{32}O_{10}S_2$	496	Anti-bacterial activity Anti-tumor (Padmashree et al., 2018)
37	10.03	Curan-17-oic acid, 19,20-dihydroxy-, methyl ester, (19S)-	$C_{20}H_{26}N_2O_4$	358	No Activity Reported
38	10.03	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	$C_{19}H_{36}O_3$	312	No Activity Reported
39	11.6	Pregna-4,6-diene-21-carboxylic acid, 17-hydroxy-3-oxo-, (17α)-	$C_{22}H_{30}O_4$	358	No Activity Reported
40	11.6	1-Bromobenzene, 4-(4-bromobenzylideneamino)-	$C_{13}H_9Br_2N$	337	No Activity Reported
41	11.6	4-O-Tosyl-2,3-O-isopropylene-d-mannosan	$C_{16}H_{20}O_7S$	356	No Activity Reported
42	11.6	3,4-Difluorobenzoic acid, 2,2,3,3,4,4,4-heptafluorobutyl ester	$C_{11}H_5F_9O_2$	340	No Activity Reported
43	11.6	2,1,3-Benzothiadiazole, 4,7-dibromo-5-nitro-	$C_6HBr_2N_3O_2S$	337	No Activity Reported
44	11.6	benzoic acid, 2-[2-phenyl-4H-1-benzopyran-4-ylidene]hydrazide	$C_{22}H_{16}N_2O_2$	340	No Activity Reported
45	11.6	N-(4-Bromo-2,5-dimethylphenyl)-2-chlorobenzamide	$C_{15}H_{13}BrClNO$	337	No Activity Reported

46	11.6	N-(4-Bromo-2,6-dimethylphenyl)-2-chlorobenzamide	$C_{15}H_{13}BrClNO$	337	No Activity Reported
47	11.6	6-(4-Nitrophenyl)benzo[4,5]imidazo[1,2-c]-quinazoline	$C_{20}H_{12}N_4O_2$	340	No Activity Reported
48	11.6	Pyrimidin-2(1H)-one, 6-trifluoromethyl-4-[2-(3-ethoxy-4-methoxyphenyl)ethenyl]-	$C_{16}H_{15}F_3N_2O_3$	340	No Activity Reported
49	12.03	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	Antioxidant, Decrease blood cholesterol anti-inflammatory, Nematicide (Al-Gara, 2019)
50	12.03	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	Anti-fungal, Antimicrobial & Antioxidant activity (Karthikeyan, 2017)
51	12.03	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284	No Activity Reported
52	12.03	Tetradecanoic acid, 12-methyl-, methyl ester	$C_{16}H_{32}O_2$	256	No Activity Reported
53	12.03	Methyl 3-methyl-pentadecanoate	$C_{17}H_{34}O_2$	270	No Activity Reported
54	12.67	Oleic Acid	$C_{18}H_{34}O_2$	282	Anti-bacterial, cancer preventive (Malathi, 2016)
55	12.67	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$	256	Anti-asthmatic, activities (Ramya et al., 2015)
56	12.67	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	$C_{26}H_{50}O_2$	394	No Activity Reported
57	12.67	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326	Greatest antimicrobial activity (Rakkimuthu et al., 2023)
58	12.67	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Anti-inflammatory, Anti-oxidant, Nematicide, Pesticide (Gideon, 2015)
59	12.67	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	No Activity Reported
60	12.67	2-Bromotetradecanoic acid	$C_{14}H_{27}BrO_2$	306	No Activity Reported
61	12.67	Eicosanoic acid	$C_{20}H_{40}O_2$	312	Anticancer, Anti-inflammatory, Cardio protective (Murugesan et al., 2011)
62	12.67	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	Anti-allergic, Anti carcinogenic, Anti diabetic, Antiprotozoal (Hugar et al., 2017)
63	14.6	13,16-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	No Activity Reported

64	14.6	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	$C_{24}H_{46}O_2$	366	No Activity Reported
65	14.6	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis	$C_{19}H_{36}O_3$	312	Anti-oxidant (Amudha et al., 2014)
66	14.6	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans	$C_{20}H_{38}O_2$	310	No Activity Reported
67	14.6	Octadecanoic acid, 9,10-dihydroxy-, methyl ester	$C_{19}H_{38}O_4$	330	No Activity Reported
68	14.6	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans	$C_{19}H_{36}O_3$	312	No Activity Reported
69	14.6	13-Tetradecynoic acid, methyl ester	$C_{15}H_{26}O_2$	238	No Activity Reported
70	14.6	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis	$C_{19}H_{36}O_3$	312	No Activity Reported
71	14.6	Dodecanoic acid, 3-hydroxy	$C_{12}H_{24}O_3$	216	Anti-bacterial Antitumor & Anti-oxidant (Rajendran et al., 2017)
72	14.6	12-Tridecynoic acid, methyl ester	$C_{14}H_{24}O_2$	224	No Activity Reported
73	15.81	2-Hexadecanol	$C_{16}H_{34}O$	242	Antimicrobial (Sharath et al., 2017)
74	15.81	Geranylisovalerate	$C_{15}H_{26}O_2$	238	Anti-viral, Anti-inflammatory Antioxidant (Abubakar, 2016)
75	15.81	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	254	Cytotoxicity (Kadhim et al., 2016)
76	15.81	Hexadecane, 1,1-bis(dodecyloxy)-	$C_{40}H_{82}O_2$	594	No Activity Reported
77	15.81	1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	228	No Activity Reported
78	15.81	1-Hexadecanol, 2-methyl	$C_{17}H_{36}O$	256	No Activity Reported
79	15.81	5-Octadecenal	$C_{18}H_{34}O$	266	No Activity Reported
80	15.81	4-Octadecenal	$C_{18}H_{34}O$	266	No Activity Reported
81	15.81	tert-Hexadecanethiol	$C_{16}H_{34}S$	258	Anti-bacterial, Enzyme activators (Semwal et al., 2018)
82	15.81	E-8-Methyl-9-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	No Activity Reported
83	16.6	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	Anti-inflammatory Anti diabetic (Ragunath et al., 2020)
84	16.6	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	Anti-cancer, Anti-inflammatory, Hepatoprotective (Gheda & Ismail, 2020)
85	16.6	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	310	Antifungal (Karthi et al., 2018)

86	16.6	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetat	$C_{16}H_{28}O_3$	268	No Activity Reported
87	16.6	Oleic Acid	$C_{18}H_{34}O_2$	282	No Activity Reported
88	16.6	cis-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310	No Activity Reported
89	16.6	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	No Activity Reported
90	16.6	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	Antibacterial Hypolipidemic (Kumar, 2018) Anticancer (Muthulakshmi et al., 2012)
91	16.6	cis-10-Nonadecenoic acid	$C_{19}H_{36}O_2$	296	Potential antitumor activity, Inhibit p53 activity (Baba et al., 2021)
92	16.6	Erucic acid	$C_{22}H_{42}O_2$	338	No Activity Reported
93	17.32	2-Hexadecanol	$C_{16}H_{34}O$	242	No Activity Reported
94	17.32	1-Hexadecanol, 2-methyl	$C_{17}H_{36}O$	256	No Activity Reported
95	17.32	Hexadecane, 1,1-bis(dodecyloxy)	$C_{40}H_{82}O_2$	594	No Activity Reported
96	17.32	tert-Hexadecanethiol	$C_{16}H_{34}S$	258	Antioxidant, Antibacterial (Venkatachalapathi, 2021)
97	17.32	1,2-Propanediol, 3-(hexadecyloxy)-, diacetate	$C_{23}H_{44}O_5$	400	No Activity Reported
98	17.32	1,2-Propanediol, 3-(tetradecyloxy)-	$C_{17}H_{36}O_3$	288	No Activity Reported
99	17.32	1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	228	No Activity Reported
100	17.32	Ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	314	Antimicrobial (Gheda et al., 2021)
101	17.32	1,2-Propanediol, 3-(octadecyloxy)-, diacetate	$C_{25}H_{48}O_5$	428	No Activity Reported
102	17.32	Octadecanoic acid, 3-hydroxy-, methyl ester	$C_{19}H_{38}O_3$	314	No Activity Reported
103	18.1	Octadecanoic acid, 3-hydroxy-, methyl ester	$C_{19}H_{38}O_3$	314	No Activity Reported
104	18.1	9,12,15-Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester, (Z,Z,Z)-	$C_{25}H_{40}O_6$	436	No Activity Reported
105	18.1	Octadecanoic acid, 3-hydroxy-, methyl ester	$C_{19}H_{38}O_3$	314	No Activity Reported
106	18.1	Oxiraneoctanoic acid, 3-octyl-, cis	$C_{18}H_{34}O_3$	298	No Activity Reported
107	18.1	Octadecanal, 2-bromo	$C_{18}H_{35}BrO$	346	Antibacterial, Antifungal & Antimicrobial (Kim et al., 2018)

108	18.1	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	280	No Activity Reported
109	18.1	Decanoic acid, 3-hydroxy-, methyl ester	$C_{11}H_{22}O_3$	202	No Activity Reported
110	18.1	Octadecanoic acid, 3-hydroxy-, methyl ester	$C_{19}H_{38}O_3$	314	No Activity Reported
111	18.1	9-Octadecenoic acid (Z)-, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	$C_{25}H_{44}O_6$	266	No Activity Reported
112	19.4	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	Anti-microbial, Anti-inflammatory, Antiasthma (Fenical, 1997)
113	19.4	1-Heptatriacotanol	$C_{37}H_{76}O$	536	Antimicrobial, anticonvulsant, antidepressant, anti-inflammatory Dermatological disorder
114	19.4	Cholestan-3-ol, 2-methylene-, (3β,5α)-	$C_{28}H_{48}O$	400	Antioxidant (Mayer et al., 2001)
115	19.4	Oxiraneoctanoic acid, 3-octyl-, cis	$C_{18}H_{34}O_3$	298	No Activity Reported
116	19.4	Ursodeoxycholic acid	$C_{24}H_{40}O_4$	392	Ursodeoxycholic acid (UDCA) is a metabolic by-product of intestinal bacteria, showing hepatoprotective effects [51]
117	19.4	7,10,13-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	320	No Activity Reported
118	19.4	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	$C_{20}H_{38}O_2$	310	No Activity Reported
119	19.4	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	No Activity Reported
120	19.4	Cholest-5-en-3-ol (3β)-, tetradecanoate	$C_{41}H_{72}O_2$	596	No Activity Reported
121	19.4	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy	$C_{30}H_{52}O_2$	444	No Activity Reported
122	20.6	Cholestane-3,5-diol, 5-acetate, (3β,5α)-	$C_{29}H_{50}O_3$	446	No Activity Reported
123	20.6	26-Nor-5-cholesten-3β-ol-25-one	$C_{26}H_{42}O_2$	386	No Activity Reported
124	20.6	Cholesterol	$C_{27}H_{46}O$	386	No Activity Reported
125	20.6	Cholesterol	$C_{27}H_{46}O$	386	No Activity Reported
126	20.6	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a] phenanthren-3-ol	$C_{27}H_{46}O$	386	No Activity Reported
127	20.6	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	No Activity Reported
128	20.6	Cholesterol	$C_{27}H_{46}O$	386	No Activity Reported

129	20.6	Cholesterol margarate	$C_{44}H_{78}O_2$	638	No Activity Reported
130	20.6	1-Methyl-7-azabicyclo[4.1.0]hepta-2,4-diene-7-carboxylic acid, 3,17-diacetoxy-4,4,10,13 tetramethylhexadecahydrocyclopenta [a]phenanthrene	$C_{33}H_{47}NO_6$	553	No Activity Reported

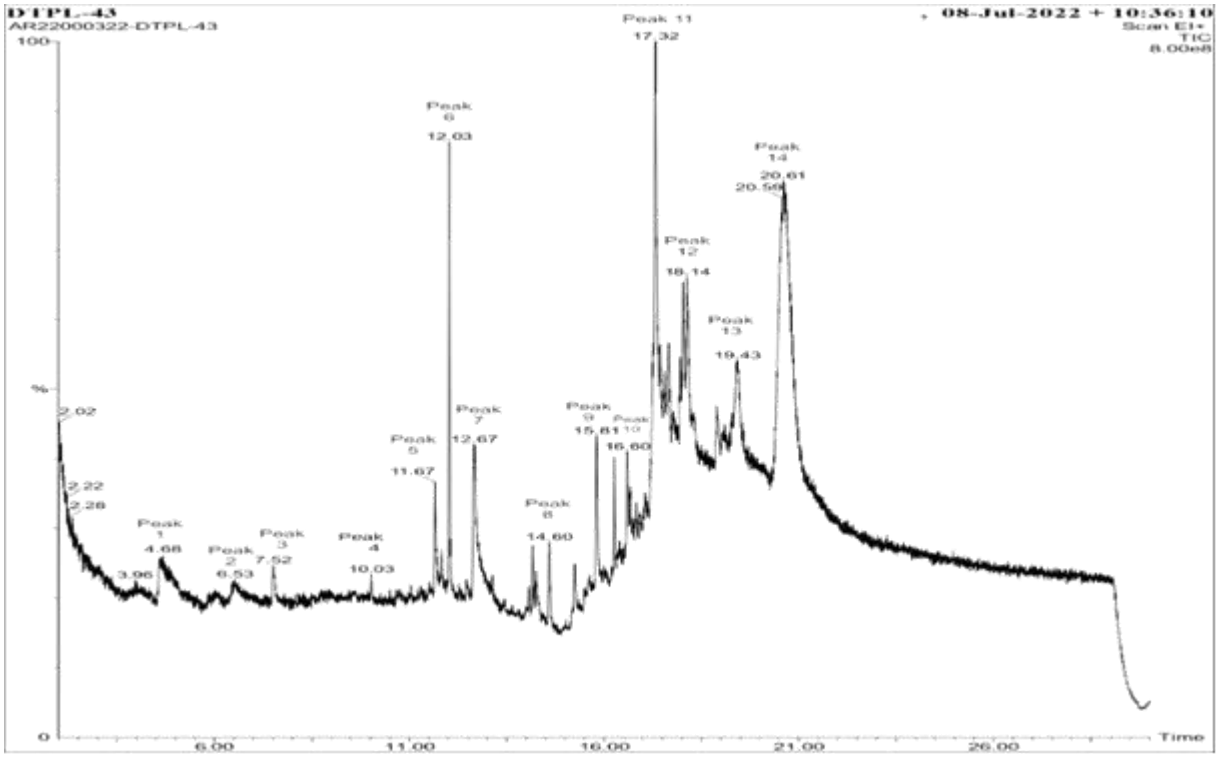

Figure 3. Chromatogram (GC-MS) of the methanol extract of *Planaxis sulcatus* whole tissue extract

Antibacterial activity

Minimum Inhibitory Concentration

For *S.aureus,* the MIC values were determined for various concentrations of methanol extract, with greater concentrations exhibiting lower MIC values as compared to pure antibiotic. *P.sulcatus* was shown to have MICs of 1.72 g/ml, 1.87 g/ml, 1.87 g/ml, and 1.99 g/ml *against S.aureus, P.auroginosa, E. coli*, and *K.pneumonia*, respectively (Table 3).

Table 3: MIC value of *P.sulcatus* methanol extract of test organisms in µg/ml

Sl. no	Test Organism	MIC value (µg/ml)
1	*Staphylococcus aureus*	1.72
2	*Pseudomonas auroginosa*	1.87
3	*Klebsiella pneumonia*	1.87
4	*Escherichia coli*	1.99

Determination of the Minimum Bactericidal Concentration (MBC)

The growth of test organisms is evaluated and colony forming unit (CFU) was calculated. Table 4 depicts the Minimum Bactericidal Concentration.

Table 4: CFU value of test organisms against *P.sulcatus* methanol extract

Concentration (µg/ml)	**MIC – CFU/mL**			
Organism Name	***S.aureus***	***P.auroginosa***	***K.pneumonia***	***E.coli***
20	9	35	TNTC	44
25	8	34	TNTC	38
30	4	20	TNTC	28
35	4	18	TNTC	14
40	0	3	TNTC	10
45	0	2	TNTC	0
50	0	0	TNTC	0
55	0	0	TNTC	0
60	0	0	TNTC	0
+Ve control	183	205	TNTC	167

TNTC- Too numerous to count

Disc Diffusion Method

The antibacterial activity of methanolic extract of *P.sulcatus* against the tested bacteria is shown in Table 5. The antibacterial activity was conferred by the methanol extracts of *P.sulcatus* was able to inhibit *Staphylococcus aureus* with maximum zone of 30 mm at concentration of 40µg followed by *Escherichia coli* 25mm. Minimum activity was noted in *Pseudomonas auroginosa* 21 mm, 20mm at concentration 160µg and 320µg respectively. Whereas, extract did not inhibit growth of *Klebsiella pneumonia* at all concentration levels Table 6, Figure 4.

Table 5: Antibacterial activity of *P.sulcatus* methanol extract with concentration ranging from 5 to 40 µg/ml of test organisms

Sl.no	Test Organism	Zone of Inhibition (mm) **Extract µg/ml**							
		5		10		20		40	
		1	2	1	2	1	2	1	2
1	***S. aureus***	18	17	24	24	27	26	30	30
2	***Escherichia coli***	0	0	15	15	17	16	25	26

Table 6: Antibacterial activity of *P.sulcatus* methanol extract with concentration ranging from 5 to 320 µg/ml of test organisms

Sl.no	Test Organism	Zone of Inhibition (mm) **Extract µg/ml**													
		5		10		20		40		80		160		320	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	***Pseudomonas auroginosa***	0	0	9	8	10	10	12	11	13	13	20	19	21	20
2	***Klebsiella pneumonia***	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The positive control (Antibiotic Ampicilin) was showed activity against all the bacteria strains tested. The maximum activity against *Klebsiella pneumonia* and *Staphylococcus aureus* was found to

be15mm and 13mm respectively.The antibacterial agent of ampicillin showed activity against all the tested bacterial strains Figure 5.

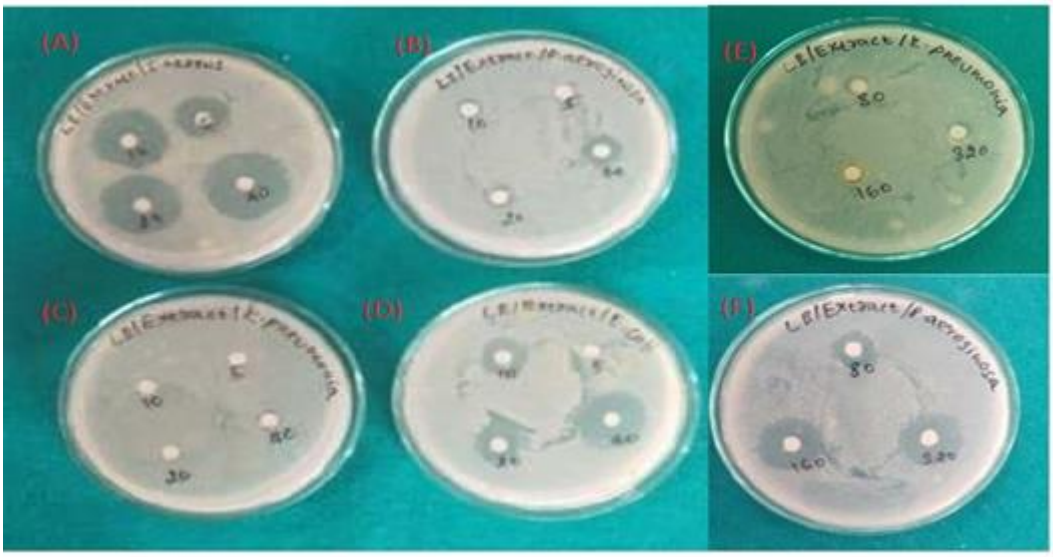

Figure 4. Disc diffusion method *P sulcatus* methonolic extract with concentration ranging from 5, 10, 20, and 40 µg/ml against

A) *Staphylococcus aureus*, B) *Pseudomonas auroginosa*, C) *Klebsiella pneumonia*, D) *Escherichia coli*, *P sulcatus* methonolic extract with concentration ranging from 80, 160, and 320µg/ml against E) *Klebsiella pneumonia*, F) *Pseudomonas auroginosa*.

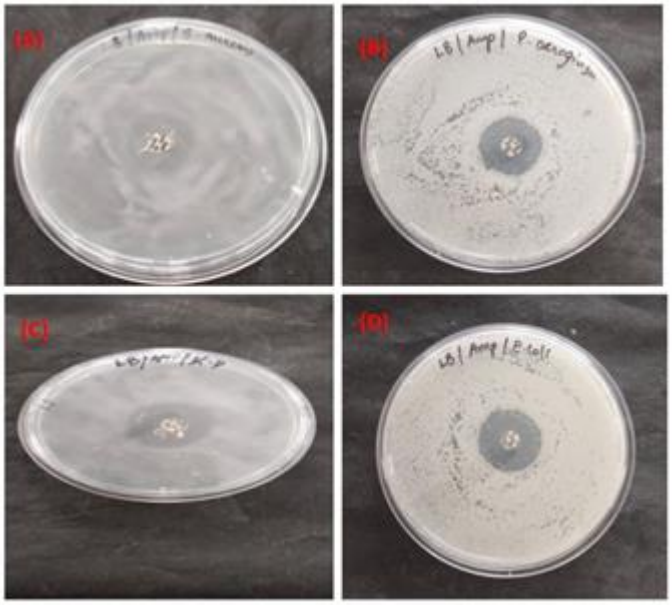

Figure 5: Disc diffusion method against Ampicilin A) *Staphylococcus aureus*, B) *Pseudomonas auroginosa*, C) *Klebsiella pneumonia*, D) *Escherichia coli*

About half of all living things are marine species, which makes them a rich source for finding natural bioactive compounds. Significant progress has been made in marine drug discovery since 1970. The promise of the oceans became apparent in 1989 when academics and pharmacologists started working together (Pati et al., 2015). Since then, several rare bioactive compounds have been isolated from marine plants and animals. Nearly every class of marine organisms exhibits a diversity of molecules

with distinctive structural characteristics as a result of the physical and chemical circumstances in the marine environment. As previously indicated, microbes have developed resistance to numerous antibiotic medications as a result of the indiscriminate use of antimicrobial drugs. They decreased antibiotic susceptibility and made it more challenging to cure infectious illnesses. In comparison to bioactive, naturally produced medications, the expense of producing synthetic drugs is also considerable, and they may also result in unfavourable side effects. Therefore, it is necessary to replace pharmaceutical treatments with naturally derived agents that have minimal adverse effects.

In terms of pharmacologically potent marine chemicals, bacteria, fungus, algae, sponges, soft corals, tunicates, molluscs, and bryozoans are among the most intriguing organisms (Fenincal, 1997). The marine invertebrates include, the Molluscs are with a long evolutionary history, and are the second most successful organisms on earth, potential sources of bioactive substances (Fenincal & Faulkner, 2003). The bioactive substances that were extracted from the gastropods are thought to play a part in the animals' chemical defenses against their predators. From marine creatures, such as *Manoalide* (Stallard & Faulkner, 2003), *Pseudopterosins*, *Topsentins*, and *Scytonemin* (Potts et al., 1992), many intriguing lead compounds have been discovered. Likewise some important lead compounds with promising antibacterial activity have been identified by various methods.

The results of the GC-MS analysis of *Planaxis sulcatus* indicate the existence of antibiotic chemicals with signals at various molecular weights. When compared to the control (antibiotic), the gastropod *P. sulcatus* crude extracts from the methanol solvent had substantial antibacterial activity. It is worthy to note that the product from the gastropod *P.sulcatus*is good for health and devoid of side effects for humans. The present study can be speculated that the extracts of *P.sulcatus* may have many biologically active principles, which need further purification study in future. Thus the importance of biologically active mixture compounds present in the extracts of *P.sulcatus*. The zoochemical and GC-MS spectrum consist of samples from body tissue in *P.sulcatus* showed the number of constituents and peaks. This proves the presence of the bioactive compounds in gastropods which could be used as potent sources.

In recent decades, attempts have been made to produce anti-bacterial medications to treat and stop the occurrence and spread of infectious diseases that affect humans and are brought on by germs. Many of these anti-microbial medications are either made in the lab utilising secondary metabolites that have been extracted from organisms or using cues from their biochemical and physiological processes. Many substances with anti-microbial activity, including chlorinated acetylenes, indole alkaloids, glycoproteins, and peptides, have been identified from marine molluscs (Mayer & Lehmann, 2001).

The antibacterial activity was conferred by the methanol extracts of *P.sulcatus* was able to inhibit *Staphylococcus aureus*, with maximum zone of 30 mm at concentration of 40µg followed by *Escherichia coli* 25mm. Minimum activity was noted in fresh methanol extract against *Klebsiella pneumoniae*(0mm). Highest concentration 160 and 320 µg respectively of Methanol extracts showed inhibition of *Pseudomonas auroginosa* 20 and 21mm respectively. The maximum activity against *Klebsiella pneumoniae* (15mm) and *Staphylococcus aureus* (13mm) were observed in positive control antibiotic Ampicilin. In the present study, a new molluscan extracts exhibited levy interesting control effect on the growth of *S. aureus, E.coli and P. aeruginosa.* The MIC of gastropod against *S. aureus, P. auroginosa, K.pneumonia* and *E. coli* was found at 1.72, 1.87, 1.99 and 1.87 µg/ml of extract. The maximum MIC antibacterial activity from methanol crude extracts of *P.sulcatus* against human pathogen *K.pneumonia* at 1.99µg/ml. The ability of the extracted chemicals and the solvents to extract them may account for variations in the antibacterial effectiveness of mollusc extract.

According to recent findings by Kumaran *et al*., (2011), two species of marine molluscs, *Thais tissoti* and *Babylonia spirata*, have bioactive substances with potent anti-microbial activity against human diseases *Klebsilla pneumonia* and *Proteous mirabilis*. At the same time, Santhi *et al.*, (2011) found secondary metabolites in the deep-water mollusk *Tonna galea*, suggesting promising potential for anti-microbial activity against the human pathogens *Vibrio cholera* and *Aeromonas hydrophila*. At the same time, bioactive substances with antibacterial activities against *K. pneumoniae* and *S. typhi* have been found in the extract of the marine mollusk *Melo melo*, according to Sivasubramanian *et al*., (2011).The current study supports the bioactive potential of molluscs with shells and offers a baseline

of information for the separation and characterization of the elements that are active. The sea molluscs employed in this investigation have only been used in a very small number of investigations. Little to no information is available on the other species, with the exception of *Nerita sp., T.radiatus, T.galea, T. tissoti,* and *B. spirata*. The preliminary studies' antibacterial activity demonstrates the bioactive potential of intertidal molluscs, making a quick examination of all available intertidal fauna essential.

4. Conclusion

In the current analysis, some bacterial pathogenic strains showed strong antibacterial activity when extracted in methanol. Since *Planaxis sulcatus* lives in an intertidal rocky shore, it's possible that it produces compounds that could prevent bacterial growth either out of food or defense against its predators. These antimicrobial chemicals are comparatively easy to synthesize, modify chemically, examine, and manipulate. The antibacterial compounds from *Planaxis sulcatus* play a significant role in bridging the gap between bioactive drugs and molecular genetics, though these compounds are also primarily translational products of genes with strong biological activity and can be altered by techniques of modern molecular genetics.

References:

Abubakar, M. N., & Majinda, R. R. T. (2016). GC-MS analysis and preliminary antimicrobial activity of Albizia adianthifolia (Schumach) and Pterocarpus angolensis (DC). Medicines, 3(3).

Al-Gara, N. I. (2019). Analysis of bioactive phytochemical compounds of Cyperus alternifolius L. using gas chromatography–mass spectrometry. IOP Conference Series: Materials Science and Engineering, 571. DOI: 10.1088/1757-899X/571/1/012047

Al-Marzoqi, A. H., Hadi, M. Y., & Hameed, I. H. (2016). Determination of metabolite products by Cassia angustifolia and evaluate antimicrobial activity. Journal of Pharmaceutical and Pharmacological Research, 8(2), 25-48. DOI: 10.5897/JPP2015.0367

Al-Rubaye, A. F., Kaizal, A. F., Hameed, I. H. (2017). Phytochemical Screening of Methanolic Leaves Extract of Malva sylvestris. International Journal of Pharmacognosy and Phytochemical Research, 9(4), 537-552. DOI: 10.25258/phyto.v9i2.8127

Al-Tameme, H. J., Hadi, M. Y., & Hameed, I. H. (2015). Phytochemical analysis of Urtica dioica leaves by Fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. Journal of Pharmacognosy and Phytochemistry, 7(10), 238-252. DOI: 10.5897/JPP2015.0361

Amudha, M., Rani, S., & Shanmugam, R. (2014). Assessing the bioactive constituents of Cadaba fruticosa (L.) Druce through GC-MS. International Journal of Pharmacy and Pharmaceutical Sciences, 6(2).

Anbuselvi, S., Chellaram, C., Sreenivasan, R. S., et al. (2009). Bioactive potential of coral-associated gastropod, Trochus tentorium of Gulf of Mannar, Southeastern India. Journal of Medical Sciences, 9(5), 240-244.

Baba, H., Kashimawo, A. J., & Ibe, A. C. (2021). Phytochemical Evaluation and GC-MS Profiling of the Dichloromethane and Ethanol extracts of Ocimum gratissimum L. and Lasianthera africana. BEAUV, Journal of Pharmacognosy and Phytotherapeutics, 20*(2), 640–655.

Benkendorff, K. (2010). Molluscan biological and chemical diversity: Secondary metabolites and medicinal resources produced by marine mollusks. Biological Reviews, 85(4), 757-775.

Chellaram, C., Mary Elizabeth, K. G., et al. (2004). Antibacterial activity of the winged oyster Pteria chinensis (Pterioida: Pteridae). Indian Journal of Medical Sciences, 33(4), 369-372.

Chia, Y. Y., Kanthimathi, M. S., Khoo, K. S., Rajarajeswaran, J., et al. (2015). Antioxidant and cytotoxic activities of three species of tropical seaweeds. BMC Complementary and Alternative Medicine, 15, 339. DOI: 10.1186/s12906-015-0860-z

Clinical and Laboratory Standards Institute. (2019). M100 Performance Standards for Antimicrobial Susceptibility Testing, 29th ed.

Eahamban, K., & Antonisamy, J. M. (2012). Preliminary phytochemical, UV-VIS, HPLC, and anti-bacterial studies on Gracilaria corticata J.Ag. Asian Pacific Journal of Tropical Biomedicine, 2, 574.

Elaiyaraja, A., Chandramohan, G. (2018). Comparative phytochemical profile of crinum defixum ker-gawler leaves using GC-MS. Journal of Drug Delivery & Therapeutics, 8(4), 365-380.

Fenical, W. (1997). New pharmaceuticals from marine organisms. Trends in Biotechnology, 15(9), 339-341. DOI: 10.1016/S0167-7799(97)01081-0

Fenical, W. (1997). New pharmaceuticals from marine organisms. Trends in Biotechnology, 15(9), 339-341. DOI: 10.1016/S0167-7799(97)01081-0

Fenical, W., & Faulkner, D. J. (2003). Marine Natural Products Chemistry and Marine Chemical Ecology. Angewandte Chemie International Edition, 42(13), 1438-1439. DOI: 10.1002/anie.200390369

Fenical, W., & Faulkner, D. J. (2003). Marine Natural Products Chemistry and Marine Chemical Ecology. Angewandte Chemie International Edition, 42(13), 1438-1439. DOI: 10.1002/anie.200390369

Gheda, S. F., & Ismail, G. A. (2020). Antioxidant and antihyperglycemic activity of Arthrospira platensis (Spirulina platensis) methanolic extract: In vitro and in vivo study. Egyptian Journal of Botany.
Gheda, S. F., Abo-Shady, A. M., Abdel-Karim, O. H., & Ismail, G. A. (2021). Antioxidant and Antihyperglycemic Activity of Arthrospira platensis (Spirulina platensis) Methanolic Extract: In vitro and in vivo Study. Egyptian Journal of Botany.
Gideon, V. A. (2015). GC-MS analysis of phytochemical components of Pseudoglochidion anamalayanum Gamble: An endangered medicinal tree. Asian Journal of Plant Science Research, 5, 36–41.
Harborne, J. B. (2005). Phytochemical Methods. Springer (India) Pvt. Ltd.
Hugar, A. L., Kanjikar, A. P., Londonkar, R. L. (2017). Bioactive Compounds Investigation From Methanol Bark Extract of Pterocarpus marsupium Using GC-MS Analysis. International Journal of Pharmaceutical Quality Assurance, 8(3), 104-110. DOI: 10.25258/ijpqa.v8i03.9571
Hussein, H. M. (2016). Analysis of trace heavy metals and volatile chemical compounds of Lepidium sativum using atomic absorption spectroscopy, gas chromatography-mass spectrometric, and Fourier-transform infrared spectroscopy. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7(4), 2555.
Kadhim, M. J., Mohammed, G. J., & Hameed, I. H. (2016). Oriental journal of chemistry, 32(3), 1329-1346.
Kailas, A. P., & Nair, M. S. (2015). Saponins and the in vitro bioactivities of different solvent extracts of some tropical green and red seaweeds. Journal of Coastal Life Medicine, 3, 931-943.
Karthi, S., Somanath, B., & H, A. J. (2018). Efficacy of Methanolic Extract of a Marine Ascidian, Lissoclinum bistratum for Antimicrobial Activity. Journal of Chemical, Biological and Physical Sciences, 5(4), 4119-4125.
Karthikeyan, A. V. P., Sudan, I. (2017). GC-MS profile of in vivo and in vitro shoots of Cleome gynandra L. International Journal of Pharmacy and Pharmaceutical Sciences, 9(11).
Kelman, D., Kashman, Y., Rosenberg, E., et al. (2001). Antimicrobial activity of the reef sponge Amphimedon viridis from the Red Sea: evidence for selective toxicity. Aquatic Microbial Ecology, 24, 9–16.
Kim, D. J., Yoon, S., et al. (2018). Ursodeoxycholic acid improves liver function via phenylalanine/tyrosine pathway and microbiome remodelling in patients with liver dysfunction. Scientific Reports, 8, 11874.
Kiuru, P., Valeria D'Auria, M., Müller, C. D., et al. (2014). Exploring marine resources for bioactive compounds. Planta Medica, 80(16), 1234–1246. DOI: 10.1055/s-0034-1383001
Kumar, G. D., M., & Rajakumar, R. (2018). GC-MS analysis of bioactive compounds from ethanolic leaves extract of Eichhornia crassipes (Mart) Solms. and their pharmacological activities. The Pharma Innovation Journal, 7(8), 459-462.
Li, C. H., Zhao, J. M., & Song, L. S. (2009). A review of advances in research on marine molluscan antimicrobial peptides and their potential application in aquaculture. Molluscan Research, 29, 17-26.
Lorenz, H. M., Schmitt, W. H., et al. (2011). Treatment of active lupus nephritis with the novel immunosuppressant 15-deoxyspergualin: an open-label dose escalation study. Arthritis Research & Therapy, 13(2), R36.
Lumeran, B. T. (2019). Assemblage of gastropods in the rocky intertidal zone of Asry Beach, Kingdom of Bahrain. DOI: 10.5772/intechopen.87772.
Malathi, K., Anbarasu, A., Sureshkumar, K. (2016). Ethyl Iso-allocholate from a Medicinal Rice Karungkavuni Inhibits Dihydropteroate Synthase in Escherichia coli: A Molecular Docking and Dynamics Study. Indian Journal of Pharmaceutical Sciences, 78(6), 780-788. DOI: 10.4172/pharmaceutical-sciences.1000184
Mayer, A. M. S., & Lehmann, V. K. B. (2001). Marine pharmacology in 1999: antitumour and cytotoxic compounds. Anticancer Research, 21, 2489-2500.
Mayer, A. M. S., & Lehmann, V. K. B. (2001). Marine pharmacology in 1999: antitumour and cytotoxic compounds. Anticancer Research, 21, 2489-2500.
Mishra, M. P., Rath, S., Swain, S. S., et al. (2015). In vitro antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing MDR bacteria. Journal of King Saud University-Science, 29(1), 84–95.
Murugesan, A., Vijayakumar, R., Panneerselvam, A. (2011). Evaluation of Phytochemical Constituents from the Leaves of Memecylon umbellatum Burm.f. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2(4), 1145.
Muthulakshmi, A., Jothibai Margret, R., & Mohan, V. R. (2012). GC-MS Analysis of Bioactive Components of Feronia elephantum Correa (Rutaceae). Journal of Applied Pharmaceutical Science, 02(02), 69-74.
Padmashree, M. S., Ashwathanarayana, R., Raja Naika, Roopa, B. (2018). Antioxidant, cytotoxic, and nutritive properties of Roem & Schult. Ipomoea staphylina plant extracts with preliminary phytochemical and GCMS analysis. Asian Journal of Pharmacy and Pharmacology, 4(4), 473-492.
Pati, P., Sahu, B. K., & Panigrahy, R. C. (2015). Review article, Department of marine sciences, Berhampur University, Berhampur, Odisha, India. Indian Journal of Geo-Marine Science, 44(7), 961-970.

Potts, B. C., Faulkner, D. J., Jacobs, R. S. (1992). Phospholipase A2 inhibitors from marine organisms. Journal of Natural Products, 55(12), 1701-1717. DOI: 10.1021/np50090a001

Potts, B. C., Faulkner, D. J., Jacobs, R. S. (1992). Phospholipase A2 inhibitors from marine organisms. Journal of Natural Products, 55(12), 1701-1717. DOI: 10.1021/np50090a001

Proksch, P., Edrada, R. A., & Ebel, R. (2002). Drugs from the seas: Current status and microbiological implications. Applied Microbiology and Biotechnology, 59, 125-134.

Ragunath, C., Kumar, Y. A. S., Kanivalan, I., & Radhakrishnan, S. (2020). Phytochemical screening and GC-MS analysis of bioactive constituents in the methanolic extract of Caulerpa racemosa (Forssk.) J. Agardh and Padina boergesenii Allender & Kraft. Current Applied Science and Technology, 20(3), 380–393.

Rajendran, P., Bharathidasan, R., Sureshkumar, K. (2017). GC-MS Analysis of Phyto-Components in Raw and Treated Sugarcane Juice. International Journal of Current Microbiology and Applied Sciences, 6(7), 51-61.

Rakkimuthu, R., Ananthi, P., Sathishkumar, P., et al. (2023). Chemical profiling of fern Cheilosoria mysurensis (Wall. ex Hook.) Ching & Shing and its biological activity. Plant Science Today, 10, 91–95. DOI: 10.14719/pst.1872.

Raman, N. (2006). Phytochemical Technique. New Indian Publishing Agencies.

Ramya, B., Malarvili, T., Velavan, S. (2015). GC-MS analysis of bioactive compounds in Bryonopsis laciniosa fruit extract. International Journal of Pharmaceutical Sciences and Research, 6(8), 3375-3379.

Semwal, P., Painuli, S., Badoni, H., & Bacheti, R. K. (2018). Screening of phytoconstituents and antibacterial activity of leaves and bark of Quercus leucotrichophora A. Camus from Uttarakhand Himalaya. Clinical Phytoscience, 4(1).

Sharath, K. P., Naika, R., (2017). Chemical profiling, in vitro antibacterial, and cytotoxic properties of Elytranthe parasitica (L.) Danser – A hemiparasitic Indian mistletoe. Journal of Applied Biology & Biotechnology, 11(3), 238-244.

Stallard, M. O., & Faulkner, D. J. (1974). Chemical constituents of the digestive gland of the sea hare Aplysia californica: I. Importance of diet. Comparative Biochemistry and Physiology Part B, 49B, 25-35.

Stallard, M. O., & Faulkner, D. J. (1974). Chemical constituents of the digestive gland of the sea hare Aplysia californica: I. Importance of diet. Comparative Biochemistry and Physiology Part B, 49B, 25-35.

Tulika, T., Agarwal, M. (2017). Phytochemical Screening and GC-MS Analysis of Bioactive Constituents in the Ethanolic Extract of Pistia Stratiotes L. Journal of Pharmacognosy and Phytochemistry, 6(1), 195-206.

Venkatachalapathi, A. (2021). GC-MS analysis of ethanolic aerial part extract of important medicinal plant Cyclea peltata (lam.) Hook. F. Thoms. (menispermaceae). International Journal of Scientific Research, 10(11).