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Extraction, Isolation and Characterization of Bioactive Compound from Tissue of Fresh Water Crab *Barytelphusa cunicularis* from Northern Region of Maharashtra

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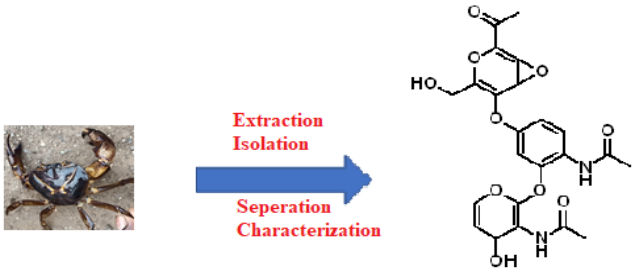
Abstract:-



The fresh water biodiversity is an unpredictable for natural product research from northern region, India, *Barytelphusa cunicularis* is major species, the Ethanol crude extract of tissue has been utilized for this study the isolation, separation and Characterization by using reputed methods. The biological activity of the crude ethanol extract was examined then this fresh water crab was resulted antibacterial and antifungal activity it means the bioactive compounds have been present in this crab. TLC was performed to set the solvent system for column chromatography while purity level to be checked by HPLC, it is 93% pure compound. Then the characterization of the pure compound by elemental analysis, UV, FTIR, ^{1}HNMR, ^{13}CNMR, LC-MS spectroscopy data analysis was revealed to found N-(2-((3-acetamido-4-hydroxy-4H-pyran-2-yl) oxy)-4-((2-acetyl-4-(hydroxymethyl)-3,7 dien-5-yl) oxy) phenyl) acetamide.

Keyword:- *Barytelphusa Cunicularis, Freshwater Crab, Unpredictable, Bioactive Compounds, Crude Extract.*

I. INTRODUCTION

Most of the animals have different biodiversity. The first biodiversity was found in water that resulted in new research studies on marine biodiversity. During this period adaptation of marine animal have been changing and there's a requirement for the development of the bioactive compounds, which are utilized for a different function like growth, reproduction, locomotion, communication, and defence etc. Bioactive compounds have found complex structures which is important for human needs. Thus, from ancient time marine animals are the source of medicine in the pharmaceutical world. Studies of various relevance shown that the extracts of marine animals cure the disease. The structure and function of bioactive compounds become known in the pharmaceutical field. Due to the fact, the majority of pharmaceutical companies are engaging to isolate the bioactive compounds from marine biota.

Marine non-chordates are important sources for drug design due to their fewer side effects, this enhanced their production on a large basis. However, this strategy is not sufficient for drug discovery. Thus, researchers have to follow the semi-synthetic pathway and need to utilize artificial compounds for medicine. Marine biodiversity provides the novel compound; enhances drug design and development to the new therapeutic drugs. It is a great success of the natural product field to lead the drug discovery (1). The drug approval between 1981 to 2014 of natural products and structure determination, which have the lead important role in drug development. The review summarises the role of natural products in hepatoprotective drug derived (1). The result shows that there is an increase in novel drug discovery from marine natural products reported every year, where 1340 new compounds were reported in 2015 (2).

Marine biodiversity provides an enormous source of bioactive compounds. These compounds are active against different therapeutic diseases. The marine environment is a prolific resource for the isolation of less exploited organisms and microorganisms. There are actually untapped habitats in the sea with unique characteristics. After intensive studies on the terrestrial microorganism, consequent attention has been focused on other ecosystems, especially those subjected to extreme environmental conditions such as desert hot springs and the sea. Most of the bioactive compounds were studied from the marine ecosystem. In the addition, the potential contribution of the freshwater ecosystem has been studied the discovery of natural products remains left behind. Crab is the best source of bioactive compounds after shrimp and lobster to provide protein, vitamins, and fatty acids to improve the health benefits of the human being. The components present in crab showed biological activity such as antioxidant,

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anticancer, antidiabetic, antimicrobial, and anticoagulant function. The above activity is also shown by the bioactive compounds obtained from the tissue of Portunid crabs (3). However, on the basis of recent advances in the research field, the following definition has been proposed for the freshwater ecosystems: "natural products from the freshwater ecosystem that have beneficial effects on the health and wellbeing of hosts".

Freshwater biodiversity is an unpredictable research area due to various reasons. The presence of species in this biota traditionally utilized for food purposes. *Barytelphusa cunicularis* (freshwater crab) is major crab species found in freshwater biota and utilized as the food of slum people and due to this reason, the population of this species tremendously increases. When there is starvation or dryness or hot condition occurs, this leads to the death of crabs which ultimately results in the decomposition of dead crab species. According to a literature survey, the freshwater crab has been less predicted than marine crab, but now the scenario has changed and more research attention is given toward the *Barytelphusa cunicularis* from various regions. The work is related to the freshwater crab such as the structure and a property of the exoskeleton of the sheep crab has been showed. The crab oil has a good saponification value i.e., 126 mg KOH/gm. The peroxide value of crab oil is less i.e., 4.3 meq/kg though the crab oil is the source of food (4). The biochemical and functional property of chitin and chitosan (obtained from *Barytelphusa cunicularis*) has been studied. It has shown the specific applications in drug delivery, tissue engineering, functional food, food preservative, biocatalyst, immobilization, wastewater treatment, molecular imprinting, and metal nanocomposites. Chitin is mainly derived from the shell of *Barytelphusa cunicularis*. The chemical composition of chitin is B-(1-4)2acetamido2-deoxy-β-D-glucose (N-acetyl glucosamine). It is insoluble in an aqueous medium and soluble in acidic medium due to the free portable amino group present in D-glucosamine unit (5).

The aim of the research is to study the novel bioactive compounds isolated from freshwater biota which is being also the interest of research and is not yet focused because of the instability in ecosystem, variables in temperature, pressure, and humidity which are highly depends on seasonal conditions. *Barytelphusa cunicularis* is freshwater crab species and is completely unknown to researchers as compared to other freshwater animals. Actually, this is an edible species, the biodiversity of this species is widely studied by many researchers however the food property is not reported yet (this is first time we have reported and investigated in this study) (6). This is the main crab found in northern region of Maharashtra in India. The different researcher has been conducted on this species which includes study of crab shell, obtained chitin and chitosan have been explored, whereas the tissue part is not studied yet (7). Tissue is the main part of the crab and utilized as food due to it high nutritive value. Especially amino acids, liver protein is found in crab's tissue. The free portable amino group present is crab tissue is focused of our study. These proteins are utilized as hepatoprotective or any therapeutic drug (8). If thinking through biodiversity conservation purpose, the population of the freshwater crab species is high as discussed previously (because of less utilization and variation of the seasonal condition results in death and decomposition of dead crabs) (9). This decomposition leads to loss of essential bioactive compounds from the crab and the analysis is very important (10). In future freshwater natural products would have a huge contribution in the pharmaceutical industry and the drug design strategy will forward to clinical drug standards (11).

II. MATERIAL AND METHODS

2.a. Sample collection:

Barytelphusa cunicularis was collected from the northern region of Maharashtra state, India. The adult species was used for extraction, isolation, and further study. The reason for selecting adult species is because these species have more bioactive compounds as compared to juveniles (11). After collection, the species were transferred alive in refrigerated containers of the laboratory. The crab species has proceeded for mercy killing (mechanical treatment) by thermal shock for 15 minutes at -300 ^{0}C. Each collected species was dissected individually and the tissue was collected from the abdomen cavity of the crab species. The crude extract was prepared from this tissue and was stored in a dip freezer and used for further study (12).

2.b. Preparation of extraction:

The tissue material was transferred to the microwave for complete drying to obtain the samples in powder form. Further, the powder was dipped into 500 ml of ethanol for 21 days. The overall process is called a cold maceration process because all bioactive compounds are stable at low temperatures (13). This method performed at room temperature and the material dipped in an airtight container. Occasionally sample was stirred for high speed for higher extraction. later the suspension was allowed to settle down. This leads to the diffusion of bioactive compounds from the tissue cell until the equilibrium (14). After 21 days the extract was filtered. It was further separated from the bulk material. After that fresh ethanol were added to the bulk immediately for high extraction (15). The procedure was repeated many times to ensure the maximum extraction yield. All extract was concentrated with rotator evaporators to remove the excess amount of ethanol. Although this method is time-consuming however it gives higher yields of bioactive compounds. it is less expensive and can be adaptable at any place. This method is best for thermally unstable compounds. The best thing is that It reduces environmental pollution (16).

2.c. Subsequent isolation of crude extract:

Bioactive compounds were isolated by using a solvent extraction process (using separating funnel) (17). Initially, the macerated ethanol extract was isolated in ethanol (which contains a group of bioactive compounds), this extract was taken into the water and left for 15 min. Further hexane was added to the mixture of water-ethanol in a separating funnel. The ratio of the water-ethanol mixture and hexane was kept constant as 1:2. The extract was conducted by shaking and allowed to stand for 10 min. Two layers are separated, the top hexane layer was removed and more hexane was added, this process was repeated 5-6 times. All collected hexane was

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recovered by rotary evaporated to obtain hexane fraction. The remaining aqueous layer was subjected to the same procedure with a solvent like an ethyl acetate, chloroform, ether. Further process was guided by bioassay fractions (18).

2.d. Column chromatography:

The column chromatography technique was used for the separation of compounds obtained from the extraction process mentioned above. The extract was adsorbed on silica gel (60-120 Mesh) and the column was eluted by n-hexane. The slight polarity was increased by adding ethyl acetate. 40 fractions were collected and stored in a 500 ml reagent bottle (borosilicate). Each fraction was subjected to TLC using a solvent system (ethyl acetate and diethyl ethyl ether). Total 100 ml fraction was passed over the column using solvent system (9.5:0.5, 9.0:1.0, 8.5:1.5, 4:1). These fractions were separately analysed in HPLC analysis to check the purity level of the isolated compound (19).

III. RESULT AND DISCUSSION

3.a. TLC analysis result of ethanol extract under UV light (370 nm):

The ethanol extract was analyzed by thin layer chromatography (TLC) on analytical plates above the solvent system were utilized for analysis on the basis of polarity, in each solvent system spot were visualized under UV light (370 nm). When TLC analysis maximum spots were visualized in seven solvent systems in ethanol extract of *Barytelphusa Cunicularis* (freshwater crab), the compounds were separated in that solvent systems.

3.b. HPLC analysis of isolated compound:

The analytical HPLC chromatogram of isolated compound S_1 showed 93% purity level, meaning that highly pured. The chromatogram was obtained by compound S_1 with acetonitrile solution with requisite time.

3.c. Elemental Analysis of isolated compound:

Elements showed in isolated compound C, H, O and N. The elemental chromatogram was obtained by isolated compound S_1 with retention time.

Calculated Anal: C- 56.79; H- 4.56; N- 5.76, O, 32.89
Found: C- 55.99; H- 5.403; N- 5.142

3.d. UV analysis of isolated compound:

The UV-visible analysis of the isolated compound S_1 (fig. 3.5.1.3), revealed that, 771.50 nm at 0.0022 abs, 322.00 nm at 0.5889 abs, 308.50 nm at 0.3983 abs, 253.00nm at 0.1664 abs, 212.00 nm at 0.1396 abs. In the UV-visible analysis the red colour absorbed at the wavelength of light 771.50 nm absorbed in which green colour experimentally observed. 212 nm indicates the presence of ketonic >C=O group, 253.00 nm indicates the –$NHCOCH_3$, 308.50 nm indicates –OH group involved in the parent chromosphere.

3.e. IR spectroscopic data of isolated compound

The FTIR (ATR) spectral data of isolated compounds of isolated compound frequencies indicated. Aromatic ring (3-Peaks): 1460.16 cm^{-1}, 1535.39 cm^{-1} and 1648.23 cm^{-1}, Ar-OH: 3634.01 to 3580.97 cm^{-1}, stretching of N-H: 2954.08 $cm^{-1}$to 2850.88 cm^{-1}, conjugated ketone >C=O: 1710.92 cm^{-1}, strong C-O stretching aromatic ester: 1260.52 to 1311.64 cm^{-1}, aromatic compound C-H bending 2016.92 cm^{-1} weak overtone, 1116.82 to 1081.44 cm^{-1} strong C-O stretching secondary alcohol (fig. 3.5.1.4). In the view of IR bands at 1460.16 cm^{-1}, 1535.39 cm^{-1} and 1648.23 cm^{-1} indicated the presence of aromatic ring, 2954.08 $cm^{-1}$to 2850.88 cm^{-1} shows the stretching of N–H group, strong C–O stretching aromatic ester: 1260.52 to 1311.64 cm^{-1}, aromatic compound C–H bending 2016.92 cm^{-1} weak overtone, 1116.82 to 1081.44 cm^{-1}strong C–O stretching secondary alcohol are observed in the compound.

3.f. ^{1}HNMR spectroscopic data of isolated compound:

The ^{1}HNMR (400MHZ DMSO-d^6 δ ppm) spectra of the isolated compound S_1 showed the peaks 7.86-6.84 (m, 6H, Ar-H); 2.51-2.50 (s, 2H, -NH); 8.26-8.23 (s, 1H, -OH); 1.62 (s, 3H, Ar-CO-R); 1.08-1.05 (s, 6H, -NH-CO-CH_3); 3.39 (s, 2H, CH_2)(fig.3.5.1.5).In the observation of NMR spectrum in 6.86-7.84 δ values, six protons found multiplets which indicate Ar-H; 2.51- 2.50 δ, two proton found singlet shows -NH group; 8.26-8.23 δ, two proton found singlet that confirms -OH group; 1.62 δ, three protons designated singlet of Ar-CO-Ar; 3.39 δ two protons found singlet that that indicate -CH_2; 1.08-1.05 δ, six proton found singlets that shows NH-CO-CH_3 groups.

3.g. ^{13}CNMR spectroscopic data of isolated compound:

The ^{13}CNMR (400MHZ DMSO-d^6 δ ppm) spectroscopic data of isolated compound S_1 reveled as19.05, 26.31, 39.38, 39.59, 40.02, 40.44, 40.65, 56.50, 127.46, 128.45, 129.29, 131.28 and 175.66.

Prediction of ^{13}C NMR spectrum, the chemical shifts when carbon is coupled to their hydrogens, carbon follows N+1 rule. The chemical shifts observed in the range of 19.05-26.31ppm indicates -CH_3 group, 56.50 ppm shows -CH_2, 127.46 – 131.28 ppm indicates unsaturated -C=C-and aromatic ring carbon and 175.66 ppm >C=O carbonyl carbon.

3.h. LC-MS spectroscopic data of isolated compound:

The mass spectra of the isolated compound S_1 shows projected structure molecular weight, indicated as LC-MS (ES+, 8.78e4): m/z = 485.40 (M^+) for $C_{23}H_{22}N_2O_{10}$

Considering all predicted data of the isolated compound, structure of the organic compound was elucidated and IUPAC name as

N-(2-((3-acetamido-4-hydroxy-4H-pyran-2-yl) oxy)-4-((2-acetyl-4-(hydroxymethyl)-3,7 dien-5-yl) oxy) phenyl) acetamide.

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IV. PROPERTIES ISOLATED COMPOUND

Molecular Formula: $C_{23}H_{22}N_2O_{10}$
Physical Appearance: Yellow Solid
Molecular weight: 486

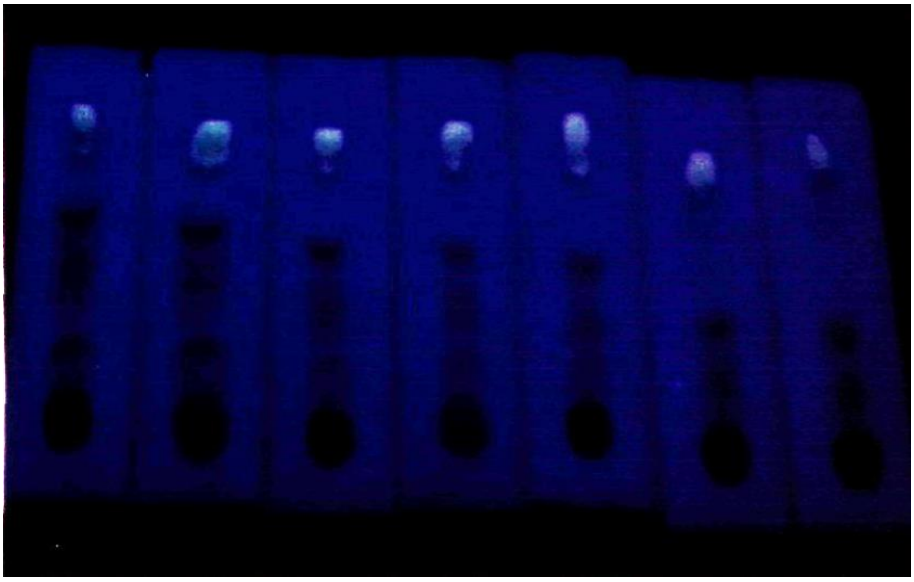
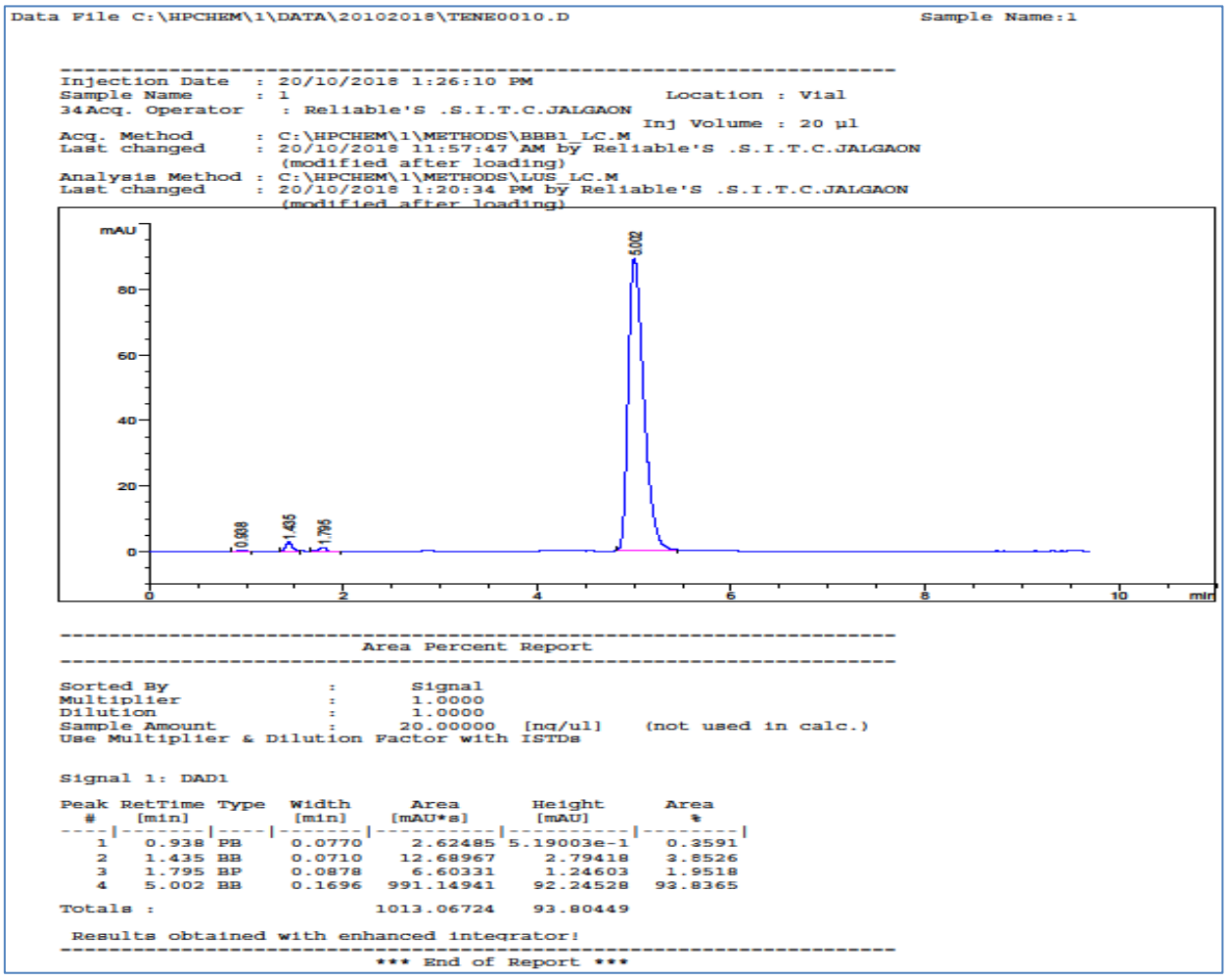


Figure. 1 TLC results for Ethanol extract, tissue of *Barytelphusa C.* at different concentration plates visualized under UV light.



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34Acq. Operator  : Reliable'S .S.I.T.C.JALGAON
                                                      Inj Volume : 20 µl
Acq. Method      : C:\HPCHEM\1\METHODS\BBB1_LC.M
Last changed     : 20/10/2018 11:57:47 AM by Reliable'S .S.I.T.C.JALGAON
                   (modified after loading)
Analysis Method  : C:\HPCHEM\1\METHODS\LUS_LC.M
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                   (modified after loading)
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                          Area Percent Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :     20.00000  [ng/ul]    (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.938 PB    0.0770    2.62485 5.19003e-1   0.3591
   2   1.435 BB    0.0710   12.68967    2.79418   3.8526
   3   1.795 BP    0.0878    6.60331    1.24603   1.9518
   4   5.002 BB    0.1696  991.14941   92.24528  93.8365

Totals :                   1013.06724   93.80449

 Results obtained with enhanced integrator!

                          *** End of Report ***
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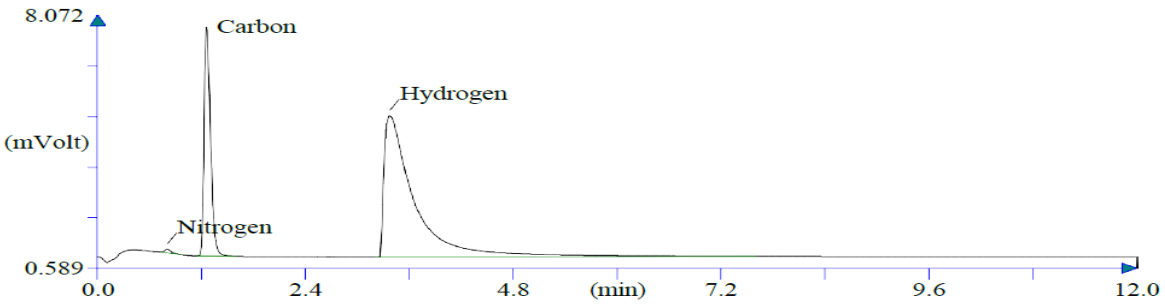
Figure. 2 HPLC results for compound S_1 isolated from *Barytelphusa Cunicularis* tissue.

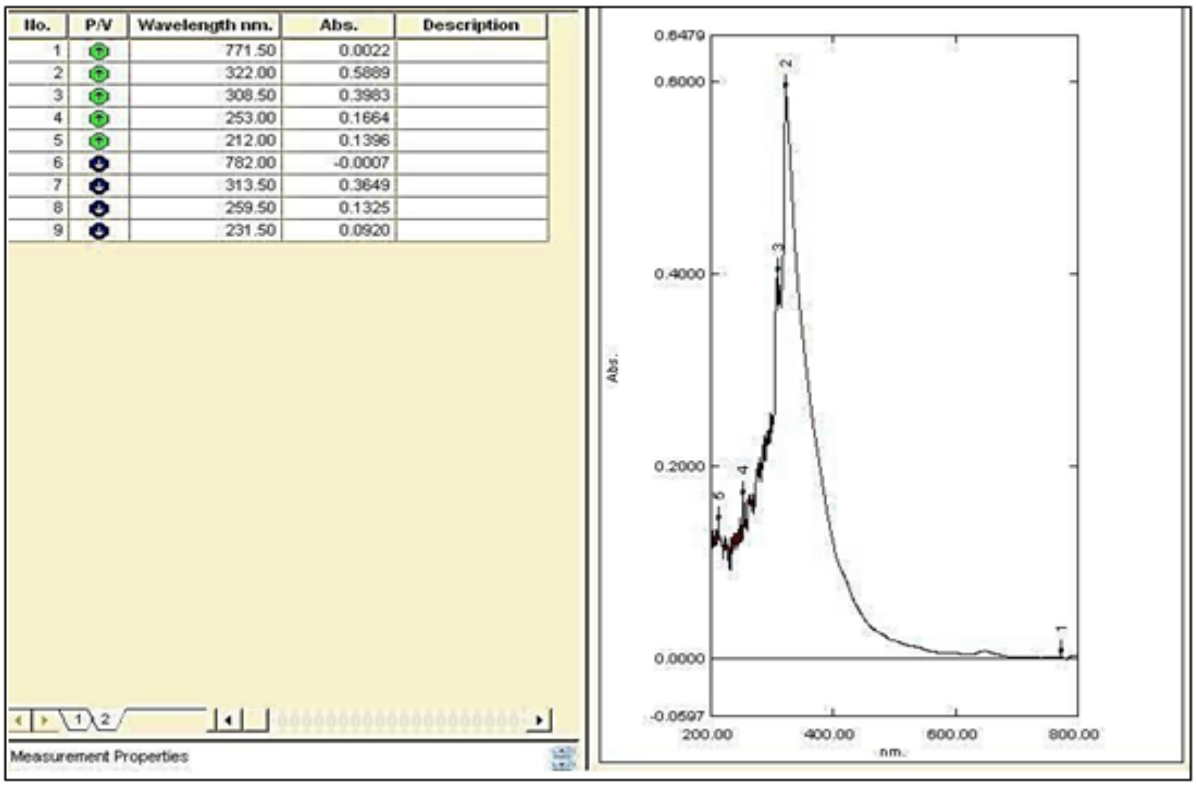
CHN Lab , Panjab University , Chandigarh

Company name:	ThermoFinnigan
Method filename:	C:\SAIF\CHNS\User - 2019\May\1-5-2019\N C H S systemmmmmmmmmm
Method name:	NCHS
Analysed:	01-05-2019 15:43
Printed:	05-01-2019 19:17
Sample ID:	**S1**
Analysis type:	UnkNown
Chromatogram filename:	C:\SAIF\CHNS\User - 2019\May\1-5-2019\21.DAT
Calibration method:	K Factors
Sample weight:	1.856



Retention Time (min)	Component Name	Element %
0.808	Nitrogen	5.142
1.258	Carbon	55.99
3.375	Hydrogen	5.403
		66.535

Figure.3 Elemental analysis results for compound S_1 isolated from *Barytelphusa Cunicularis* tissue.



No.	P/V	Wavelength nm.	Abs.	Description
1		771.50	0.0022	
2		322.00	0.5889	
3		308.50	0.3983	
4		253.00	0.1664	
5		212.00	0.1396	
6		782.00	-0.0007	
7		313.50	0.3649	
8		259.50	0.1325	
9		231.50	0.0920	

Figure.4 UV-visible analysis result for isolated compound S_1 of *Barytelphusa Cunicularis.*

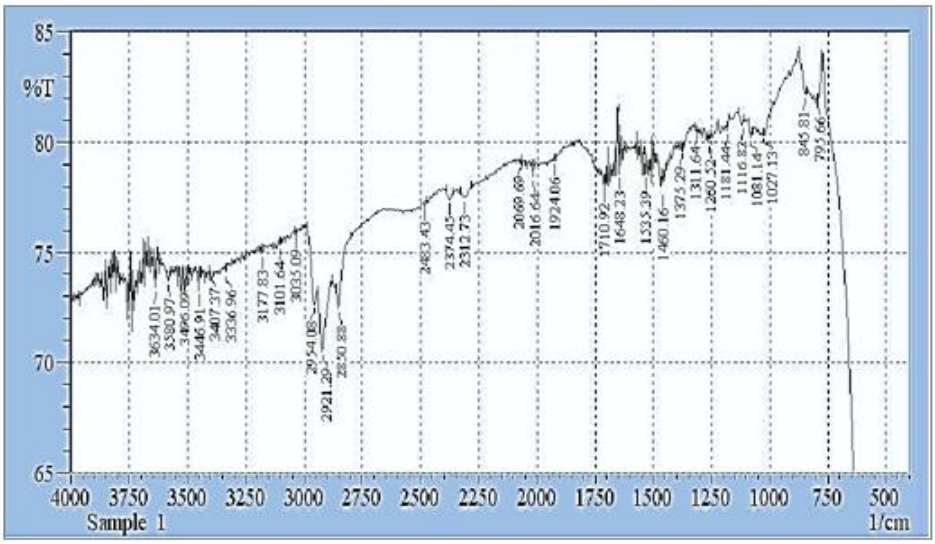


Figure.5 FTIR analysis results for isolated compound S_1 of *Barytelphusa Cunicularis* tissue.

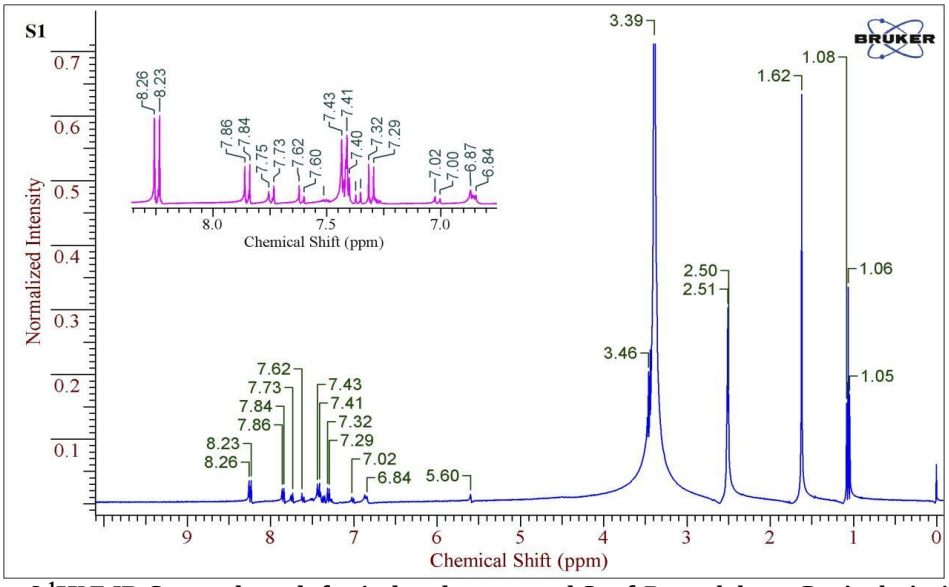


Figure.6 ^{1}HNMR Spectral result for isolated compound S_1 of *Barytelphusa Cunicularis* tissue.

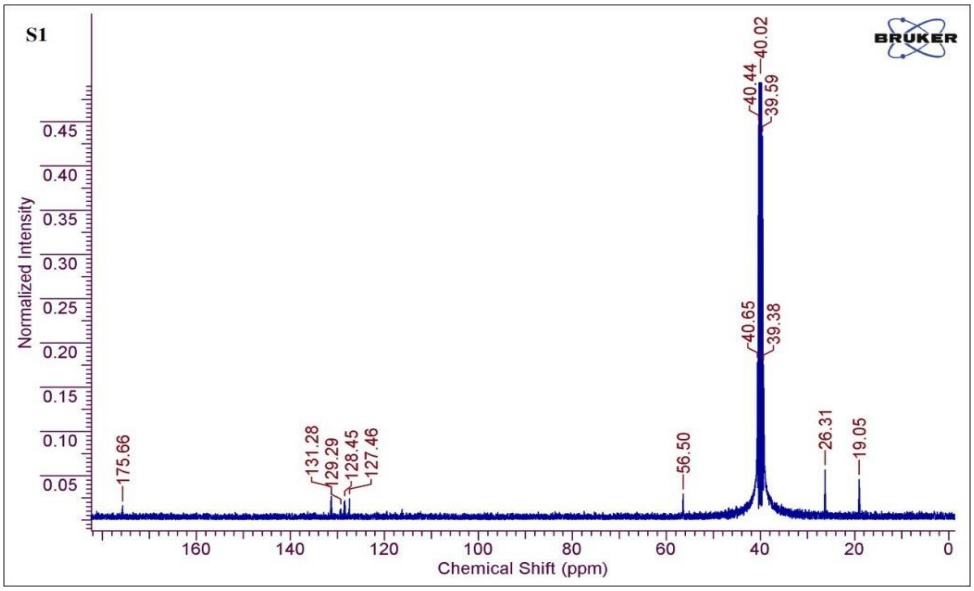


Figure. 7 ^{13}CNMR spectral result for isolated compound S_1 of *Barytelphusa Cunicularis tissue.*

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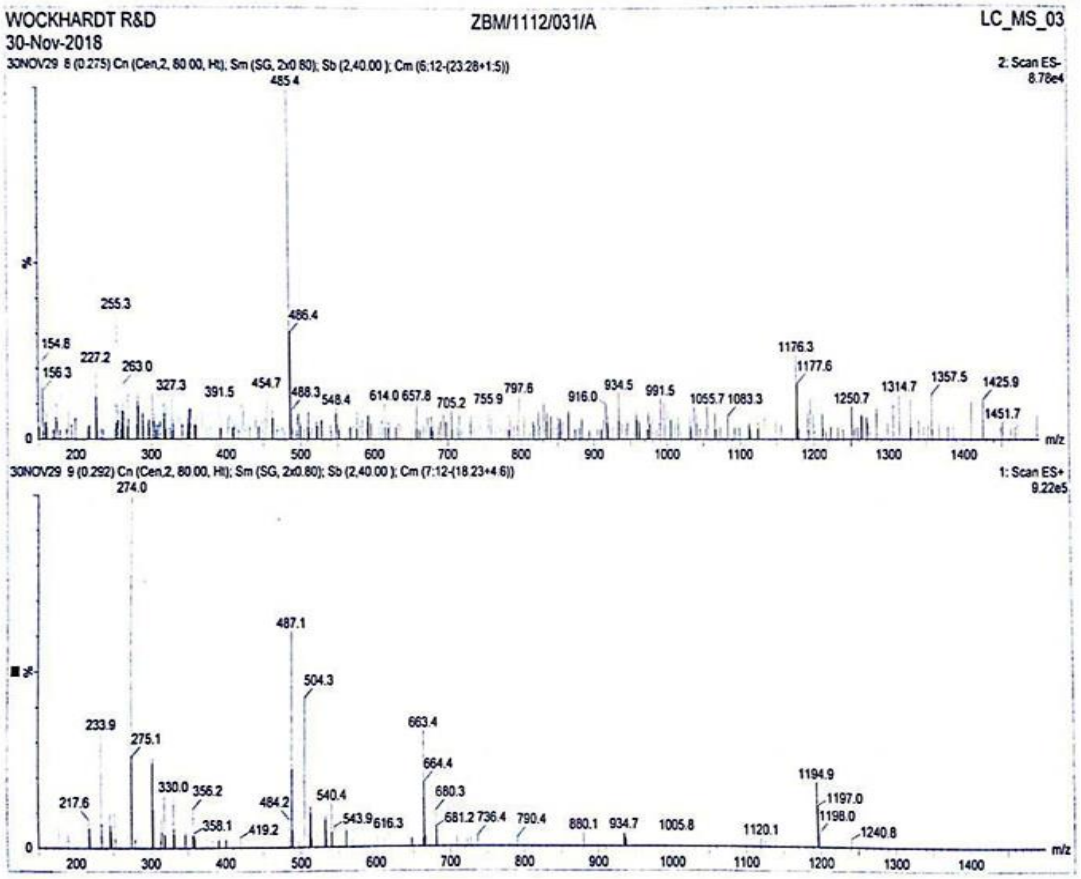
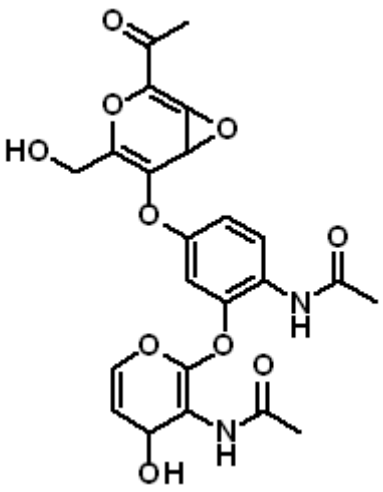


Figure. 8 LC-MS spectral data for isolated compound S ₁of *Barytelphusa Cunicularis tissue.*

V. STRUCTURE OF ISOLATED COMPOUNDS



VI. CONCLUSION

The above discussion gives a detailed literature survey on the bioactive compounds from marine biodiversity, which has been a major contribution in drug development. It also explains that the active ingredients are present in bioactive compounds, its application in medicinal chemistry and new drugs were added every year and most drugs being discovered form secondary metabolites. The isolation of bioactive compounds is possible due to the HTS-advanced technique, which has been used in the pharmaceutical field. The study was continuing due to the relationship between combinatorial chemistry and natural product chemistry, which highlights the importance of the work. Furthermore, throughout the survey of the literature, many examples have shown by a systematic study on metabolic fingerprinting analysis and Bioinformatics tools have been a strong backbone of drug development from secondary metabolites and millions of drugs has been incorporated in the world. Now the aim of this thesis is attended to, the freshwater ecosystems have been more unpredictable to researcher bioactivity research and this study will help in the future the great debates conferences will be arranged by researchers in this remote research area. Although it has been presented widely, still many challenges are to be answered and it is natural product isolation gave the unpredictable results, so present review shows the semisynthetic pathway is important for drug development, but without isolation of natural product synthesis or semi synthesis is impossible. Therefore, extraction and isolation are important in drug design and emitting the novel compounds which can be used for the clinical trials for feature drug. The results of bioactivity and molecular docking give the confirmation that, the crude ethanol extract from tissue of *Barytelphusa cunicularis* showed antibacterial and antifungal activities while molecular docking study of the isolated compound shows different binding interaction with the protein DNA gyrase. The present study, all the spectral data revelled the bioactive compound N-(2-((3-acetamido-4-hydroxy-4H-pyran-2-yl) oxy)-4-((2-acetyl-4-(hydroxymethyl)-3,7 dien-5-yl) oxy) phenyl) acetamide is present in tissue of fresh water

crab *Barytelphusa cunicularis* form northern region of Maharashtra, India and it is also confirming this bioactive compound is feature drug against the therapeutic diseases.

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REFERENCES

[1]. Newman D.J., Gragg G.M., Natural products and sources of new drug from 1981 to 2014., Journal of Natural Products 79(3):629-661, 2016.
[2]. Blunt J.W., Copp B.R., Keyzers R.A., Munro M.H.G., Prinsep M.R., Marine natural products, Natural Product Reports,33,382-431, 2016.
[3]. Laith A. A., Ambak M., Abol M. A. B., Nurhafizah W. W. I., Metabolomic analysis of marine and mud crab based on antimicrobial activity, Aquac.Rep.7:7-15, 2017.
[4]. Mohammd M. M., Vishal L. Vaidya P., Characterization of oil extract from freshwater edible crab (Barytelphusa cunicularis), International Journal of Fauna and biological studies, 4(2):88-90, 2017.
[5]. Balkhand J. V., Ratnakar P. U., Extraction and FTIR analysis of chitosan from freshwater crab Barytelphusa cunicularis and freshwater Prown Macrobarchium Rosenberg gii, Journal of Advanced Scientific Research, 10(4):870-374, 2019.
[6]. Parmesan C., Ecological and evolutionary responses to recent climate change. Annu. Rev.Ecol.Evol.Syst. 37:637–669, 2006.
[7]. Seidel, V., Initial and bulk extraction. In: Sarker, S. D., Latif, Z. & Gray, A. I. (eds.) Natural Products Isolation. New Jersey: Humana Press., 2006
[8]. Association of Analytical Chemist (AOAC), Official method of analysis, Agricultural chemicals, contaminants, drug, 15(1):69-88, 1990.
[9]. Cheong A. M., Tan C. P., Nyam K. L., Stability of bioactive compounds and antioxidant activities of Kenaf Seed oil-in-water nanoemulsions under different storage temperatures. Journal of Food Science, 2018. doi:10.1111/1750-3841.14332
[10]. Malviya Nilesh, Malviya, Sapna, Bioassay guided fractionation-an emerging technique influence the isolation, identification and characterization of lead phytomolecules, International Journal of Hospital Pharmacy, 2017, DOI: 10.28933/ijhp-2017-07-0901
[11]. Harborne J.B., A Guide to Modern Techniques of Plant Analysis. London. Published by Chapman and Hall, 6-7, 1998.
[12]. Zhang W.Q., Lin G.L. and Ye C.W., Techniques for extraction and isolation of natural products: a comprehensive review. Chinese Medicine, 13(20):1-26, 2018. https: //doi.org/10.1186/s13020-018-0177-x.
[13]. Mohmmad G. R., Extraction, Isolation and Characterization of Natural Products from Medicinal plants, International Journal of Basic Science and Applied Computing (IJBSAC), 2(6), 2018.
[14]. Njila N.I.M, Mahdi E., Lembe M.D., Nde Z. and Nyonseu D., Review on extraction and isolation of plant secondary metabolites, 7th Int'l Conference on Agricultural, Chemical, Biological and Environmental Sciences, 67-72, 2018, https://doi.org/10.15242/IIE.C051720.
[15]. Zote, Pasari A.K., Zaithanpuia, Nayak C., Kumar N., Allah E.A., Hashem A., Al Qarawi A.A., Malik J.A., Singh B.P., Polygenic affiliation and determination of bioactive compounds of bacterial population associated with organs of mud crab, Scylla olivacea,Saudi Journal of Biological Science, 25(8):1743-1754, 2018. doi: https://doi.org/10.1016/j.sjbs.2018.08.025
[16]. Balkhand J. V., Ratnakar P. U., Extraction and FTIR analysis of chitosan from freshwater crab Barytelphusa cunicularis and freshwater Prown Macrobarchium Rosenberg gii, Journal of Advanced Scientific Research, 10(4):870-374, 2019.
[17]. Hamdi, M., Nasri, R., Dridi, N., Li, S., & Nasri, M., Development of novel high-selective extraction approach of carotenoproteins from blue crab (Portunus segnis) shells, contribution to the qualitative analysis of bioactive compounds by HR-ESI-MS. Food chemistry, 302, 125334., 2020.
[18]. Narayanasamy, A., Balde, A., Raghavender, P., Shashanth, D., Abraham, J., Joshi, I., & Nazeer, R. A., Isolation of marine crab (Charybdis natator) leg muscle peptide and its anti-inflammatory effects on macrophage cells. Biocatalysis and Agricultural Biotechnology, 25, 101577., 2020
[19]. Narayanasamy, A., Balde, A., Raghavender, P., Shashanth, D., Abraham, J., Joshi, I., & Nazeer, R. A., Isolation of marine crab (Charybdis natator) leg muscle peptide and its anti-inflammatory effects on macrophage cells. Biocatalysis and Agricultural Biotechnology, 25, 101577., 2020.
[20]. Nanda, P. K., Das, A. K., Dandapat, P., Dhar, P., Bandyopadhyay, S., Dib, A. L., & Gagaoua, M., Nutritional aspects, flavour profile and health benefits of crab meat based novel food products and valorisation of processing waste to wealth: A review. Trends in Food Science & Technology., 2021
[21]. Karnila, R., & Ramadhani, N. R., Antioxidant Activity of Astaxanthin Flour Extract of Mud Crab (Scylla Serrata) with Different Acetone Concentrations. In IOP Conference Series: Earth and Environmental Science (Vol. 695, No. 1, p. 012047)., 2021 IOP Publishing.
[22]. de Silva, M. K. S., & Senaarachchi, W. A. R. K., Efficiency of biotransformation of shellfish waste to carotenoprotein by autolysis and crab-shrimp endo-enzymes. Journal of Aquatic Food Product Technology, 1-9., 2021.

[23]. Hajji, S., Kchaou, H., Bkhairia, I., Salem, R. B. S. B., Boufi, S., Debeaufort, F., & Nasri, M., Conception of active food packaging films based on crab chitosan and gelatin enriched with crustacean protein hydrolysates with improved functional and biological properties. Food Hydrocolloids, 116, 106639., 2021.
[24]. Maalej, H., Maalej, A., Affes, S., Hmidet, N., & Nasri, M., A Novel Digestive α-Amylase from Blue Crab (Portunus segnis) Viscera: Purification, Biochemical Characterization and Application for the Improvement of Antioxidant Potential of Oat Flour. International Journal of Molecular Sciences, 22(3), 1070., 2021
[25]. Ó Fearghail, F., Behan, P., Engström, N., & Scheers, N., A LCMS Metabolomic Workflow to Investigate Metabolic Patterns in Human Intestinal Cells Exposed to Hydrolyzed Crab Waste Materials. Frontiers in Bioengineering and Biotechnology, 9, 101., 2021