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DNA BARCODING, DETERMINATION OF BIOACTIVE COMPOUNDS, ANTIOXIDANT AND ANTI-DIABETIC PROPERTY IN EDIBLE GASTROPOD *BROTIA COSTULA* (RAFINESQUE, 1833) OF DIMAPUR DISTRICT, NAGALAND

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Keywords:

α-amylase, Anti-diabetic activity, Antioxidant activity, DNA barcoding, DPPH, GC-MS, Molluscs

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ABSTRACT: The present study evaluated the bioactive components, antioxidant and anti-diabetic potential in the methanolic extract of edible *Brotia costula*. The identification of the sample was confirmed with the help of the DNA barcoding technique. The COI gene sequence was submitted in NCBI and obtained the accession number OM056887. Bioactive compounds present in the sample were evaluated and identified with the help of the Gas chromatography-mass spectroscopy (GC-MS) technique. The methanolic extract was tested for antioxidant capacity by its scavenging activity against Diphenylpicrylhydrazyl **(**DPPH), and its anti-diabetic activity was measured by the methanolic extract Alpha- amylase inhibition method. The GC-MS study revealed the presence of 76 bioactive components, and the compound with the highest peak area is phenol, 2, 4-bis (1, 1-dimethylethyl)-, phosphite. An inhibition percentage of 69.89 % in 500 µg against DPPH of the sample was observed as compared to 85.77% in ascorbic acid. The sample showed a good anti-diabetic potential with an inhibition percentage of 87.80 % in 500 µg of the sample. The findings of this study indicate that the methanolic extract of *Brotia costula* contains bioactive components and shows efficient antioxidant and anti-diabetic activity, which could be further utilized for various pharmaceutical purposes.

INTRODUCTION: In India, the majority of the population of tribal and coastal communities is known to rely on edible gastropods as food and in traditional medicinal practices [1]. Nagaland, a state in India, is known for its rich biodiversity, being home to a wide variety of plants and animals, molluscs being one of them. The freshwater edible gastropod *Brotia costula* is a member of the family Pachychilidae and is distributed in most parts of India, including Uttar Pradesh, Andhra Pradesh, West Bengal, and Northeast India.

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It is extensively used as food and is also believed to cure several ailments such as gastritis, arthritis, hypertension, and post-operative care, which is still in practice today [2]. Molluscs are usually benthic in nature and tend to have limited mobility in their habitat. This exposes them to extreme water conditions such as pollution and also attacks by pathogenic microbes.

According to current research, molluscs contain an incredible arsenal of inbuilt bioactive components secreted through various biochemical pathways and glandular secretions that act as a highly effective chemical defense system, enabling the molluscs to adapt and protect themselves since they are devoid of any physical defense system [3]. The bioactive components are known to possess antitumor, antimicrobial, antioxidant, anti-cytotoxicity, and anti-inflammatory properties [4].

This has attracted many researchers to tap into these bioactive components of molluscs. Many novel classes of bioactive compounds isolated from molluscs have proven to be pharmacologically significant [5].

In the present day, with the introduction of various artificial cosmetics, processed food, pollution, and radiation, the body is exposed to a lot of harmful toxic substances which produce unfavorable effects on the body. It affects the biochemical pathways and causes several redox reactions of various compounds, which eventually increase the free radicals within the body [6]. The free radicals possess free electrons in the outermost orbit and are highly reactive. The free radicals thus produced are known to cause notable damage to the body, such as oxidative stress, cancer, cardiovascular diseases, arthritis, and DNA damage. In order to reduce the impact of the radicals in the body, many synthetic antioxidants are used. However, the unreliability of synthetic antioxidants regarding human health drives the quest for natural substrates with possible antioxidant action as a replacement for synthetic molecules [7].

According to the International Diabetes Federation, diabetes affects around 382 million people worldwide, which is anticipated to double by 2030. Many aquatic organisms have been screened to evaluate anti-diabetic properties [8]. It is of utmost importance as there are a limited number of anti-diabetic drugs compared to the increasing number of diabetic patients. In a number of research and reviews, bioactive components such as phenolics have been shown to have potential therapeutic effects in treating diabetes and obesity problems [9].

In the present age, edible molluscs are of great interest and are widely investigated to obtain dietary supplements used in pharmaceuticals and cosmetics [10]. Though snail meat is highly favored in Nagaland as it is believed to cure many ailments, less work has been carried out in the state to study the implications of snail meat on human health. Thus, the present study investigates bioactive components using GC-MS analysis. The antioxidant activity was determined by examining the sample extract's ability to scavenge diphenylpicrylhydrazyl (DPPH) radical. The synthetic drugs available in the market have a strong inhibitory action against alpha-amylase. Thus the study also aims at testing the samples for anti-diabetic potential using alpha-amylase activity.

MATERIALS AND METHODS:

Study Area: The current field survey was conducted in the Dimapur district of Nagaland, along the Dhansiri River and its tributaries, from October 2021 to May 2022. It lies between the coordinates 26˚39'59.99" N latitude and 93˚44'59.99" E longitude; at an average altitude of 145m above sea level. The river Dhansiri originates from the Laisang peak in Nagaland and joins the Brahmaputra. It is slow to the moderately fast-flowing river. The present study area and collection site are sandy, muddy, and pebbled substratum.

Identification:

Morphological Identification: The collected samples were cleaned thoroughly, and measurement was taken using Dial Caliper, Mitutoyo 505-633-50 (Japan). The specimens were identified according to Köhler *et al*., (2006) [11] and Ramakrishna and Dey (2007) [12]. The identification of the collected samples was further confirmed by Scientist-E, NERC, ZSI, Shillong.

Molecular Identification: Snails were dissected to remove the foot muscle, cut into small pieces of $2mm^3$ and preserved in 100% ethanol until further use. The Genomic DNA was isolated from the tissues using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions [13]. The sequencing was carried out in 5' → 3' direction in a small fragment of COX1 gene. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems), and Sequence alignment editing of the obtained sequences were carried out using Geneious Pro v5.1 [14].

Phylogenetic Tree Analysis: Phylogenetic tree was drawn based on neighbour tree-joining method and fast minimum evolution in Geneious v.9.0.2 [15]. BLAST analysis tool of NCBI was used to determine the MSA sequence, taxonomic classification, database indexing and FASTA format of the COI gene was submitted in NCBI to gain accession number [16].

Sample Preparation: The edible parts of the snails were removed by breaking the shell, washed thoroughly in distilled water, and dried at 60°C overnight using a hot air oven. The air-dried sample was then powdered using a mortar and pestle and stored in an air-tight container till further use.

Preparation of Methanol Extract: Methanolic extract was prepared by adding 2g of the powdered sample to 20 ml of Methanol and kept undisturbed for 72 hours. The sample was then filtered using Whatman No.1 filter paper and stored at 4°C until further use [17].

Gas - chromatography - Mass Spectroscopy Method: GC-MS analysis was carried out using GC-MS (QP2010 PLUS Shimadzu, Japan). The column oven temperature was 40.0°C, and the injection temperature was 270°C. A pressure of 49.5 kPa was maintained with a total flow of 14.0 mL/min and a column flow of 1.00 mL/min. The linear velocity was 36.1 cm/sec, plurge flow of 3.0 mL /min and a split ratio of 10.0. The GC program ion source temperature was 230.00°C, interface temperature 300.00°C with a solvent cut time of 3.00 min. The MS program start time was 3.00 min and ended at 40.00 min. The event time was 0.30 sec at a scan speed of 1666µl/sec. Mass spectra were recorded, and the range was m/z 30-500 amu. The total running time was 40 minutes.

Identification of Components: The National Institute of Standards and Technology's (NIST) database and WILEY 8 were used to interpret the mass spectrum of the GC-MS. The names, structures, and molecular weights of the components present were ascertained. The percentage of each compound present was calculated by comparing the individual peak area to the total area.

Antioxidant Test:

DPPH Test:

Procedure: Radical scavenging activity in the sample was measured using DPPH as described in Xiong *et al*., (1996) [18] and Blois (1958) [19].

Antidiabetic Test (α- amylase Test): The α-amylase inhibitory activity was assessed by the method described by Dong *et al*., (2012) [20] with suitable modification.

RESULTS AND DISCUSSION:

Morphological identification:

Shell morphology: The shell is medium to large, up to 12 whorls. The spire is pyramidal and very high; whorls are rounded in diameter and separated by a well-defined thin suture. The shell is solid but not very thick, often coated with the dark epidermis, and uniformly coloured, ranging from dark grey to black. The shell is sculptured with spiral ridges and regularly prominent axial ribs. The axial ribs very often support small, spiny nodules, arranged in a spiral band; however, some specimens are smooth. The aperture is wide, ovate, angled above, and produced below. Shell height is 42-63mm, shell diameter is 15-21 mm, aperture height is 14-20 mm, and width aperture is 9-11 mm. The operculum is slightly oval with 4-6 whorls and a central nucleus.

Molecular Identification: DNA was extracted from the tissue, and the sample's cytochrome oxidase I (COI) gene was successfully examined. An average length of 680 bp was amplified and sequenced. The amplified sequences were subjected to BLAST to infer the homology between the study sample and those in GenBank. This aids in the proper identification of similar sequences across genomes. The sample showed 100.00% similarity with *Brotia* sp of voucher FJ377244.1 and 99.70% with voucher FJ377243.1 **Table 1** in BLAST format. The COI gene sequence was submitted to NCBI to obtain the accession number (OM056887).

The present findings are well supported by the morphological and molecular identification done by Köhler and Glaubrecht (2004) 21 who had done extensive work on the southeast Asian freshwater gastropod Brotia by sequencing fragments of 646 bp cytochrome oxidase gene and 826 bp 16S rRNA and is supported by the work done in the revised edition of the species Brotia by Köhler and Glaubrecht (2006) 22. DNA studies backed up the species designation based on shell characteristics.

The phylogenetic tree was constructed using Neighbor-Joining method and fast minimum evolution with a maximum sequence difference of 0.75 **Fig. 1.**

TABLE 1: BLAST ANALYSIS OF THE COI GENE OF 8 GASTROPOD AND BIVALVE SPECIES SHOWING THE SIMILARITY PERCENTAGE IN GENBANK

Studied species	GenBank (BLASTN)	Total score	Query cover	Evalue	Similarity % GenBank (BLASTN)	Accession number
Brotia costula	*Brotia costula*	1219	100%	0.0	100.00%	FJ377244.1
	Brotia costula	1208	100%	0.0	99.70%	FJ377243.1

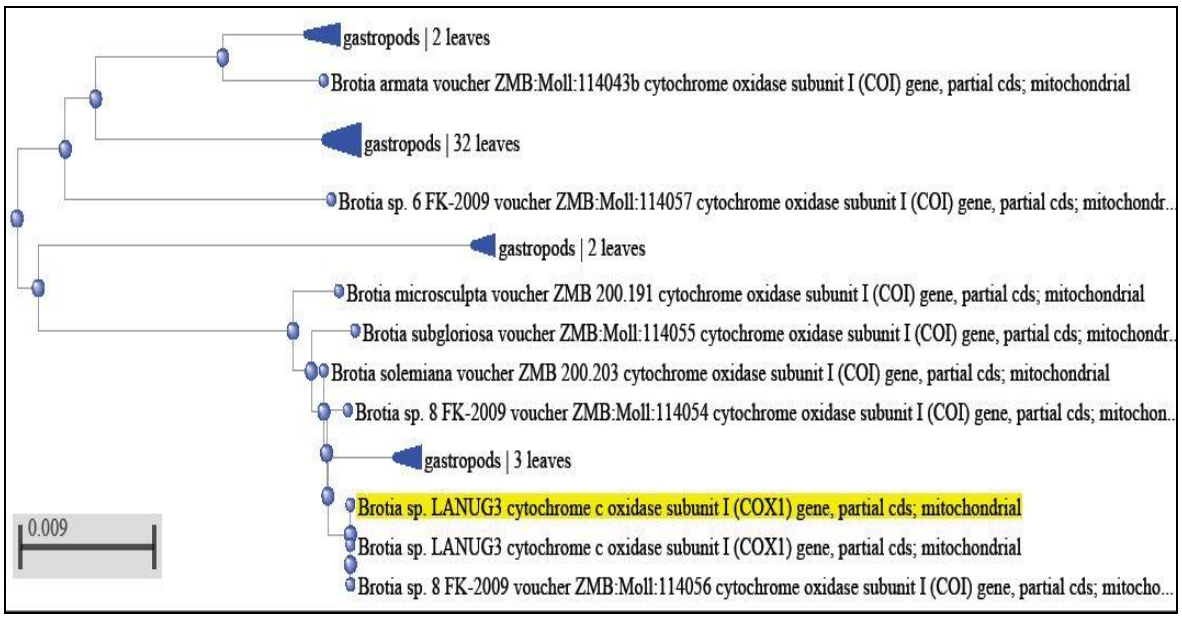

FIG. 1: MOLECULAR PHYLOGENETIC ANALYSIS USING FAST MINIMUM EVOLUTION TREE OF *BROTIA COSTULA*

Gas Chromatography-Mass Spectroscopy (GC-MS): GC-MS analysis is used to identify the presence of volatile compounds and is an excellent tool for identifying bioactive compounds. According to studies conducted from 1984 to 2019, more than 1334 bioactive components have been reported to aid Molluscan-derived therapeutics [23].

The GC-MS analysis of the present study revealed the presence of seventy-six compounds in *Brotia costula*, identified and separated by different retention times. The retention time, compound name, molecular formula, molecular weight, and peak area are presented in **Table 2**. **Fig. 2** represents the GC-MS chromatogram of *Brotia costula* showing 76 bioactive components. The most prevailing compound by the peak area obtained was identified as Phenol, 2, 4-bis (1, 1-dimethylethyl)-phosphate. The first compound identified with the least retention time (3.022 min) was hydroxyacetic acid, hydrazide, whereas the compound that took the longest retention time (39.501 min) was phenol, 2, 4 - bis (1, 1-dimethylethyl) - phosphate. The following components identified in the sample namely 1,2-benzenedicarboxylic acid; eicosanoic acid, methyl ester; 11-octadecenoic acid, methyl ester; 3-cyclopentylpropionic acid, 2-dimethylamino is in consistent with the bioactive components identified in a bivalve *Parreysia corrugate* [24]. The compound methyl 9-octadecanoate in the sample was reported in the Giant African Snail *(Archachatina maginata)* haemolymph as well [25].

The compound Octadecanoic acid is known to have anti-inflammatory and anti-arthritic properties [26]. Eicosanoic acid, 2-hydroxyethyl is known to have anti- cancer properties and also asthma preventing properties [27]. Phenol, 2, 6-bis (1,1-dimethylethyl) phosphite identified in *Brotia costula* is known to posses antioxidant and antibacterial properties [28]. The compound nonadecane is known to be a major component of essential oils [29]. Various studies have confirmed the medicinal properties, such as antitumor, antimicrobial, anti-inflammatory, and antioxidant of the bioactive components in snail meat [30, 31]. *Perna canaliculus*, a bivalve, demonstrated activity against inflammatory enzymes that can help in alleviating the symptoms related to joints, tissues and can be used for treating arthiritis while *P. viridis* showed potential activities against 5-LOX and COX-2 [32]. A study conducted

on the Giant African Land snail proves to be a therapeutic target for cancer, producing faster-acting insulin [33]. Thus, the presence of these bioactive compounds in the sample justifies the use of snail meat by traditional medicinal practitioners in treating various ailments.

TABLE 2: GC-MS ANALYSIS OF *BROTIA COSTULA*

Peak	Retention time	Compound name	Molecular formula	Molecular weight	Peak area (%)
1	3.022	hydroxyacetic acid, hydrazide	$C_2H_6N_2O_2$	90	0.02
2	4.025	o-ethylhydroxylamine	C_2H_7NO	61	0.54
3	4.218	3-dimethylamino-2,2-dimethylpropionaldehyde	$C_7H_{15}NO$	129	0.40
4	4.484	ethanamine, 2-chloro-n,n-dimethyl-	$C_4H_{10}ClN$	107	0.71
5	5.431	hexane, 1-(ethenyloxy)-	$C_8H_{16}O$	180	0.62
6	6.266	1-butanamine, n-butylidene	$C_8H_{17}N$ C	127	0.06
7	7.791	Nonanal	$C_9H_{18}O$	142	0.05
8	9.110	2-propenamide,n-ethyl-	C_5H_9NO C	99	0.02
9	9.616	1-octene	$C_8H_{15}D$	113	0.01
10	10.616	3-dimethylsilyloxytridecane	$C_{15}H_{34}O$	258	0.11
11	11.060	1,2,3-propanetriol	$C_3H_8O_3$	92	6.00
12	12.175	1-ethyl-2-pyrrolidinone	$C_6H_{11}NO$	113	0.01
13	12.682	5-ethyl-2-heptanone	$C_9H_{18}O$	142	0.02
14	12.944	cyclopropyl carbinol	C_4H_8O	72	0.22
15	13.496	5-hepten-2-one,6-methyl-	$C_8H_{14}O$	126	0.02
16	14.071	4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6	$C_6H_8O_4$	144	0.37
17	14.325	allyloxydi(tert-butyl)silane	$C_{11}H_{24}OSi$	200	0.10
18	14.839	9-heptadecanone	$C_{17}H_{34}O$	254	0.03
19	15.420	2-nitrohexane	$C_6H_{13}NO_2$	131	0.05
20	16.016	2-(tert-butylsulfanyl)-4-methyl-1	$C_9H_{13}NO_2S_2$	231	0.02
21	16.430	1-heptanol, 2-propyl	$C_{10}H_{22}O$	158	0.03
22	17.360	2-bromononane	$C_9H_{19}Br$	206	0.02
23	18.035	2-nitro-2-ethyl-1,3-propanediol	$C_5H_{11}NO_4$	149	0.09
24	18.367	3-methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihy	$C_{14}H_{16}O_2S_2$	280	0.21
25	18.737	Tridecane	$C_{13}H_{28}$	184	0.04
26	19.039	pentadecafluorooctanoic acid, 2-methylpent-3-yl	$C_{14}H_{13}F_{15}O_2$	498	0.05
27	19.658	1,5-hexadiene-3,4-diol, 3,4-dimethyl-	$C_8H_{14}O_2$	142	0.01
28	19.865	3-methyl-4-nonanone	$C_{10}H_{20}O$	156	0.00
29	20.202	1-tridecyn-4-ol	$C_{13}H_{24}O$	196	0.03
30	20.534	6-dodecanone	$C_{12}H_{18}O$	178	0.12
31	20.971	2,4-di-tert-butylphenol	$C_{14}H_{22}O$	206	0.12
32	21.226	octane, 6-ethyl-2-methyl-	$C_{11}H_{24}$	156	0.05
33	21.521	2(4h)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,	$C_{11}H_{16}O_2$	180	0.03
34	22.363	1,2-benzenedicarboxylic acid	$C_{12}H_{14}O_4$	222	0.56
35	23.167	octadecanoic acid, (2-phenyl-1,3-dio	$C_{28}H_{46}O_4$	446	0.01
36	23.896	oxirane, 2,2'-(1,4-butanediyl)bis-	$C_8H_{14}O_2$	142	0.03
37	23.896	6-dodecanone	$C_{12}H_{18}O$	178	0.06
38	24.238	tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382	0.06
39	25.105	1-dodecanol	$C_{12}H_{26}O$	186	0.06
40	25.458	3-buten-2-ol, 2-methyl-4-(1,3,3-trimethyl-7-oxa	$C_{14}H_{24}O_2$	224	0.06
41	25.740	tetradecanoic acid, 12-methyl-, me	$C_{16}H_{32}O_2$	256	0.04
42	26.058	6-dodecanone	$C_{12}H_{18}O$	178	0.04
43	26.281	2-methyl-3-pentyloxirane #	$C_8H_{16}O$	128	0.02
44	26.586	1-hexadecanol	$C_{16}H_{16}O_2$	240	0.22
45	27.179	eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	0,82
46	27.401	4-methylpentyl 3-hydroxy-2-methylenebutanoate	$C_{11}H_{20}O_3$	200	0.15
47	28.043	pentadecanoic acid, 14-methyl-, me	$C_{17}H_{34}O_2$	270	0.27
48	28.264	2,7-octadiene, 1-butoxy-	$C_{12}H_{22}O$	182	0.09
49	28.552	tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382	0.02
50	28.909	(4z)-4-decenal	$C_{10}H_{18}O$	154	0.03
51	29.332	bis(2-(dimethylamino)ethyl) ether	$C_8H_{20}N_{2O}$	160	0.05
52	29.559	11-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	0.35
53	29.826	methyl stearate	$C_{19}H_{38}O_2$	298	0.28

54	30.788	cholest-5-ene, 3-methoxy-, (3.beta.)-	$C_{28}H_{48}O$	400	14.48
55	31.579	6,9,12-octadecatrienoic acid, methyl	$C_{19}H_{32}O_2$	292	0.24
56	31.806	3-cyclopentylpropionic acid, 2-dimethylamino	$C_{12}H_{23}NO_2$	213	0.53
57	32.005	methyl 9-octadecenoate	$C_{19}H_{36}O_2$	296	0.75
58	32.549	heptacosane	$C_{27}H_{56}$	380	1.25
59	33.197	hexanoic acid, octadecyl ester	$C_{24}H_{48}O_2$	368	8.60
60	33.476	2-iso-propyl-3-amino-1-thia-3-aza-c	$C_6H_{14}N_2S$	146	1.43
61	33.846	fumaric acid, 2-dimethylaminoethyl nonyl ester	$C_{17}H_{31}NO_4$	313	1.72
62	34.251	hexanoic acid, octadecyl ester	$C_{24}H_{48}O_2$	368	3.79
63	34.832	1,1-diethoxy-2-ethylhexane	$C_{12}H_{26}O_2$	202	1.66
64	35.166	5-cholestene-3-ol, 24-methyl-	$C_{28}H_{48}O$	400	5.83
65	35.521	3-phenylpropionic acid, 2-dimethylaminoethyl	$C_{13}H_{19}NO_2$	221	0.98
66	36.052	heptacosane, 1-chloro-	$C_{27}H_{55}Cl$	414	2.85
67	36.258	tetracosane	$C_{24}H_50$	338	2.89
68	36.611	dotriacontyl isopropyl ether	$C_{35}H_{72}O$	508	1.51
69	36.970	sulfurous acid, decyl pentyl ester	$C_{15}H_{32}O_3S$	292	1.86
70	37.253	Tricosane	$C_{23}H_{48}$	324	3.51
71	37.490	cholest-5-ene, 3-ethoxy-, (3.beta.)-	$C_{29}H_{50}O$	414	2.35
72	37.837	hexatriacontane	$C_{36}H_{74}$	506	3.69
73	38.232	tetracosane	$C_{24}H_50$	338	3.67
74	38.636	cholesta-4,6-dien-3-ol, benzoate	$C_{34}H_{48}O_2$	488	2.30
75	38.857	1-iodo-2-methylnonane	$C_{10}H_{21}I$	268	1.78
76	39.501	phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite	$C_{42}H_{63}O_3P$	646	18.91

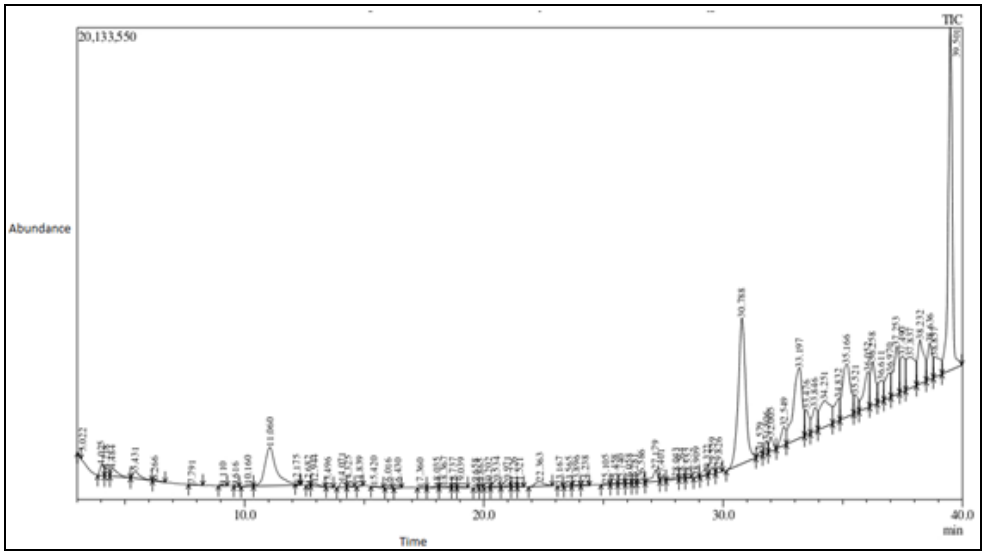

FIG. 2: GC-MS CHROMATOGRAM OF *BROTIA COSTULA*

Antioxidant Test:

Diphenylpicrylhydrazyl (DPPH) Radical Scavenging Activity: Diphenylpicrylhydrazyl **(**DPPH) is one of the few stable and commercially available organic nitrogen radicals. It can be efficaciously scavenged by antioxidants [34] and accepts a hydrogen atom to get converted to 1, 1-dipehnyl-2-picrylhydrazine. The antioxidant effect is proportional to the disappearance of DPPH in test samples and shows maximum absorbance at 517 nm **Fig. 4**. In the present study, the percentage of inhibition (scavenging activity) of the methanolic extract was tested at different concentration in triplicates and ascorbic acid was used as the standard. As depicted in **Fig. 3** the DPPH radical scavenging activity for the methanol extract increased with increase in concentration. It increased from 57.66% inhibition in 10µg/ml to 69.89% in 500µg/ml as compared to 85.77% inhibition in Ascorbic acid. Ascorbic acid is a natural antioxidant used as a food preservative and is also found in artificially synthesized vitamin C tablets [35]. A similar study on *Perna viridis*, a marine bivalve, also showed comparable results with a good antioxidant potential [36]. Similarly, the antioxidant activity of *Globularia alypum*, a traditional medicinal plant of northeast Morocco showed a high antioxidant potential [37]. The result

of the present study is comparable to that examined in *Pila virens*, with a scavenging activity of 67.09% in 250µg/ml [38]. Two marine bivalves *Meretrix meretrix* and *Meretrix casta* showed lower antioxidant scavenging potential than the present study, with antioxidant activity of 34.56% and 32.2% in 100µg/ml, respectively [39]. The methanol extract of gastropod *Pila ampullacea* showed 50.84% inhibition in 200µg/ml [40] and a similar study of antioxidant potential conducted in ale-ale shellfish showed a strong antioxidant potential as well [41]. A study conducted on the peptides of the spotted Babylon snail (*Babylonia areolata*) showed good antioxidant potential and displayed cytoxicity against human colon adenocarcinoma (Caco-2) cells [42]. According to a study by Sotiropoulou *et al.*, the extraction temperature, duration, and solvent quantity are all key elements to consider when evaluating antioxidant properties [43]. The methanolic extract of *Brotia costula* in the present study showed lower activities than the control (ascorbic acid); however it suggests that the methanolic extract of species *Brotia* has good antioxidant potential.

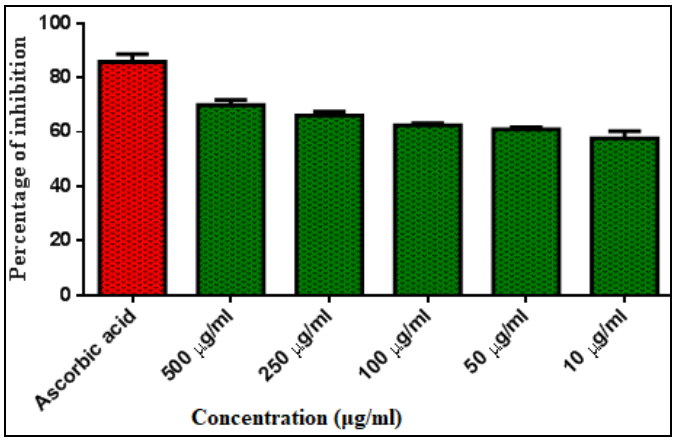

FIG. 3: DPPH RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACT OF *BROTIA COSTULA* AND ASCORBIC ACID

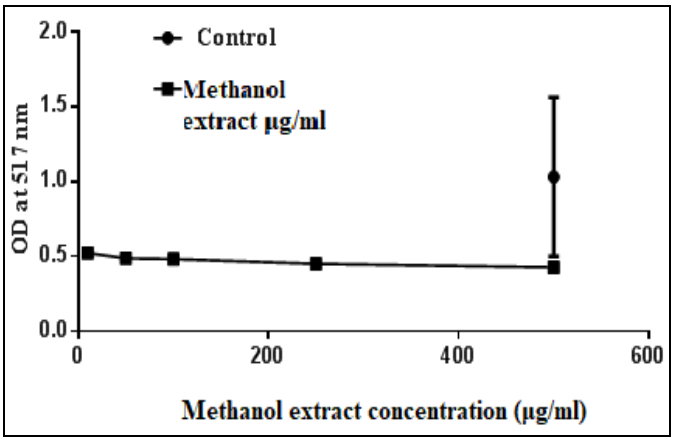

FIG. 4: DPPH RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACT OF *BROTIA COSTULA* AND ASCORBIC ACID SHOWING ABSORBANCE AT 517 NM

Antidiabetic Test:

Alpha-amylase Test: The Alpha- amylase inhibition method can measure Anti-diabetic activity. Alpha-amylase enzyme operates on 1,4-glycosidic linkages of starch and converts it into glucose. Lowering the catalytic properties of this enzyme decreases glucose synthesis in the postprandial period, which is a potential way of managing type-2 diabetes [44].

As indicated in **Fig. 5**, an increased inhibition percentage with increasing concentration was exhibited. The inhibition percentage increased from 70.293% at 10µg/ml to 87.808% at 500µg/ml; on the other hand the inhibition percentage of Acarbose is 97.278 %. Acarbose is an anti-diabetic drug used to treat diabetes mellitus type 2. The present study thus shows that the methanol extract of *Brotia costula* has good anti-diabetic potential as it shows comparable inhibition activity to that of Acarbose **Fig. 6.** Studies on two molluscs *Hemifusus pugilinus* and *Natica didyma* also shows good anti-diabetic potential [45].

This was supported by the study that was conducted on a sea slug *Aplysia* sp. that showed a high alpha-amylase inhibitory activity at 93% and *Kalinga ornata* a nudibranch showed a low inhibition activity at 49.03% [46]. The methanol extract of mangrove gastropod *Cerithidea obtuse* showed a percentage of α-glucosidase inhibition at 40.10% [47]. A study conducted on the leaf extracts of *Hellenia speciosa* exhibited maximum inhibition of 63.2% showing good anti-diabetic potential [48]. Bioactive components such as phenolics have been shown to have potential therapeutic effects in the treatment of diabetes and obesity problems in a number of research and reviews [49]. A similar study was conducted on three seaweed extracts, *Undaria pinnatifida sporophyll* (UPS), *Codium fragile* (CF), and *Gracilaria verrucosa* (GV) against mouse cells and showed good anti-diabetic potential [50]. Thus, we can consider utilizing the huge quantities of natural bioactive components found in freshwater and marine organisms that are yet to be explored and extracted.

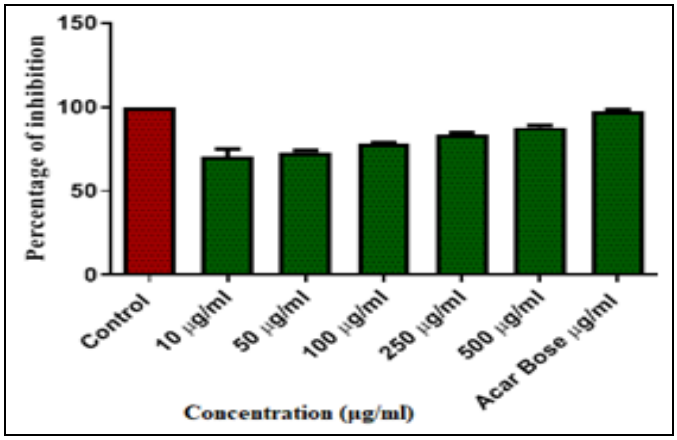

FIG. 5: ALPHA AMYLASE INHIBITION ACTIVITY OF METHANOL EXTRACT OF BROTIA COSTULA AND ACARBOSE

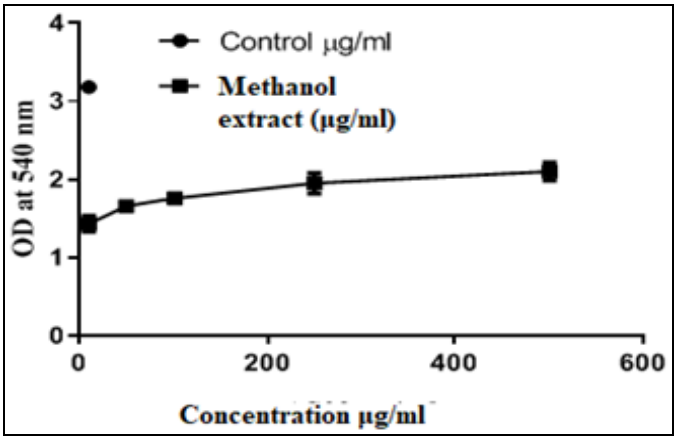

FIG. 6: ALPHA AMYLASE INHIBITION ACTIVITY OF BROTIA COSTULA AND ACARBOSE SHOWING ABSORBANCE AT 540 NM

CONCLUSION: Oxidative stress of the cells has become the leading factor known to cause damage to lipids, proteins, and DNA of the body. In normal conditions, the by-products produced through various biochemical pathways are stabilised by the body's antioxidants. However, with the advancement of technologies, consumption of highly processed foods, artificial food colorings and cosmetics, our bodies undergo a lot of oxidative stress due to the free radicals that are produced as a by-product of the biochemical pathways, leading to health disorders such as diabetes mellitus, neurodegenerative diseases, cancer, inflammatory diseases, amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD) [51].

Many synthetic antioxidants such as BHA, BHT and other microbial agents are used to reduce oxidative stress. Still, with continuous use they are known to cause cancer, liver damage, and some other health issues, such as skin allergies, gastrointestinal tract problems, and other diseases [52]. Thus, procuring natural drugs becomes very crucial. The current study indicates that the sample extract contains bioactive components possessing antioxidant and anti-diabetic properties that could be used in various therapeutic interventions, cosmetics and nutraceutical preparations and thus justifies the folkloric use of snail meat to treat and relieve various medical conditions. However, further work is needed to isolate and characterize *Brotia costula*.

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