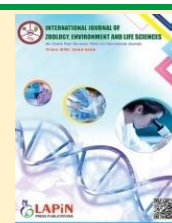




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MOLECULAR PHYLOGENY, AND INTEGRATED NUTRITIONAL, ANTIOXIDANT, ANTIDIABETIC, AND ANTICANCER ASSESSMENT OF *LITTORARIA SCABRA* FROM CORINGA MANGROVES

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ABSTRACT

The present study investigates the nutritional composition, antioxidant, antidiabetic, and anticancer properties of the estuarine gastropod *Littoraria scabra*, collected from the Coringa mangroves of Andhra Pradesh, India. Molecular identification using the mitochondrial cytochrome oxidase subunit I gene confirmed species identity and revealed a close phylogenetic relationship with other *L. scabra* isolates, forming a distinct clade within the genus. Nutritional analysis of the muscle tissue showed high moisture (76.87 ± 5.68 g/100 g FW) and protein content (17.83 ± 2.56 g/100 g FW), with moderate levels of ash, carbohydrates, lipids, and free amino acids. Methanolic extracts of *L. scabra* muscle exhibited concentration-dependent antioxidant activities, as demonstrated by increasing free radical scavenging capacities in DPPH, ABTS, FRAP, superoxide, and nitric oxide assays. The extracts also demonstrated significant in vitro antidiabetic potential, including enhanced glucose adsorption, inhibition of hemoglobin glycosylation, increased glucose uptake by yeast, and α -amylase inhibition, all with favourable EC_{50} values. Furthermore, the methanolic extracts showed dose-dependent cytotoxicity against MCF-7 and HL-60 cell lines, with IC_{50} values of 6.91 mg and 10.26 mg, respectively. These findings highlight the nutritional value of *L. scabra* and demonstrate its promising bioactive properties, supporting its potential as a source of natural antioxidants, antidiabetic agents, and anticancer compounds. This study contributes valuable insights for future research on estuarine gastropods as alternative food sources and reservoirs of therapeutic compounds.

Keywords: *Littoraria scabra*, nutritional value, antioxidant activity, antidiabetic activity, anticancer activity.

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INTRODUCTION

The extensive use of natural resources results in irreversible alterations and the exhaustion of traditional food sources [1]. Therefore, it is crucial to monitor the environment and explore the potential of using biologically active compounds as alternative food sources. Furthermore, pathogenic bacteria pollution has grown to be a major public health concern on a global scale, affecting several industries, including agriculture, food production, medicine, and the environment, and resulting in severe human illnesses and significant financial losses [2]. Approximately 80% of pathogenic infections are attributed to biofilms, which are difficult to eliminate and significantly enhance the resistance of pathogenic and spoilage bacteria to conventional antibiotics and disinfectants, rendering them 10 to 1000 times more resistant to these interventions [3]. The rapid development and spread of

antimicrobial resistance (AMR) represent a critical global health emergency, undermining the effectiveness of infectious disease treatment and increasing the risk of outbreaks, prolonged illness, and mortality [4]. Conventional antibiotics are losing efficacy due to overuse and misuse, leading to multidrug-resistant bacterial strains and other ailments [5] Moreover, oxidative stress, arising from an imbalance between the generation of reactive oxygen species (ROS) and the organism's capacity to neutralize these reactive intermediates, is associated with the development of various chronic and degenerative diseases, such as cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders [6]. Reactive oxygen species, including superoxide anions, hydroxyl radicals, and hydrogen peroxide, can impair cellular components such as lipids, proteins, and DNA, resulting in cellular malfunction and apoptosis [7]. The increasing threat of

antimicrobial resistance and oxidative stress has intensified the search for new therapeutic agents from natural sources, especially estuarine habitats, which are a promising reservoir of bioactive compounds with antimicrobial, antidiabetic and anticancer properties.

Estuarine ecosystems, defined by the junction of freshwater and seawater, are dynamic habitats that sustain a diverse array of creatures, including gastropods. Gastropods in these environments encounter diverse environmental stressors, including salinity variations, pollution, and microbial threats, prompting the evolution of distinctive physiological and biochemical responses [8]. The ability of marine gastropod extracts to provide synergistic effects—either through the interaction of several bioactive components within the extract or by strengthening the effectiveness of conventional medications when used in combination therapy [9]. Gastropods are a very diversified and ecologically important group of invertebrates residing in mangrove estuarine ecosystems. Precise identification of these species is crucial for comprehending their ecological functions, evaluating biodiversity, and directing conservation initiatives [10]. The conventional morphological identification of gastropods in these environments is frequently impeded by phenotypic plasticity, cryptic species, and convergent evolution, resulting in potential misidentification or underestimation of biodiversity [11]. Molecular identification enhances species delimitation accuracy, aids in uncovering cryptic variety, and supports ecological and conservation research in intricate ecosystems like mangrove estuaries [12]. Recently, molecular techniques—especially DNA barcoding utilizing mitochondrial genes such as cytochrome c oxidase subunit I (COI)—have become effective instruments to augment and refine taxonomic resolution [13]. Therefore, the present study focused on the evaluation of nutritional, antimicrobial, antidiabetic and anticancer properties from the estuarine gastropod *Littoraria scabra*.

Littoraria scabra, or the rough periwinkle, is a small marine gastropod in the Littorinidae family, found along rocky shores in temperate and subtropical regions within the intertidal zone [14]. It exhibits a broad distribution across temperate and subtropical coastal regions, particularly favoring rocky intertidal zones. This species is commonly found along the eastern Atlantic coasts, including the shores of Western Europe, the British Isles, and extending southward to the coasts of North and West Africa [14, 15]. *L. scabra* is also present in parts of the Mediterranean and is noted for its ability to withstand variable environmental conditions, such as fluctuating salinity and temperature, which facilitates its wide geographic range [16]. In India, this species has been recorded along both the eastern and western coastal regions, where it inhabits rocky intertidal zones exposed to tidal fluctuations and wave action [17, 18]. Specifically, along the coast of Andhra Pradesh, *L. scabra* is commonly found on rocky shores and breakwaters, particularly in areas such as

Visakhapatnam and Kakinada. The species' presence in these locations is indicative of its adaptability to varying salinity, substrate types, and tidal exposures characteristic of the region [19]. This species features a robust shell with spiral ridges that provide protection against predators and environmental stresses [15]. It primarily grazes on microalgae and biofilms on rocks, fulfilling an important ecological role in its habitat. *L. scabra* is well-adapted to endure desiccation, temperature changes, and wave action, which supports its survival in challenging coastal environments [20].

MATERIALS AND METHODS

Sample Collection

Healthy *L. scabra* samples were collected from the estuarine areas of the Coringa mangroves, which are situated in northeastern Andhra Pradesh, bordering to the Bay of Bengal, Kakinada, India (Long: 18°33' 52" to 18°32' 11"; N; Lat: 84°21' 26" E to 84°18' 22" E). After being collected, the samples were aseptically transferred into zip pouches and brought to the lab. The samples were cleaned with tap water and then with distilled water. Following that, the samples were carefully dissected, and the tissue was separated. The isolated tissue was preserved at -20°C for subsequent studies.

Molecular Identification

The collected *L. scabra* samples were characterised molecularly to identify the species by using mitochondrial cytochrome oxidase gene sequence (mtCOI). The molecular identification was carried out by the isolation of genomic DNA, Amplification of mtCOI gene by PCR, Sequencing of amplified mtCOI gene, multiple sequence alignment and phylogenetic tree construction. The genomic DNA was carried out by the phenol-chloroform-isoamyl alcohol method as described by the methodology of Sambrook and Russell [21]. The quality of extracted DNA was evaluated by agarose gel electrophoresis and then visualised by using UV-transilluminator and photographed by gel imaging system.

The amplification of the mtCOI gene was performed using two primers such as Cyt oxidase Forward (5' TCAACCAACCACAAAGACATTGGCAC-3') and Cyt oxidase Reverse (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'). The following procedure was used for mtCOX1 gene amplification in a thermocycler: an initial denaturation step was performed at 95°C for 5 min, and then PCR cycles were adjusted to 40. In each cycle, denaturation was conducted at 95°C for 30 sec, annealing was conducted at 60°C for 15 sec, and extension was conducted at 72°C for 30 sec. The final elongation step was performed for 10 minutes at 72°C, followed by incubation at 12°C. The amplified PCR products' quality was evaluated using electrophoresis on a 1.5% agarose gel that included 0.5 µg/mL ethidium bromide, and the gel images were captured. The PCR products were then sequenced using Sanger's di-deoxy method. The sequencing was performed in both forward and

reverse orientations using an ABI Prism 3700 DNA analyser. The large dye terminator v3.1 cycle sequencing kit from Applied Biosystems Inc., USA, was used for the sequencing.

The BiologicsCorp web server (<https://www.biologicscorp.com/tools/GCCContent/>) was used to compute the nucleotide composition of the mtCOI gene. The mtCOI gene sequence was analysed using the NCBI server's BLASTn to detect homologous species. Depending on the degree of homology with the query gene sequence, similar species were selected from the NCBI database. The homology and phylogenetic parameters of each gastropod species mtCOI gene sequence was analysed by multiple sequence alignment (MSA) using an advanced cluster approach UPGMA. A maximum-parsimony phylogenetic tree was generated using MEGA-X and the tree was subjected to 1000 replicates of the bootstrap method.

Evaluation of Nutritional Properties

The moisture and ash content in the tissue of *L. scabra* was estimated by the AOAC [22] methodology. Hodge and Hofreiter [23], Miller [24], and Varkonyi et al. [25] methodologies were used for the determination of nonreducing, reducing sugars, and glycogen content. The total protein content from the muscle tissue was extracted by using 0.1 M phosphate buffer (pH-7.4) and the total protein concentration in each test sample was determined using the Lowry et al. [26] methodology. The methodology of Bligh and Dyer [27] was employed to extract total lipids and the estimation of lipid concentration done by Knight et al. [28] method using phosphovanillin reagent. The free amino acids content was determined by the methodology of Moore and Stein [29].

Evaluation of Antioxidant Properties

The antioxidant potential of *L. scabra* was evaluated at five different concentrations of methanolic extracts such as 10, 7.5, 5.0, 2.5, and 1 mg/mL. The DPPH scavenging efficacy of *L. scabra* methanolic extract was assessed by Mensoret al. [30] method. The ABTS radical scavenging activity was assessed according to Shirwaikaret al. [31] methodology. The ferric ion reducing ability of methanolic extracts was determined by Benzie and Strain [32] method. The superoxide scavenging activity of methanolic extracts was assessed using NBT, as described by Winterbourn et al. [33]. The nitric oxide scavenging activity was determined by the Balakrishnan et al. [34] method using sodium nitroprusside.

Screening of In vitro Antidiabetic Potential

Glucose adsorption capacity of methanolic extracts from *L. scabra* was determined by the methodology of Ou et al. [35]. The effect of *L. scabra* methanolic extracts on haemoglobin glycosylation was determined by Adisa et al. [36] methodology. The effect of extract on in-vitro glucose diffusion was determined by Ahmed et al. [37] methodology. The effect of extract on glucose uptake in yeast cells was done by using the methodology of Pitchaipillai and Ponniah [38]. The α -

amylase inhibitory assay for the methanolic extract was evaluated by the Malik and Singh [39] method.

Determination of In vitro Cytotoxic Activity

The in vitro anticancer activity for the *L. scabra* crude methanolic extracts was evaluated by the MTT assay using various human cancer cell lines, including breast cancer (MCF-7) cell line, and human promyelocytic leukemia (HL-60) cell line. The selected cancer cell lines were cultured in various mediums according to the type of cell line. The MCF-7 cell lines were cultured in DMEM medium, HL-60 cell lines were cultured in RPMI 1640 medium. The cultures of all the selected cell lines were recovered by discarding the culture medium and the cultures were subjected to trypsinization to prepare cell suspension. After trypsinization, the disaggregated cells were suspended in the liquid medium. The cytotoxic activity of crude methanolic extract was performed with MTT to determine the viability of cells according to the methodology described by Sudha and Selvam [40].

Statistical Analysis

All the results were given as Mean \pm Standard Deviation (SD) obtained from three independent experiments, and the data was assessed by one way analysis of variance (ANOVA). The 'p' value between greater than 0.01 and less than 0.05 was considered as significant difference.

RESULTS AND DISCUSSION

Molecular Identification

Molecular identification facilitates ecological monitoring by acting as genetic indicators for research on population connectivity, tracking invasive species, and evaluating the effects of habitat disturbance [41]. In the present study, genomic DNA was successfully extracted from *L. scabra*, and the extracted DNA exhibited absorbance ratio of A260/A280 between 1.7 to 1.9 which indicates the purity of extracted DNA. Furthermore, the quantity of total DNA was found to be 456 ± 18 μ g/gm respectively. William et al. [42] stated that the 260/280 nm absorbance ratio was used to assess the quality of isolated DNA and RNA. The A260/A280 ratio of pure DNA falls within the range of 1.7 to 2.0, while RNA often has a ratio larger than 2.0. Figure 1 shows the PCR amplified mtCOI gene band from *L. scabra* in a 1.5% agarose gel. The amplified mtCOI genes exhibited their size in the range of 600 to 700 bp length.

The advancement of the mtCOI gene, which exhibits high variability, along with advances in statistical techniques for analysing genetic data, has allowed for a detailed comprehension of population characteristics such as dispersal and genetic structures [43]. Kartavtsev et al. [44] relied on cyt b and 16S rRNA sequences to deduce the evolutionary relationships of *Liobagrus obesus*, a species of bullhead torrent catfish. Cytochrome b is highly valuable in determining the evolutionary connections between species within genera and families due to its significant sequence diversity [45].

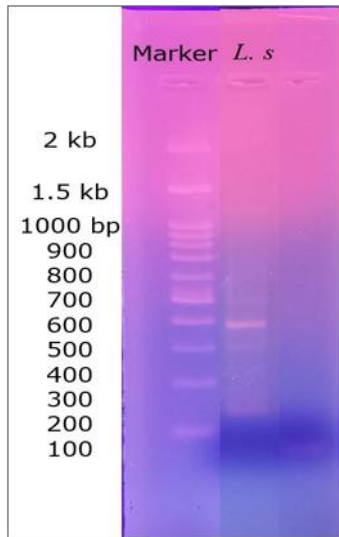


Figure 01: PCR amplified mtCOI gene product on 1.5% agarose gel.

Sequence Analysis

The PCR amplified sequences of mtCOI gene from *L. scabra* have total 654 nucleotides with 158 bp of adenine (24%), 226 bp of thymine (36%), 122 bp of guanine (18%), and 148 bp of cytosine (22%). The G+C content was calculated to be 40%. Figure 2 illustrates the GC distribution across the amplified mtCOI gene sequence of *L. scabra*. Current research indicates that the mtCOI gene from the three gastropod species demonstrates a comparatively low GC content. These findings correspond with other research, including Grande et al. [46] which indicated GC levels ranging from 27% to 34% in the mtCOI gene across different gastropod lineages. Research by Powell et al. [47] and Grande et al. [46] indicates that the mitochondrial genomes of gastropods, comparable to other mollusks, typically display a low GC content, usually between 25% and 40%.

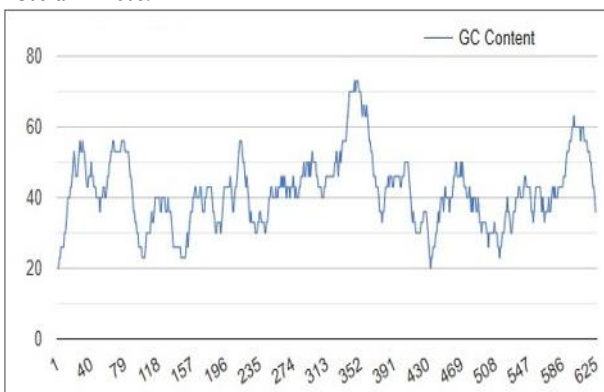


Figure 02: GC distribution over the amplified mtCOI gene sequence of *L. scabra*.

Phylogenetic Analysis

The phylogenetic tree (Figure 3) illustrates the evolutionary relationships among various isolates and haplotypes within the genus *Littoraria*. The results show that the collected *Littoraria scabra* isolate DSA.1 exhibited a close cluster with the *L. scabra* isolates

including SCA.VAT.1, and SCA.DIL.1, indicating a high degree of genetic similarity. These isolates, along with the haplotypes H22 and H19 as well as SCA.ERP.1, form a distinct clade, demonstrating their close evolutionary relationships. In contrast, *L. angulifera* isolates (LAN01, ANG.SIN.1, and ANG.CAR.1) constitute a separate branch, signifying clear species-level differentiation from *L. scabra*. Other species such as *L. pallescens* (PAL.MAK.1), *L. intermedia* (INT.KAN.1), and *L. cingulata* (CIN.KAR.1) also appear as distinct branches on the tree, highlighting their genetic divergence from both *L. scabra* and *L. angulifera*. Overall, the phylogenetic tree underscores both the close relationships among *L. scabra* isolates and the broader genetic separation between different *Littoraria* species. The current results align with the findings of Reid et al. [48], who indicated that COI-based molecular analyses of mangrove-associated gastropods have yielded substantial insights into species delimitation, population structure, and biogeographical patterns. Studies by Strong et al. [49] indicated that *Littoraria* and *Cerithidea* occupy separate clades within the superfamily Cerithioidea, a classification robustly corroborated by COI and other mitochondrial markers.

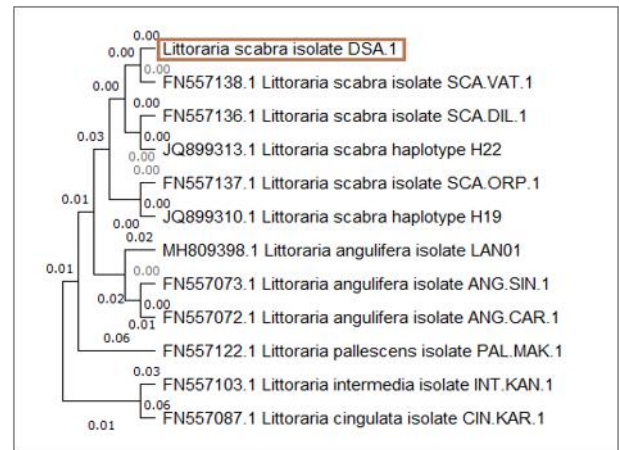


Figure 03: Maximum Parsimony tree of *L. scabra* isolate and other relative species based on the mtCOI gene.

Nutritional Properties

The nutritional analysis of *Littoraria scabra* muscle tissue, as presented in the figure 4, shows a high moisture content of 76.87±5.68 g/100 g fresh weight. The ash content, representing total mineral matter, is 0.98±0.09 g/100 g. Nonreducing sugars and reducing sugars are present at 1.18±0.07 g and 0.46±0.02 g/100 g, respectively. Glycogen content is measured at 0.62±0.04 g/100 g, indicating the presence of stored carbohydrates. The total protein content is relatively high at 17.83±2.56 g/100 g, suggesting the tissue is a good protein source. Total lipid content is 1.89±0.86 g/100 g, and free amino acids are present at 0.38±0.05 g/100 g. Overall, the muscle tissue of *L. scabra* is characterized by high moisture and protein content, with moderate levels of carbohydrates, lipids, and minerals.

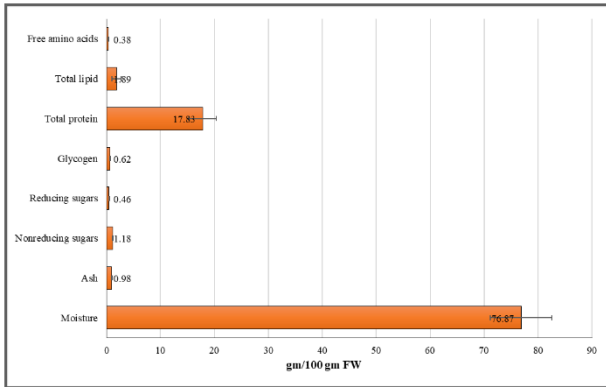


Figure 04: Different nutritional parameters of *L. scabra* tissue.

Moisture is one of the most important components in the food material which can decide the quality of food because, it affects the physical, chemical aspects of food which relates with the freshness and stability [50]. The moisture content observed in the present study correlates with prior research of Odaibo and Olayinka [51] who discovered that the moisture content of edible land snails ranged from 76% to 85%. The amount of ash in food is measured as part of its proximate composition during nutritional assessment. The present findings of ash align with prior research, like Margret et al. [52] which indicated that the ash concentration in gastropods was minimal, around 1.18% in *B. zeylonica*, followed by other gastropods.

Reducing sugars, primarily glucose, are essential for energy metabolism in gastropods and are present in the hemolymph and tissues; their concentrations fluctuate with physiological conditions such as food consumption and starvation [53]. Glycogen, the main polysaccharide that stores energy, builds up in the hepatopancreas, mantle, and muscle when there is a lot of food and is quickly released when the body needs more energy, like when larvae are growing or when they are starving [54]. Quantifying protein in estuarine snails has garnered significant interest recently due to their nutritional and ecological significance. The current findings align with the research conducted by Adeyeye et al. [55] on *Littorina littorea* and *Pachymelania aurita* from Nigerian waters indicated protein concentrations ranging from 17.0 to 19.5 g/100 g wet weight, illustrating the species' dietary preferences and their adaptability to intertidal habitats. Lipids constitute the

third most prevalent component in aquatic creatures, comprising 6% to 20% of their composition, primarily located in the subcutaneous tissue, liver, muscles, mesenteric tissue, abdominal flap, and head [56]. Tasbozan and Gokce [57] asserted that numerous variables, including diet, species, seasons, temperature, geographical features, and salinity, account for variations in lipid proportions.

The accumulation of free amino acids in the cytoplasm is crucial for regulating the organism's osmotic balance [58]. Carter et al. [59] indicated that the concentration of free amino acids is influenced by developmental stage, starvation, reproductive migration, seasonal variations, water temperature, and hardness. Ranjan and Babu [60] found free amino acid concentrations between 0.55 and 0.78 g/100 g FW in *T. telescopium* and *C. obtusa*, values that closely align with those observed in the current study. The present study indicates that the examined estuarine snails have significant nutritional reserves, with quantitative studies elucidating their nutritional worth and prospective use in the food and feed industries.

Antioxidant Properties

The results of the antioxidant potential of muscle methanolic extracts from *Littoraria scabra* are summarized in Table I. The data show that the percentage of free radical scavenging activities, measured by DPPH, ABTS, FRAP, SO, and NO assays, increases with higher extract concentrations. Specifically, DPPH scavenging activity rose from 8.12±1.67% at 1 mg/ml to 58.97±3.26% at 10 mg/ml, while ABTS activity increased from 13.78±1.63% to 69.34±3.08% over the same concentration range. Similarly, FRAP values increased from 11.89±1.07% to 57.08±2.55%. The superoxide (SO) scavenging activity ranged from 2.81±0.83% to 34.63±2.36%, and nitric oxide (NO) scavenging ranged from 21.32±2.74% to 67.41±3.26% as concentration increased. The EC₅₀ values, which indicate the concentration required to achieve 50% scavenging activity, were 8.11 mg for DPPH, 6.71 mg for ABTS, 8.75 mg for FRAP, 13.89 mg for SO, and 6.17 mg for NO. These results suggest that the methanolic extract of *L. scabra* muscle exhibits concentration-dependent antioxidant activities with significant free radical scavenging potential, particularly evident in the ABTS and NO assays.

Table 01: Percentage of different free radical scavenging activities from the *L. scabra* methanolic extract with increasing concentrations.

S. No.	Extract Conc. (mg/ml)	% of scavenging activities (Mean ± SD)				
		DPPH	ABTS	FRAP	SO	NO
1	1	8.12±1.67	13.78±1.63	11.89±1.07	2.81±0.83	21.32±2.74
2	2.5	18.28±2.16	20.39±2.21	19.86±2.43	7.32±1.36	32.68±1.86
3	5	34.61±2.59	41.33±1.98	28.62±2.73	15.47±1.55	47.82±3.04
4	7.5	47.52±2.11	56.73±2.07	44.04±3.21	23.88±2.43	56.55±2.81
5	10	58.97±3.26	69.34±3.08	57.08±2.55	34.63±2.36	67.41±3.26
6	EC ₅₀	8.11 mg	6.71 mg	8.75 mg	13.89 mg	6.17 mg

P<0.05 was considered as significant difference.

DPPH (2,4-diphenyl-picryl-hydrazyl) is a free radical that can take an electron or hydrogen radical, resulting in the formation of a stable diamagnetic molecule [61]. The antioxidant activities of *L. scabra*, align closely with the findings of Senevirathne et al. [62], which indicated approximately 60% DPPH inhibition at comparable concentrations in marine mollusc extracts. Kumaran and Karunakaran [63] documented IC₅₀ values between 7 and 9 mg/mL for methanolic extracts of diverse marine gastropods, underscoring comparable antioxidant capacities. Krishnaraju et al. [64] demonstrated that the efficacy of antioxidants is often associated with their ability to destroy stable, highly reactive free radicals. The ability of extracts to quench proton radicals is measured by using ABTS radical scavenging activity [65]. The present results align with the findings of Shantha et al. [66] who observed IC₅₀ values ranging from 2–3 mg/ml in methanolic extracts of marine gastropods, suggesting significant antioxidant potential. The FRAP assay is a widely employed method for assessing the antioxidant capacity of dietary polyphenols, evaluating the sample's ability to reduce ferric ions [67]. Rajauria et al. [68] documented 85–92% FRAP inhibition in marine mollusc extracts at comparable dosages. Priyanka et al. [69] suggested that elevated FRAP activities are associated with enhanced antioxidant capacities. Shirwaikaret al. [31] identified IC₅₀ values of 6–7 mg/ml for superoxide scavenging activity from analogous extracts. Reactive nitrogen species, including nitric oxide are free radicals that arise from the reaction of NO with other reactive oxygen species and molecular oxygen [70]. Ravikumar et al. [71] documented considerably less NO

scavenging activity, achieving a maximum inhibition of approximately 45% at 10 mg/ml for alternative marine molluscs, signifying markedly worse antioxidant capacity. Chanda and Dave [72], who documented 70–75% inhibition at comparable dosages for marine mollusc extracts.

In vitro Antidiabetic Potentials

The results for the antidiabetic potential of muscle methanolic extracts from *Littoraria scabra* reveal a concentration-dependent increase in activity across all tested parameters. As the extract concentration increased from 1 to 10 mg/ml, the percentage of glucose adsorption rose from 4.92±1.26% to 56.78±2.71%, indicating enhanced glucose binding at higher concentrations. Similarly, inhibition of hemoglobin glycosylation increased from 28.66±3.62% to 63.22±3.26%, suggesting a protective effect against protein glycation. The glucose diffusion retardation index (GDRI) also improved, ranging from 47.82±4.12% at the lowest concentration to 73.45±6.21% at the highest. Glucose uptake by yeast was elevated from 9.86±1.52% to 50.04±2.53%, while α-amylase inhibition rose from 3.11±1.21% to 33.48±2.41%. The EC₅₀ values for each assay were 7.89 mg for glucose adsorption, 6.02 mg for Hb glycosylation inhibition, 0.83 mg for GDRI, 9.86 mg for glucose uptake, and 14.73 mg for α-amylase inhibition. These findings indicate that *L. scabra* muscle methanolic extract exhibits significant in vitro antidiabetic activities, particularly in glucose adsorption, glycosylation inhibition, and enzyme inhibition assays.

Table 02:Percentage of different In vitro antidiabetic potentials from the *L. scabra* methanolic extract with increasing concentrations.

S. No.	Extract Conc. (mg/ml)	(Mean ± SD)				
		% of Glucose adsorption	% of Hb glycosylation inhibition	% GDRI	% Glucose uptake by yeast	% of α-amylase inhibition
1	1	4.92±1.26	28.66±3.62	47.82±4.12	9.86±1.52	3.11±1.21
2	2.5	10.26±2.11	37.72±3.11	56.13±4.09	14.78±2.64	7.83±2.03
3	5	23.22±2.93	48.65±2.81	63.42±3.24	25.21±2.39	14.81±1.09
4	7.5	41.68±3.23	56.27±4.07	69.78±5.23	41.02±3.16	23.71±1.89
5	10	56.78±2.71	63.22±3.26	73.45±6.21	50.04±2.53	33.48±2.41
6	EC ₅₀	7.89 mg	6.02 mg	0.83 mg	9.86 mg	14.73 mg

P<0.05 was considered as significant difference.

The current findings showed that the extract was capable of binding glucose at lower concentrations, as a consequence, the glucose binding ability of the extract may greatly decrease the amount of glucose. Sharma et al. [73] reported similar concentration-dependent inhibition. Das and Devi [74] reported that the glucose adsorption by extracts reduces the possibility of postprandial blood sugar levels. The present results are in agreement with previous studies that highlighted the potent antidiabetic and enzyme inhibitory activities of methanolic marine extracts. For instance, Patra

and Muthuraman [75] reported significant glucose diffusion inhibition in methanol extracts of marine gastropod *Babylonia spirata*, emphasizing their therapeutic potential in diabetes management. Similarly, studies of Kim et al. [76] and Zhang et al. [77] revealed that the polysaccharide rich extracts from marine gastropods such as *Turbo cornutus* and *Haliotis discus* greatly decreased the glucose diffusion which is associated with the viscosity of extracts. The glucose uptake into the yeast cells was increased with increased extract concentrations used in the study.

The present results correspond with those of Thippesh [78] who similarly noted substantial stimulation of glucose uptake in yeast cells by 38% at 40mg/ml and 60% at 100mg/ml in *M. casta* methanolic extract at 5mM displaying promising outcomes in facilitating yeast cell glucose uptake, suggesting a potential role in regulating glucose metabolism. The present findings of α -amylase inhibitory activity align with those of Sadhasivam et al. [79] who observed a similar inhibition of α -amylase activity by 70.6% and 49.03% in methanolic extracts of the nudibranch species *Bursatellaleachii* and *Kalinga ornata*, demonstrating heightened sensitivity even at low concentrations. Ravi et al. [80] similarly reported that the acetone extracts of *H. pugilinus* and *N. didyma* demonstrated α -amylase inhibitory activities of $72.23 \pm 0.44\%$ and $51.23 \pm 0.44\%$, respectively, at a concentration of 50 μ L.

Anticancer Potentials

The methanolic extracts of *Littoraria scabra* demonstrated notable anticancer properties in vitro, as indicated by their cytotoxic effects on MCF-7 (breast cancer) and HL-60 (leukemia) cell lines. The results show a clear concentration-dependent increase in cytotoxicity (Table 3). For the MCF-7 cell line, cytotoxicity increased from 25.62% at 1 mg/mL to

59.82% at 10 mg/mL extract concentration, while for the HL-60 cell line, cytotoxicity ranged from 13.92% to 47.86% across the same concentration range. The IC_{50} values, representing the concentration required to inhibit 50% of cell viability, were 6.91 mg for MCF-7 cells and 10.26 mg for HL-60 cells. These findings indicate that the methanolic extract of *L. scabra* muscle possesses significant cytotoxic activity against both cancer cell lines, with a stronger effect observed on MCF-7 cells compared to HL-60 cells. Figure 4 and 5 shows the microscopic images of MCF-7 and HL-60 cell lines under treatment of 1 mg/mL and 10 mg/mL *L. scabra* methanolic extract.

The methanolic extracts of *L. scabra* show significant, dose-dependent cytotoxicity against both MCF-7 and HL-60 cell lines. These findings are consistent with previous studies such as Alkassar et al. [81] and Kour et al. [82] who reported that marine gastropods, such as *Rapana venosa* and *Conus* species, have shown cytotoxicity against a variety of cancer cell lines, including MCF-7 and HL-60. Similarly, Righi et al. [83] reported that the extracts of *Helix aspersa* have demonstrated antiproliferative effects on HepG2 (liver cancer) and HeLa cell lines.

Table 03: In vitro cytotoxic activity of *L. scabra* methanolic extracts on cancer cell lines.

S. No	Extract. Conc. (mg/mL)	% of cytotoxicity (Mean)	
		MCF-7	HL-60
1	1	25.62	13.92
2	2.5	33.42	20.68
3	5	46.28	31.62
4	7.5	52.68	40.22
5	10	59.82	47.86
6	IC_{50}	6.91 mg	10.26 mg

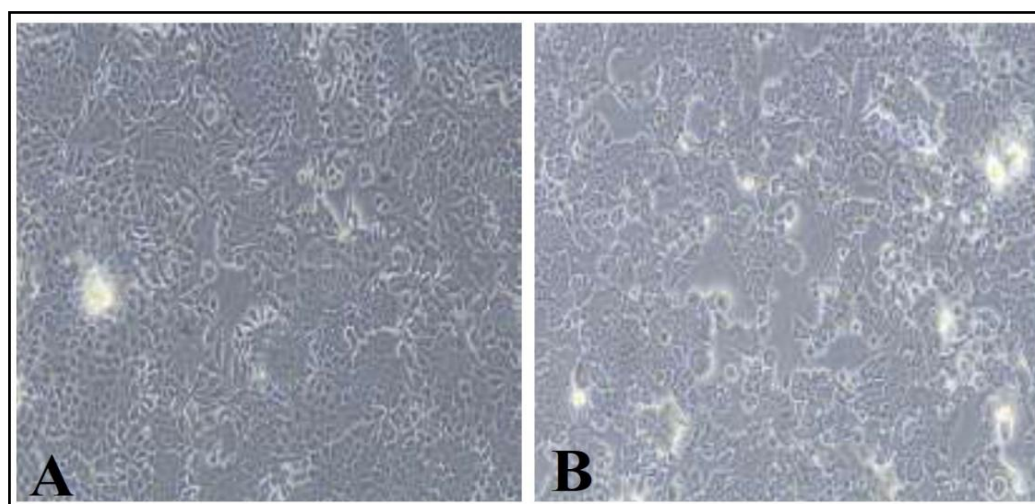


Figure 05: Microscopic images of MCF-7 cell lines under treatment of A) 1 mg/mL *L. scabra* methanolic extract B) 10mg/mL *L. scabra* methanolic extract.

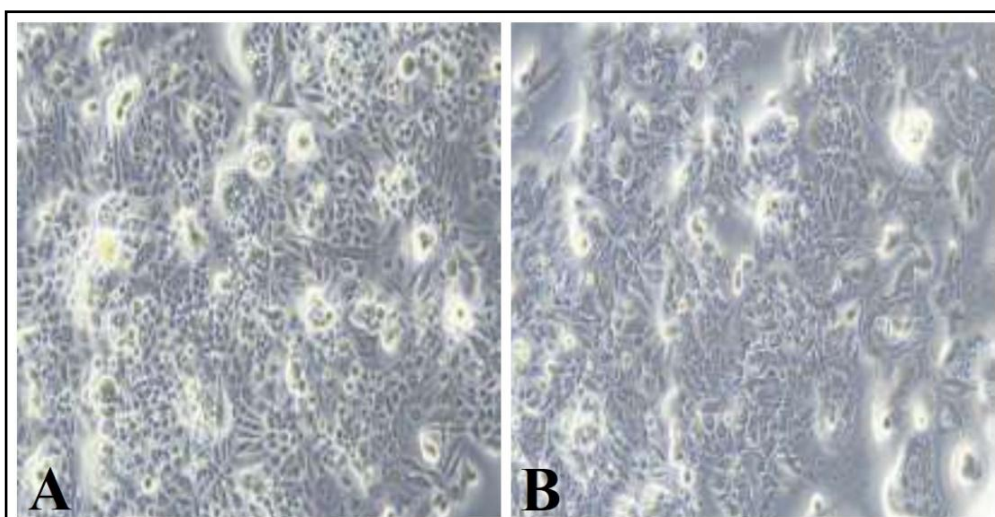


Figure 06: Microscopic images of HL-60 cell lines under treatment of A) 1 mg/mL *L. scabra* methanolic extract B) 10mg/mL *L. scabra* methanolic extract.

Conclusion

This study demonstrates that *Littoraria scabra* from the Coringa mangroves possesses valuable nutritional content and exhibits significant antioxidant, antidiabetic, and anticancer activities in vitro. Molecular analysis confirmed its taxonomic identity and phylogenetic placement among related species. The methanolic extracts showed strong free radical scavenging, glucose regulatory, and cytotoxic properties, highlighting the species' potential as a source of natural health-promoting compounds. These findings support the potential application of *L. scabra* in functional foods or pharmaceutical development and emphasize the importance of further exploring estuarine gastropods for novel bioactive substances.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Author Contributions

Suneetha Yedla: Conceptualization, sample collection, molecular analysis, laboratory investigations, data analysis, manuscript preparation, and interpretation of results.

Manjulatha Chapara: Study supervision, methodology development, data validation, critical review, manuscript editing, and final approval of the manuscript.

Both authors have read and approved the final version of the manuscript.

Ethical Statement

The specimens of *Littoraria scabra* used in this study were collected from the estuarine regions of the Coringa mangroves, Andhra Pradesh, India, following applicable institutional and environmental guidelines. The study involved molecular, nutritional, antioxidant,

antidiabetic, and in vitro anticancer investigations using gastropod samples. No human participants, vertebrate animals, or clinical specimens were involved in this research. Therefore, approval from an Institutional Ethics Committee was not required.

Informed Consent Statement

Not applicable. This study did not involve human participants or human biological materials.

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