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EXPLORING THE NUTRITIONAL COMPOSITION AND MULTIFUNCTIONAL HEALTH BENEFITS OF *LITTORARIA PALLESCENS* FROM ESTUARINE MANGROVES

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ABSTRACT

The increasing global prevalence of chronic diseases such as cancer and diabetes, often linked to oxidative stress, underscores the need for novel, safe, and effective therapeutic agents from natural sources. This study evaluated the nutritional content, antioxidant, antidiabetic, and anticancer properties of the estuarine gastropod *Littorariapallescens*. Specimens were collected from the mangrove regions of the Coringa Wildlife Sanctuary, India, and identified through molecular analysis of the mtCO1 gene, which revealed high genetic similarity with other *L. pallescens* isolates. Nutritional analysis of muscle tissue indicated high moisture (78.29 g/100 g) and protein (15.62 g/100 g) content, with moderate levels of carbohydrates, lipids, and minerals. Methanolic extracts of *L. pallescens* exhibited significant, concentration-dependent antioxidant activity, as evidenced by DPPH, ABTS, FRAP, superoxide, and nitric oxide scavenging assays, with the highest efficacy observed in nitric oxide inhibition (EC_{50}: 3.03 mg/ml). The extract also demonstrated notable antidiabetic potentials: glucose adsorption, hemoglobin glycosylation inhibition, glucose diffusion retardation, glucose uptake by yeast, and α-amylase inhibition all increased with extract concentration. EC_{50} values ranged from 5.56 to 10.46 mg across these assays. Furthermore, the extract showed dose-dependent cytotoxicity against MCF-7 and HL-60 cell lines, with IC_{50} values of 11.86 mg and 8.67 mg, respectively, indicating a higher sensitivity in HL-60 cells. Collectively, these findings highlight *L. pallescens* as a promising source of bioactive compounds with nutritional, antioxidant, antidiabetic, and anticancer properties for potential pharmaceutical and nutraceutical applications.

Keywords: *Littorariapallescens, nutritional value, antioxidant activity, antidiabetic activity, anticancer activity.*

INTRODUCTION

The rising incidence of chronic diseases, including cancer, diabetes, and conditions associated with oxidative stress, has emerged as a significant worldwide health issue. The World Health Organization (WHO) reports that non-communicable diseases (NCDs) constitute over 70% of global mortality, with cancer and diabetes being significant contributors [1]. The pathogenesis of these disorders is frequently linked to elevated oxidative stress, disrupted glucose metabolism, and unregulated cell proliferation [2]. Notwithstanding considerable progress in synthetic drug research, persistent challenges remain, such as drug resistance, unpleasant side effects, and the elevated expense of prolonged therapy [3]. This has heightened the quest for innovative, efficacious, and safer medicinal agents, particularly from natural origins. Natural items have traditionally been essential in the discovery and development of pharmaceuticals.

Approximately 50% of contemporary pharmaceuticals are obtained, either directly or indirectly, from natural origins [4]. Plants, microbes, and marine life have yielded a variety of bioactive compounds with medicinal promise. The maritime ecosystem, encompassing more than 70% of the Earth's surface, contains a diverse range of unique species, much of which is still inadequately studied [5]. Marine species, such as gastropods, encounter severe and competitive conditions, resulting in the development of distinctive chemical defense mechanisms and bioactive metabolites [6].

Recent investigations have revealed several metabolites from marine gastropods exhibiting varied bioactive qualities, rendering them significant candidates for research. Oxidative stress, arising from an imbalance between the generation of reactive oxygen species (ROS) and cellular antioxidant defense, is pivotal in the onset and advancement of numerous diseases, including

diabetes and cancer [7]. Marine gastropods, including snails and slugs, are known to synthesize several antioxidant chemicals, such as phenolics, carotenoids, and peptides [8, 9]. Moreover, research has discovered various bioactive peptides and polysaccharides derived from marine gastropods that demonstrate inhibitory effects on critical enzymes in glucose metabolism, including α-glucosidase and α-amylase [10, 11]. Marine gastropods have produced a variety of secondary metabolites, including terpenoids, alkaloids, and polyketides, exhibiting significant anticancer properties [12]. These chemicals may trigger apoptosis, suppress angiogenesis, and obstruct metastasis, providing multi-faceted strategies for cancer treatment [6, 12]. This study evaluated the nutritional, antibacterial, antidiabetic, and anticancer effects of the estuarine gastropod *Littorariapallescens*.

L. pallescens is a marine gastropod mollusk of the family Littorinidae, generally referred to as periwinkles. Extensively distributed in the Indo-Pacific region, particularly along the coasts of Southeast Asia, encompassing Malaysia, Indonesia, Thailand, and the Philippines, and extending eastward to Papua New Guinea and northern Australia [13, 14]. This littorinid snail is generally located in the roots and lower trunks of mangrove trees within coastal habitats [14]. Morphologically, *L. pallescens* possesses a slender, elongated shell characterized by a pale hue, differentiating it from other *Littoraria* species [13]. This species has been recorded in significant mangrove areas of India, such as the Sundarbans (West Bengal), the Mahanadi delta (Odisha), the Godavari and Krishna deltas (Andhra Pradesh), and the mangrove regions of Tamil Nadu and Gujarat [15-17]. *L. pallescens* is prevalent in the expansive mangrove forests of the Godavari and Krishna estuaries in Andhra Pradesh, which are among the most affluent mangrove habitats in India [15]. The estuary mangroves, especially in the vicinity of Coringa Wildlife Sanctuary and the Krishna mangroves near Machilipatnam, offer optimal habitats for the species due to the prevalence of appropriate host mangrove trees like *Avicennia* and *Rhizophora* [17].

MATERIALS AND METHODS

Sample Collection

Healthy *L. pallescens* samples were taken from the estuarine regions of the Coringa mangroves, located in northeastern Andhra Pradesh, adjacent 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). Upon collection, the samples were aseptically transported to the laboratory and samples were rinsed with tap water followed by distilled water. Subsequently, the samples were meticulously dissected, and the extracted tissue was stored at -20°C.

Molecular Identification

The molecular identification of *L. pallescens* was carried out by the isolation of genomic DNA, Amplification of mtCO1 gene by PCR, Sequencing of amplified mtCO1 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 [18]. Thequality and quantity of extracted DNA was evaluated by agarose gel electrophoresis.

The mtCO1 gene was amplified using two primers: Cyt oxidase Forward (5' TCAACCAACCACAAAGACATTGGCAC-3') and Cyt oxidase Reverse (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'). In a thermocycler, mtCOX1 gene amplification initiated with 5 min of denaturation at 95°C, then 40 PCR cycles. Each cycle included 30 seconds of denaturation at 95°C, 15 seconds of annealing at 60°C, and 30 seconds of extension at 72°C. Final elongation was 10 minutes at 72°C, followed by 12°C incubation. The quality of amplified PCR products was assessed using electrophoresis on a 1.5% agarose gel with 0.5 µg/mL ethidium bromide, and gel pictures were taken. The PCR products were sequenced using Sanger's di-deoxy technique. Reverse and forward sequencing were done using an ABI Prism 3700 DNA analyzer

The BiologicsCorp web server (https://www.biologicscorp.com/tools/GCContent/) was used to calculate the nucleotide composition of the mtCO1 gene. To find comparable species, the mtCO1 gene sequence was examined using the NCBI server's BLASTn. Species having varying degrees of similarity to the query gene sequence were selected from the NCBI database. The homology and evolutionary characteristics of mtCO1 gene sequences were analyzed by multiple sequence alignment (MSA) employing the sophisticated clustering method UPGMA. A maximum-parsimony phylogenetic tree was constructed utilizing MEGA-X and performed 1000 bootstrap repetitions.

Evaluation of Nutritional Properties

The AOAC [19] approach was used to determine the moisture and ash content of *L. pallescens* tissue. The techniques of Hodge and Hofreiter [20]; Miller [21]; and Varkonyi et al. [22] were employed to ascertain the levels of nonreducing sugars, reducing sugars, and glycogen content. The total protein content from the muscle tissue was quantified employing the methodology of Lowry et al. [23]. The approach of Bligh and Dyer [24] was utilized to extract total lipids, and lipid content was estimated using the method of Knight et al. [25] using phosphovanillin reagent. The concentration of free amino acids was assessed using the methods established by Moore and Stein [26].

Evaluation of Antioxidant Properties

The antioxidant properties of *L.pallescens*were assessed at five distinct concentrations of methanolic extracts: 10, 7.5, 5.0, 2.5, and 1 mg/mL. The DPPH scavenging potential was evaluated by Mensor et al. [27] method. The ABTS radical scavenging activity was evaluated using the methods of Shirwaikar et al. [28]. The ferric ion reduction capacity was assessed using by Benzie and Strain [29] method. The superoxide scavenging activity was evaluated using NBT, as outlined by

Winterbourn et al. [30]. The nitric oxide scavenging activity was assessed using the method of Balakrishnan et al. [31] with sodium nitroprusside.

Screening of In vitro Antidiabetic Potential

The glucose adsorption capability of methanolic extracts from *L. pallescens*was assessed using the method of Ou et al. [32]. The impact of extract on hemoglobin glycosylation was assessed using the methods of Adisa et al. [33]. The impact of the extract on in-vitro glucose diffusion was assessed using the method of Ahmed et al. [34]. Pitchaipillai and Ponniah [35] methodology was used to examine the effect of extracts on glucose absorption in yeast cells. The α-amylase inhibitory assay was assessed using the Malik and Singh [36] method.

Determination of In vitro Cytotoxic Activity

The in vitro anticancer efficacy of *L.pallescens* crude methanolic extracts was assessed using the MTT assay on human cancer cell lines, including the breast cancer (MCF-7) and human promyelocytic leukemia (HL-60) cell lines. The MCF-7 cell lines were grown in DMEM medium, while the HL-60 cell lines were cultured in RPMI 1640 medium. The cultures of all selected cell lines were retrieved by removing the culture media, followed by trypsinization to prepare a cell suspension. Following trypsinization, the disaggregated cells were suspended in the liquid media. The cytotoxic activity of the crude methanolic extract was assessed using MTT to evaluate cell viability, following the approach outlined by Sudha and Selvam [37].

Statistical Analysis

All the results were given as Mean ± 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

In the present study, genomic DNA was successfully extracted from *L. pallescens*, 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 433±8 µg/gm respectively. Willian et al. [38] 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 mtCO1 gene band from *L. pallescens* in a 1.5% agarose gel. The amplified mtCO1 genes exhibited their size in the range of 600 to 700 bp length.

The advancement of the mtCO1 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 [39].Kartavtsev et al. [40] 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 [41].

Sequence Analysis

The PCR amplified sequences of mtCO1 gene from *L. pallescens*have total 601 nucleotides with 151 bp of adenine (25%), 208 bp of thymine (36%), 105 bp of guanine (17%), and 137 bp of cytosine (22%). The G+C content was calculated to be 39%. Figure 2 illustrates the GC distribution across the amplified mtCO1 gene sequence of *L. pallescens*. Current research indicates that the mtCO1 gene from the three gastropod species demonstrates a comparatively low GC content. These findings correspond with other research, including Grande et al. [42] which indicated GC levels ranging from 27% to 34% in the mtCO1 gene across different gastropod lineages. Research by Powell et al. [43] and Grande et al. [42] indicates that the mitochondrial genomes of gastropods, comparable to other molluscs, typically display a low GC content, usually between 25% and 40%.

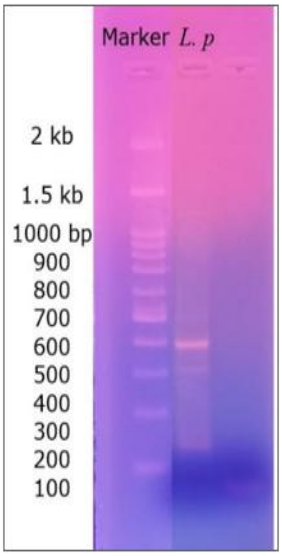

Figure 01:PCR amplified mtCO1 gene product on 1.5% agarose gel.

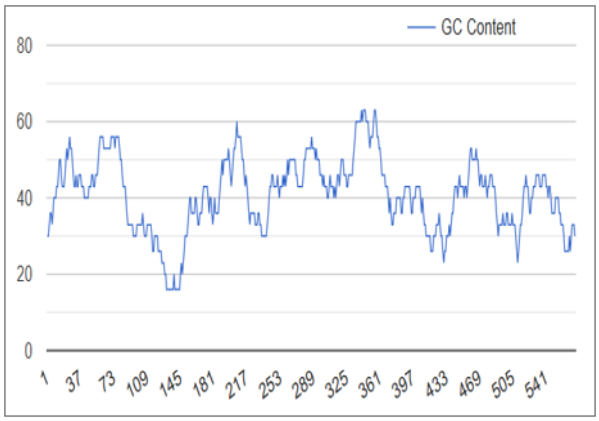

Figure 02:GC distribution over the amplified mtCO1 gene sequence of *L. pallescens*.

Phylogenetic Analysis

Figure 3 illustrates the phylogenetic tree of the various isolates and haplotypes of *L. pallescens*. From these results, it was observed that the collected *L. pallescens* isolate DSA.1 is closely related to several other *L. pallescens* isolates, including MG826437.1 (isolate aoc40), MG826456.1 (isolate abl59), and MG826413.1 (isolate pbc16). All of these isolates are grouped together in a major clade with a genetic distance of 0.0000, indicating a very high similarity.Other closely related isolates in this main clade include MG826494.1 (ffa97), MG826501.1 (ffi104), and MG826498.1 (ffe101), which also show minimal genetic divergence.Furthermore, a separate subclade consists of MG826512.1 (ffu115), MG826515.1 (ffz118), MG826530.1 (pff133), and MG826522.1 (hff125), with a slightly higher genetic distance (0.0017) from the main clade containing DSA.1.Overall, the genetic distances between all isolates are very low (ranging from 0.0000 to 0.0017), indicating that these *L. pallescens* isolates are genetically very similar and likely share a recent common ancestor. The phylogenetic tree demonstrates that *L. pallescens isolate DSA.1* is highly similar to other isolates of the same species, with negligible genetic differentiation.

The current results align with the findings of Reid et al. [44], 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. [45] indicated that *Littoraria* and *Cerithidea* occupy separate clades within the superfamily Cerithioidea, a classification robustly corroborated by COI and other mitochondrial markers.

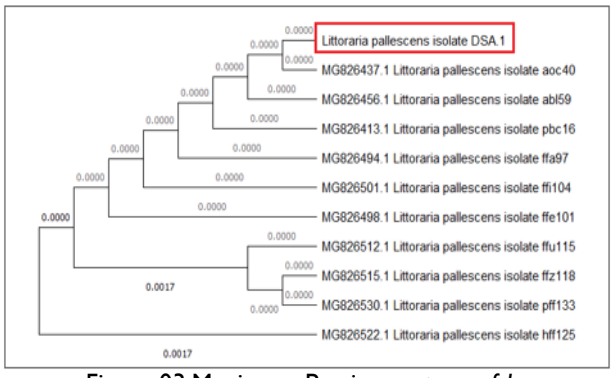

Figure 03:Maximum Parsimony tree of *L. pallescens*isolate and other relative species based on the mtCO1 gene.

Nutritional Properties

The nutritional analysis of *L. pallescens*muscle tissue, as presented in the figure 4, shows a high moisture content of 78.29±6.28 g/100 g fresh weight. The ash content, representing total mineral matter, is 1.08±0.04 g/100 g. Nonreducing sugars and reducing sugars are present at 1.24±0.06 g and 0.37±0.02 g/100 g, respectively. Glycogen content is measured at 0.73±0.03 g/100 g, indicating the presence of stored carbohydrates. The total protein content is relatively high at 15.62±1.54 g/100 g, suggesting the tissue is a good protein source. Total lipid content is 1.42±0.05 g/100 g, and free amino acids are present at 0.41±0.02 g/100 g. Overall, the muscle tissue of *L. pallescens*is characterized by high moisture and protein content, with moderate levels of carbohydrates, lipids, and minerals.

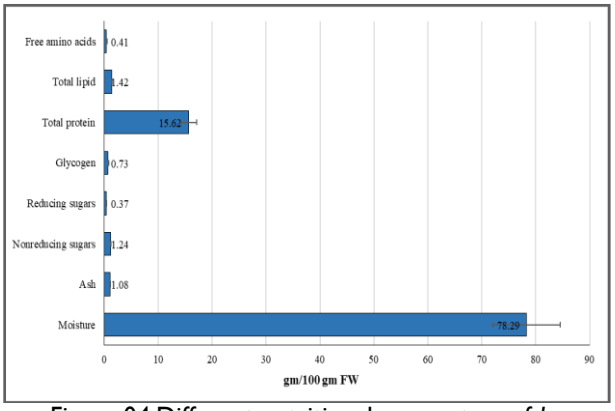

Figure 04:Different nutritional parameters of *L. pallescens*tissue.

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 Murray et al. [46]. The moisture content observed in the present study correlates with prior research of Odaibo and Olayinka [47] 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. [48] 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 [49]. 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 [50]. 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. [51] 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 [52]. Tasbozan and Gokce [53] 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 [54]. Carter et al. [55] 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 [56] 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 *Littorariapallescens*are summarized in Table 1. From these results, it was observed that the methanolic extract of *L. pallescens* demonstrated significant and concentration-dependent antioxidant potentials, including DPPH, ABTS, FRAP, superoxide (SO), and nitric oxide (NO) scavenging activities. As the extract concentration increased from 1 mg/ml to 10 mg/ml, there was a notable rise in the percentage of free radical scavenging activities. Specifically, the DPPH activity increased from 18.21% to 63.74%, ABTS from 22.8% to 72.04%, FRAP from 18.97% to 67.22%, SO from 4.88% to 56.29%, and NO from 33.56% to 81.54%. The extract exhibited the highest antioxidant activity in the NO assay, with an EC_{50} value of 3.03 mg/ml, indicating strong efficacy against nitric oxide radicals. Similarly, the ABTS assay showed a relatively low EC_{50} value of 5.43 mg/ml, followed by FRAP (6.53 mg/ml), DPPH (7.33 mg/ml), and SO (8.04 mg/ml). These results suggest that *Littorariapallescens* methanolic extract possesses substantial antioxidant properties, with the most pronounced effect observed in its nitric oxide scavenging capacity.

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

S. No.	Extract Conc. (mg/ml)	% of scavenging activities (Mean±SD)				
		DPPH	ABTS	FRAP	SO	NO
1	1	18.21±2.48	22.8±3.41	18.97±1.35	4.88±1.21	33.56±4.55
2	2.5	24.62±3.11	37.22±3.68	27.23±2.68	10.23±2.52	49.81±3.74
3	5	37.81±3.42	48.45±4.13	41.41±3.07	22.79±1.86	67.22±4.88
4	7.5	51.02±3.97	63.45±3.77	58.29±4.22	39.82±2.24	72.61±5.01
5	10	63.74±2.56	72.04±4.82	67.22±3.09	56.29±3.92	81.54±4.82
6	EC_{50}	7.33 mg	5.43 mg	6.53 mg	8.04 mg	3.03 mg
P<0.05 was considered as significant difference.						

The antioxidant activities of *L. pallescens*, align closely with the findings of Senevirathne et al. [57], which indicated approximately 60% DPPH inhibition at comparable concentrations in marine mollusc extracts. Kumaran and Karunakaran [58] documented IC_{50} values between 7 and 9 mg/mL for methanolic extracts of diverse marine gastropods, underscoring comparable antioxidant capacities. Krishnaraju et al. [59] 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 [60]. The present results align with the findings of Shantha et al. [61] who observed IC_{50} 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 [62]. Rajauria et al. [63] documented 85–92% FRAP inhibition in marine mollusc extracts at comparable dosages. Priyanka et al. [64] suggested that elevated FRAP activities are associated with enhanced antioxidant capacities. Shirwaikaret al. [28], identified IC_{50} 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 [65]. Ravikumar et al. [66] 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 [67] who documented 70–75% inhibition at comparable dosages for marine mollusc extracts.

In vitro Antidiabetic Potentials

Table 2 depicts the in vitro antidiabetic potentials from the *L. pallescens* methanolic extract with increasing concentrations. These results demonstrated that the *L. pallescens* extract exhibited significant, concentration-dependent antidiabetic potential. As the extract concentration increased from 1 to 10 mg/ml, glucose adsorption rose from 6.79±1.07% to 47.82±3.42%, while inhibition of hemoglobin glycosylation increased from 31.12±3.42% to 64.22±3.92%. Similarly, the

glucose diffusion retardation index (GDRI) improved markedly from 14.84±2.52% to 72.47±4.84%. The percentage of glucose uptake by yeast cells also increased significantly, ranging from 7.72±1.86% at the lowest concentration to 46.72±2.98% at the highest. Inhibition of α-amylase activity followed the same trend, increasing from 9.28±1.36% to 61.65±4.78%. The EC_{50} values for these parameters were 10.46 mg for glucose adsorption, 5.56 mg for Hb glycosylation inhibition, 5.63 mg for GDRI, 10.36 mg for glucose uptake by yeast, and 7.12 mg for α-amylase inhibition. These findings indicate that the *L. pallescens* methanolic extract possesses promising antidiabetic properties, with statistically significant effects observed across all tested concentrations.

Table 02:Percentage of different In vitro antidiabetic potentials from the *L. pallescens* 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	6.79±1.07	31.12±3.42	14.84±2.52	7.72±1.86	9.28±1.36
2	2.5	18.28±2.12	42.68±4.05	31.86±3.11	18.94±1.25	27.84±2.34
3	5	27.01±2.08	49.08±3.78	52.28±4.56	27.81±2.07	43.42±4.05
4	7.5	36.53±3.14	56.72±4.86	64.91±5.08	39.43±3.55	54.18±3.22
5	10	47.82±3.42	64.22±3.92	72.47±4.84	46.72±2.98	61.65±4.78
6	EC_{50}	10.46 mg	5.56 mg	5.63 mg	10.36 mg	7.12 mg
P<0.05 was considered as significant difference.						

The present 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. [68] reported similar concentration-dependent inhibition. Das and Devi [69] 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 [70] 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. [71] and Zhang et al. [72] 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 [73] 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. [74] 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. [75] similarly reported that the acetone extracts of *H. pugilinus* and *N. didyma* demonstrated α-amylase inhibitory activities of 72.23±0.44% and 51.23±0.44%, respectively, at a concentration of 50 µL.

Anticancer Potentials

Table 3 depicts the anticancer potentials from the *L. pallescens* methanolic extract with increasing concentrations. These results demonstrated that the *L. pallescens* extract exhibited notable anticancer potential against MCF-7 (breast cancer) and HL-60 (leukemia) cell lines. The cytotoxic effect increased in a concentration-dependent manner for both cell lines. For MCF-7 cells, cytotoxicity ranged from 11.26% at 1 mg/ml to 42.65% at 10 mg/ml, with an IC_{50} value of 11.86 mg. Similarly, for HL-60 cells, cytotoxicity increased from 12.55% at 1 mg/ml to 56.78% at 10 mg/ml, with a lower IC_{50} value of 8.67 mg, indicating a higher sensitivity compared to the MCF-7 cell line. These results suggest that the *L. pallescens* methanolic extract possesses significant cytotoxic and potential anticancer activities, with efficacy increasing alongside concentration. Figure 4 and 5 shows the microscopic images of MCF-7 and HL-60 cell lines under treatment of 1 mg/mL and10 mg/mL *L. pallescens* methanolic extract.

The methanolic extracts of *L. pallescens* 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. [76] and Kour et al. [77] 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. [78] 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. pallescens* methanolic extracts on cancer cell lines.

S. No	Extract. Conc. (mg/mL)	% of cytotoxicity (Mean)	
		MCF-7	HL-60
1	1	11.26	12.55
2	2.5	13.19	21.45
3	5	21.77	36.78
4	7.5	35.99	41.22
5	10	42.65	56.78
6	IC_{50}	11.86 mg	8.67 mg

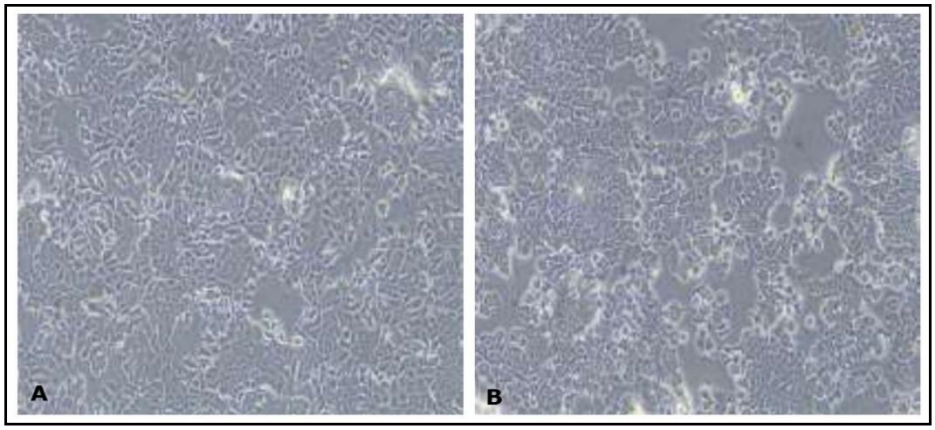

Figure 05: Microscopic images of MCF-7 cell lines under treatment of A) 1 mg/mL *L. pallescens* methanolic extract B) 10mg/mL *L. pallescens* methanolic extract.

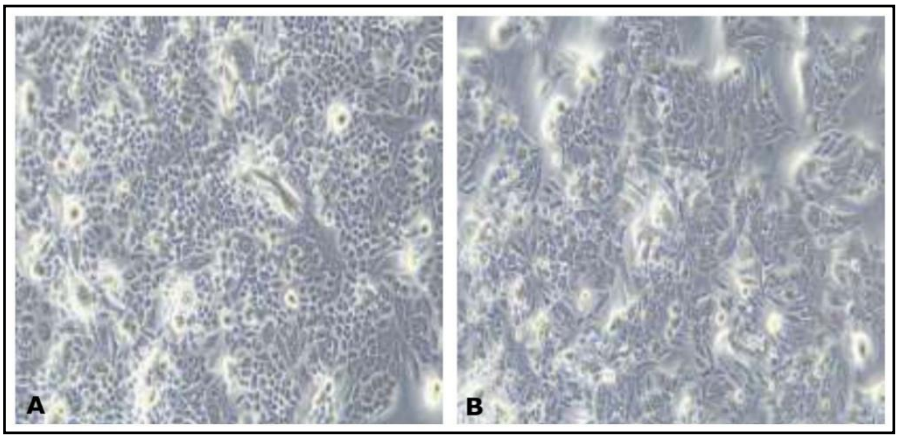

Figure 06: Microscopic images of HL-60 cell lines under treatment of A) 1 mg/mL *L. pallescens* methanolic extract B) 10mg/mL *L. pallescens* methanolic extract.

CONCLUSION

This study demonstrates that the methanolic extract of *L. pallescens* demonstrates significant nutritional value and potent bioactive properties, including antioxidant, antidiabetic, and anticancer activities. The extract exhibited strong free radical scavenging, effective inhibition of key diabetic enzymes, and notable cytotoxicity against cancer cell lines, all in a dose-dependent manner. These findings highlight the potential of *L. pallescens* as a valuable natural source for the development of novel nutraceuticals and therapeutic agents targeting chronic diseases such as diabetes and cancer. Further studies are recommended to isolate active compounds and evaluate their mechanisms of action in vivo.

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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, laboratory experiments, data analysis, manuscript preparation, and interpretation of results.

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

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

Ethical Statement

The specimens of Littorariapallescens used in this study were collected from the estuarine mangrove regions of Coringa Wildlife Sanctuary, Andhra Pradesh, India, following the applicable institutional and environmental guidelines. The study did not involve human participants, vertebrate animals, or clinical samples. Therefore, formal 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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