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Original Research Article

Pharmacological profiling of the marine squid *Sepioteuthis lessoniana*: Elemental, chemical and antimicrobial assessment

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Abstract

Cephalopods, especially Sepioteuthis lessoniana, are ecologically vital and economically important marine molluscs. This study investigates their hidden biomedical potential, focusing on chemical composition and antibacterial properties. Antibacterial assays against five pathogens showed inhibition zones for all except E. coli, indicating selective resistance. Bioactive compounds in squid extract were identified using UV-Vis and FTIR spectroscopy. Further analysis through FESEM, EDAX, and XRD revealed detailed microstructural and elemental profiles, emphasizing the chemical richness and antimicrobial potential of Sepioteuthis lessoniana extracts for biomedical applications. This multidisciplinary investigation unveils both the structural and functional attributes of bioactive compounds derived from S. lessoniana, establishing it as a promising candidate in the search for novel antimicrobial agents. In the face of rising global antibiotic resistance, marine bioprospecting emerges as a vital strategy for discovering new therapeutic options. S. lessoniana, with its rich chemical complexity and potent bioactivity, serves as a compelling example of the untapped potential within marine organisms. This study not only highlights its biomedical significance but also encourages further research into cephalopod chemistry as a foundation for innovative pharmaceutical developments and next-generation antimicrobial therapies.

Keywords: *Sepioteuthis Lessoniana*, Chemo-complexity, Bacterial-resist, Bioprospection, Therapeutic action

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1. Introduction

Marine ecosystems host an astonishing array of biodiversity, offering a unique and often untapped source of bioactive compounds with significant pharmacological and biotechnological applications. Among the vast diversity of marine fauna, cephalopods occupy a prominent ecological and commercial niche. Belonging to the phylum *Mollusca* and class *Cephalopoda*, cephalopods are characterized by bilateral body symmetry, a distinct head, and a set of specialized appendages that aid in locomotion, feeding, and interaction with their environment.[1] Squids, a major group within cephalopods, exhibit remarkable morphological features such as large, well-developed eyes, elongated soft bodies, and lateral fins, which are critical for their survival and adaptability in marine habitats.

In recent decades, there has been a global surge in the harvesting and consumption of cephalopods, primarily driven by their high nutritional value. These marine organisms are rich in essential proteins, lipids, and micronutrients, making them a sought-after food source.[2-3] Their palatable flavour, minimal inedible parts, and versatility in culinary applications have further contributed to their increasing commercial value. Consequently, cephalopods have become integral not only to marine ecosystems but also to global fisheries and aquaculture industries.[4-5] *Sepioteuthis lessoniana* found abundantly in the Indian Ocean[6] increasing global demand for their availability in various processed forms such as frozen, chilled, and ready-to-eat products, expanding their consumer base beyond traditional markets.[7] Beyond its culinary appeal, *S. lessoniana* holds promising potential in biomedical and pharmaceutical research.

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Taxonomically, *S. lessoniana* belongs to the order *Myopsida* and the family *Loliginidae*. Despite sharing some external characteristics with cuttlefish, species within the genus *Sepioteuthis* are easily distinguishable due to specific morphological and anatomical features[8] recognized as one of the most commercially valuable among loliginid squids.[9-10] The biochemical richness of *S. lessoniana* is attributed to the complex array of secondary metabolites they produce, which serve ecological roles such as potent biological activities, including antimicrobial, antioxidant, anticancer, and anti-inflammatory properties. Marine squids may synthesize or accumulate flavonoids, phenolic compounds, quercetin and rutin, making them an attractive subject for biochemical screening and antimicrobial evaluation.[11-12]

Marine bioprospecting in the exploration of marine based novel bioactive substances is highly recognized as a frontier for pharmaceutical innovation led to the development of structurally distinct compounds not commonly found in terrestrial life. These compounds hold immense promise as future therapeutics, cosmetics, dietary supplements, molecular probes, and agrochemicals.[13] Numerous studies have also emphasized the relevance of marine species in traditional Indian medical systems, further reinforcing their biomedical potential.[14]

This study aims to biochemically profile and evaluate the antimicrobial activity of *S. lessoniana*. Bioactive compounds from squid tissues were characterized using UV-Vis, FT-IR, XRD, SEM, and EDAX analyses. The antibacterial potential was tested against clinical pathogens such as *E. coli*, *S. aureus*, *B. subtilis*, *B. cereus*, and *P. aeruginosa*. These bacteria are increasingly resistant to existing antibiotics, highlighting the need for alternative agents. The study demonstrates the pharmaceutical and nutraceutical promise of *S. lessoniana* and supports marine biotechnology role in addressing antimicrobial resistance through the sustainable use of marine bioresources and advanced analytical approaches.

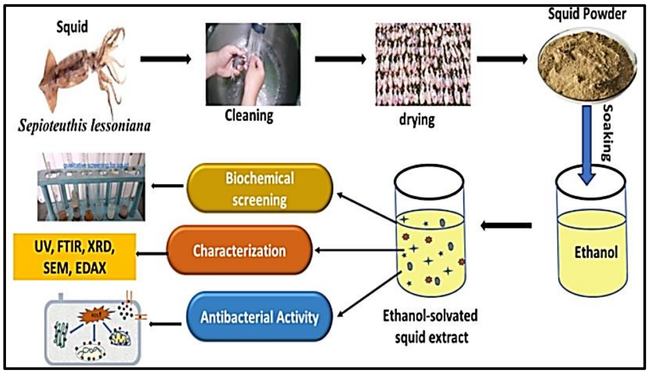


Figure 1: Schematic process carried out by *Sepioteuthis Lessoniana*

2. Materials and Methods

2.1. Sample preparation

The marine squid *Sepioteuthis lessoniana* were collected from the Threspuram Market in Thoothukudi. The freshly procured specimens were immediately transferred to the laboratory in sterile, airtight containers to preserve their integrity. Initially, the squids were rinsed with seawater to eliminate external debris such as sand, pebbles, and shell fragments. This was followed by thorough washing with running tap water and deionized water to ensure complete cleanliness. Internal components such as the ink sac, cartilage, skull, and viscera were removed. The cleaned squid bodies were then cut along the dorsal side, flattened to ensure uniform drying, and sliced into smaller segments. Blanching was carried out in boiling water (80–90 °C) for 30–60 seconds to improve texture, reduce microbial load, and inactivate enzymes. The samples were then shade-dried at room temperature. Once dried, a tissue blender was used to pulverize the material into a fine powder, which was stored under refrigeration for future use.

3. Preparation of Extract and Chemical Screening

To prepare the extract, five grams of dried squid powder were homogenized with ten volumes of 80% ethanol. The mixture was centrifuged, and the supernatant was collected. The residue was re-extracted with ethanol, centrifuged again, and both supernatants were pooled and evaporated for further analysis. The resulting ethanolic extract was subjected to preliminary phytochemical screening following the protocol outlined by Trease and Evans[15] to detect the presence of bioactive compounds.

4. Characterization Studies

UV-Vis, FTIR, XRD, SEM and EDAX were used to characterize the *Sepioteuthis Lessoniana* extract. Using UV-Vis (JASCO V-650), the absorption spectra of this species were acquired. The absorptions were measured between 350 and 750 nm. Using dried potassium bromide powder and a Fourier Transform Infrared Spectrometer SHIMADZU device, the functional groups in the sample were identified. A disc containing the mixture was scanned between 4000-500 cm^{-1}. The Panalytical X'pert powder X'Celerator Diffractometer was used to determine the sample's size and crystalline phase. Analysis of the sample's morphology was done using a JEOL-JSM-5610LV Scanning Electron Microscope. The ESEM Quanta 200, an FEI Energy Dispersive X-ray Spectroscopy (EDAX) device was used to assess the sample's purity.

4.1. Antibacterial assay

The antibacterial activity of Sepioteuthis lessoniana was evaluated using the disc diffusion method on Muller Hinton Agar. Clinical strains of Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus, E. coli, and Bacillus subtilis were maintained at 4 °C. Sterilized media (25 mL, pH 7.2–7.4) was poured into plates to a depth of 4 mm. Sterile discs infused with squid extract or standard antibiotics were placed 15 mm from the edge. Plates were left at room temperature for 30 minutes, then incubated at 35–37 °C for 16–18 hours. The diameter of inhibition zones was measured in millimetres to assess antibacterial efficacy.[16] Active Index (AI) was calculated using the following formula.

Active Index (AI) =	Inhibition zone of the test sample	x 100
	Inhibition zone of the standard	

5. Results and Discussion

5.1. Biochemical screening

The qualitative phytochemical screening of *Sepioteuthis lessoniana* tissue extracts revealed a diverse array of bioactive constituents. Tests conducted using various solvents such as petroleum ether, benzene, chloroform, ethanol, and water showed the presence of several primary and secondary metabolites. These included alkaloids, proteins, phenols, flavonoids, tannins, lipids, steroids, saponins, quinones, anthraquinones, and carbohydrates, highlighting the biochemical richness of the squid (**Table 1**). The presence of various bioactive compounds is highly detected in ethanol extracting mode which supports in analgesic, antibacterial, and antimalarial activities, consistent with earlier findings on marine molluscs.[17] Flavonoids with antioxidant and anti-inflammatory effects may aid in biological protection and human health.[18] Saponins and tannins, found in moderate to high amounts, exhibit cytotoxic and antimicrobial potential.[19] Steroids and terpenoids contribute to membrane functions and may serve as drug precursors.[20]

Table 1: Preliminary chemical screening of sample *Squid Sepioteuthis lessoniana*

S. No	Biochemical Screening	Ethanol extract	
		Qualitative analysis	Quantitative Analysis
1	Alkaloids	+	+
2	Terpenoids	+	-
3	Steroids	+	+
4	Coumarin	-	-
5	Tannins	+	+
6	Saponins	-	-
7	Flavonoids	+	+
8	Quinones	+	+
9	Antroquinones	+	+
10	Phenols	+	3.9 %
11	Catechins	+	-
12	Aromaticacids	+	+
13	Proteins	+	14.3 %
14	Lipids	+	11.8 %
15	Carbohydrate	+	40 %

5.2. UV-visible Spectroscopy

The UV-Vis spectral analysis of *S. lessoniana* indicated a no **Table 1** absorption peak at approximately 279 nm. This absorbance is typically associated with the presence of proteinaceous and aromatic compounds, which may reflect the physiological and ecological functions of the squid's biochemical components (**Figure 2**). UV-Vis analysis showed a major absorption peak at 279 nm, suggesting low levels of aromatic amino acids like tryptophan, tyrosine, and phenylalanine.[21] The presence of UV-absorbing compounds in molluscs supports their role in photoprotection, possibly sourced from algae or synthesized internally.[22] These compounds may protect soft tissues and serve as chemotaxonomic or stress indicators. Seasonal variations, especially higher summer absorbance, highlight solar radiation's impact on molluscan metabolism.[23]

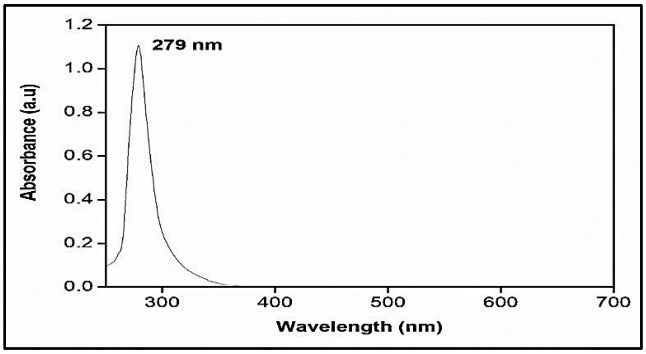


Figure 2: UV-visible spectral analysis of the *Sepioteuthis Lessoniana*

5.3. FT-IR spectroscopy

FT-IR spectral data provided insights into the functional groups present in the squid tissue. Prominent absorption bands included peaks at 3357.29 cm^{-1} (O-H and N-H stretching), 2923.46 cm^{-1} (C-H stretching), 1652.88 cm^{-1} and 1541.79 cm^{-1} (Amide I and II bands, indicating proteins), 1400.40 cm^{-1} and 1082.85 cm^{-1} (carbonate groups). These spectral signatures confirm the presence of water, polysaccharides, proteins, lipids, and carbonate minerals in the sample (**Figure 3**). The FTIR spectrum analysis showed characteristic bands indicating the presence of polysaccharides, with a broad absorption between 3600 and 3000 cm^{-1} due to O-H stretching in alcohols and hydroxyl groups, and N-H stretching in amines appearing around 3200–3600 cm^{-1}.[24] These spectral features confirm that biogenic calcium carbonate is the dominant inorganic component in the tissue. The variations in band intensity reflect mineralization differences. FTIR proves valuable for assessing biomineralization and environmental impacts on marine organisms.[25]

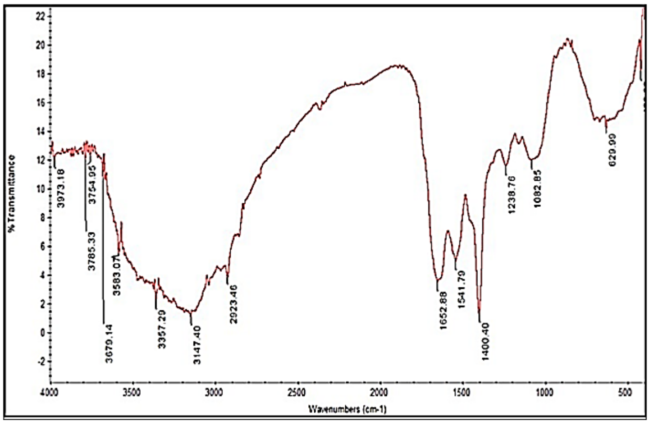


Figure 3: FTIR spectrum of squid *Sepioteuthis lessoniana*

X-ray diffraction

X-ray diffraction analysis revealed that the crystalline structure in the squid tissue matched typical aragonite patterns, with strong peaks at 2θ values of 77.7300, 44.1400, and 64.4500 corresponding to the (311), (200), and (220) planes. These values align with the face-centered cubic (FCC) structure, consistent with aragonite-type calcium carbonate biomineralization (**Table 2** and **Figure 4**). X-ray diffraction (XRD) analysis of mollusc tissues, such as oyster shells, enables identification of crystalline phases by comparing observed diffraction peaks with standardized reference patterns maintained by the Joint Committee on Powder Diffraction Standards (JCPDS), now part of the ICDD. Oyster shells primarily consist of calcium carbonate in the form of aragonite or calcite, each producing distinct XRD patterns. Peaks matching specific JCPDS cards aragonite: 01-062-0336 and calcite: 05-0586 confirm the mineral phase present. This technique aids in determining mineral composition, comparing species-specific crystalline structures, and understanding biomineralization and ecological adaptations in marine organisms.[26] The crystallinity index (CI) determined using peak sharpness and intensity showed that mature shells had a high degree of crystallinity. Juvenile and environmentally stressed specimens had broader, less intense peaks, indicating more amorphous and weakly crystalline material. These findings are consistent with earlier research that has examined ontogenetic and environmental impacts on shell mineralogy.[27] The XRD findings thus support the mollusk mineral composition which is species-specific and modified by the environment. Such variations have significance for paleoenvironmental reconstruction, biomineralization research and conservation efforts in marine ecosystems.[28]

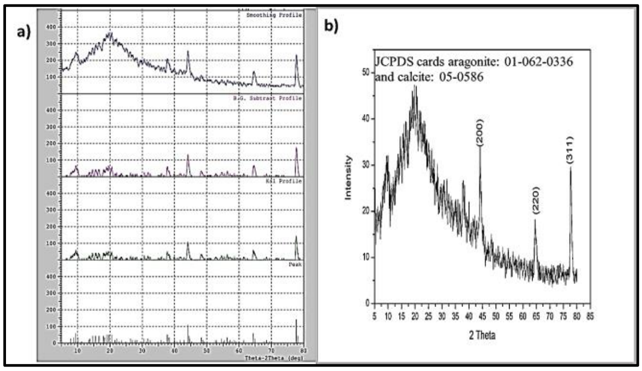


Figure 4: XRD pattern of squid *Sepioteuthis lessoniana* ***a)*** *Scanned data with peak pattern* ***b)*** *Interpolated plane of aragonite and calcite XRD pattern of Squid*

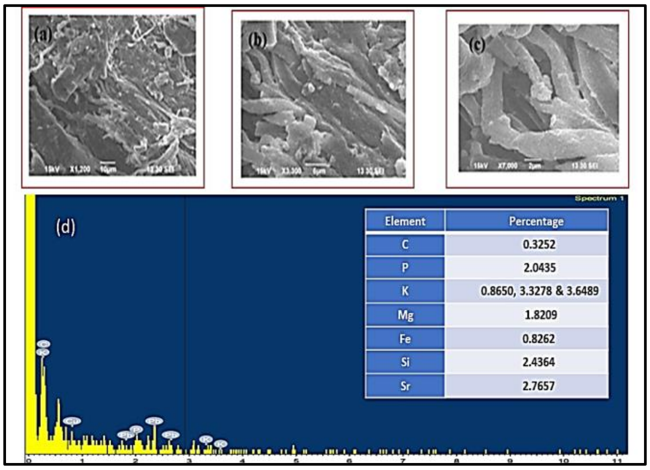
Table 2: XRD Studies of squid *Sepioteuthis lessoniana*

Peak No	2Theta(deg)	θ	Sin θ	$Sin^2θ$	d (A)	Ratio	FWHM (deg)	Plane	a (nm)
63	77.7300	38.865	0.6275	0.40	1.22759	11	0.46000	311	0.40480
39	44.1400	22.070	0.3757	0.14	2.05009	4	0.40000	200	0.40457
58	64.4500	32.225	0.5332	0.29	1.44455	8	0.30000	220	0.40471

6. Scanning Electron Microscopy with EDAX

Scanning Electron Microscopy (SEM) analysis of *Sepioteuthis Lessoniana* tissues showed a fibrous surface morphology with aggregated microfibres measuring 2 to 10 µm in diameter, typical of molluscan structural tissues. **Figure 5** a, b, c exhibit the images at 2, 5, and 10 µm of the nacreous layer well-organized, polygonal aragonite tablets arranged in a rod-like pattern, while the prismatic layer featured needle-like calcite crystals-oriented perpendicular to the surface. These structural features support mechanical strength and flexibility. The organic matrix visible between mineral layers likely contributes to biomineralization and crack resistance.[29]

Energy Dispersive X-ray Spectroscopy (EDAX) identified major elements carbon and oxygen, with magnesium, strontium, silicon, iron, and potassium present in trace amounts likely from organic residues, and phosphorus mainly in soft tissues. Magnesium variations, affecting shell strength through lattice substitution, align with environmental factors like temperature and salinity. Trace silicon and iron suggest possible environmental pollution, while strontium acts as a calcium substitute and paleoenvironmental marker. These elemental variations reflect biological and environmental influences, aiding ecological monitoring and paleoclimate studies.[30-31]



Element	Percentage
C	0.3252
P	2.0435
K	0.8650, 3.3278 & 3.6489
Mg	1.8209
Fe	0.8262
Si	2.4364
Sr	2.7657

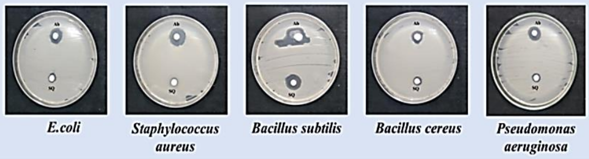
Figure 5: SEM micrographs of squid *Sepioteuthis lessoniana* a) 10 µm scale b) 5 µm scale c) 2 µm scale d) EDAX pattern with insert table of elemental composition percentage

7. Antibacterial Activity

The ethanol extract of *Sepioteuthis lessoniana* demonstrated significant antibacterial potential against five clinically relevant bacterial strains: *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. *Sepioteuthis Lessoniana* demonstrated increased antibacterial activity against five human diseases, with zones of inhibition ranging from 6 to 17 mm. In this species, *Escherichia coli* organisms have greater ampicillin resistance values, but *Bacillus subtilis* has lesser resistance. Exploration of the marine environment for numerous, often quite complex chemical compounds is a current focus in natural-source medicine development.[32] Antimicrobial medication development is primarily reliant on marine natural product research. Recent advances in selective organic synthesis, ribosome crystallography, chemical biology approaches for target elucidation, and creative ways for identifying new natural products suggest that this study will continue to produce new medications for unmet medical needs. Numerous investigations have demonstrated that marine bacteria produce distinct secondary metabolites as a pathogenic invasion resistance strategy.[33-36]

Research on antimicrobial chemicals isolated from marine sources is limited due to non-specific toxicity in humans, unclear biosynthetic pathways, and low yields. [37] However, future research should focus on optimizing fermentation conditions that have been shown to be bioactive, in order to increase the yield of active substances synthesized by microbes as well as searching for the regulatory gene.

a)



b)

Bacterial strains	Inhibition zone in mm	
	Ampicillin	Sample
E.coli	10	6
Staphylococcus aureus	14	-
Bacillus subtilis	15	14
Bacillus cereus	10	7
Pseudomonas aeruginosa	11	7

Figure 6: Antibacterial assay of squid *Sepioteuthis Lessoniana*

8. Conclusion

The multidisciplinary investigation of *Sepioteuthis lessoniana* tissue revealed significant biochemical and mineralogical complexity. Phytochemical screening confirmed the presence of diverse bioactive compounds, notably alkaloids, flavonoids, and saponins, especially abundant in ethanol extracts, supporting therapeutic potential. UV-Vis and FTIR spectroscopy confirmed proteinaceous, aromatic, and carbonate functional groups, indicating complex biochemical composition and biomineralization traits. XRD analysis identified aragonite as the primary crystalline form of calcium carbonate, characteristic of molluscan shells, with high crystallinity levels suggesting structural maturity. SEM imaging corroborated this with fibrous microstructures and organized mineral layers, while EDAX profiling revealed essential elements (C, O, Mg, Sr) and trace pollutants (Fe, Si), highlighting both biological function and environmental interactions. Additionally, the ethanol extract displayed notable antibacterial activity against multiple pathogens, indicating pharmaceutical relevance. These findings collectively underscore the ecological adaptability and biomedical promise of *S. lessoniana*, offering valuable insights into marine biochemistry, biomineralization, and bioactivity for applications in environmental monitoring and natural product development.

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10. Source of Funding

None.

11. Conflicts of Interests

None.

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