Pharmacogn. Res., 2026; 18(3):863-869.
https://www.phcogres.com

Original Article

# Characterization and Therapeutic Potential of Marine-Derived *Loligo duvauceli* Ink Powder

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## ABSTRACT

**Background:** This research work focuses on the bioactive components of squid (*Loligo duvauceli*) ink, a naturally occurring marine fluid that is potentially defensive and has therapeutic qualities. This study investigates the phytochemical compounds, functional groups, and antibacterial activity of squid ink powder. **Materials and Methods:** Squid ink was collected and processed from a fresh squid sample, and evaluated for phytochemical screening, UV-vis spectrophotometry, FTIR, GC-MS analysis, and antimicrobial assays. **Results:** The existence of alkaloids, phenols, saponins, tannins, and flavonoids in a range of solvents was demonstrated by phytochemical analysis. Melanin and conjugated substances are characterized by a significant absorption between 200 and 400 nm in the UV-vis spectrum. Functional groups that are suggestive of bioactive compounds, such as hydroxyl, amine, carbonyl, and aromatic rings, were validated by FTIR analysis. Trimethyl phosphate was the main component of the chemicals found by GC-MS profiling, coupled with bioactive phenolic and quinonoid derivatives. There was a significant zone of inhibition against *E. coli* and *S. aureus*, which was evident in antibacterial tests. **Conclusion:** The results highlight *L. duvauceli* ink's potential as a useful source of organic antioxidant and antibacterial compounds for application in pharmaceutical and nutraceutical formulations.

**Keywords:** Antibacterial, Biological activities, Functional group, Pharmaceutical, Phytochemical screening.

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**Received:** 15-12-2025;
**Revised:** 09-01-2026;
**Accepted:** 23-03-2026.

## INTRODUCTION

A prominent head, bilateral symmetry, and several arms or tentacles derived from the molluscan foot are characteristics of the diverse class of marine mollusks known as cephalopods, which includes squids, octopuses, cuttlefish, and nautiluses (Schmidt & Mouritsen, 2022). According to taxonomy, they are separated into two subclasses that are currently in existence: Nautiloidea, which includes shelled nautiluses, and Coleoidea, which includes octopuses, squids, and cuttlefish which usually have internalized or non-existent shells (Sanchez *et al.*, 2018). Despite the dominance of wild fisheries, demand and sustainability concerns are driving the emergence of specialized aquaculture initiatives that concentrate on species such as *Octopus vulgaris, Sepia officinalis,* and *Sepioteuthis lessoniana* (Vidal *et al.*, 2014).

Important to the economy are India's exports of squid and cuttlefish, primarily frozen products that are sent to the United States, China, Japan, and the European Union (Bhoir & Kumar, 2024). Because of their complex biology which includes sophisticated cognition, dynamic camouflage, rapid jet propulsion, and unique ink chemistry as well as their commercial and cultural uses, cephalopods are a unique subject at the intersection of evolutionary biology, biotechnology, fisheries economics, and sustainable seafood practices.

The Indian squid, or *Loligo duvauceli*, is a short-lived mollusk species that is highly significant both ecologically and commercially in the Indo-Pacific area. Its quick growth rate, distinct reproductive technique, and adaptability make it widely distributed over the Indian coastline and greatly enhance marine biodiversity and coastal fisheries. About 22% of the protein in *L. duvauceli's* mantle is myofibrillar, accounting for nearly 60% of its protein content, underscoring its nutritional and biochemical significance (Collagen and protein studies) (Raman & Mathew, 2014). Addressing the biology, population dynamics, and potential consequences of cephalopods is crucial for sustainable collection and use as the demand for these animals rises worldwide. One of *L. duvauceli's* interesting by-products is its ink, melanin-rich secretion that is mostly employed to evade predators. Along with melanin, this ink also contains proteins, lipids, tyrosinase, and free amino acids like taurine and glutamate, which work together to impair predators' senses and provide chemical defense (Derby *et al.*, 2007).

**DOI:** 10.5530/pres.20260231

Additionally, the ink has the broadest variety of therapeutic applications and multiple major roles in the field of alternative medicine (Girija *et al.*, 2014). Squid ink is becoming more well-known for its biological activity, such as antibacterial, antioxidant, and anti-inflammatory qualities, in addition to its ecological role. As such, it is a promising option for new medicinal and nutraceutical uses (Nadarajah *et al.*, 2017).

The amazing potential of eumelanin in squid ink as a natural antioxidant, illuminating its wide range of uses. Interestingly, it has the potential to be a valuable addition to the field of antioxidant food additives, which can enhance the preservation and health benefits of various food items. Additionally, because of its therapeutic potential, it is a good candidate to be included in pharmaceutical and nutritional substances, which could improve people's health and well-being in a variety of ways (Song *et al.*, 2023).

Thus, *L. duvauceli* ink was chosen for the purpose of research in order to examine its bioactive properties. We must first understand the components of squid ink in order to effectively utilise it. The goal of this work was to analyse the phytochemicals, UV-vis, GC-MS, and FTIR properties of *L. duvauceli* ink, as well as its antibacterial properties.

## MATERIALS AND METHODS

### Experimental Sample Collection

The fresh squid (Figure 1) was procured in March 2025 from Adirampattinam, Tamil Nadu, India, and had the same size and physical appearance. The squids were kept at 4°C while being transported. This was determined by the Department of Zoology PSG College of Arts & Science in Coimbatore.

### Processing of Squid Ink

Squid (*L. duvauceli*) were dissected and the ink sacs were extracted. The ink duct was severed with sterile scissors, and the sac was gently squeezed, releasing the ink. The ink glands were then transferred to clean plastic containers before being stored in the blast freezer.

A modified version of Jeyasanta *et al.*, (2020) was utilized. The squid ink extract was isolated from the dry powder (Figure 2) after the ink had been completely dried in a hot air oven. A mortar and pestle were used to grind 50 g of powder. The parallel extraction procedure was used in sterile glass bottles to mix 25 g of powder and 75 mL of methanol. A sterilized glass rod was used to mix the powder and methanol gradually. Following 72 hr of chilling at 4°C, the mixture was shaken for 8-10 hr at room temperature. The supernatant was generated by centrifugation of the methanol extract. The crude methanol extracts were collected, weighed, and their sterility was tested for approximately two hours under a UV light. The extract was kept in brown bottles at 4°C.

### Phytochemical Analysis

The preliminary phytochemical evaluation for the powder sample of (*L. duvauceli*) was done with solvents such as aqueous, ethanol, methanol, acetone, and chloroform. This test was carried out to identify the presence of specific secondary metabolites in the sample. The sample was screened qualitatively for the phytochemical constituents like alkaloids, phenols, saponins, flavonoids, steroids and tannins.

### UV-vis Spectral analysis

A spectrophotometer was used to record UV-vis absorption spectra ranging from 200 to 400 nm. Absorbance measurements were taken in a 1 cm quartz cuvette using the Beer-Lambert equation to calculate the concentration of the absorbing species.

### FTIR Analysis

FTIR is a crucial instrument for identifying and characterizing chemical functional groups and bonds. A compound's chemical bond can be established by reading its infrared absorption spectra. FTIR spectrum with a scan range of 200-4000 $cm^{-1}$ and a resolution of 4 $cm^{-1}$ were recorded with 100 scans for the sample. The functional groups were identified comparing the peaks with the library.

### GC-MS Analysis

A Gas chromatograph analyser (Shimadzu GC-MS 2010 QP plus) Specifications: Column name and dimension: Rxi 5sil Ms, 30 m, 0.25 mmID, 0.25 um $d_f$, Carrier gas: Helium 5.5 grade, Column Oven Temp.: 70.0°C Injection Temp.: 280.00°C Injection Mode: Split Flow Control Mode: Linear Velocity Pressure: 63.3 kPa Total Flow: 14.4 mL/min Column Flow: 1.02 mL/min Linear Velocity: 37.1 cm/sec, Purge Flow: 3.0 mL/min, Split Ratio: 10.1, Ion SourceTemp: 200.00°C, Interface Temp. 280.00°C, Solvent Cut Time: 2.00 min. was used to perform GC-MS analysis for the squid (*L. duvauceli*) ink powder. Correlating with the library (NIST library: NIST 2020 version), GC-MS revealed the names, molecular weights, and structures of the test material's components. Each component's relative percentage quantity was computed by dividing its average peak area by the overall area.

### Anti-Bacterial Activity

The antibacterial activity was determined by the agar well-diffusion method against the test organisms, *E. coli* and *S. aureus*. The 24 hr bacterial culture was prepared and transferred to sterile petri plate with Mueller Hinton agar medium (Hi-Media) and was spread with a sterile spreader to create a lawn. Wells of 5 mm was punctured into the previously seeded Mueller Hinton agar plates using sterile cork borer. About 200 µL of the ink was placed in to wells and allowed to diffuse for 2 hr at 4°C and then plates were incubated at 37°C for 24 hr. The activity was determined by measuring the diameter of the inhibition zones for each well and expressed in millimetre.

### Statistical Analysis

The data represent the mean of three replicates ± Standard Deviation (SD). Results were performed using SPSS version 20.0 (Statistical Package for the Social Sciences, Inc., Chicago, IL, United States).

### Ethical Statement

Ethical review and approval were not required for this study.

## RESULTS

### Phytochemical Analysis

Several bioactive chemicals were found in the following Table 1, whereas powder sample of (*L. duvauceli*) was subjected to phytochemical screening using a variety of solvents, including water, methanol, ethanol, acetone, and chloroform. Alkaloids, carbohydrates, proteins, amino acids and tannins were present in all the solvents.

Their absence in chloroform indicates that they are soluble in polar solvents and may have structural advantages. A wide range of solvents, with the exception of acetone, contained proteins and amino acids, which are crucial for biological processes and enzymatic activity found in aqueous, and were tannins, which have astringent and antioxidant qualities and show good extractability across polarity.

The aqueous extract contained saponins, which have antibacterial and immune-stimulating properties. All solvent extracts lacked glycosides, phytosterols, and fats/fixed oils, indicating that they are either not present in squid ink powder or cannot be extracted under the test conditions.

### UV-vis Spectroscopy Analysis

The UV-vis spectrum (Figure 3) in the above image indicates a sample's absorbance from 200 to 400 nm. In this current study spectrum shows significant absorption in the UV region with numerous variations and peaks suggesting the existence of several electronic transitions. The greatest measured UV absorption value, 9.0000, was found at several wavelengths, especially between 200 and 300 nm. The maximal absorption wavelength of melanin ink ranges between 196 and 300 nm (Pralea *et al.*, 2019). Beyond 300 nm, there is a dramatic reduction in the absorbance, indicating that the sample has reached its absorption edge. This pattern is characteristic of substances with high UV absorption, which could indicate the presence of conjugated systems or other chromophoric groups which includes proteins, metal chelates, and melanin, which contribute to its potent UV-absorbing capabilities and photoprotective role, is consistent with this spectral behavior.

### FTIR-Fourier Transform Infrared Spectroscopy Analysis

Figure 4 shows suggestive of bioactive substances that are responsible for their antibacterial and antifungal activity. The existence of hydroxyl groups from phenolic compounds and amine groups commonly present in proteins and melanin is confirmed by a large absorption band at about 3400 $cm^{-1}$, which corresponds to O-H and N-H stretching vibrations. Peaks seen between 2920 and 2850 $cm^{-1}$ are ascribed to aliphatic chains ranging from C to H, indicating the presence of lipids and fatty acids, which are known to disrupt microbial membranes. A prominent peak in the 1740-1650 $cm^{-1}$ range denotes C=O stretching vibrations, which are linked to carbonyl groups from peptide bonds, carboxylic acids, or ketones-essential building blocks of bioactive compounds (Karpagam *et al.*, 2019). Furthermore, the C=C

**Figure 1:** Fresh Squid sample (Loligo duvauceli).

**Table 1: Phytochemical screening of squid (*L. duvauceli*) ink powder.**

| Sl. No. | Phytochemical constituents | Aqueous | Methanol | Ethanol | Acetone | Chloroform |
|---|---|---|---|---|---|---|
| 1 | Alkaloids | + | + | + | - | - |
| 2 | Flavonoids | - | - | + | - | - |
| 3 | Carbohydrates | + | + | + | + | - |
| 4 | Proteins and Amino Acids | + | + | + | - | + |
| 5 | Phenols | - | + | + | - | - |
| 6 | Tannins | + | + | - | + | + |

**Figure 2:** Extracted Squid ink powder (*L. duvauceli*).

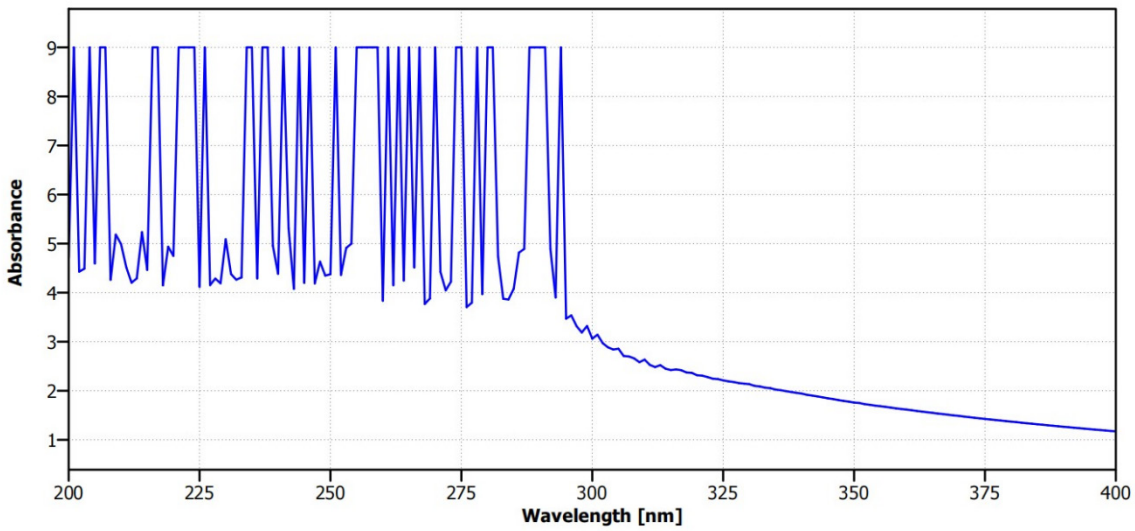


**Figure 3:** UV-vis Spectrum of squid (*L. duvauceli*) ink powder.

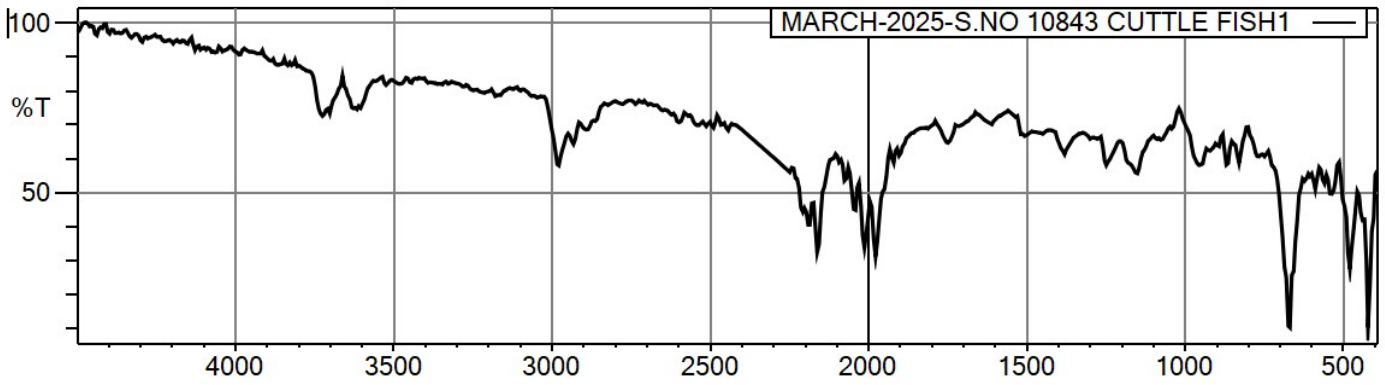


**Figure 4:** FTIR spectrum of squid (*L. duvauceli*) ink powder.

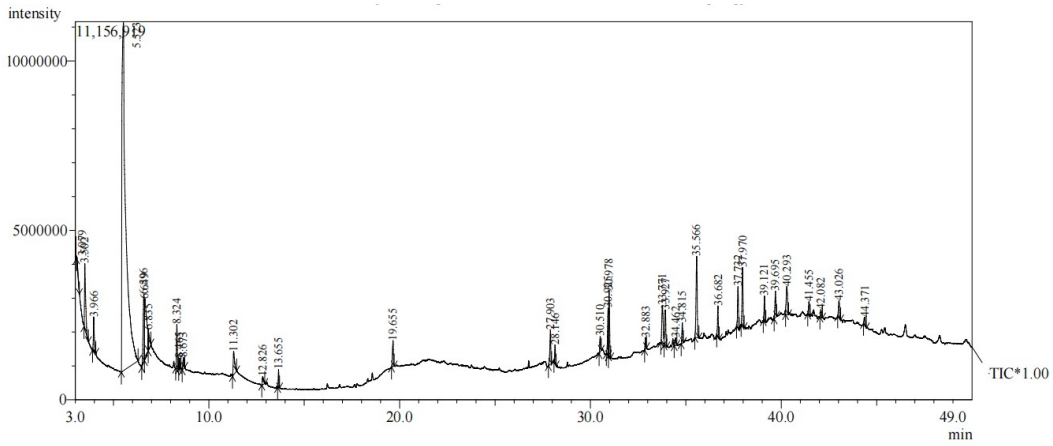


**Figure 5:** GC-MS analysis of squid (L. duvauceli) ink powder.

stretching or amide II bands, which indicate aromatic structures or protein content, match to the band approximately 1600-1500 $cm^{-1}$. While peaks in the 600-900 $cm^{-1}$ range are suggestive of aromatic ring vibrations, which are frequently linked to phenolic and quinonoid compounds, absorptions between 1100 and 1000 $cm^{-1}$ are linked to C-O and C-N stretching, indicating the presence of alcohols, ethers, and alkaloids. All of these functional groups work together to verify that the powdered squid (*L. duvauceli*) ink contains a complex matrix of bioactive compounds via various mechanisms, including oxidative stress induction, enzyme inhibition, and membrane disruption.

## GC-MS Analysis

The GC-MS analysis (Figure 5) of the sample revealed a rich diversity of bioactive compounds indicative of phytochemical potential. The predominant component identified was Trimethyl phosphate, with a retention time of 5.52 min, contributing approximately 55.15% of the total peak area, indicating its dominant presence in the sample. Compounds such as 2,4-Dihydroxy-3-methylbenzaldehyde (2TMS derivative) and Violaceol I (4TMS derivative) are notable for their phenolic and quinonoid structures, which are well-documented for their antimicrobial and antioxidant properties (Pralea *et al.*, 2019).

## Anti-Bacterial Activity

Antibacterial activity of squid (*L. duvauceli*) ink powder was demonstrated by the zone of inhibition around the wells in the petri plates, where the squid ink extract was added. A clear zone of inhibition was observed against *E. coli* and *S. aureus*. The clear zone of inhibition was found to be 22±1.2 mm and 25±2.4 mm against *E. coli* and *S. aureus*, respectively. The melanin, peptides, and quinonoid compounds present as bioactive substances in squid ink were responsible for having strong antibacterial properties.

# DISCUSSION

*Loligo duvauceli* ink powder was phytochemically screened, and the results showed a rich presence of bioactive chemicals in several solvent extracts, with polar solvents exhibiting noticeably increased extractability (Sukmiwati *et al.*, 2023; Kamyab *et al.*, 2024). The majority of solvents contained alkaloids, proteins, carbohydrates, amino acids, and tannins, demonstrating their strong polarity and solubility in alcoholic and aqueous conditions (Sukmiwati *et al.*, 2023; Liu *et al.*, 2023). While the total lack of phytochemicals like glycosides, phytosterols, and fixed oils indicates their limited or non-extractable character in squid ink powder, the presence of flavonoids only in ethanol suggests that medium-polar solvents are more effective for extracting

these compounds (Sukmiwati *et al.*, 2023; Martinez *et al.*, 2024). Saponins were also detected in aqueous extracts, supporting the traditional use of squid ink for its immunomodulatory and antibacterial qualities. Overall, the findings suggest the presence of a variety of biologically active substances with polarity in squid ink (Kamyab *et al.*, 2024; Khandelwal *et al.*, 2023), indicating its potential therapeutic utility and encouraging further pharmacological and biochemical research.

## CONCLUSION

The present research highlights the varied bioactive potential of (*L. duvauceli*) ink powder through comprehensive phytochemical, spectroscopic (UV-vis, FTIR), chromatographic (GC-MS), and antimicrobial investigations. Numerous secondary metabolites with established biological and therapeutic properties, including proteins, tannins, phenols, and alkaloids, were identified through phytochemical screening. The presence of proteins, melanin, and other chromophoric compounds was confirmed by UV-vis and FTIR investigations, which also revealed distinctive absorption patterns and functional groups. Trimethyl phosphate was shown to be the predominant constituent by GC-MS profiling, along with several phenolic and quinonoid derivatives, confirming the bioactive substance of the squid (*L. duvauceli*) ink powder. Additionally, the antibacterial tests showed notable inhibitory effects against *S. aureus* and *E. coli*, highlighting the squid (*L. duvauceli*) ink powder ink's encouraging antimicrobial activities. These results provide a solid scientific foundation for the potential use of squid ink in the food, pharmaceutical, and nutraceutical industries in addition to confirming its traditional medical properties. Other marine-derived cephalopods investigations were required further research in order to open up new therapeutic opportunities based on natural products.

## ACKNOWLEDGEMENT

The authors would like to thank the Management of PSG College of Arts & Science, Coimbatore - 641 014, for providing infrastructure support and the Central Research Laboratory (CRL), PSG College of Arts & Science, Coimbatore - 641 014 for GC-MS analysis and results interpretation.

## ABBREVIATIONS

**FTIR:** Fourier Transform Infrared Spectroscopy; **UV-vis:** Ultraviolet-visible; **GC-MS:** Gas Chromatography-Mass Spectrometry; **NIST:** National Institute of Standards and Technology; **TMS:** Trimethylsilyl; **ID:** Internal Diameter; **DF:** Film Thickness: **RH:** Running Head; ***E. coli*:** *Escherichia coli*; ***S. aureus*:** *Staphylococcus aureus*; **SD:** Standard Deviation; **SPSS:** Statistical Package for the Social Sciences; **UV:** Ultraviolet; **ORCID:** Open Researcher and Contributor ID.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## FUNDING

This work is a part of research work under PSG Institutional Seed Grant (Ref No: PSGCAS/IRSG/2024-2025/CDF/029) and the authors would like to thank PSG College of Arts & Science, Coimbatore for providing Institutional Seed Grant.

## AUTHOR CONTRIBUTIONS

S Ragavi and P Karpagam contributed to the study conception and design, Manuscript preparation, data collection, and analysis. Manuscript draft correction, GC-MS analysis and interpretation was performed by K M Sakthivel and K Pavithra.

## SUMMARY

This study assessed the bioactive potential of *Loligo duvauceli* squid ink powder using phytochemical, spectroscopic, chromatographic, and antibacterial studies. Ink extracted from squid was tested for their phytochemical screening, which revealed the presence of alkaloids, proteins, amino acids, carbohydrates, tannins, saponins, and phenolic chemicals, with polar solvents showing increased extractability. UV-vis spectroscopy revealed high absorption in the 200-300 nm range, which is typical of melanin and conjugated chromophores, while FTIR analysis identified functional groups associated with proteins, phenolics, lipids, and quinonoid chemicals. Trimethyl phosphate was found as the main compound using GC-MS profiling, coupled with phenolic and quinonoid derivatives known to have antibacterial action. The squid ink extract had substantial antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, as demonstrated by distinct zones of inhibition. Overall, the findings indicate *L. duvauceli* ink powder as a rich source of bioactive chemicals with excellent antibacterial properties, indicating potential uses in the pharmaceutical, nutraceutical, and functional food industries.

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**Cite this article:** Ragavi S, Karpagam P, Sakthivel KM, Pavithra K. Characterization and Therapeutic Potential of Marine-Derived *Loligo duvauceli* Ink Powder. Pharmacog Res. 2026;18(3):863-9.