

January - June 2026

Volume 35

Issue 1

PRINT ISSN: 2277-1867

ONLINE ISSN: 2277-8853



JOURNAL OF FORENSIC MEDICINE SCIENCE AND LAW

Official Publication of Medicolegal Association of Maharashtra

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**MULTISPECIALITY, MULTIDISCIPLINARY, NATIONAL
PEER REVIEWED, OPEN ACCESS, MLAM (SOCIETY) JOURNAL
Indexed with Scopus (Elsevier)**

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JOURNAL OF FORENSIC MEDICINE SCIENCE AND LAW

(Official Publication of Medicolegal Association of Maharashtra)

Email.id: mlameditor@gmail.com

PRINT ISSN:

2277-1867

ONLINE ISSN:

2277-8853

Case Series

Preliminary Thin Layer Chromatography Analysis of *Cerbera Odollam* in Oral Fluid

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Article Info

Received on: 27.11.2025

Accepted on: 08.04.2026

Key words

TLC,
Cerbera odollam,
oral fluid,
Solvent system,
Forensic.

Abstract

The detection and recovery of toxic compounds is a critical aspect of **forensic toxicology**. This study investigates the use of Thin Layer Chromatography (TLC) for the detection of toxic compounds in the *Cerbera odollam* plant, known for its traditional use in various medicinal applications but also associated with potential poisoning incidents. We aimed to isolate and identify the phytochemicals in saliva, a vital matrix for assessing poisoning cases. Oral fluid samples were collected from 20 healthy individuals and prepared using appropriate extraction techniques, followed by **TLC analysis** with optimized solvent systems. This study underscores the importance of TLC as a rapid screening tool in toxicological assessments, offering valuable insights for further investigations into the safety and pharmacological properties of *Cerebra odollam*. The developed chromatograms were analyzed for specific retention factors (R_f values) ranging between 0.3-0.5, enabling the identification of compounds with potential toxicological significance. The results demonstrate that TLC is a valuable tool for the rapid screening of saliva in forensic toxicology. Our findings also highlights the potential of using **saliva** as a reliable biological matrix for the detection of poisons, paving the way for further research into its application in toxicological screenings.

1. Introduction

Human contact with potentially toxic plants, which may occur through abuse or by accident or attempted suicide, is frequent and sometimes results in clinically significant toxicity. Plants have evolved the strategy to produce bioactive natural products as a means of defense. Some plants produce toxins that can severely damage or kill a human.¹

Despite intensified efforts; intoxication by toxins is still significant threat to public health. In forensic toxicology, plant poison detection is

essential for solving criminal cases involving poisoning.² A diverse range of plants contain toxic compounds, including alkaloids, glycosides, and proteins, many of which can be lethal even in small amounts. Detecting and identifying these poisons in biological samples (blood, urine, tissues) is a critical aspect of forensic investigations.³

Forensic toxicology involves the detection, identification, and quantification of drugs, poisons, and other chemical substances in biological specimens to aid in legal investigations.⁴ Saliva has

How to cite this article: Vijayan A, Philip RS. Preliminary Thin Layer Chromatography Analysis of *Cerbera Odollam* in Oral Fluid. J Forensic Med Sci Law. 2026;35(1):39-43. doi: [10.59988/jfmsl.vol.35issue1.8](https://doi.org/10.59988/jfmsl.vol.35issue1.8)

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emerged as an important biological matrix in forensic toxicology due to its unique advantages, ease of collection, and relevance in reflecting the presence of various substances in the human body. Saliva, a clear, slightly alkaline fluid secreted by salivary glands, plays a crucial role in oral health and digestion.⁵ Beyond its physiological functions, saliva serves as a valuable biological specimen in forensic science due to its ability to mirror the free (non-protein-bound) concentrations of substances present in blood plasma. One of the most significant advantages of using saliva is the non-invasive nature of its collection.⁶ Saliva primarily reflects recent substance use, with drugs being detectable for a shorter duration than in urine or hair. This correlation arises from passive diffusion, making saliva a suitable alternative to more invasive samples like blood and urine.⁷

Cerbera odollam, often called the "suicide tree," is infamous for its potent toxicity. Its seeds contain cerebrin, a cardiac glycoside that can cause fatal heart arrhythmias.⁸ In India, this tree's toxicity has historical, cultural, and forensic significance. The widespread presence of *Cerbera odollam* in coastal and tropical regions of the country has made it both accessible and frequently implicated in cases of poisoning, particularly suicides and homicides. The tree grows abundantly in the coastal states of Kerala, Tamil Nadu, and Karnataka, and is also found in other tropical regions. In Kerala, where it is colloquially known as "odollam," the plant is often planted as a natural fence due to its dense foliage and salt tolerance.⁹ However, its proximity to human settlements also increases the risk of its seeds being used for self-harm or harm toward others. India records a high incidence of poisoning cases, with *Cerbera odollam* playing a notable role in the southern states. Its availability, coupled with the lack of awareness about its toxicity, contributes to its frequent use in poisoning incidents. Homicidal use of the seeds has also been documented. The ease of concealing the seeds in food and the relatively delayed onset of symptoms make it an attractive option for malicious intent.¹⁰

Cases of poisoning are frequent in our country therefore toxicological analysis aims in identifying the toxic principles in the biological fluids. Thin Layer Chromatography (TLC) offers a fast, reliable, and cost-effective method for the detection and identification of plant-derived toxins. Saliva as a biological fluid is gaining popularity amongst researchers especially in

the field of forensic science. It is a complex biological fluid medium of choice for all forensic and criminal investigations because of its ease of collection and safety in handling. They reveal the presence of recent toxic alkaloids and free alkaloid concentration in the plasma. In this study, we have emphasized on the role of saliva in toxicological analysis of *Cerbera odollam* poisoning.¹¹

2. Materials and methods

Samples

Cerbera odollam fruits were collected from the Kottayam district in Kerala. The samples were shade-dried and stored in a dark, low-moisture environment.

Extraction of seed extract

A total of 12 g of ripe fruit was collected and extracted with one liter of boiling water, then left to cool to room temperature. The aqueous extract was combined with isobutyl alcohol to induce aqueous phase separation. The butanol phase was isolated by centrifugation at 3000 rpm for 30 minutes. The resulting extract was dried, dissolved in a small amount of methanol, and subjected to TLC analysis.¹²

Oral fluid sample preparation

Oral fluid was spiked with the seed extract and subjected to the same extraction procedures mentioned in the above. Twenty healthy saliva samples from the anonymous donors within the age group of 20-35 years with due approval of the institutional ethical committee. (Ethics clearance approval – PHD/EC/SC/F/008-AUG2022)

Chromatographic Isolation Techniques

Pre-prepared TLC plates were utilized for the analysis. The fruit extract and saliva samples were applied along the baseline of the plates using a fine capillary tubes, which were subsequently placed in a TLC chamber for development using various solvent systems. Five runs for each solvent system was done to obtain a standard mean.¹²

Solvent systems

Solvent system used in this study is mentioned in the below **table- 1**

Table 1: Various solvents were tested to optimize the separation process.

Mobile phase	Ratio
Butanol: Acetic Acid: Water	5:1:1
Dichloromethane: Ethylacetate	5:1
Chloroform: Methanol	9:1
Chloroform: Methanol: Water	9:0.5:1
Methanol: n-Hexane	8:2
Toluene : Ethyl Acetate : Formic Acid	5:4:1
Ethyl Acetate : Methanol : Water	10:2.5:1

Methanol : Ammonia	9:1
Ethyl Acetate : Methanol : Water	8:0.5:8
Toluene : Acetic Acid: Formic Acid	5:4:1
Butanol : Ammonia: Water	4:1:5
Chloroform : Acetone : Water	6:3:1

Visualization

The TLC plate was developed until three-quarters of its length, then removed and dried at room temperature. Visualization was performed using iodine vapors and UV-VIS chamber.

3. Results

Percentage of yield extracts

To evaluate the effectiveness of the extraction process and to demonstrate that the method can be consistently reproduced, the percentage yield of plant extract was determined by dividing the weight of the dried crude extract by the weight of the powdered plant material before extraction, and then expressing the result as a percentage. The percentage yield provides a quantitative measure of the effectiveness of the solvent used in the extraction process, and of the performance of the extraction itself. With respect to thin-layer chromatography (TLC), the percentage yield must be determined prior to the application of the extract onto the TLC plate in order to standardize the concentration of the extract (**Table 2,3**). This proper standardization of extracts will produce uniform spot intensity on the TLC plate, and improve the resolution of the phytochemical constituents, thereby allowing for valid qualitative comparisons of the chemical profiles of samples.

Table 2: Standardize the concentration of the extract on Thin Layer Chromatography (TLC).

Color of the extract	Yield of the extract (in gram)	Percentage yield (%w/w)
Pale yellowish	3	25%

Table 3: Experimental condition

Saturation time	15 min
Run time	10 min
Temperature	38 °C

In this study, spots obtained using different mobile phases were carefully recorded and mentioned in **table-3 & 4**, and their corresponding retention factor (Rf) values were calculated. This step was crucial in determining the efficiency of each solvent system in separating the target compounds. Multiple mobile phases were tested to optimize separation, with each solvent system tailored to the chemical nature of the compounds, such as glycosides, alkaloids, or phenolics.

The observation and documentation of distinct spots ensured that the compounds were effectively

separated, with clear differentiation between closely related substances. The use of visualizing agents like iodine vapor and UV-VIS fluorescence enhanced the clarity and detection of these spots, even for compounds with low concentrations. Several solvents were tested in order to optimize the extraction conditions: by adjusting the solvent ratio of **chloroform, methanol, and water** offered a unique balance of polarity and solubility making it highly effective in separating the compounds.

Recording the Rf values for each compound allowed for comparisons across different mobile phases, facilitating the selection of the most effective solvent system. The Rf value obtained ranges from 0.3-0.5.¹² This systematic approach ensured reproducibility and provided valuable data for the identification and characterization of the analytes.

Table 3: Results obtained for Cerebra Odollam extract

S. no	Mobile phase	Rf value (Mean value)	Observation
1	Butanol: Acetic Acid: Water	0	No movement of spots
2	Chloroform : Acetone : Water	0	
3	Butanol : Ammonia: Water	0	
4	Toluene : Ethyl Acetate : Formic Acid	0	
5	Dichloromethane: Ethylacetate	0	Spots migrated tailing
6	Chloroform: Methanol	0	
7	Toluene : Ethyl Acetate : Formic Acid	0	
8	Toluene : Acetic Acid: Formic Acid	0	
9	Methanol: n-Hexane	0	Spots moved along the solvent
10	Methanol : Ammonia	0	
11	Ethyl Acetate : Methanol : Water	0	
12	Chloroform: Methanol: Water	0.5	Spots migrated cleanly without any tailing

Table 4: Results obtained for oral fluid sample spiked with Cerebra Odollam

Sl no	Mobile phase	Rf value (Mean value)	Observation
1	Chloroform: Methanol: Water	0.5	Spots migrated cleanly without any tailing

4. Discussion

In the current study of *Cerebra odollam* (known as the suicide tree), the results are similar to those of previous research on the toxicology and phytochemistry of this plant, particularly in the chromatographic analysis of the cardiac glycoside cerberin. Furthermore, many investigators have utilized other preliminary analytical procedures, such as thin layer chromatography (TLC), in the

identification of toxins derived from plant sources and have indicated that when sophisticated instrumental methods are not available, they can be used as a screening technique for many types of toxins, including plant derived toxins. Similarly, in this study, the bioactive components isolated from the extracts of *C. odollam* were separated and produced unique Rf values which can be used as probable indicators of toxicity. Similar investigations have reported requiring TLC to identify cardiac glycosides present in plant materials, and while the ability of those studies to detect total cardiac glycoside concentrations using TLC may not have been extremely sensitive, they did indicate the utility of TLC as a means to conduct a preliminary toxicological screening.¹³ Additionally, the extraction efficiency and chromatographic behavior of the extracts from *C. odollam* were consistent with data previously reported regarding the influence of solvent polarity on the isolation of cerberin and other related components of a similar chemical structure.

Detecting plant-based toxin from a plant species, such as *Cerbera odollam*, in an individual's saliva creates important forensic implications, particularly because of the difficulty of identifying a plant-based poison postmortem. *Cerbera odollam*, as it undergoes rapid metabolism, contains the toxic chemical cerberin, which does not remain present in an individual's blood or tissues for long after death and therefore cannot be detected in those biological specimens. Saliva can provide a viable alternative specimen to conduct early detection of cerberin or its metabolites in postmortem testing by forensic investigators during cases of suspected suicide or homicide poisoning.^{14,15} These factors make saliva sampling advantageous when conducting field forensic testing and mass screening since saliva does not necessarily require a medically trained professional to obtain a sample. The interplay between toxicology and legal systems has become increasingly vital due to the rising complexity of environmental, pharmaceutical, and industrial challenges.¹⁶⁻¹⁹

There are multiple limitations associated with TLC when compared to advanced analytical methods such as HPLC and LCMS in regards to the ability to identify the toxins in *Cerbera odollam*.²⁰ The most significant disadvantage of TLC is that it has a low sensitivity, which makes the detection of cerberin at trace levels in biological specimens, such as saliva where the concentrations of the analytes are typically

very low, very difficult. Conversely, HPLC has higher resolution and sensitivity and provides much more accurate separation and quantitation of compounds. In addition to the higher resolution separation and sensitivity of HPLC versus TLC, LCMS provides further enhancement of the analytical capabilities by providing information regarding the molecular weight of the analytes and will provide some structural information about the analytes being analyzed. In addition, LCMS will provide a clear identification of the toxins being analyzed, even when they are in a complex matrix. Another disadvantage of TLC is that the detection methods used to identify the analyte include visual detection (i.e., UV light and/or chemical reagents) methods which may not be specific and can lead to a misinterpretation of the results. Another limitation of TLC is that the reproducibility of the TLC method is poor because factors including but not limited to plate quality, solvent composition and environmental conditions can influence Rf values. In contrast, the HPLC and LCMS are very reproducible and can be automated making them more appropriate for confirmatory analysis in a forensic laboratory.²¹

5. Conclusion

The developed TLC technique has demonstrated significant potential in effectively analyzing and identifying compounds in both raw plant samples and biological fluids such as oral fluid. This advancement represents a major step forward in simplifying the detection and analysis of bioactive compounds, including plant poisons, with applications in toxicology, pharmacology, and forensic investigations.

For biological fluids such as oral fluid, TLC offers a non-invasive and cost-effective method to detect the presence of plant toxins and other compounds. Oral fluid, being a readily accessible bio-fluid, can be an excellent medium for forensic and toxicological studies. The developed TLC technique has demonstrated its capability to separate and identify compounds present in saliva samples with minimal preprocessing. This is particularly significant in forensic investigations, where rapid and reliable detection of toxins can provide crucial evidence. The adaptability of TLC to analyze small volumes of oral fluid without extensive sample preparation enhances its practicality in resource-limited settings. The portability and low cost of TLC equipment further extend its applications to field studies and on-site analysis.

In conclusion, the developed TLC technique has shown promising results in separating and identifying compounds in raw plant samples and biological fluids like saliva. Its cost-effectiveness, simplicity, and adaptability make it a powerful tool for research and diagnostic purposes. As further advancements in TLC technology emerge, its applications are expected to expand, offering even greater accuracy, sensitivity, and efficiency in chemical analysis.

Future research

Future advancements in TLC include incorporating advanced detection methods, such as fluorescence detection, to enhance sensitivity, and adopting automated systems to accelerate analysis. Moreover, combining TLC with hybrid techniques like HPLC and UHPLC offers more comprehensive analysis and improved identification of plant toxins.

Contributor ship of Author: All authors equally contributed. **Ethical Clearance:** Yes.

Conflict of interest: None to declare.

Source of funding: None to declare.

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