Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
www.plantsjournal.com
JMPS 2024; 12(6): 19-22

Received: 24-09-2024
Accepted: 21-10-2024

Pratibha Thakre
Department of Botany, Faculty of Science, Rabindranath Tagore University, Bhopal, Madhya Pradesh, India

Prachi Tadge
Advanced Materials Research Centre, Rabindranath Tagore University, Bhopal, Madhya Pradesh, India

Suchi Modi
Department of Botany, Faculty of Science, Rabindranath Tagore University, Bhopal, Madhya Pradesh, India

Sudeshna Ray
Advanced Materials Research Centre, Rabindranath Tagore University, Bhopal, Madhya Pradesh, India

Corresponding Author:
Sudeshna Ray
Advanced Materials Research Centre, Rabindranath Tagore University, Bhopal, Madhya Pradesh, India

Investigation of alkaloid's in hemlock by thin layer chromatography for forensic significance

Pratibha Thakre, Prachi Tadge, Suchi Modi and Sudeshna Ray

DOI: https://doi.org/10.22271/plants.2024.v12.i6a.1761

Abstract
Medicinal plants are a two-edged sword that might be used as a medicine for several diseases as well as deadly poisonous substances to commit murder or suicide when administered in high doses. In order to provide the self-defense against threats, a number of toxins from the medicinal plants are generated which are the produced secondary metabolites such as 'alkaloids', glycosides, 'saponins', 'resinoids'. Importantly, plants having toxic properties may be used as biological weapons and have their significant application in 'Forensic Botany, which is composed of the scientific use of plant materials to help to solve crimes and to gain information regarding possible crimes. This paper reports the investigation of phytochemicals of 'Hemlock' leaves and flowers using 'Thin layer chromatography' (TLC). A 'Soxhlet' extractor was used to extract the hemlock leaves and flowers for TLC. Using a solvent system consisting of Glacial acetic acid: Acetone in a ratio of 10:90 and Ethyl acetate: Methanol: Water in a ratio of 50:10:40, a qualitative analysis of the extracts of Hemlock leaves and flowers was carried out via TLC. The TLC chromatogram was obtained displaying the Rf values of the generated content in the crude leaves and crude flowers. Interestingly, TLC based finding substantiates the existence of the lethal alkaloid 'Coniine' in both Hemlock leaves and flowers collected for this research.

Keywords: *Conium maculatum* L. (Poison hemlock), TLC plates, alkaloids, coniine

1. Introduction
Conium maculatum L. (poison hemlock) is a perennial, herbaceous, flowering plant belonging to the family Apiaceae, quite poisonous, common in many parts of India. Some plants are poisonous in nature but play an important role in healing also [1-3]. Hemlock juice or extract has been accused of being used for criminal purposes and is a deadly poison that the Greek philosopher Socrates was sentenced to drink (399 BC). Hemlock is most poisonous in the early stages of spring growth but is dangerous in all stages of growth [4, 5]. Toxins in hemlock have been implicated in death by wild birds that eat hemlock seeds. Hemlock is native to Europe and western Asia and was introduced to North America as an ornamental plant. Hemlock often grows near fences, roads, streams, construction sites, farms, crops, and fields, where it can be mistaken for harmless plants. Such extracts are used both as sedatives and antispasmodic. The most poisonous of these is *Conium maculatum*, an annual or biennial plant, which is an annual plant under suitable conditions. *Conium maculatum* is characterized by many secondary metabolites such as alkaloids, phenols, steroids. *Conium maculatum* has been used as a traditional medicine and its extracts have been shown to have anti-allergic and anti-inflammatory properties [6]. This effect can be attributed to the presence of bioactive anti-inflammatory and analgesic compounds. The bitter juice of the plant is mixed with the seeds of stachys (Stachys officinalis) and fennel (Foeniculum vulgare) against the bites of rabid dogs. In later periods of history, grape juice was used as a last resort as an antidote to strychnine and other poisons. In the 15th and 16th centuries, religious orders used the roasted root to relieve gout pain. Conium is still a classic homeopathic medicine with many uses. It is said to be a long-term treatment and is especially beneficial to those with weakened vital organs in old age. It has been used in cancer treatment. Therefore, it is necessary to isolate, characterize and evaluate the biological activity of aluminum to facilitate drug development. Analytical methods have attracted great attention for several years because they play an important role in drug discovery, development and production. Traditional methods for the study of natural products include the classification of compounds, the separation and separation of individual products using column chromatography and elucidation techniques using various spectroscopic methods.

For further analysis, it is necessary to isolate and separate bioactive compounds. After drying, the toxicity of plant material is greatly reduced which means that toxic substances may change or become unstable [7].

Ten volatile alkaloids have been identified till date, among which the most abundant are trypanine and coneconine, which are responsible for most of the serious and chronic diseases in plants [8-10]. Symptoms of hemlock poisoning include first irritation of the extremities and central nervous system, then paralysis and depression, slow movement, strength, then rapid pulse, hyperventilation, urination, and finally coma and death. Structure → activity (Toxicity, teratogenic effects) relationship studies of *C. maculatum* alkaloids show that the side chain of the piperidine moiety must have at least one propyl group in order to be effective [11]. Recently, the analgesic effect of theobromine has also been studied in thermal and chemical pain models and the results of two experiments showed that theobromine has an antinociceptive effect. It has been reported that the analgesic effect of morphine is provided by conidine, while the effect of conidine is inhibited by the nicotinic receptor blocker mecamylamine [12]. The medicinal value of hemlock (i.e. immature *C. maculatum* seeds are dried and stored for use as antiseptic, disinfectant or antiseptic is very limited because therapeutic and toxic effects are very close [13, 14]. Previous studies have shown that all tissues of *C. maculatum* are rich in alkaloids. They are more abundant in the fruits with 1% (w/w) of all alkaloids [15]. This study varies depending on the ecological conditions and phenological developmental stages [16]. However, most of the studies on the elucidation of alkaloids (Now considered classical and probably outdated) were performed in the 1950s and 1960s, until the end of biosynthetic research in the 1970s [16, 17]. It has been observed that the illegal consumption or illegal use of phytotoxic substances is increasing;

Recently, the use of chemical signatures has facilitated the detection of poisonous plants in forensic medicine [18-20]. This concept leads to the conclusion that medicinal plants play an important role in human life due to their biological properties. 'Toxicology' is the basic science that studies poisons. It is generally believed that serious injury or death occurs as a result of the physical and biochemical interaction of the poison with living tissue. Phytotoxicity is an ever-expanding field based on technology and assessment methods, research strategies, scientific understanding and strategic analysis. Lack of understanding and forensic skills in the investigation of poisonous plants is responsible for the large epidemic in poor countries. Poisoning from plant substances is the least studied and is poorly understood. Botanical evidence is less used in forensic investigations due to the lack of botanical knowledge and scientific standards of those involved in illicit clinical, clinical and forensic investigations.

On the other hand, phytotoxic chemistry and biology experts and forensic medicine experts have different perspective and applications. To date, there is very little research and information on poisonous plants. Primary metabolism produces the most important biomolecules (Lipids, carbohydrates, and proteins) necessary for plant growth and expansion, while secondary metabolism produces various products called secondary metabolites (SM), which are involved in defense mechanisms and communication with the environment. SM contributes to the plant's odor, color, and aroma, as well as its resistance to external factors. SM is responsible for biological defense. Drought, temperature, salinity, and UV stress are all variables that can be reduced by SM. Other metabolic pathways of SM include the shikimate pathway to produce phenolic compounds, the mevalonate pathway to produce terpenes, and the Krebs cycle pathway to produce nitrogenous molecules.

Phytotoxic substances are generally produced by enzyme-catalyzed secondary metabolic pathways involving primary metabolites such as sugars and amino acids [21-23]. These SMs are mainly used to protect insects and animals. Some of these antibiotics, such as cyanogenic glycosides, alkaloids or phenols, have a bitter taste, which is a warning to animals and poisonous plants. Animals often eat dead plants and die from pyrrolizidine alkaloid poisoning. In recent years, medicinal plants have proliferated as people have become more aware of their protective and preservative properties. However, the number of deaths from medicinal plants has increased in recent years, probably due to improper use of the plants and their associated products.

2. Materials and Methods

2.1 Collection of plant material

Fresh leaves and flowers of Hemlock plant were collected from Raisen district near Bhopal city of Madhya Pradesh. The images of fresh leaves and flowers are shown in Fig. 1. The leaves are needle-like with flat appearance and rounded tips. The white flowers are having small erect clusters. In this study, fresh leaves and flowers were washed to remove the dirt and then stored in aluminum foil at 40 °C.

Fig 1: Images of Hemlock leaves and flowers

Extraction of Hemlock leaves and flowers were carried out as shown in Fig. 2. The extraction steps are as follows [23]:

- **Step 1:** The plants were shade dried for one week and pulverized in pulverize.
- **Step 2:** This dried powder was packed in filter paper thimble and kept in Soxhlet apparatus, below which, round bottom flask containing chloroform (Ranbaxy Fine Chemicals, New Delhi, India) placed and set in heating mantle.
- **Step 3:** The temperature was set to 80-110 °C.
- **Step 4:** The extract is obtained by concentrating to one fourth of its original volume under reduced pressure.

Fig 2: Extraction of Hemlock leaves and flowers for TLC

3. Results and Discussion

3.1 TLC of plant extracts

TLC plates were cut into strips. A straight line was drawn with a pencil to mark the chloroform extract dissolved in chloroform and petroleum ether and the solvent produced on the edge is also signature with a straight line. The chromatography chamber is 1 cm below the source and contains a saturated solvent mixture containing ethyl acetate: methanol: water in a ratio of 50: 10: 40. Then the chloroform was extracted to the source, the bands were placed in the chromatographic chamber and allowed to run. The solvent rises along the plate with the help of capillary action until it reaches the edge of the solvent. Some compounds have a better inclination for silica, while others exhibit a better affinity for solvents. Due to the difference between the two, they started to move to the end of the difference. The strips were then collected from the chamber, dried and sprayed with methanol and sulfuric acid. The strips were kept in a hot oven and the position of the mobile phase was marked. The chloroform extract was spotted on the origin this TLC plate and was put in chromatographic chamber and allowed to run. The solvent travelled up the plate by capillary action till it reached the solvent front. The TLC plate was taken out from the developing chamber and visualized under UV lamp (TLC detector) as shown in Fig. 3.

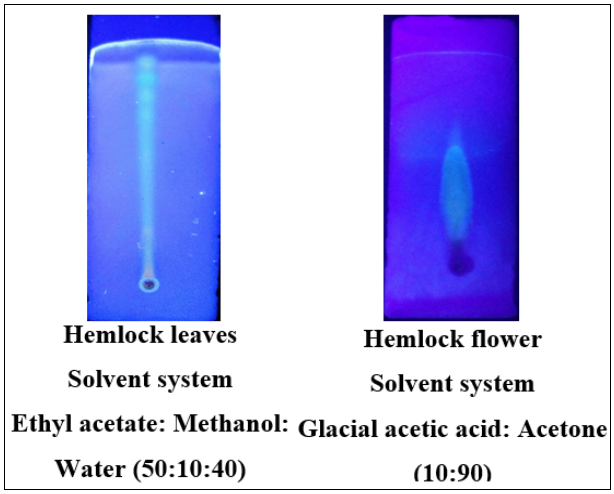

Fig 3: Chromatography of the extracts of Hemlock leaves and flowers

The retention factor (Rf) value for all elements is determined by the following formula:

Retention Factor = Distance traveled by the extract / Distance traveled by the solvent. Rf values for Hemlock leaves was found to be 0.97 and Rf values for Hemlock flower was 0.42.

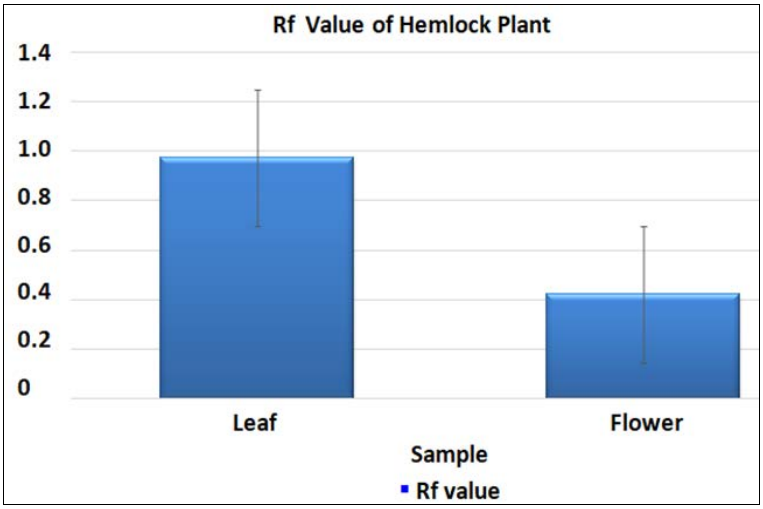

Fig 4: Comparison of the Rf values of the extracts of Hemlock leaves and flowers

Viscera sample of a Hemlock victim is also analyzed for alkaloid analysis by TLC to get the key forensic evidence. Qualitative analysis of Hemlock leaves and flowers has been carried out by TLC (Thin layer chromatography) using Ethyl acetate: Methanol: Water in a ratio of 50:10:40 for Hemlock leaves and Glacial acetic acid: Acetone (10:90) for Hemlock flowers.

The extract shows the presence of 'coniine'. Coniine is most lethal component found in hemlock (*Conium maculatum*). Rf value of produced content 0.97 in crude of leaves and Rf value of produced content was 0.42 in produced crude of flowers. The Rf value is a ratio that shows the relative speed of a moving point compared to the distance travelled by the eluent. Rf of 0.97 means that the spot has moved 97% more with respect to the distance travelled by the eluent. Rf of 0.42 indicates that the spot has moved 42% more with respect to the distance travelled by the eluent. This method is an excellent method for assessing the amount of major alkaloids. The TLC chromatogram obtained and the calculation of Rf values [0.97 for Hemlock leaves and 0.42 for Hemlock flowers] were carried out and the results were matched with the standard value. The extract shows the presence of 'coniine' [24, 25]. Coniine is most deadly component found in Hemlock (*Conium maculatum*).

4. Conclusion

In this paper, we detail about the collection of fresh Hemlock leaves and flowers from Raisen district. After the collection, Hemlocks leaves and flowers underwent extraction for TLC using a Soxhlet extractor. A qualitative analysis of the extracts of Hemlock leaves and flowers was conducted via TLC utilizing a solvent system comprised of Ethyl acetate: Methanol: Water in a ratio of 50:10:40 and Glacial acetic acid: Acetone (10:90). The TLC chromatogram was acquired. From the chromatogram, the Rf value of the produced content in the crude leaves was found to be 0.97 and the Rf value of the produced content in the crude flower was observed to be 0.42. The obtained results were compared with the standard value of 0.9. The analysis revealed the presence of 'Coniine', the most lethal compound in Hemlock (*Conium maculatum*). This finding substantiates the existence of the lethal alkaloid coniine in both Hemlock leaves and flowers collected for this

research. The study also authenticates the use of TLC in identifying toxic alkaloids in Hemlock extracts for the application in 'Forensic Botany'.

References

1. Greval SD. Lyon's Medical Jurisprudence for India. 10th ed. India: Law Publishers (India) Pvt. Ltd; c1953.
2. Narayan RKS. Medical Jurisprudence and Toxicology (Law Practice & Procedure). India: ALT Publications; c2005.
3. Begum SB, Mastan M. Phytochemical screening, chromatographic analysis of chloroform extract of *Conium maculatum*. Int Res J Biol Sci. 2015;4:27-29.
4. Khajja BS, Sharma M, Singh R, *et al*. Forensic study of Indian toxicological plants as botanical weapons: A review. J Environ Anal Toxicol. 2011;1:112.
5. Murari A, Sharma GK. A comparative study of poisoning cases autopsied in LHMC New Delhi and JIP-MER Pondicherry. J Forensic Med Toxicol. 2002;19:19-21.
6. Ballantyne B, Mars TC, Turner P. Fundamentals of toxicology. Gen AppTox. USA: McMillan Press; c1995.
7. Viswanathan N, Joshi BS. Toxic constituents of some Indian plants. Curr Sci. 1983;52:1-8.
8. Subrahmanyam BV. Modi's Medical Jurisprudence and Toxicology. 22nd ed. India: Butterworths; c1999.
9. Boesche R. Kautilya's *Arthasastra* on war and diplomacy in ancient India. J Milit Hist. 2003;67:9-37.
10. Parikh CK. Textbook of Medical Jurisprudence and Toxicology. 6th ed. India: CBS Publishers; c1999.
11. Lin CC, Yang CC, Phua DH, *et al*. An outbreak of foxglove leaf poisoning. J Chin Med Assoc. 2010;73:97-100.
12. Vyas A, Bachani N, Thakur H, *et al*. Digitalis toxicity: ECG vignette. Indian Heart J. 2016, 68.
13. Solomon FE, Sharada AC, Devi PU. Toxic effects of crude root extract of *Plumbago rosea* (Rakta chitraka) on mice and rats. J Ethnopharmacol. 1993;38:79-84.
14. Bandara V, Weinstein SA, White J, *et al*. A review of the natural history, toxinology, diagnosis and clinical management of *Nerium oleander* (Common oleander) and *Thevetia peruviana* (Yellow oleander) poisoning. Toxicon. 2010;56:273-281.
15. de Lima JM, de Freitas FJ, Amorim RN, *et al*. Clinical and pathological effects of *Calotropis procera* exposure in sheep and rats. Toxicon. 2011;57:183-185.
16. Matthai TP, Date A. Renal cortical necrosis following exposure to sap of the marking-nut tree (*Semecarpus anacardium*). Am J Trop Med Hyg. 1979;28:773-774.
17. Adhikari KM. Poisoning due to accidental ingestion of *Dieffenbachia* plant (Dumb cane). Indian Pediatr. 2012;49:247-248.
18. Mocking RJ, Schene KM, Maingay-Visser DA. Dieffenbachia poisoning in an infant. Ned Tijdschr Geneeskd; c2015 .p. 160.
19. Altin G, Sanli A, Erdogan BA, *et al*. Severe destruction of the upper respiratory structures after brief exposure to a *Dieffenbachia* plant. J Craniofac Surg; c2013 .p. 24.
20. Pack WK, Raudonat HW, Schmidt K. Lethal poisoning with hydrocyanic acid after ingestion of bitter almonds (*Prunus amygdalus*). Z Rechtsmed. 1972;70:53-54.
21. Hodjat M, Rezvanfar MA, Abdollahi M. A systematic review on the role of environmental toxicants in stem cells aging. Food Chem Toxicol. 2015;86:298-308.
22. Riou B, Kongolo G, Djeddi D. Nicotine poisoning from patch application: a pediatric case report. Arch Pediatr. 2015;22:895-896.
23. Demirhan A, Tekelioğlu ÜY, Yıldız İ, *et al*. Anticholinergic Toxic Syndrome caused by *Atropa belladonna* fruit (Deadly Nightshade): A case report. Turk J Anaesthesiol Reanim. 2013;41:226-228.
24. Glatstein M, Danino D, Wolyniez I. Seizures caused by ingestion of *Atropa belladonna* in a homeopathic medicine in a previously well infant: case report and review of the literature. Am J Ther. 2014;21.
25. Vetter J. Poison hemlock (*Conium maculatum* L.). Food Chem Toxicol. 2004;42:1373-1382.