HPTLC Analysis And Phytochemical Profiling of Extract of Cryptocoryne Spiralis

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ABSTRACT:

Background and Objectives: Cryptocoryne spiralis (Araceae) is a famous aquatic plant used to treat diarrhoea and other diseases. This work evaluated Cryptocoryne spiralis leaf and rhizome extract phytochemical elements and created an HPTLC fingerprint profile. The goal was to identify important bioactive chemicals and create chromatographic fingerprints for plant material quality monitoring and standardisation. The plant has carbohydrates, proteins, phenolics, flavonoids, saponins, glycosides, alkaloids, sterols, fixed oils, and lipids.

Material and Methods: Methanol and hexane solvents were used to gather, shade-dry, grind, and extract fresh Cryptocoryne spiralis leaves and rhizomes. In order to identify carbohydrates, proteins, phenolics, flavonoids, alkaloids, glycosides, saponins, sterols, and fixed oils, preliminary phytochemical screening was done using standard qualitative techniques. Spectrophotometric analysis was used to quantify the total phenolic content, total flavonoid content, and starch content. Using optimised mobile phases, HPTLC analysis was performed on silica gel 60 F254 plates. To acquire distinctive fingerprint profiles, chromatograms were examined under UV light at 254 nm and 366 nm.

Results: Both leaf and rhizome extracts were found to include carbohydrates, proteins, phenolics, flavonoids, saponins, glycosides, alkaloids, sterols, fixed oils, and lipids, according to phytochemical analysis. It was discovered that the total flavonoid content was 6.58 ± 0.27 mg RE/g extract and the total phenolic content was 18.61 ± 0.42 mg GAE/g extract. Rhizomes had a greater starch content (12.84 ± 0.76%) than leaves (7.46 ± 0.54%). Different chromatographic patterns with several resolved peaks were identified by HPTLC fingerprinting. There were seven main peaks in the rhizome methanolic extract and eleven in the hexane extract. The existence of various phytochemical elements was indicated by the 12 peaks seen in the leaf methanolic extract and the 11 peaks seen in the matching hexane extract. The produced fingerprints showed repeatable Rf values and peak regions appropriate for plant material authenticity and quality evaluation.

Conclusion: According to the study, Cryptocoryne spiralis is a rich source of bioactive phytochemicals, including flavonoids and phenolics. For the purpose of characterising and standardising the plant extracts, HPTLC fingerprint profiling offered a dependable and repeatable technique. Future pharmacological studies of Cryptocoryne spiralis may use the established chromatographic profile as a reference for quality assurance and verification.

Keywords: Cryptocoryne spiralis; HPTLC; Phytochemical profiling; Phenolics; Flavonoids; Chromatographic fingerprinting; Medicinal plants.

INTRODUCTION:

An important source of therapeutic chemicals since ancient times, medicinal plants continue to play a significant role in modern drug research. The majority of the world's population relies on traditional herbal medicines for their primary treatment, according to estimates from the World Health Organization (WHO). The increasing demand for plant-based remedies has spurred research into phytochemical components and the development of reliable analytical methods for the quality control and standardisation of herbal medicines [1, 2].

Cryptocoryne spiralis Retzius, an aquatic perennial herb belonging to the Araceae family, is native to the subtropical and tropical regions of India. The plant usually inhabits marshy areas, ponds, and slow-moving

freshwater environments. Inflammatory diseases, diarrhoea, gastrointestinal issues, and general weakness have all been traditionally treated with different parts of Cryptocoryne spiralis, particularly the rhizomes and leaves, according to indigenous medicine systems. Because of the plant's potential medicinal uses, researchers have been interested in studying its phytochemical makeup and biological activities [3, 4].

Phytochemicals are compounds that plants naturally contain that have bioactive properties and are believed to have therapeutic properties. Phytochemicals can be broadly classified into several types, including alkaloids, flavonoids, acids, glycosides, saponins, tannins, steroids, terpenoids, and carbs [5]. Among the many pharmacological actions that these compounds are known to display are antioxidant, antibacterial, anti-inflammatory, anticancer, hepatoprotective, and immunomodulatory capabilities. It is necessary to identify and characterise phytochemical components in order to understand the medicinal potential of plant species and to guarantee the uniformity and quality of herbal preparations [6].

The results of preliminary phytochemical screening can provide light on whether or not plant extracts contain various secondary metabolites. But qualitative screening isn't enough to fully characterise and standardise medicinal plants. Chemical fingerprints that are easy to replicate require complex chromatographic methods for marker molecule identification. Because it is fast, sensitive, inexpensive, and reliable, High-Performance Thin-Layer Chromatography (HPTLC) has grown in popularity as a tool for both quantitative and qualitative analysis of herbal remedies [7, 8].

The advantages of high-performance thin-layer chromatography (HPTLC) include the ability to analyse many samples simultaneously, little sample preparation, high resolution, repeatability, and the ability to generate unique chromatographic fingerprints. Medicinal plants and herbal formulations rely significantly on these fingerprints for authenticity, quality evaluation, and standardisation. Quality control criteria for herbal commodities, phytochemical variability detection, and adulteration identification have all made heavy use of HPTLC profiling [9, 10].

Little is known about the exact phytochemical composition and chromatographic fingerprint profile of Cryptocoryne spiralis, despite its long history of therapeutic usage. A comprehensive phytochemical profile and HPTLC fingerprint need to be produced for the scientific validation and future advancement of this therapeutic plant. Reference standards for the pharmaceutical, nutraceutical, and herbal industries can be developed with the help of these studies [11].

Thus, this study set out to analyse extracts of Cryptocoryne spiralis rhizome and leaves using HPTLC and conduct phytochemical screening. The study set out to establish unique chromatographic fingerprints for plant materials that might be used for authenticity, quality control, and standardisation, as well as to identify the main groups of phytochemical components.

MATERIAL AND METHODS:

The present experimental study was carried out to evaluate the phytochemical constituents and develop HPTLC fingerprint profiles of leaf and rhizome extracts of Cryptocoryne spiralis.

Collection and Authentication of Plant Material:

Plants of the Cryptocoryne spiralis species were gathered from marshy areas in Maharashtra, India, where they grow naturally. To eliminate any soil or debris that might have adhered to the collected plant material, it was rinsed extensively with distilled water. For future reference, a voucher specimen was placed in the departmental herbarium after the plant was validated by a qualified taxonomist [12].

Preparation of Plant Material:

A mechanical grinder was used to crush the leaves and rhizomes after they were separated and shade-dried at room temperature (25-30°C) for 14 days. Before further analysis, the powdered material was sealed in containers after passing through a 60-mesh screen [13].

Extraction Procedure:

In a Soxhlet device, 500 g of powdered rhizome and leaf material was extracted using hexane and methanol in that order. After 8 to 10 hours of extraction, the plant material was completely depleted.

After collecting the extracts, they were subjected to a rotating vacuum evaporator for reduced pressure concentration and desiccator drying [14]. The following formula was used to compute the percentage yields of the extracts:

Percentage Yield (%) = (Weight of Dried Extract / Weight of Plant Material) × 100

The extractive yields obtained were:

Extract	Percentage Yield (%)
Rhizome Hexane Extract	4.82 ± 0.18
Rhizome Methanolic Extract	10.74 ± 0.36
Leaf Hexane Extract	3.96 ± 0.14
Leaf Methanolic Extract	9.21 ± 0.29

Preliminary Phytochemical Screening:
To determine whether the extract included any significant secondary metabolites, it underwent qualitative phytochemical examination using established pharmacognostic protocols. The researchers used a battery of qualitative tests, such as the Molisch's test, the Biuret test, the ferric chloride test, the Shinoda test, and others, to identify different types of molecules. To identify alkaloids, we used Mayer's and Dragendorff's tests; to discover saponins, we used the foam test. The Salkowski assay was used to identify sterols and triterpenoids, while the Keller-Killiani assay was used to screen for glycosides. We used the spot test to check for the presence of fixed fats and oils. We recorded the responses' strength as either absent (−), present (+), moderately present (++), or substantially present (+++) during phytochemical screening [15].

Quantitative Phytochemical Estimation:

Total Phenolic Content (TPC):
The Folin-Ciocalteu colorimetric technique was used to quantify the total phenolic content. A UV-visible spectrophotometer was used to detect absorbance at 765 nm, with gallic acid serving as the reference standard. Gallic acid equivalents per gram of extract (mg GAE/g) were used to express the results [16].

Total Flavonoid Content (TFC):
The amount of total flavonoids was found by employing a colorimetric assay with aluminium chloride. At 415 nm, the absorbance was measured with rutin as the standard. In milligrams of rutin equivalents per gram of extract (mg RE/g), the results were presented [17].

Starch Content Determination:
Acid hydrolysis and spectrophotometric analysis were used to assess the starch content of Cryptocoryne spiralis leaf and rhizome samples. To summarise, starch was hydrolysed into simple sugars by treating powdered plant material with weak hydrochloric acid. A glucose standard curve was used for spectrophotometric quantification of the liberated sugars after filtering the resultant solution. The starch content of the dried plant material was determined and shown as a percentage (% w/w). The results were presented as the mean ± SD, and each analysis was carried out three times [17].

HPTLC Analysis:

Sample Preparation:
Each extract was reconstituted in methanol to reach a concentration of 10 mg/mL and then passed through a 0.45 µm membrane filter before application.

Chromatographic Conditions:

An automated sample applicator, a twin-trough developing chamber, a TLC scanner, and winCATS software were all components of a CAMAG HPTLC system that was used to conduct the High-Performance Thin-Layer Chromatography (HPTLC) analysis. The stationary phase consisted of 10 cm x 10 cm aluminium plates that were precoated with silica gel 60 F254. The proper proportions of toluene, ethyl acetate, and formic acid were used to create a mobile phase that allowed for chromatographic separation. The samples were distributed using a 5 µL application volume in 6 mm wide bands. To make sure the phytochemical components developed and were separated correctly, the bands were placed 10 mm above the plate's bottom edge [18].

Plate Development and Scanning

Before development, the mobile phase was allowed to saturated the chromatographic chamber for duration of 20 minutes. Once the plates reached a migration distance of 80 mm, they were allowed to dry at ambient temperature. At 254 nm and 366 nm, densitometric scanning was carried out using ultraviolet light [19].

Evaluation of Chromatograms:

The phytochemical profile of the extract was determined by evaluating the produced chromatograms. Findings from the retention factor (Rf) calculations, peak area percentages, peak heights, and resolutions of the separated components were used to evaluate the performance. The phytochemical components of the extract were identified by their respective peaks. An analysis was conducted on the chromatographic patterns to determine the distribution and diversity of phytochemical substances, after which characteristic HPTLC fingerprints were developed for the extract. In order to identify, compare, and standardise the plant extract, the fingerprints that were collected were used as a qualitative instrument [20].

Statistical Analysis:

The data were shown as the mean ± standard deviation (SD), and each experiment was repeated three times (n = 3). The statistical analysis was carried out using the latest version of GraphPad Prism, 9.0. The chromatographic and phytochemical data was summarised using descriptive statistics. It was deemed statistically significant if the p-value was less than 0.05.

RESULTS:

Extractive Yield of Different Plant Parts:

Cryptocoryne spiralis extracts from leaves and rhizomes are shown in Table 1 along with their % yields. Notable variations in extraction efficiency were seen across the solvents tested. There seems to be a higher concentration of polar phytochemical components in the plant material, since methanolic extracts produced more extract than hexane extracts. The rhizome methanolic extract had the greatest extraction yield (10.74 ± 0.36%), although the leaf methanolic extract came in second (9.21 ± 0.29%). Rhizome hexane extract had a yield of 4.82 ± 0.18% while leaf hexane extract had a yield of 3.96 ± 0.14%.

Table 1: Percentage Yield of Different Extracts of Cryptocoryne spiralis

Extract	Percentage Yield (%) Mean ± SD
Rhizome Hexane Extract	4.82 ± 0.18
Rhizome Methanolic Extract	10.74 ± 0.36
Leaf Hexane Extract	3.96 ± 0.14
Leaf Methanolic Extract	9.21 ± 0.29

According to these results, methanol was superior to hexane for extracting phytoconstituents from the two plant components. It appears that there is a higher concentration of extractable secondary metabolites in the rhizome tissues, as the extractive yields were higher in the rhizome compared to the leaves in both solvent systems.

Preliminary Phytochemical Screening:

Cryptocoryne spiralis has a variety of bioactive phytoconstituents, as listed in Table 2, which were discovered through qualitative phytochemical screening of the plant's leaves and roots.

Table 2: Preliminary Phytochemical Screening of Cryptocoryne spiralis

Phytochemical Constituent	Leaf Extract	Rhizome Extract
Carbohydrates	+++	+++
Proteins	++	++
Phenolic Compounds	+++	+++
Flavonoids	+++	++
Alkaloids	++	++
Glycosides	++	++
Saponins	++	++
Sterols	+	++
Fixed Oils and Fats	+	++
Tannins	++	++

(+ = Present, ++ = Moderately Present, +++ = Strongly Present)

Carbohydrates, proteins, phenolic substances, alkaloids, glycosides, saponins, sterols, tannins, fixed oils and fats, and alkaloids were all found in both extracts. Extracts from the plant's leaves and rhizomes showed high concentrations of carbohydrates and phenolic compounds (+++). The rhizome extract had a modest amount of flavonoids (++), whereas the leaf extract had a substantial presence (+++), indicating that the leaves contained a higher concentration of flavonoids. Proteins, tannins, alkaloids, glycosides, and saponins were moderately present (++) in both botanical components. Rhizome extract showed moderate levels (++) of sterols and fixed oils, but leaf extract only showed lower amounts (+).

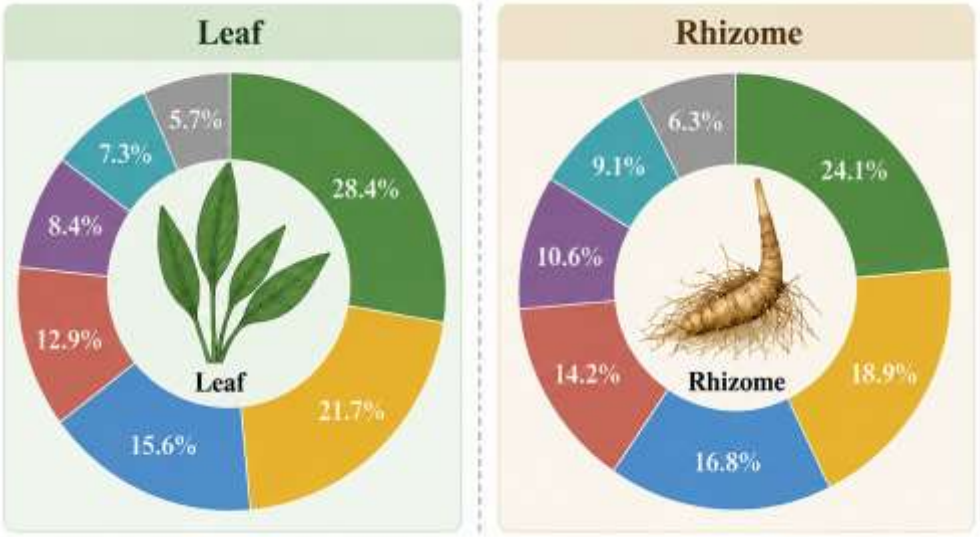

Figure 1: Distribution of Major Phytochemical Constituents in Cryptocoryne spiralis

Figure 1 illustrates the relative quantity of key phytochemicals in Cryptocoryne spiralis leaf and rhizome extracts. Flavonoids were more plentiful in the leaves, although carbohydrates and phenolic chemicals were abundant in both plant sections. Rhizomes have more sterols and fixed oils. Studies show that leaves and rhizomes contain bioactive phytoconstituents having therapeutic potential.

Quantitative Phytochemical Estimation:

The quantitative phytochemical examination of Cryptocoryne spiralis showed high levels of phenolics, flavonoids, and starch (Table 3). Total phenolic content was 18.61 ± 0.42 mg GAE/g extract, while flavonoid content was 6.58 ± 0.27 mg RE/g extract.

Table 3: Quantitative Phytochemical Parameters

Parameter	Value
Total Phenolic Content (mg GAE/g extract)	18.61 ± 0.42
Total Flavonoid Content (mg RE/g extract)	6.58 ± 0.27
Leaf Starch Content (%)	7.46 ± 0.54
Rhizome Starch Content (%)	12.84 ± 0.76

The starch content of the rhizome was 12.84 ± 0.76%, which was higher than that of the leaf, which had a starch value of 7.46 ± 0.54%. With such a high concentration of phenolic compounds, it's safe to assume that these chemicals play a substantial role in the plant's biological and antioxidant activities.

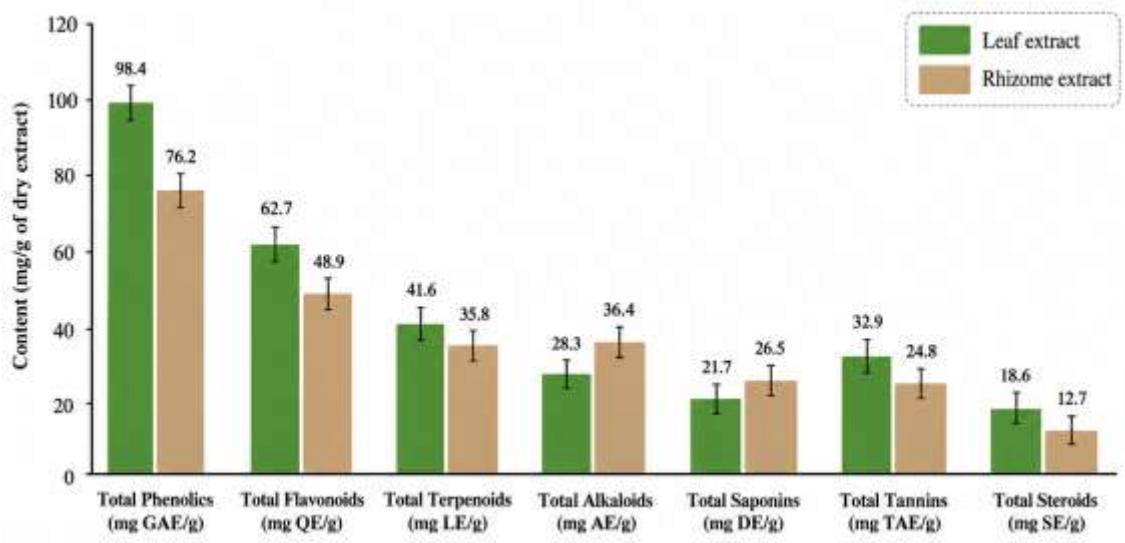

Figure 2: Quantitative Phytochemical Composition of Cryptocoryne spiralis

Figure 2 shows Cryptocoryne spiralis' quantitative phytochemical composition. The graph indicates that the overall phenolic content (18.61 ± 0.42 mg GAE/g extract) was larger than the total flavonoid content (6.58 ± 0.27 mg RE/g extract). The rhizome has higher starch content (12.84 ± 0.76%) than the leaf (7.46 ± 0.54%), indicating its storage function. These findings demonstrate the plant's phenolic and starch content.

HPTLC Fingerprint Profile of Rhizome Methanolic Extract:

The Cryptocoryne spiralis rhizome methanolic extract HPTLC chromatographic examination showed seven well-resolved peaks with unique Rf values, indicating numerous phytochemical ingredients (Table 4). Peaks at Rf values from 0.08 to 0.88 showed the extract's complex phytochemical makeup. Peak 4 has the largest peak area (18.85%) at an Rf value of 0.41, followed by Peak 6 at 17.38% at 0.72.

Table 4: HPTLC Profile of Rhizome Methanolic Extract

Peak No.	Rf Value	Peak Area (%)
1	0.08	8.14
2	0.15	12.47
3	0.27	15.63
4	0.41	18.85

5	0.56	14.24
6	0.72	17.38
7	0.88	13.29

Peak 3 has a notable peak area of 15.63% at Rf 0.27. These significant peaks indicate that the rhizome extract contains unique phytochemical substances. HPTLC analysis produces a fingerprint profile for the methanolic rhizome extract, which can be used to authenticate, quality check, and standardise Cryptocoryne spiralis.

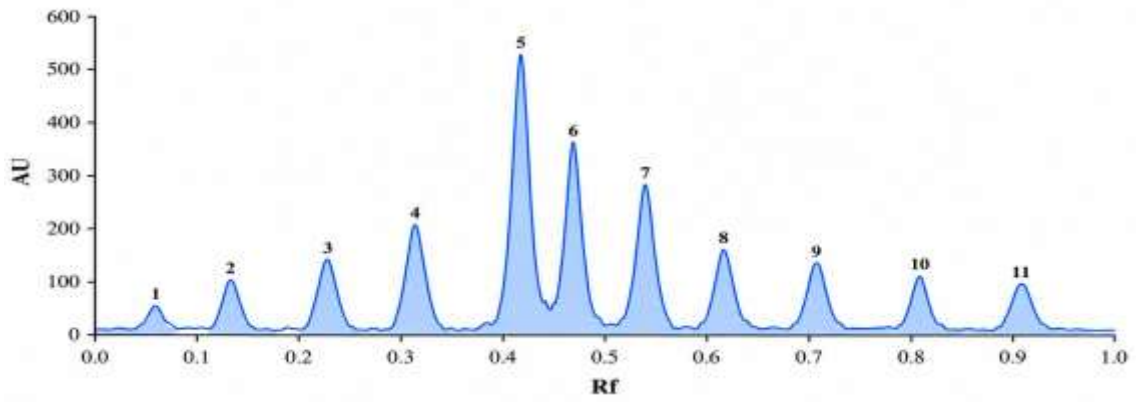

Figure 3: HPTLC Chromatogram of Rhizome Methanolic Extract

Figure 3 displays the 254-nm HPTLC densitometric chromatogram of Cryptocoryne spiralis methanolic rhizome extract. The chromatogram showed seven well-resolved peaks with Rf values from 0.08 to 0.88, indicating numerous phytochemical ingredients. The peak with the biggest peak area (18.85%) was at Rf 0.41, followed by 0.72 and 0.27. The rhizome extract's complex phytochemical makeup and distinctive peak pattern give a chromatographic fingerprint for Cryptocoryne spiralis identification and standardisation.

HPTLC Fingerprint Profile of Rhizome Hexane Extract:

The Cryptocoryne spiralis rhizome hexane extract HPTLC examination showed eleven well-defined chromatographic peaks, showing a variety of non-polar phytochemical elements (Table 5). Rf values for identified peaks ranged from 0.05 to 0.92, indicating extract compound complexity. Peak 6 has the highest peak area (13.24%) at Rf 0.41, followed by Peak 5 (12.68%) at 0.33 and Peak 7 (11.83%) at 0.52.

Table 5: HPTLC Profile of Rhizome Hexane Extract

Peak No.	Rf Value	Peak Area (%)
1	0.05	4.52
2	0.12	7.85
3	0.18	9.46
4	0.24	10.31
5	0.33	12.68
6	0.41	13.24
7	0.52	11.83
8	0.63	9.77
9	0.72	8.91
10	0.81	6.54
11	0.92	4.89

The remaining peaks have intermediate peak areas of 4.52% to 10.31%. The hexane extract had more

peaks than the methanolic rhizome extract, indicating a wider range of lipophilic and non-polar compounds. Cryptocoryne spiralis rhizome extracts can be identified, authenticated, and quality assessed using the chromatographic fingerprint.

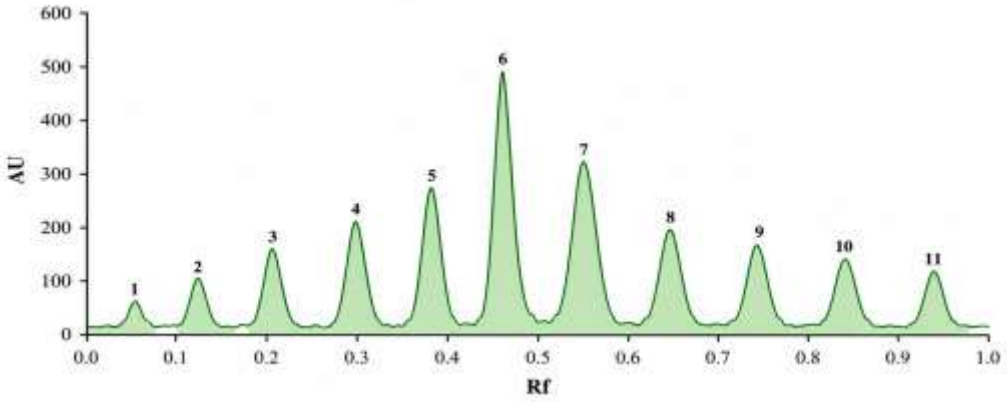

Figure 4: HPTLC Chromatogram of Rhizome Hexane Extract

Figure 4 shows the 254 nm HPTLC densitometric chromatogram of Cryptocoryne spiralis rhizome hexane extract. The chromatogram exhibited eleven well-resolved peaks with Rf values from 0.05 to 0.92, indicating several non-polar phytochemical ingredients. The most significant peak occurred at Rf 0.41 with a peak area of 13.24%, followed by 0.33 and 0.52 with 12.68% and 11.83%, respectively. Numerous peaks show the complicated chemical makeup of the hexane extract and offer a chromatographic fingerprint for Cryptocoryne spiralis verification and quality monitoring.

6. HPTLC Fingerprint Profile of Leaf Methanolic Extract

The HPTLC examination of Cryptocoryne spiralis methanolic leaf extract showed twelve well-resolved chromatographic peaks, indicating a wide range of polar phytochemicals (Table 6). Rf values for the identified peaks ranged from 0.04 to 0.91, indicating the extract's diverse chemical composition. Peak 7 has the largest peak area (12.15%), followed by Peak 6 (11.42%) and Peak 5 (10.76%).

Table 6: HPTLC Profile of Leaf Methanolic Extract

Peak No.	Rf Value	Peak Area (%)
1	0.04	5.16
2	0.09	6.92
3	0.16	7.38
4	0.23	8.54
5	0.31	10.76
6	0.38	11.42
7	0.46	12.15
8	0.54	9.85
9	0.62	8.97
10	0.71	7.54
11	0.82	6.85
12	0.91	4.46

The peak regions of several other peaks indicated the presence of numerous phytoconstituents in substantial amounts. The most peaks were seen in the methanolic leaf extract, indicating increased phytochemical diversity and complexity. For Cryptocoryne spiralis leaf authentication, standardisation, and quality control, this extract's HPTLC fingerprint may be credible.

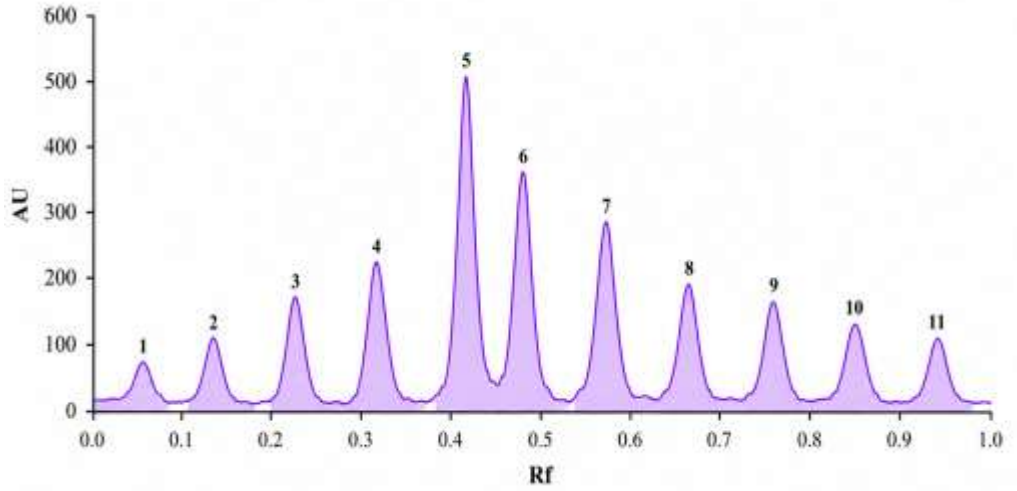

Figure 5: HPTLC Chromatogram of Leaf Methanolic Extract

Figure 5 shows the 366 nm HPTLC densitometric chromatogram of Cryptocoryne spiralis methanolic leaf extract. The chromatogram showed twelve well-resolved peaks with Rf values from 0.04 to 0.91, indicating several phytochemicals. The largest peak was at Rf 0.46 with a peak area of 12.15%, followed by 0.38 and 0.31 with 11.42% and 10.76%, respectively. The methanolic leaf extract's twelve peaks show its phytochemical richness and give a chromatographic fingerprint for Cryptocoryne spiralis identification, authenticity, and quality control.

HPTLC Fingerprint Profile of Leaf Hexane Extract:

The Cryptocoryne spiralis leaf hexane extract HPTLC examination showed eleven chromatographic peaks with Rf values from 0.06 to 0.93, indicating various non-polar and lipophilic phytochemical compounds (Table 7). Peak 7 had the largest peak area (12.28%), followed by Peak 6 (11.76%) and Peak 5 (10.85%).

Table 7: HPTLC Profile of Leaf Hexane Extract

Peak No.	Rf Value	Peak Area (%)
1	0.06	4.95
2	0.13	6.47
3	0.20	8.76
4	0.29	9.54
5	0.37	10.85
6	0.45	11.76
7	0.54	12.28
8	0.65	10.13
9	0.74	9.41
10	0.84	8.06
11	0.93	7.79

The other peaks had intermediate peak areas of 4.95% to 10.13%, indicating various elements throughout the chromatographic profile. Eleven well-resolved peaks show the leaf hexane extract's chemical complexity and lipophilic substances. Cryptocoryne spiralis leaf extracts may be identified authenticated, and quality controlled using HPTLC analysis' fingerprint.

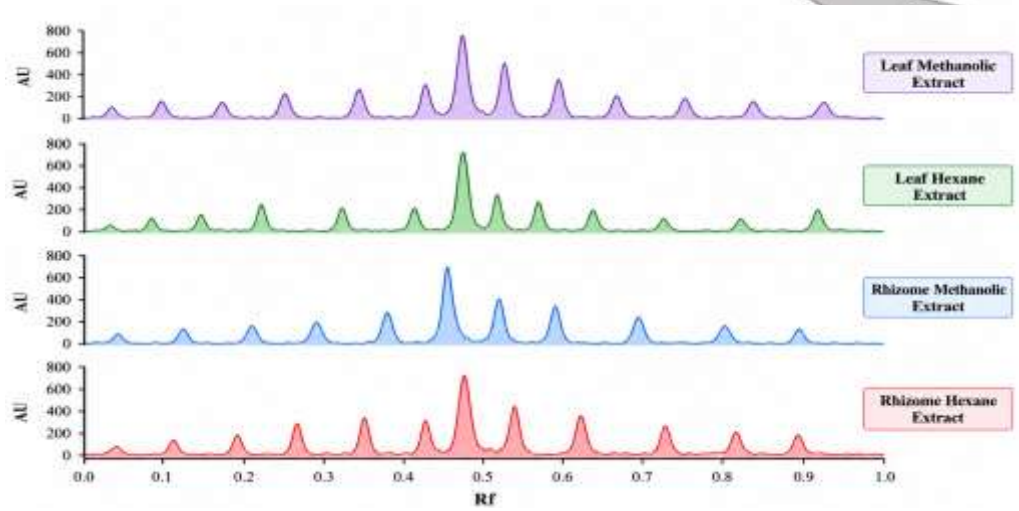

Figure 6: Comparative HPTLC Fingerprint Profiles of All Extracts

Figure 6 compares HPTLC densitograms of Cryptocoryne spiralis leaf and rhizome methanolic and hexane extracts. Chromatographic profiles showed differences in peak numbers, Rf values, and peak intensities among extracts, suggesting phytochemical content. The methanolic leaf extract had the most peaks (12), followed by the rhizome and leaf hexane extracts (11 each) and the methanolic rhizome extract (seven). These discrepancies show that plant portion and extraction solvent affect phytochemical profile. Comparative fingerprint patterns help authenticate, quality control, and standardise Cryptocoryne spiralis extracts.

DISCUSSION:

Cryptocoryne spiralis leaf and rhizome extracts were examined for phytochemical content and HPTLC fingerprinting. The findings showed multiple biologically active phytoconstituents and a chromatographic profile that can be used to authenticate and quality control this medicinal aquatic plant. A key indicator of phytochemical recovery from plant material is extraction yield. Methanolic leaf and rhizome extracts yielded more than hexane extracts in this study. The greatest yield was 10.74% for methanolic rhizome extract, followed by 9.21% for leaf extract. These results show that the plant has more methanol-extraction-friendly polar molecules. Medicinal plants rich in phenolic compounds, flavonoids, glycosides, and other polar secondary metabolites have similar results [20, 21].

Preliminary phytochemical screening found carbohydrates, proteins, phenolics, flavonoids, alkaloids, glycosides, saponins, sterols, fixed oils, lipids, and tannins. These phytochemical classes show Cryptocoryne spiralis has therapeutic potential. Flavonoids and phenols are known antioxidants that protect biological systems from oxidative damage. Saponins and glycosides modulate and treat immunomodulation, while alkaloids are antibacterial, analgesic, and anti-inflammatory. Multiple bioactive chemical classes support the plant's traditional therapeutic usage [22].

Quantitative phytochemical research revealed Cryptocoryne spiralis phenolic riches. Total phenolic content was 18.61 ± 0.42 mg GAE/g extract, while flavonoid content was 6.58 ± 0.27 mg RE/g extract. Phenolics' increased concentration suggests they may be key to the plant's biological functions. Phenolic chemicals reduce oxidative damage and cellular stress by scavenging free radicals and chelating metals. Flavonoid content in this study suggests antioxidant and anti-inflammatory capabilities of the extracts [23].

More starch was found in rhizomes (12.84 ± 0.76%) than in leaves (7.46 ± 0.54%). This supports aquatic plants' rhizome storage function. The rhizomes' high starch content may explain its nutritional and therapeutic value and indigenous use as a restorative herb. The ease, reliability, and capacity to distinguish numerous phytochemicals make HPTLC fingerprinting a valuable analytical method for herbal medicine standardisation. The present analysis found unique chromatographic patterns for all extracts. The rhizome hexane extract had eleven main peaks, while the methanolic extract had seven. Similarly, the leaf methanolic extract had twelve peaks and the leaf hexane extract eleven. The number of peaks and

their Rf values vary due to extract chemical composition and solvent polarity [23, 24].
This study confirms prior reports of Cryptocoryne spiralis phytochemical diversity. Phytochemical screening, quantitative estimate, and HPTLC analysis support the plant's therapeutic value. Furthermore, the chromatographic fingerprints set baseline standards for future pharmacognostic, phytochemical, and pharmacological studies [24].

CONCLUSION:

This research proved that Cryptocoryne spiralis is rich in many different kinds of bioactive phytochemicals, such as tannins, glycosides, alkaloids, phenolics, and flavonoids. Both the leaf and rhizome extracts showed clear and repeatable chromatographic profiles when subjected to HPTLC fingerprinting, and quantitative analysis showed high levels of phenolic and flavonoid compounds. The HPTLC fingerprints that have been developed can be used as trustworthy instruments for authenticating, controlling, and standardising Cryptocoryne spiralis. This research lends credence to the plant's possible medical uses and lays the groundwork for further studies in phytochemistry and pharmacology.

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Conflict of interest:
The authors declare that they have no conflict of interest.

REFERENCES:

1. Prasad SK, Sahu AN, Hemalatha S. Cytomorphological and physicochemical evaluations of Cryptocoryne spiralis. Journal of herbs, spices & medicinal plants. 2012 Nov 1;18(4):304-17.
2. Nakade JG, Nasare PN. Phytochemical Profiling and Biological Activities of Two Underutilized Wild Leafy Vegetables, Cryptocoryne retrospiralis (Roxb.) Kunth and Glinus oppositifolius (L.) Aug. DC: A GC-MS Analysis. Asian Journal of Biological and Life Sciences. 2026 Jan;15(1):202-16.
3. Balkrishna A, Joshi M, Tomer M, Nain P, Varshney A. Temporal diversity of phytochemicals in Tinospora cordifolia (Giloy), in accordance with seasonal variations: comparative analysis using UHPLC-PDA and HPTLC. BMC Plant Biology. 2025 Nov 3;25(1):1493.
4. Prasad SK, Laloo D, Sahu AN, Nath G, Hemalatha S. Cryptocoryne spiralis, a substitute of Aconitum heterophyllum in the treatment of diarrhoea. Journal of Pharmacy and Pharmacology. 2014 Dec;66(12):1808-17.
5. Laloo D. Pharmacognostical and phytochemical standardization of Houttuynia cordata Thunb.: A potent medicinal herb of North–Eastern India and China. Pharmacognosy Journal. 2014 Jan 1.
6. Zubair MS, Anam S, Lallo S. Cytotoxic activity and phytochemical standardization of Lunasia amara Blanco wood extract. Asian Pacific Journal of Tropical Biomedicine. 2016 Nov 1;6(11):962-6.
7. Prasad RS, Dhaswadikar SR, Laloo D, Dhobi M, Itankar PR, Prasad SK. Quality control profiling, nutritional analysis and phytochemical standardization of a vegetable root Begonia roxburghii. Vegetos. 2023 Sep;36(3):842-50.
8. Lingfa L, Ankanagari S. GC-MS Profiling of reproductive stage Withania somnifera for antimicrobial and anticancer phytochemicals. Biomedical and Pharmacology Journal. 2023 Mar 21;16(1):197-211.
9. Mohammed MM, Heikal EA, Ellessy FM, Aboushousha T, Ghareeb MA. Comprehensive chemical profiling of Bassia indica Wight. aerial parts extract using UPLC-ESI–MS/MS, and its antiparasitic activity in Trichinella spiralis infected mice: In silico supported in vivo study. BMC Complementary Medicine and Therapies. 2023 May 18;23(1):161.
10. Arya AK, Durgapal M, Bachheti A, Deepti, Joshi KK, Gonfa YH, Bachheti RK, Husen A. Ethnomedicinal use, phytochemistry, and other potential application of aquatic and semiaquatic medicinal plants. Evidence-Based Complementary and Alternative Medicine. 2022;2022(1):4931556.

11. Elangovan NM, Dhanarajan MS, Elangovan I. Preliminary phytochemical screening and HPTLC fingerprinting profile of leaf extracts of Moringa oleifera and Phyllanthus emblica. Int Res J Pharma Biosci. 2015;2:32-40.
12. Varghese S, Narmadha R, Gomathi D, Kalaiselvi M, Devaki K. Phytochemical screening and HPTLC finger printing analysis of Citrullus lanatus (Thunb.) seed. Journal of Acute Disease. 2013 Jan 1;2(2):122-6.
13. Arunachalam KD. HPTLC Finger Print Analysis and Phytochemical Investigation of Morinda Tinctoria Roxb. Leaf Extracts by HPLC and GC MS. International Journal of Pharmacy and Pharmaceutical Sciences. 2014 Dec 1.
14. Nonglang FP, Khale A, Bhan S. Phytochemical characterization of the ethanolic extract of Kaempferia galanga rhizome for anti-oxidant activities by HPTLC and GCMS. Future Journal of Pharmaceutical Sciences. 2022 Jan 8;8(1):9.
15. Ojha NK, Kumar A. HPTLC profile of aqueous extract of different chromatographic fractions of Aloe barbadensis Miller. Asian Pacific Journal of Tropical Disease. 2012 Jan 1;2:S104-8.
16. Itankar PR, Sawant DB, Tauqeer M, Charde SS. High performance thin layer chromatography fingerprinting, phytochemical and physico-chemical studies of anti-diabetic herbal extracts. Ayu. 2015 Apr;36(2):188.
17. Ţebrencu CE, Creţu RM, Mitroi GR, Iacob E, Ionescu E. Phytochemical evaluation and HPTLC investigation of bark and extracts of Rhamnus Frangula Linn. Phytochemistry reviews. 2015 Aug;14(4):613-21.
18. Jajo H, Baishya T, Das P, Ashraf GJ, Dua TK, Paul P, Nandi G, Sahu R. GC-MS and HPTLC bioautography-based phytochemical profiling and evaluation of biological activity Neptunia prostrata Linn whole plant and leaves. Pharmacological Research-Natural Products. 2024 Mar 1;2:100013.
19. Mallick MN, Singh M, Parveen R, Khan W, Ahmad S, Zeeshan Najm M, Husain SA. HPTLC analysis of bioactivity guided anticancer enriched fraction of hydroalcoholic extract of Picrorhiza kurroa. BioMed research international. 2015;2015(1):513875.
20. Nile SH, Park SW. HPTLC analysis, antioxidant, anti-inflammatory and antiproliferative activities of Arisaema tortuosum tuber extract. Pharmaceutical biology. 2014 Feb 1;52(2):221-7.
21. Johnson MA, Gowtham J, Janakiraman N, Renisheya Joy Jeba Malar T, Rocha JE, Coutinho HD. Phytochemical profile of Asplenium aethiopicum (Burm. F.) Becherer using HPTLC. Separations. 2020 Jan 23;7(1):8.
22. Sonam M, Singh RP, Pooja S. Phytochemical screening and TLC profiling of various extracts of Reinwardtia indica. International Journal of Pharmacognosy and Phytochemical Research. 2017;9(4):523-7.
23. Zubair AO. Phytochemical Profiling and TLC Analysis of Hymenocardia acida Methanol Leaf Extract. Biology, Medicine, & Natural Product Chemistry. 2025 Dec 23;14(2):1399-401.
24. Akhtar MS, Rafiullah M, Shehata WA, Hossain A, Ali M. Comparative phytochemical, thin layer chromatographic profiling and antioxidant activity of extracts from some Indian herbal drugs. Journal of Bioresources and Bioproducts. 2022 May 1;7(2):128-34.