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Microscopic characterization and preliminary Pharmacognostical evaluation of *Cryptocoryne retrospiralis* (Roxb) Kunth

Jayendra G, Nakade*[1] and Praveenkumar N. Nasare[2]

[1]*IHLR & SS Nilkanthrao Shinde Science & Arts College Bhadrawati Dist-Chandrapur Maharashtra, & Department of Botany Bhawabhuti Mahavidhyalaya Amgaon Dist Gondia Maharashtra, 441209, India*

[2]*Department of Botany, Nilkanthrao Shinde Science & Arts College, Bhadrawati Dist-Chandrapur Maharashtra, 442902, India*

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*Corresponding Author: **Jayendra G, Nakade** | Email Address: **jayendranakade26@gmail.com**

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Abstract

Cryptocoryne retrospiralis (Roxb) Kunth, locally known as "pakanbhed" in the Vidarbha region of Maharashtra, is a traditionally utilised leafy vegetable, particularly during winter. It thrives in specific habitats, including river edges, sandy soils, and open plateaus. The leaves hold significant value in folk medicine, employed by traditional healers to treat conditions such as diarrhoea, fever, jaundice, burns, boils, cough, and abdominal issues. The current study focuses on the detailed pharmacognostic and anatomical characterization of C. retrospiralis leaves to establish robust identification parameters for both fresh and dried crude drug samples. Macroscopic, microscopic, fluorescence, and phytochemical analyses were conducted, adhering to WHO-recommended standardization methods. Phytochemical screening was performed to determine the presence of phytoconstituents, which revealed the presence of carbohydrates. Alkaloids, flavonoids. Anatomical & Powder microscopy of leaves show paracytic stomata & fluorescence analysis of the crude powder shows fluorescent activity. These comprehensive observations will help to distinguish closely related species of cryptocoryne, and researchers with drug development.

Keywords: *Cryptocoryne retrospiralis, Pharmacognostic, Phytochemical Screening, fluorescence analysis, leafy vegetables.*

Introduction

Herbal materials necessitate thorough authentication and quality evaluation, primarily conducted through pharmacognosy, which utilises both macroscopic and microscopic characteristics [1]. A substantial amount of research is dedicated to confirming the accurate plant source to distinguish among the various available options [2]. Recently, bioactive compounds obtained from plants have garnered significant attention due to their diverse applications across different sectors of the contemporary world. The medicinal importance of plants largely arises from the presence of bioactive compounds, including alkaloids, glycosides, volatile oils, tannins, phenolics, flavonoids, and other phytochemicals. [3]

The Araceae family, commonly referred to as the arum family, is a vast and varied family of flowering plants comprising over 4,000 species across 140 genera. This family is distributed globally, except for the driest deserts and the coldest regions. While the majority of Araceae species are terrestrial, several aquatic members exist, thriving in a range of habitats, including ponds, lakes, rivers, and streams. [4]

Cryptocoryne is the largest genus among the aquatic plants, with 150 species. It belongs to the Araceae family. These plants are native to tropical Asia, covering regions from India to Southeast Asia and New Guinea, and are typically found in slow-moving water bodies and areas that experience seasonal flooding [5]. They are often located along the banks of ditches, in sandy or gravelly riverbeds, and on open, elevated plateaus. In India, their distribution extends from Maharashtra to North Karnataka, Kerala, the Coromandel Coast, Puducherry, Bengal, and Assam. The rhizomes of C. spiralis are used to treat diarrhoea, cough, abdominal issues, fever, and vomiting in infants. The rhizome of C. retrospiralis, known locally as 'mala' in Malayalam, was historically ingested as food by early humans. [6]. The rhizome of C. retrospiralis, locally referred to as 'mala' in Malayalam, was historically consumed as food by prehistoric humans [7].

In rural India, the healthcare system has relied on medicinal plants since ancient times, utilising traditional medicines to treat various ailments, including wound healing, inflammation, diabetes, heart attacks, malaria, jaundice, microbial infections, and others.

India boasts a rich history of Ayurveda, which highlights the use of numerous medicinal herbs for addressing different pathogenic challenges throughout history. In contemporary times, ethnomedicines, supported by scientific research, are attracting the interest of the scientific community due to their lack of undesirable side effects.[8]. Considering the reported effectiveness of this plant in treating a range of health issues, this study aims to identify the classes of natural products found in the leaves of *C. retrospiralis*. For standardization and quality assurance, it is essential to verify authenticity, purity, and assay. A review of the literature has indicated a deficiency in Pharmacognostical information regarding this species. The current research endeavours to tackle the issue of controversial drugs commonly found in Ayurveda and aims to establish vital pharmacopeial standards. Consequently, this study provides detailed accounts of Morphoanatomical,& pharmacognostic Details of C. retrospiralis

Experimental Materials & Methods

Collection & Identification of Plant Material

Leaves of *Cryptocoryne retrospiralis* were collected from the Wasa Porla Wainganga Riverbelt in Gadchiroli District, Maharashtra, located at a latitude of 20.319187^0 and longitude of 79.977843^0, during September to November 2022. The identification was confirmed through existing literature, such as the Flora of Maharashtra, and by a botanist from Nagpur University. A voucher specimen (No. BMV370) has been prepared and is stored for future reference at the Herbarium of the Department of Botany, Bhawabhuti Mahavidyalaya, in Amgaon, Gondia district, Maharashtra.

Morphological, Macroscopic and microscopic studies

Morphological characteristics, such as shape, size, margin, apex, and base, were examined and recorded in the laboratory. Fresh leaf material was utilized for anatomical studies, where free-hand sections were prepared and subjected to an alcoholic dehydration process. Subsequently, a double staining technique using saffranine and light green was employed, and the sections were mounted in glycerine. Photographs were captured using a microphotography unit. Quantitative microscopy was conducted to assess surface constants, including stomatal number and stomatal index, utilizing a light microscope to record the findings. The microphotography unit was utilized to examine different diagnostic characteristics of fresh leaves. The analysis of leaf powder was performed at SAIF(Sophisticated Analytical Instrumentation Facility), Chandigarh, using FESEM (Field Emission Scanning Electron Microscopy), while light microscopy was conducted at Bhawabhuti Mahavidyalaya, Amgaon.

leaf extracts preparation

Cryptocoryne retrospiralis Shade dried powder was obtained by soaking 50 g in 250 mL of various solvents, including water, ethanol, methanol, acetone and chloroform (70% v/v), for a week with periodic stirring. The macerated solution was then filtered, evaporated, and kept in an airtight container at 4°C for future experiments. [10]

Pharmacognostical analysis

Pharmacognostic parameters, including the amount of foreign organic matter, total ash percentage, acid-insoluble and water-soluble ash values & moisture content, were assessed in accordance with the WHO standards for the quality control of plant materials. Additionally, chemical tests and fluorescence analysis were conducted.[11]

Preliminary phytochemical screening

An initial qualitative phytochemical analysis of all the extracts was conducted to identify different active compounds using standard traditional methods.

Fluorescence analysis

Different chemical and water extracts of leaf powder were used for fluorescence analysis for UV short wavelength, UV long wavelength, & Visible light. Photographs & Data were recorded.

Results

1.1 Plant Morphology & its Uses

1. *Cryptocoryne retrospiralis* (Roxb.) Kunth
Family: -Araceae

Description: -Stoloniferous herbs that inhabit marshes are characterized by an abundance of linear-oblong, pointed, glabrous leaves that can reach lengths of up to 16 cm and widths of 0.8 cm, each featuring a closed, white sheath. A slender peduncle, measuring 2-3 cm in length, supports a spathe that is 10-12 cm long and divided by a transverse diaphragm positioned above the spadix. The tube of the spathe is 6 cm long above the diaphragm, while its spirally twisted, acuminate limb is pale brown with deep brown spots and measures 3 cm in length. The spadix, which is 14 mm long, is housed within the chamber and is slender in its middle section. The floral structure comprises 4 or 5 female flowers arranged in a basal whorl, featuring connate, single-celled ovaries with numerous ovules situated on parietal placentas, along with a short, curved style. Above these are 4 to 6 neutral flowers that create a whorl of appendages. Scattered throughout are many male flowers, each containing 1 or 2 stamens, clustered in terminal, globose formations. Fig. A, B, C & D

Macroscopic Characters,

Sr.No.	Part	Characters
1	Habit	Stoloniferous marshy herb, Seasonal
2	Leaf	Radical, sessile or subsessile, many, glabrous, Acute.
3	Leaf Margin	minutely serrate, undulated on margins
4	Leaf shape	Linear-oblong,
5	Length of leaf blade	24-26 cm
6	Width Leaf blade	1.3-2.5 cm
7	Inflorescence	Spadix
8	Peduncle	2-3 cm
9	Spathe	10-12 cm
10	Flower	Unisexual,
11	Male Flower: -	Many Male flowers with terminal globose clusters; stamens 1 or 2.
12	Female Flower: -	There are 4 or 5 female flowers, arranged in a single whorl at the bottom; the ovaries are fused, with one chamber, numerous ovules positioned on parietal placentas; the style is short and curved. Neutral flowers consist of 4 to 6, located above the female flowers, forming a whorl of appendages.
13	Fruit.	Ovoid
14	Seeds	Usually 2,

Uses of Cryptocoryneretrospiralis: -It is commonly utilized as a leafy vegetable. The leaves are typically employed in the preparation of chilli (Chapati preparation mixing with leaves of *C. retrospiralis*) during the winter season. It is used in treating conditions such as antiemesis, boils, burns, and vomiting experienced during pregnancy [12,13]

Fig. A Natural Habitat

Fig B.Plant body

Fig. C. width of Lamina 1.3 cm

Fig.D. Length of Leaves 25.2 cm

Pharmacognostical analysis

The presence of foreign organic matter in the powdered plant sample was measured at 58.6±1.26%. The extractive yield of *C. retrospiralis* was found to be 14.32±0.04% when using alcohol (ethanol) and 26.31±0.12% in water. The moisture content, determined by weight loss during drying at 105°C, for CR was observed to be 93.00±0.26%. The pH level of the dried powder was recorded at 7.15±0.03, while the electric conductivity measured 1697.05±62.85 µS/cm. The nitrogen content in C. retrospiralis was 19320±371.5 mg/kg, and the protein content was determined to be 18.11±0.0007 g/kg. The dry ash's physical state was a fine, greyish-white powder that had a strong taste. The total ash content, along with the acid-insoluble and water-soluble ash components of CR, was quantified as 7.26±0.06%, 0.72±0.04%, and 4.80±0.11%, respectively.

T. S. of Leaves

The leaf blade, viewed in cross-section, displays the standard arrangement of tissues: a uniseriate upper and lower epidermis enclosing the mesophyll. Both epidermal layers are protected by a variably thick cuticle, and stomata interrupt their continuity.

The mesophyll is characterized as homogeneous and consists of loosely arranged, irregularly shaped cells with prominent intercellular spaces. This undifferentiated mesophyll, which appears similar on both the upper and lower surfaces, classifies the leaves as isobilateral, consistent with observations in other aquatic plants. Immediately beneath the upper epidermis are two to three layers of chlorenchyma tissue, containing some chloroplasts. The lower epidermis is slightly more prominent than the upper. In the midrib region, the cells are larger, possess thin cellulosic walls, fewer chloroplasts, and have relatively smaller intercellular spaces. At the leaf margins, the mesophyll narrows to approximately four cell layers, two of which are richer in chloroplasts. The midrib itself contains a closed collateral vascular bundle with limited conductive elements. The xylem, located toward the upper epidermis, comprises two to four vessels and a large protoxylem lacuna. The phloem, oriented toward the lower epidermis, is represented by a few phloem vessels. A group of sclerenchyma cells providing mechanical support is situated beneath the vascular bundle.

Fig.E. T.S. of leaf showing VB, Xy& Ph, with epidermis & Mesophyll cell

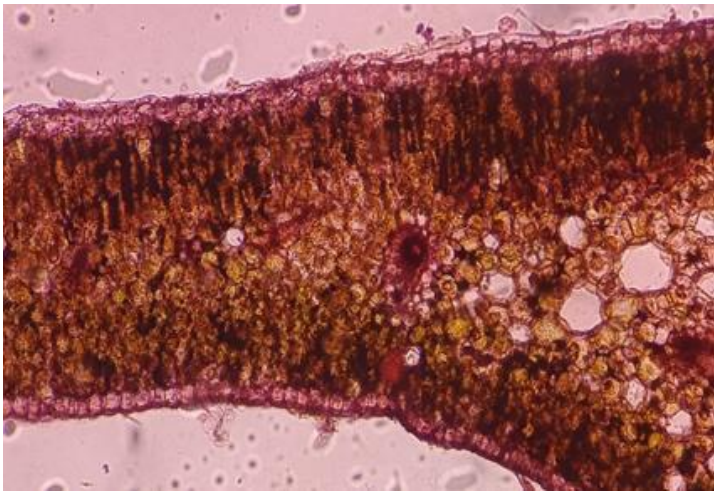


Fig. F. Lamina of leaves showing chlorenchymatous mesophyll cell

Stomatal study:-

Cryptocoryne retrospiralis: - The stomata exhibit a paracytic arrangement. The guard cells are elongated and possess a kidney-like shape, characterized by dense granular contents. Each guard cell features a robust inner and outer ledge. The subsidiary cells are elongated and semicircular, displaying thick walls. The contents of the cells are notably prominent.

Fig. F. Lamina of leaves showing chlorenchymatous mesophyll cell

Name of Plant	Epidermal cell	Epidermal shape	Stomatal type	Stomatal index	Stomatal length	Stomatal width
Cryptocoryne retrospiralis	polygonal	Elliptical circular	Paracytic	20.30	1.10µm	0.80µm

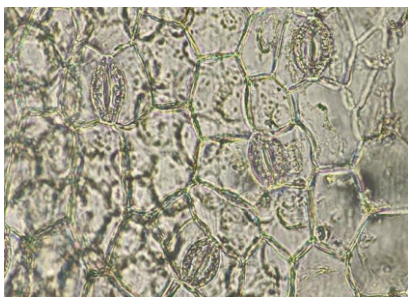


Fig. G. Upper epidermis showing paracytic stomata

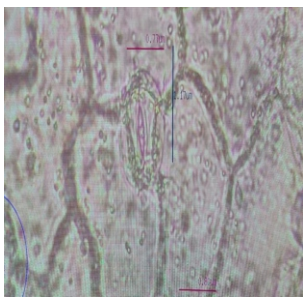
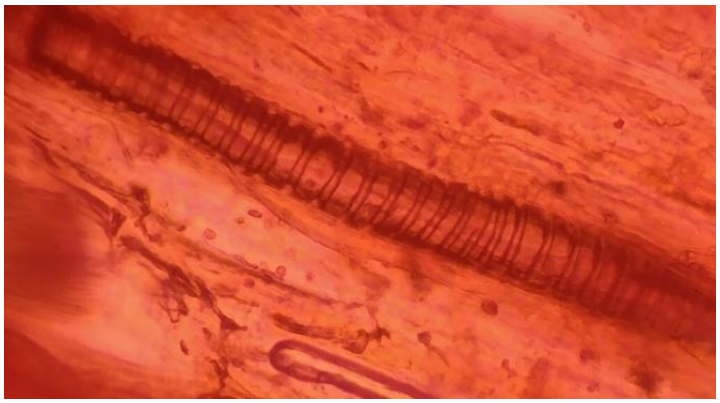


Fig. H. Measurment of stomata

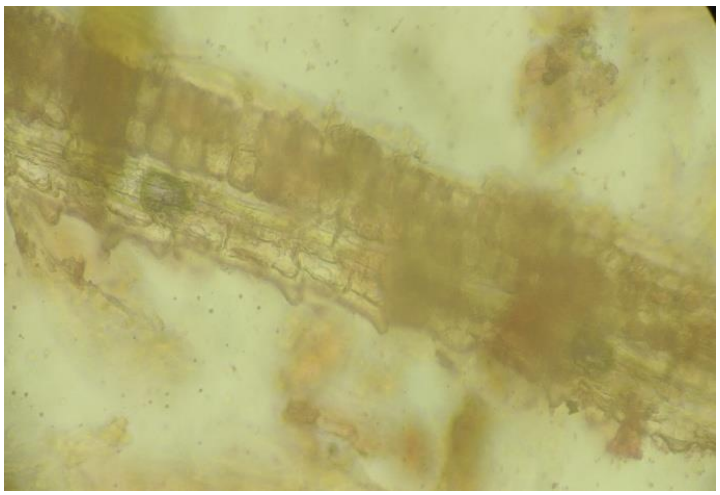
Powder microscopy

Cryptocoryne retrospiralis leaf powder as such are muddy in colour & bitter in taste. The Epidermal cells were parenchymatous, thin-walled, polygonal and tangentially elongated cells with a starch grain. Vessel's element present in leaf powder. Starch granules are simple, round & elliptical in shape. Raphides, phloem fibres were present. Leaf powder contained different tissue fragments of epidermal cells. Paracytic Circular elliptical-shaped stomata were present in

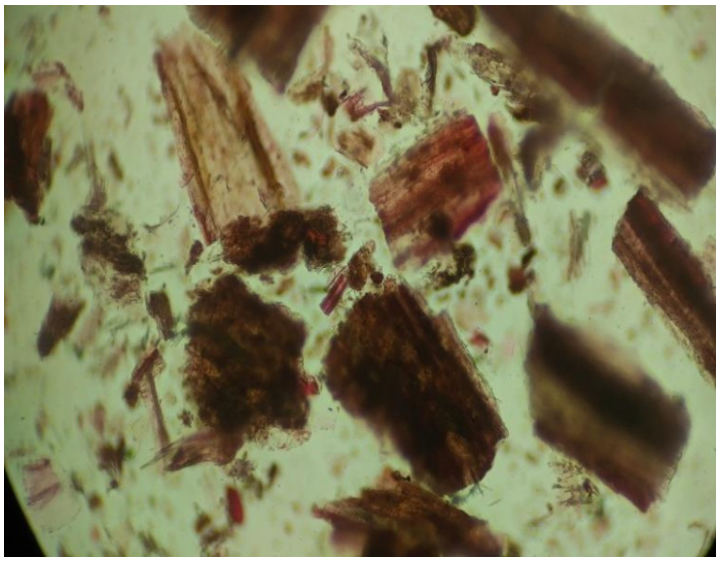
the epidermis with 3-4 wavy subsidiary cells. Xylem fibres, reticulated and thickened helical vessels in groups, annular and spiral tracheids, oilbodies in cortical cells and cystoliths were also detected in the powder of the leaf.



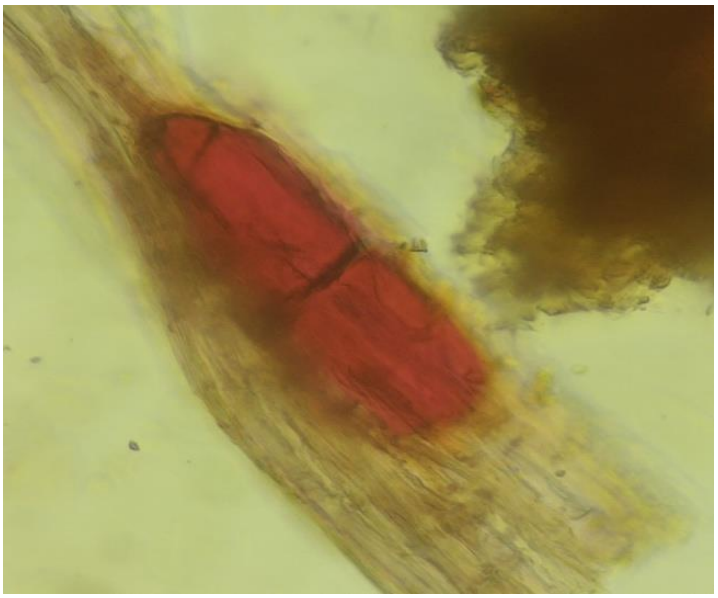
Spiral vessels



Phloem fiber



Fiber fragment



Calcium oxalate crystal

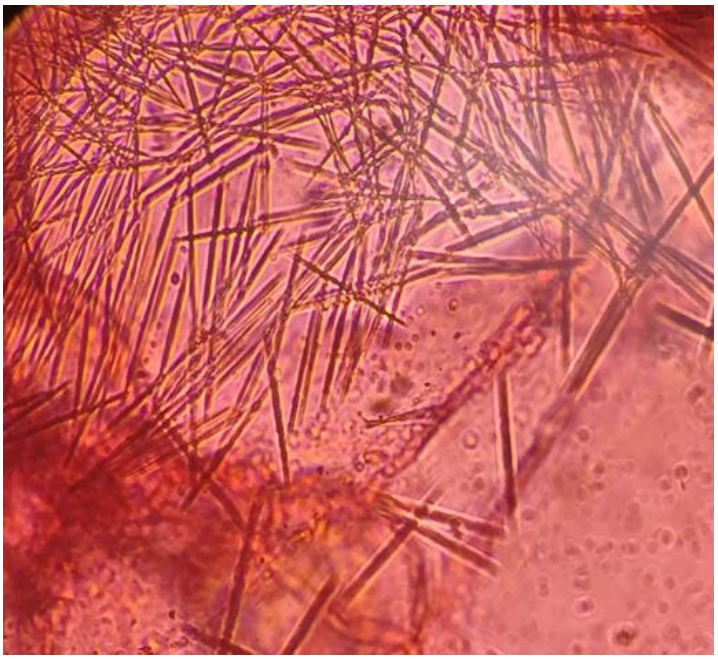
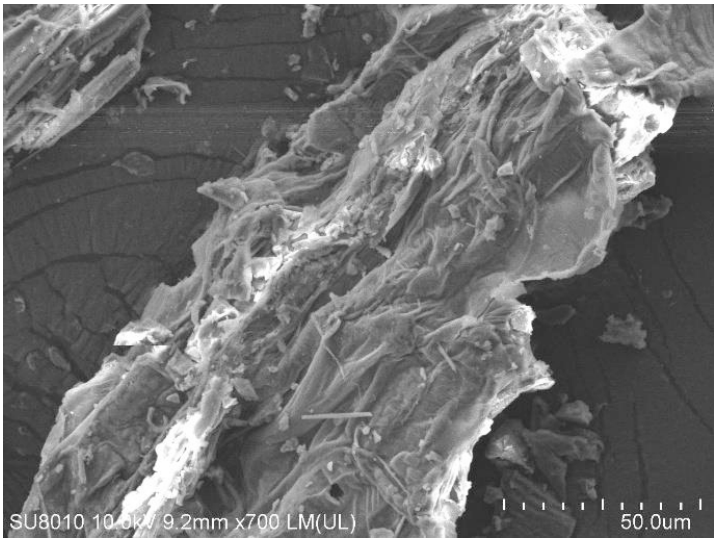
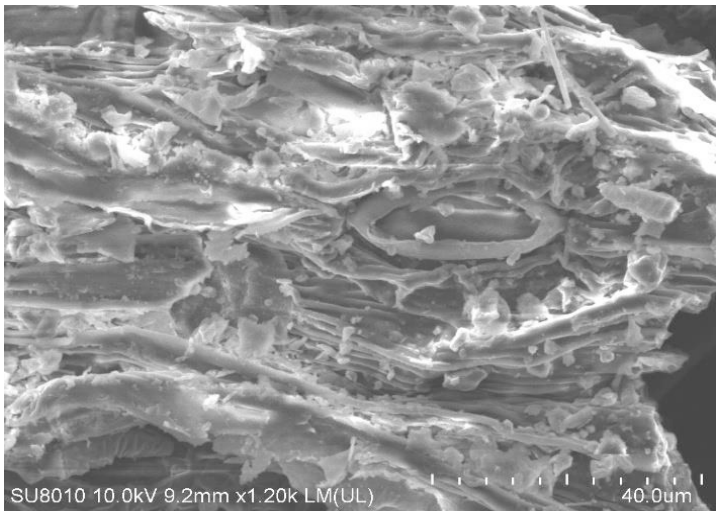


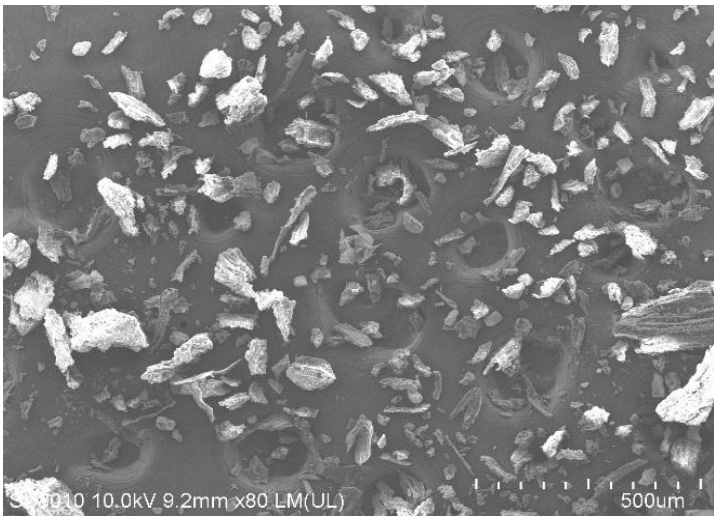
Fig Acicular Crystal



Crystal in FESEM



FESEM Showing Paracytic stomata



Mesophyll tissue

Phytochemical analysis

The results from the preliminary phytochemical analysis of water, Ethanol, methanol, Acetone, and chloroform extracts derived from the leaves of *C. retrospiralis* are summarized in Table 1. Predominantly, the phytoconstituents detected in polar solvents such as water, ethanol, and methanol consist of carbohydrates, alkaloids, proteins, glycosides, sterols, phenolics, flavonoids, tannins, as well as fat and fixed oils, which constitute the major phytochemicals found in the foliage of *C. retrospiralis*. The water extract indicates the presence of carbohydrates, proteins, phenolics, tannins, glycosides, saponins, alkaloids, and flavonoids. The methanol extract contains carbohydrates, proteins, alkaloids, phenolics, tannins, flavonoids, glycosides, saponins, and sterols. Notably, both acetone and chloroform extracts comprise fats and Fixed oils.

Table 1

Sr.No	Name of Compound	Water Extract	Ethanol Extract	Methanol Extract	Acetone Extract	Chlorofrom Extract
1	Carbohydrate	+	+	+	+	-
2	Proteins and Amino acids	-	+	+	-	-
3	Alkaloids	-	+	+	-	-
4	Phenolics compounds	+	+	+	-	-
5	Tannins	-	+	+	+	-
6	Flavonoids	-	+	+	+	-
7	Coumarine	-	+	+	+	-
8	Steroids	-	+	+	-	-
9	Glycosides	+	+	+	-	-
10	Saponins	-	+	-	-	-
11	Fats &Fixed oils	-	-	-	+	+

(+ present & - absent)

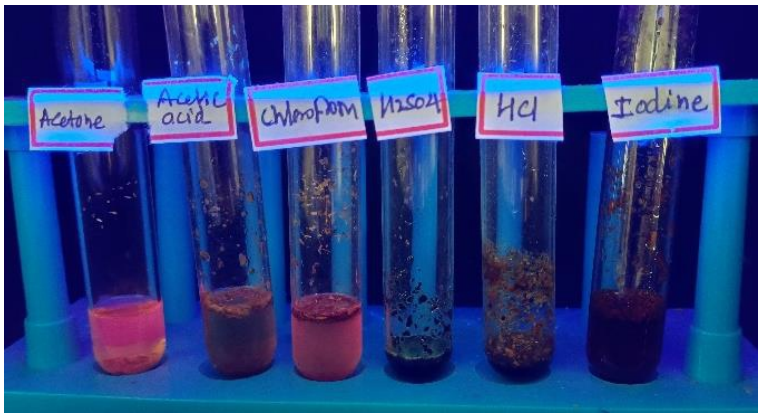
Fluorescence analysis

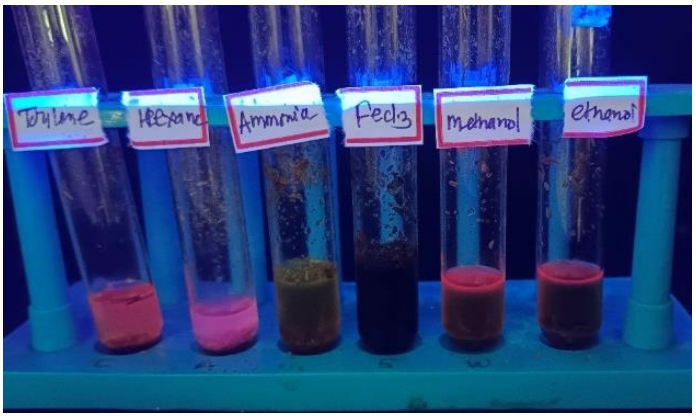
Crude powder of *Cryptocoryne retrospiralis* is exposed to ultraviolet (UV) light, under a UV & visible light chamber for identification of phytoconstitution based on fluorescence colour. Different chemicals were used for fluorescence analysis, viz. Acetone, Acetic acid, Chlorofrom, H_2SO_4, sodium hydroxide, hydrochloric acid, nitric acid, iodine, and ferric chloride and the results of different chemical reagents were noted in Table No.2

Table 2

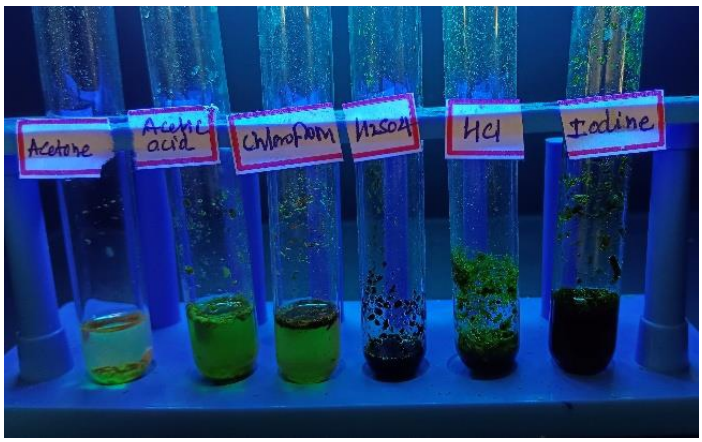
Sr. No.	Chemical	Short UV (254)	Long UV (356)	Visible Light
1	Acetone	Green	Pink	Yellowish green
2	Acetic acid	Green	Redish Brown	Green
3	Chloroform	Green	Magenta	Green
4	H2SO4	Fluorescent blue	Navy blue	Dark blue
5	HCL	Moss green	Navy blue	Green
6	Iodine	Brown	Dark blue	Dark blue
7	Toluene	Green	Redish pink	Yellowish green
8	Hexane	Light green	Pink	Pale green
9	Ammonia	Green	Tan	Green
10	Water	Pale green	Light blue	Pale green
11	FeCl3	Dark green	Dark blue	Navy blue
12	NaoH	Green	Redish Brown	Green
13	Petroleum ether	Light green	Pink	Light pink
14	KOH	Green	Redish brown	Green
15	Methanol	Green	Pinkish Red	Green with orange margin
16	Ethanol	Green	Pinkish Red	Green with orange margin

Short UV 254 nm





Long UV 356nm



Visible light

Discussion

The microscopic features of *Cryptocoryne retrospiralis* reveal the existence of paracytic elliptical and circular stomata. The anatomical characteristics of the leaves indicate that both the upper and lower epidermis contain closed collateral vascular bundles with a limited number of conducting elements. The xylem, located near the upper epidermis, displays the physiochemical properties of the plant, with a protein content recorded at 18.11±0.0007 g/kg. Moreover, the organic matter content in the powdered plant material was noted to be 58.6±1.26%. The pH level of the drug was assessed at 7.15±0.03, and the electric conductivity was measured at 1697.05±62.85 µS/cm. Initial phytochemical analysis of the water, methanol, Ethanol, Acetone and chloroform extracts from the leaves of *C. retrospiralis* highlighted the presence of carbohydrates, proteins, alkaloids, tannins, glycosides, sterols, phenolics, flavonoids, fixed oils, and fats as the primary constituents. The water extract was found to contain carbohydrates, proteins, alkaloids, phenolics, tannins, glycosides, saponins, and flavonoids. The methanol extract was identified to have carbohydrates, proteins, alkaloids, phenolics, tannins, flavonoids, glycosides, saponins, and sterols. Both acetone and chloroform extracts were observed to contain fats & fixed oils. This observation supports findings from other researchers [3]. The majority of phytochemicals were identified in water, ethanol, and methanol, suggesting that the best results were obtained using petroleum ether (non-polar), ethanol (polar), and water (even more polar) solvents based on their polarity. Similar findings were documented by other researchers regarding different plant extracts from these solvents [14]. In powder microscopy, the plant shows the presence of raphides,

calcium oxalate crystals, fibres, and spiral vessels. Additionally, fluorescence describes the phenomenon exhibited by various chemical constituents found in plant material. Certain components show fluorescence in the visible spectrum during the day. Ultraviolet light can induce fluorescence in many natural products such as alkaloids like berberine that may not exhibit visible fluorescence under normal daylight conditions. If the substances themselves do not fluoresce, they can often be converted into fluorescent derivatives or decomposition products using various reagents. Thus, some crude drugs are typically evaluated qualitatively in this manner, making it an important element of pharmacognostical assessment [15,16].

Conclusion

The Current study on *Cryptocoryne retrospiralis* leaves reveals several phytoconstituents with various pharmacological properties. This plant holds promise for the development of alternative medicines. As a leafy vegetable, it contains a significant amount of protein and fibre, indicating its suitability for nutritional purposes and its role as a source of natural antioxidants for pharmaceutical and nutraceutical uses. Therefore, it should be promoted to the public. Efforts to conserve and raise awareness about underutilized indigenous vegetables are essential. Further research is required to fully understand the potential of this plant.

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Conflict of Interest: -We do not have any conflicts of interest to report.

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