



Pharmaceutical Standardization

Pharmacognostical and phytochemical studies of *Curcuma neilgherrensis* (Wight) leaf - A folklore medicineShyam Prasad M., Anju P. Ramachandran¹, Harimohan Chandola², Harisha C. R.³, Vinay J. Shukla⁴

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Abstract

Curcuma neilgherrensis Wight is a folk medicinal plant used in the management of diabetes mellitus. The leaves of this herb are said to be successful in managing high blood glucose levels. This study is aimed at assessing the scientific appraisal of *C. neilgherrensis* in the course of pharmacognostical characters and phytochemical parameters, as these are not yet been done. Pharmacognostic study mainly covered the macroscopic and microscopic features of the leaves including powder microscopy, and revealed the presence of trichomes, spiral vessels etc. Phytochemical parameters such as pH, total ash value, water-soluble extract and MeOH extract values were assessed in the preliminary physicochemical screening. Qualitative analysis revealed the existence of certain chemical constituents such as flavonoids, tannins, organic acids and saponin glycosides. The crude extract of leaves was subjected to TLC and HPTLC for the separation of components.

Key words: *Curcuma neilgherrensis*, HPTLC, pharmacognosy, phytochemistry, TLC

Introduction

India's biodiversity offers a great deal of opportunities in health and allied sciences. The existence of traditional as well as folk medicine depends upon this biodiversity. The information of medicine, which is being transformed verbally from generation to generation and not in the course of written documentation is generally known by the name "folklore medicine". It was the only one means through which the ancestors fulfilled their medicinal needs. Even though it was evolved by the individual and ethnic experiences, it needs further investigations in stipulations of different branches of advanced scientific techniques to tackle the issues like that of identification, standardization, safety profile etc. Moreover, in the management of diseases such as diabetes mellitus, the alliance between different branches of medical sciences has much to offer for the suffering populace.

Curcuma neilgherrensis Wight (Family: Zingiberaceae); known as "Kattukalvazhai" in Tamil is a folklore medicine widely used by the tribes of Western Ghats for the management of diabetes mellitus. The traditional medical practitioners of Kodagu District of Karnataka have identified its usage in

diabetes mellitus, even so the Palliayar tribes of Tamilnadu are using its tuber for edible purposes. The leaf is considered as the useful part for counteracting the ill effects of diabetes mellitus. In spite of its reputation, it has not yet been investigated scientifically and hence its leaves were thought worth to study in detail. The available textual information regarding the herb is very minimum and inadequate. This paper reviews research on the identification, authentication and qualitative analysis. The plant is extensively distributed in the southern part of India, especially over high altitudes [Figure 1].

C. neilgherrensis is a herb with small conical rhizomes internally whitish in color, ending in root tubers, fusiform. Leaves green, lanceolate/oblong - lanceolate in shape, 25cm in length and 8 cm wide [Figures 2 and 3]. Inflorescence present in both lateral and central, long with a distinct coma. Coma bracts are oblong-lanceolate, fused only at base, light to dark pink or violet in color. Fertile bracts are fused about lower 1/3, slightly curved, margin wavy, green, green with a pink or violet spot at the tip, and densely pubescent. The bracteoles are triangular in shape. Flowers are longer than the bracts, 3-4 in each bract, and light yellow in color. Calyx three lobed at apex, violet dotted, and densely pubescent. Corolla tube light yellow in color, lobes unequal, pubescent, hooded at tip. Labellum shows a median cleft, yellow with a deep yellow median band.^[1] Flowers usually appear in the season of February-March, and the leaves are not visible at the time of flowering [Figure 4].^[2]

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Materials and Methods

Collection of plant material

The whole plant with leaves was collected in the season of June, from the natural habitat of Talakaveri forests of Kodagu District, Karnataka, a tail of Western Ghats, and accurately identified with the help of different floras^[3-5] at Pharmacognosy Laboratory, I.P.G.T. and R.A., Gujarat Ayurved University, Jamnagar. Matured leaves were separated from the plant, cut in to pieces, shade dried, coarsely powdered (10 mesh), and used for further study. The rest of leaf sample was preserved in the solution of F.A.A (70% Ethyl alcohol: Glacial acetic acid: Formalin in the ratio of 90:5:5) to study the histological profile.

Pharmacognostical studies

The plant was macroscopically studied. Free hand transverse sections of leaves were taken and examined. Surface preparation was done and both the surface of leaves were observed. Organoleptic evaluation was done for the appreciation of color, odor, and taste. The powder microscopy of dried leaves was also carried out. The microphotographs were taken using the Carl Zeiss binocular microscope.

Physico-chemical and Phytochemical studies

The physico-chemical parameters such as loss on drying, total ash content, pH, and extractive values, (water-soluble and alcohol soluble) were determined. Physico-chemical parameters were analyzed in accordance with the Ayurvedic Pharmacopeia of India. The extracts were further analyzed for the presence of constituents such as tannins, flavonoids, and alkaloids, etc. Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) were performed for the normal phase separation of components.

Results and Discussion

The macroscopic analysis revealed that the leaves of *C. neilgherrensis* are lanceolate or oblong-lanceolate in shape, measuring about 25 cm in length and 6 cm width with parallel venation.

Organoleptic characters

The nature of powder was coarse, grey in color, bitter in taste, and slightly aromatic in odor.

Surface preparation

Small pieces of (2 mm square) the leaf in the solution of chloral hydrate in a test tube were taken, boiled in water bath until get transparent material, mounted in glycerin and the following characters like trichomes, epidermal cells [Figure 5], and stomata [Figure 6] were observed under the microscope.

Transverse Section of leaf

The epidermis is found on both upper and lower surfaces of the leaf. Single-layered oval-shaped cells covered with cuticle, some cells with trichomes, stomata present at both the sides, mesophyll undifferentiated compactly arranged thin-walled isodiametric, chlorophyllous with intercellular spaces. Some cells filled with tannin materials. The vascular bundles [Figure 7] are collateral and closed. The xylum is found towards

upper side and phloem towards the lower side surrounded by bundle sheath, consisting of thin parenchyma cells loaded with starch grains. Xylem consists of vessels and phloem of sieve tubes and companion cells [Figure 8]. Sclerenchyma cells present in patches at both ends of the midrib region. It gives mechanical support to the vascular bundles and also for the leaves.

Powder microscopy

Leaves were shade dried and made in to coarse powder for the powder microscopy. The diagnostic features observed were the trichomes [Figure 9], spiral vessels [Figure 10], epidermal cells [Figure 11], chloroplasts, abundance of fibers, prismatic crystals, tannins [Figure 12], etc. which are helpful in identification and authentication of the plant material in future works [Table 1].

Phytochemical analysis

Physico-chemical parameters and qualitative tests of powder of *C. neilgherrensis* were done. The value of total ash (8.02%

Table 1: The microscopic features of the dried leaves of *C. neilgherrensis* Wight

Features identified	Result
Trichomes	++
Chloroplasts	++
Fibers	++
Prismatic crystals	++
Tannin contents	++
Spiral vessels	++
Parenchyma cells	++
Epidermal cells	++

++: Present

Table 2: Physico-chemical parameters of dried leaves of *C. neilgherrensis* Wight

Parameter	Result
Loss on drying	4.5% w/w
Determination of total ash	8.02% w/w pH 6.27
Water soluble extract	6.4% w/w
Alcohol soluble extract	8.0% w/w

Table 3: Phytochemical screening of methanol extract of *C. neilgherrensis* wight

Qualitative parameter	Results
Flavonoids	++
Alkaloids	++
Carbohydrates	++
Tannins	++
Proteins	—
Amino acids	—
Organic acids	++
Steroids	—
Cyanogenetic glycosides	—
Cardiac glycosides	—
Saponin glycosides	++

++: Present, --: Absent

w/w) shows the presence of residue after ignition, representing the plant residue, and external materials. Total ash is the gauge of non-volatile inorganic constituents remaining subsequent to incineration. It is made up of physiological and non-physiological ash. The physiological ash consists of silicates, carbonates, chlorides, nitrates, and phosphates of metals that the plant acquired when it was growing. The non-physiological ash represents the ash from external matter. pH^[6] the quantitative measure of alkalinity or acidity was studied with the help of digital pH-indicator. The pH of the water-soluble extract was observed as 6.27, suggesting that the sample is near to neutral or slightly acidic in nature. Further

analysis of crude drug yielded water-soluble extract of 6.4% w/w and alcohol-soluble extract about 8.0% w/w [Table 2]. The qualitative analysis revealed the occurrence of flavonoids, alkaloids, tannins, organic acids, and saponin glycosides that will be the land marks in authentication of this plant [Table 3].

TLC and HPTLC profiles

Thin-layer chromatography^[7] [Table 4] and high-performance thin-layer chromatography^[8] [Table 5] were executed for the normal phase separation of components of methanol extracts of leaves of *C. neilgherrensis* Wight. For TLC screening,

Table 4: TLC profile

Track 1		254 nm		366 nm	
Methanol extract	No. of spots	R_f		No. of spots	R_f
	3	0.16, 0.75, 0.85		6	0.16, 0.41, 0.66, 0.75, 0.85, 0.9

Table 5: HPTLC profile

Track 1		254 nm		366 nm		After post chromatographic deprivation
Methanol extract	No of spots	R_f		No of spots	R_f	R_f
	6	0.22, 0.30, 0.32, 0.43, 0.60, 0.68		2	0.32, 0.68	0.035, 0.10, 0.57, 0.63, 0.71, 0.98



Figure 1: Talkaveri hills



Figure 2: Plant in natural habitat



Figure 3: Leaves



Figure 4: Inflorescence

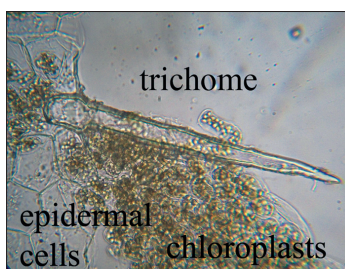


Figure 5: Upper epidermis



Figure 6: Lower epidermis

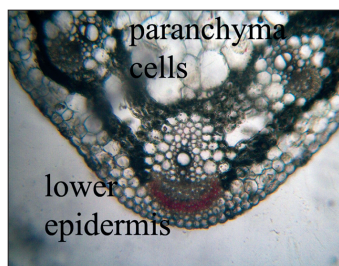


Figure 7: T.S. through mid rib

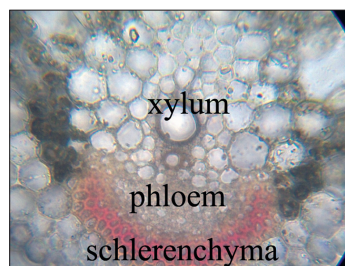


Figure 8: Vascular bundle



Figure 9: Simple trichome



Figure 10: Spiral vessel

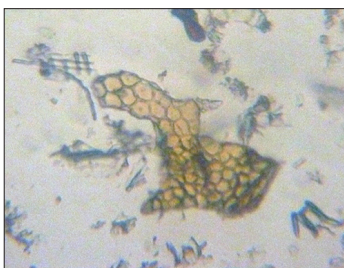


Figure 11: Epidermal cells

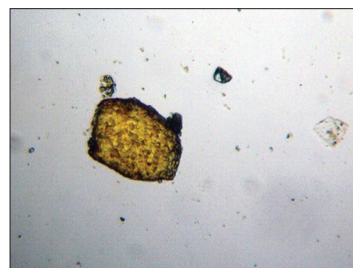


Figure 12: Yellowish-brown tanin content

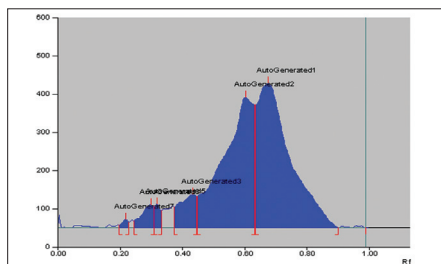


Figure 13: HPTLC densitogram at 254nm

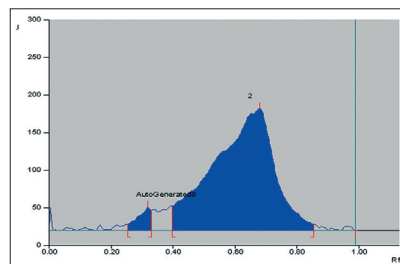


Figure 14: HPTLC densitogram at 366nm

solvent system was prepared by taking toluene and ethyl acetate in a proportion of 8:2. Stationary phase for the TLC profile was silica gel. The spots obtained from the extract were examined under ultra violet light of wavelength 254 and 366 nm. The resolution factor was calculated by using the formula $R_f = \text{distance travelled by solute} / \text{distance travelled by solvent}$.

After postchromatographic deprivation with Anisaldehyde H_2SO_4 , the R_f values were obtained at 0.16, 0.75, 0.85. The solvent system prepared by toluene and ethyl acetate in a ratio of 8:2 was used for the HPTLC [Figures 13 and 14] screening of methanol extracts of leaves of *C. neilgherrensis*.

When the sample is tested in 254 and 366 nm, two spots were identical, that is, 0.32 and 0.68. After postchromatographic deprivation with anisaldehyde H_2SO_4 , six spots were obtained with an R_f value of 0.035, 0.10, 0.57, 0.63, 0.71, and 0.98. The presence of spots after this specific spray may be suggestive of occurrence of phenolic-type compounds or tannins.

Conclusion

The available textual information regarding the herb *C. neilgherrensis* is very minimum and inadequate. As the drug is widely used in conditions like diabetes mellitus by folklore practitioners, and to adopt it in current herbal medicinal practice, it is essential to conduct the pharmacognostical and phytochemical analysis that covers the preliminary steps of standardization. Therefore, this study was carried out with the objective of claiming identification and also to investigate the

physico-chemical and qualitative analysis. The microscopic parameters and other physico-chemical reports, which are obtained in this work, can be used for the confirmation and diagnosis of this plant. TLC and HPTLC results showed the presence of certain constituents with matching R_f values when visualized in UV radiation of various wavelengths. Hope that the current study and the facts obtained here, may act as a stepping stone for further cavernous research works on *C. neilgherrensis* Wight in the field of plant science and medicine.

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हिन्दी सारांश

एक लोक औषधि *Curcuma neilgherrensis* के पत्रों का स्वरूपलक्षणात्मक एवं रासायनिक अध्ययन

श्यामप्रसाद एम., अंजु पी. रामचन्द्रन, हरिमोहन चन्दोला, हरीशा सी. आर., विनय जे. शुक्ला

Curcuma neilgherrensis Wight, मधुमेह की चिकित्सा में प्रयोग की जाने वाली एक लोकपरंपरागत औषधि है। इस औषधि के पत्रों का सफल प्रयोग रक्तशर्कराधिक्य की चिकित्सा में किया जाता है। प्रस्तुत अध्ययन का मुख्य उद्देश्य *Curcuma neilgherrensis* की गुणवत्ता का वैज्ञानिक रूप से स्वरूपात्मक लक्षण और रासायनिक मापदण्डों के आधार पर सत्यापित करना है, जो अब तक नहीं किया गया है। स्वरूप लक्षणात्मक अध्ययन में मुख्यतः पत्रों के सूक्ष्म और स्थूल लक्षणों का अध्ययन किया गया, जिसके अंतर्गत चूर्ण सूक्ष्मपरीक्षण में trichomes एवं spiral vessels, आदि पाए गए। प्रारंभिक भौतिक रासायनिक अध्ययन में रासायनिक मापदण्ड जैसे कि pH, total ash value, water soluble extract, MeOH extract, आदि का आंकलन किया गया। गुणात्मक अध्ययन में कुछ रासायनिक तत्व, जैसे flavonoid, tannin, organic acid, saponin glycosides, आदि मिले। पत्रों के घनसत्व का HPTLC एवं TLC अध्ययन घटकों के पृथक्करण हेतु किया गया।