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Original Article

Macro-Microscopic evaluation, Physicochemical analysis and HPTLC Finger printing of *Curculigo orchioides* Gaertn. Rhizome

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ABSTRACT

Introduction: *Curculigo orchioides* Gaertn. Family Hypoxydaceae (Amaryllidaceae) is a perennial herb with finger like rhizome. Its rhizomes have been significantly used in treatment of various ailments including cancer. **Methods:** Macro-microscopic analyses, physico-chemical studies and HPTLC finger printing of rhizomes of *C. orchioides* were performed according to pharmacopoeial procedures. **Results:** Microscopic analysis has shown rows of rectangular and conical cells in cork, thin walled cortex, parenchymatous cells, starch grains, crystal of calcium oxalate, mucilage cell and spiral xylem vessel in the rhizomes of *C. orchioides*. Physicochemical studies revealed loss on drying (10.469%), total ash (8.463%), acid insoluble ash (1.10%), water-soluble ash (2.163%), alcohol soluble extractive value (3.036%), and water-soluble extractive value (22.666%) of the raw drug. Ethanol extract of the plant were fingerprinted in petroleum ether and ethyl acetate (7.0:3.0) solvent system and scanned densitometrically after dipping in anisaldehyde-sulphuric acid reagent followed by heating at 105°C. **Conclusion:** These specific identities will be useful in identification and authentication of the raw drug in dried form.

Key words: *Curculigo orchioides*, HPTLC fingerprinting, Pharmacognostic, Standaridisation, Quality control.

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INTRODUCTION

Curculigo orchioides Gaertn., syn. *C. malabarica* Wight., *C. brevifolia* Dryand.[1] and *Hypoxis orchioides* Kurz.[2] is well known medicinal plant known as Kalimusli in Hindi. It is a perennial herb with a rosette of sensible with linear lanceolate, membraneous leaves and bright yellow colour flowers, closed to ground.[2] The plant is native to India[3] and distributed from sub-tropical Himalayas, West Bengal, Assam, Konkan, West peninsula to Kanyakumari.[2] Its rhizomes are widely used as a demulcent, diuretic and aromatic tonic, aphrodisiac, in the treatment of leprosy and nervous disease.[4] Rhizomes of *C. orchioides* have been reported for their medicinal properties like platelet regeneration,[5] antioxidant activity,[6] hepato-protective efficacy,[7] antipyretic activity[8] and immune stimulant properties.[9]

Vedic manuscripts described the therapeutic potential of *C. orchioides* and are well documented in the treatise of Ayurveda in the name of Talamuli. Accordingly, the synonyms and medicinal properties of *C. orchioides* are depicted in Sanskrit shlokas mentioned in Bhavprakash Nighantu (Shloka 183).[10]

Rhizomes of this plants has been described to possess medicinal properties as sweet, cooling, diuretic, aphrodisiac, anti-aging and considered as a Rasayana drug which can be used in male infertility, hemorrhoids, piles, which also increases Kapha and reduces Pitta daha (burning sensation), acts as stimulant and gives strength.

C. orchioides has been reported with constituents[11] such as flavones, glycosides, steroids (sitosterol, stigmasterol and yuccagenin),[12] terpenoids, saponins, glucose, mannose, xylose, glucoronic acid, resin, tannin, fat, mucilaginous substances[13] and other secondary metabolites. Steam distilled fraction of *C. orchioides* has reported for its antibacterial activity against Gram-negative and Gram-positive pathogens.[14] Methanolic extract of rhizomes of *C. orchioides* is reported for its immunostimulatory action against cyclophosphamide induced toxicity and neuroprotective efficacy against cyclophosphamide neurotoxicity.[15-16] Various studies have been reported for its antidiabetic activity; ethanolic extract and aqueous extract of *C. orchioides* in alloxan induced diabetes.[17-18]

With this background detailed quality control studies were undertaken for this traditional raw drug with the aim of developing standards of authenticity.

MATERIALS AND METHODS

Collection and Identification

Dried rhizomes were collected from local Ayurveda pharmacy in Gwalior, Madhya Pradesh. The plant material was authenticated at Pharmacognosy department of SDM Center for Research in Ayurveda and Allied Sciences, Udupi, Kuthpady, Karnataka and (a specimen (725/16021205) is being maintained for future reference. The dried rhizomes were cleaned, coarsely powder and used for macroscopic and microscopical characterization, phytochemical analysis and HPTLC.

Macro-microscopic analysis

Macroscopic characters of fresh rhizome and powder were keenly observed under naked eyes to record the specific botanical characters. The external features of the test samples were documented using Canon IXUS digital camera.

Rhizomes were preserved in formalin-acetic acid-alcohol preservative solution {5% formalin (5 ml), 5% acetic acid (5 ml) and 50% ethyl alcohol (90 ml)}, before 48 hours[19] of microscopic analysis. Very thin transverse sections of rhizome were obtained using sharp blade followed by safranine staining.[20] Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of figures are indicated in scale bars.

For powder microscopy, 1g of coarse powder was sift through 80 pore size mesh. A pinch of powder was mixed with drops of choral hydrate on

microscopic slides and mounted with a drop of glycerine-water. Slides were observed and characterized under Zeiss AXIO trinocular microscope. Magnifications are indicated by scale bars.[21]

Physico-chemical analysis: Physico-chemical characterization like loss on drying at 105ºC, total ash, acid insoluble ash, water soluble ash, ethanol and water soluble extractives values were determined as per Quality Standard of Indian Medicinal Plants.[22]

HPTLC Fingerprinting

One gram of powdered rhizomes were extracted with 10 ml ethanol and kept for cold percolation for 24 h and filtered. 4, 8 and 12 µl of the plant extract were applied on a pre-coated silica gel F254 on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. *C. orchioides* plate was developed in Petroleum ether: Ethyl acetate (7.0:3.0 v/v). The developed plate was visualized in UV 254 nm, 366 nm and then derivatised with anisaldehyde sulphuric acid reagent[23] and scanned under UV 254 nm, 366 nm and 620 nm post derivatisation. R_f, colour of the spots and densitometric scan were recorded using CAMAG Scanner 4.[24-25]

RESULTS

Macro-microscopic observations: Macroscopic studies showed blackish brown rhizomes about 10 to 15 cm long, cylindrical and straight to slightly curve. Transversely cut pieces are yellowish brown externally and yellowish off-white internally. External surface was prominent with wrinkled, transverse cracks with a few root scars, nodes and internodes (Figure 1a-c). Microscopic characters of transverse section of *Curculigo orchioides* with narrow strip of cork consisting of 5 to 7 rows of conical and rectangular cells, thin walled cortex consists of parenchymatous cells, densely filled starch grains and acicular crystal of calcium oxalate and vascular bundle lying underneath the endodermis (Figure 1d-1f) . Powder microscopy of coarse powder of rhizomes of *Curculigo orchioides* was dark brown, slightly bitter in taste. Powder microscopy depicts the cork in transverse and surface view (Figure 2a-c). Powder contains annular crystal of calcium oxalate, mucilage cell and spiral xylem vessel. Power microscopy also shows spiral fragments of lignified vessels of vascular bundles (Figure 2d-2l).

Physico-chemical analysis: Physico-chemical characters were performed as per Quality Standard of Indian Medicinal Plants[22] (Table 1).

HPTLC Finger printing: R_f values and colour of the spots in chromatogram developed in petroleum ether:ethyl acetate (7.0:3.0) for ethanolic extract of rhizomes were recorded (Table 2). TLC photo-documentation revealed presence of many phytoconstituents with different R_f values and HPTLC densitometric scan of the plates showed numerous bands under short UV, long UV and 620 nm (after derivatisation). On photo documentation no spots under short UV, 4 spots under long UV and 3 spots under 620 nm post-derivatisation with anisaldehyde sulphuric acid spray reagent (Figure 3a-c). Densitometric scan at 254 nm revealed 5 peaks corresponding to 5 different compounds in the ethanolic extract, compounds with R_f - 0.03 (71.37%), 0.19 (2.77%), 0.38 (18.47%), 0.76 (2.91%) and 0.88 (4.47%) in Figure 4a. Densitometric scan at 366 nm, (Figure 4b) showed 8 peaks, peak with R_f – 0.07 (9.04%), 0.11 (14.03%), 0.17 (10.40%), 0.22 (50.45%), 0.75 (5.94%), 0.86 (5.30%), 0.90 (3.38%) and 0.98 (0.45%). Figure 4c depicts 8 peaks- with R_f – 0.03 (42.03%), 0.32 (3.89%), 0.37 (6.48%), 0.41 (5.57%), 0.58 (9.36%), 0.65 (11.79%), 0.85 (10.69%) and 0.86 (10.18%) (Table 3).

DISCUSSION

C. orchioides rhizomes are mucilaginous in nature and well known Rasayana drug in Ayurveda System of Medicine. Morphological and anatomical standardization of herbal drugs needs the information from basic disciplines of plant sciences for identification of plant drug. Simultaneously for identification of chemical nature of plant in term of physico-chemical analysis, qualitative and quantitative analysis for the detection of active constituent expertise are required. According to Kunle et al,[26] standardization of herbal drug is a series of protocols which assure the quality, efficiency and safety of plant drug. Macro-microscopy helps in the identification of plant characters anatomically and helps in identification of botanical background. Present study shows a clear zone of rectangular and tangentially elongated cork cells. Thin walled oval and spherical parenchymatous cells with small intercellular spaces were recorded. Parenchymatous cells were distributed throughout the section while some parenchymatous cells show the presence of bundles and needle like crystals of calcium oxalate. These observations are in support of the previous studies on pharmacognostic evaluation of *Curculigo orchioides*.[27-29]

Standardization and authentication of plants was done by evaluating physicochemical testing.[30-32] The values obtained in the study will serve as constants for quality standard measures for standardization of drug in the dried form.

High performance thin layer chromatography (HPTLC) serves as quality assessment tool which helps in identification of variation in chemical composition plants.TLC identity is a part of every herbal monograph of international standards.[33] HPTLC fingerprinting shows different R_f values at different wavelength and reported values can be used as quality indicating fingerprint for *Curculigo orchioides* in the dried form.

Findings reported in the present investigation are in support of API[34] and QSIMP 2012[2].

CONCLUSION

Pharmacognostical characterization of the *C. orchioides* has been done as per pharmacopoeial methodology. Present study explores the botanical (in terms of macro-microscopic observations), physicochemical observation (in terms of total ash, AIA, WSA, ASE, WSE and loss on drying)

Table 1: All the experiments were performed in triplicates. Values are expressed in ± SE=3

Parameter (% w/w)	Mean value ± SE (N = 3)
Loss on drying	10.469 ± 0.002
Total Ash	8.463 ± 0.015
Acid Insoluble Ash	1.10 ± 0.005
Water soluble Ash	2.163 ± 0.086
Alcohol soluble extractive value	3.036 ± 0.056
Water soluble extractive value	22.666 ± 0.088

Table 2: R_f values of all the samples

At 254 nm	At 366 nm	After post derivatisation
-	0.16 (FL. blue)	-
-	-	0.51 (L. purple)
-	-	0.56 (L. purple)
-	0.63 (FL. blue)	-
-	0.71 (F aqua blue)	-
-	-	0.74 (L. purple)
-	0.83(FD. blue)	-

*L-Light, D-Dark, F-Fluorescence.

Table 3: Densiometric scan of ethanolic extract of *Curculigo orchioides*

254nm		366nm		620nm	
R_f	% Area	R_f	% Area	R_f	% Area
0.03	71.37	-	-	0.03	42.03
-	-	0.07	9.04	-	-
-	-	0.11	14.03	-	-
-	-	0.17	10.40	-	-
0.19	2.77	-	-	-	-
-	-	0.22	50.45	-	-
-	-	-	-	0.32	3.89
0.38	18.47	-	-	0.37	6.48
-	-	-	-	0.41	5.57
-	-	-	-	0.58	9.36
-	-	-	-	0.65	11.79
0.76	2.91	0.75	5.94	-	-
-	-	-	-	0.85	10.69
-	-	0.86	5.30	0.86	10.18
0.88	4.47	-	-	-	-
-	-	-	-	-	-
-	-	0.90	3.38	-	-
-	-	0.98	0.45	-	-

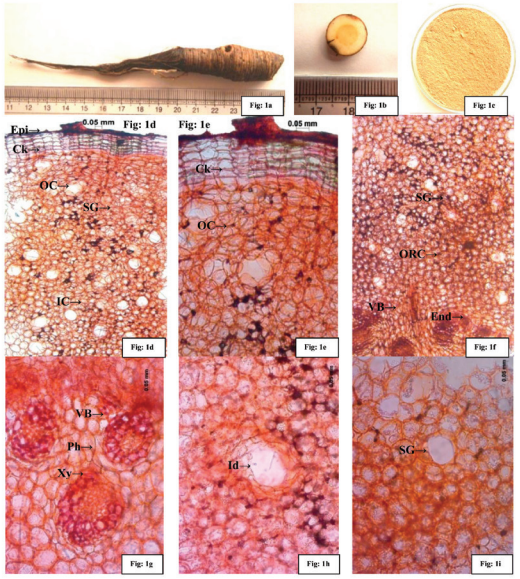

Fig. 1a- Fresh Rhizome; **1b-** TS of Fresh Rhizome**; 1c-** Powder of dried rhizome**; 1d-1e-** TS of rhizome: outer region**; 1f-1i-** TS of rhizome: inner region.

Ck – cork; **End** – endodermis; **Epi** – epidermis; **IC** – inner cortex; **Id→** Idioblast ; **OC** – outer cortex; **ORC** – oleo resin cells; **Ph** – phloem; **SG** – starch grains; **VB** – vascular bundle; **Xy** – xylem.

Figure 1: Macro-microscopic features of *rhizome* of *Curculigo orchioides.*

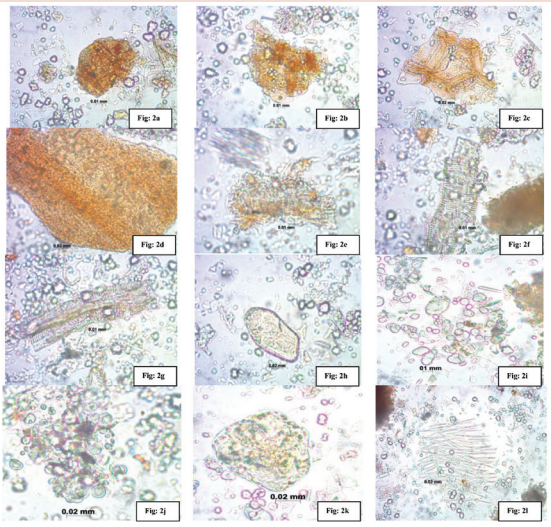

Fig. 2a, 2b, 2c: Cork cells in surface view; **2d:** Parenchyma cells; **2e:** parenchyma cells with acicular crystals; **2f:** annular and xylem vessels; **2g:** annular and spiral vessels; **2h:** pitted parenchyma cells; **2i:** isolated starch cells; **2j-2k:** parenchyma cells with starch grains; **2l:** acicular crystals of calcium oxalate.

Figure 2: Powder microscopy of *rhizome* of *Curculigo orchioides.*

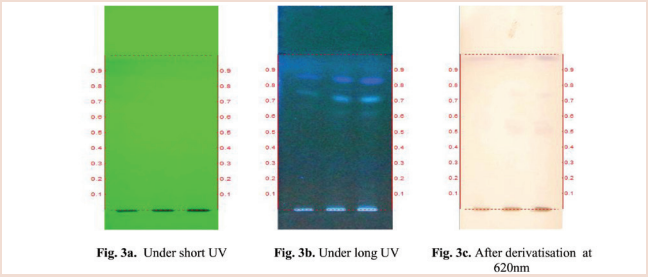

Fig. 3a. Under short UV Fig. 3b. Under long UV Fig. 3c. After derivatisation at 620nm

Figure 3: TLC Photodocumentation of ethanolic extract of *rhizome and Curculigo orchioides.*

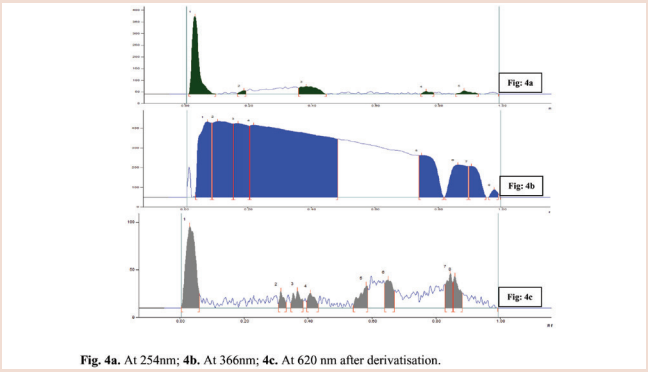

Fig. 4a. At 254nm; **4b.** At 366nm; **4c.** At 620 nm after derivatisation.

Figure 4: HPTLC Densitometric scan of ethanolic extract of *rhizome* of *Curculigo orchioides.*

identification and authentication of drug in dried form.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

ABBREVIATIONS USED

TLC: Thin Layer Chromatography; **HPTLC:** High Performance Thin Layer Chromatography; **QSIMP:** Quality Standard of Indian Medicinal Plant; **AIA:** Acid Insoluble Ash; **WSA:** Water Soluble Ash; **ASE:** Alcohol Soluble Extractive; **WSE:** Water Soluble Extractive.

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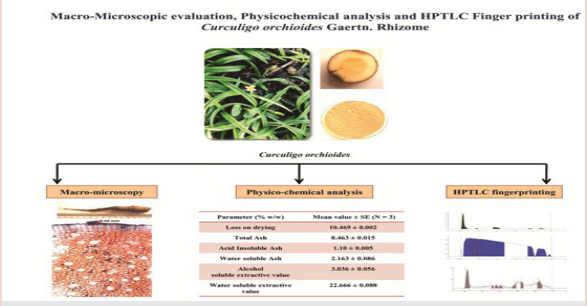
PICTORIAL ABSTRACT



- Microscopy of *Curculigo orchioides* Gaertn. showed the presence of rectangular and conical cells in cork, thin walled cortex, parenchymatous cells, starch grains, crystal of calcium oxalate, mucilage cell and spiral xylem vessel.
- Ethanolic extract of dried rhizomes of *Curculigo orchioides* Gaertn. revealed physico-chemical constants as LOD (10.469%), TA (8.463%), AIA (1.10%), WSA (2.163%), ASE (3.036%) and WSE (22.666%).
- HPTLC fingerprint profile was obtained at different wavelengths with different Rf peaks.
- Microscopy, physico-chemical constants and HPTLC fingerprint profile will be useful for identification and authentication of dried rhizomes of *Curculigo orchioides* Gaertn.

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