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

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ABSTRACT
Introduction: Curculigo orchioides Gaertn. Family Hypoxidaceae (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 (22.66%). Various studies have been reported for its antibacterial activity, demulcent, diuretic and aromatic tonic, aphrodisiac, in the treatment of leprosy and nervous disease. The plant is native to India and distributed from sub-tropical Himalayas, West Bengal, Assam, Konkan, West peninsula to Kanyakumari. Its rhizomes are widely used as a demulcent, diuretic and aromatic tonic, aphrodisiac, in the treatment of leprosy and nervous disease. Rhizomes of C. orchioides have been reported for their medicinal properties like platelet regeneration, antioxidant activity, hepatoprotective efficacy, antipyretic activity and immune stimulant properties. 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). Rhizomes of this plants has been described to possess medicinal properties (Shloka 183). 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 of microscopic analysis. Very thin transverse sections of rhizome were obtained using sharp blade followed by safranine staining. 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.

INTRODUCTION
Curculigo orchioides Gaertn., syn. C. malabarica Wight., C. brevipolia Dryand. and Hypoxis orchioides Kurz. 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. The plant is native to India and distributed from sub-tropical Himalayas, West Bengal, Assam, Konkan, West peninsula to Kanyakumari. Its rhizomes are widely used as a demulcent, diuretic and aromatic tonic, aphrodisiac, in the treatment of leprosy and nervous disease. Rhizomes of C. orchioides have been reported for their medicinal properties like platelet regeneration, antioxidant activity, hepatoprotective efficacy, antipyretic activity and immune stimulant properties. 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). 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 such as flavones, glycosides, steroids (sitosterol, stigmasteral and yuccagenin), terpenoids, saponins, glucoside, mannose, xylose, glucoronic acid, resin, tannin, fat, mucilaginous substances and other secondary metabolites. Steam distilled fraction of C. orchioides has reported for its antibacterial activity against Gram-negative and Gram-positive pathogens. Methanolic extract of rhizomes of C. orchioides is reported for its immunostimulatory action against cyclophosphamide induced toxicity and neuroprotective efficacy against cyclophosphamide neurotoxicity. Various studies have been reported for its antidiabetic activity; ethanolic extract and aqueous extract of C. orchioides in alloxan induced diabetes. 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 (a specimen (725/16021205) is being maintained for future reference). The dried rhizomes were cleaned, coarsely powder and used for macroscopic and microscopical characterisation, 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 of microscopic analysis. Very thin transverse sections of rhizome were obtained using sharp blade followed by safranine staining. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxiosCam 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
Physico-chemical analysis: Physico-chemical characterization like loss on drying at 105°C, total ash, acid insoluble ash, water soluble ash, ethanol and water soluble extractives values were determined as per Quality Standard of Indian Medicinal Plants.

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 and scanned under UV. All the experiments were performed in triplicates.

RESULTS

Macro-microscopic observations: Macroscopic studies showed blackish brown rhizomes about 10 to 15 cm long, cylindrical and straight to slightly curved. 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 (Table 1).

HPTLC Fingerprinting: \( R_f \) 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 phytocomponents 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 \( R_f \) of 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 \( R_f \) of 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 \( R_f \) of 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., 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. Standardization and authentication of plants was done by evaluating physicochemical testing. The values obtained in the study will serve as constants for quality standard measures for standardization of drug in the dried form.

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 and loss on drying)
Table 3: Densiometric scan of ethanolic extract of *Curculigo orchioides*

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

Figure 2: Powder microscopy of rhizome of *Curculigo orchioides*.
identification and authentication of drug in dried form.

ACKNOWLEDGEMENT

Authors are highly grateful to Jiwaji University, Gwalior, Madhya Pradesh for financial assistance (Dev/2016/214) dated 31/05/2016. Authors highly regard Dr. B. Ravishankar, Director, SDM Centre for Research in Ayurveda for providing the lab facilities. Suchita Prabhavalkar is gratefully acknowledged for constant support during the study.

CONFLICT OF INTEREST

The author declare no conflict of interest.

ABBREVIATIONS USED


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22. Quality standard of Indian Medicinal Plant, Indian Council of Medical Research, New Delhi; 2014.
Yadav et al.: Microscopy, physico-chemical analysis and HPTLC fingerprinting of Curculigo orchioides Gaertn.

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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