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# PHARMACOGNOSTIC EVALUATION AND HPTLC FINGERPRINT PROFILE OF CURCULIGO ORCHIOIDES GAERTN. RHIZOMES

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## ABSTRACT

*Curculigo orchioides* Gaertn. (Family: Hypoxidaceae) commonly known as Kali musli, the rhizomes of which are extensively utilized as nutritive tonic for strength and for treatment of asthma, skin diseases, bronchitis, diarrhoea, dyspepsia, etc. It also possesses anticancer and antipyretic activity. It is one of the important Rasayana drugs of Ayurvedic Materia Medica for vigor and vitality. It is also used in many Ayurvedic and Unani compound formulations as an important ingredient. Hence, the present work has been undertaken to establish the requisite pharmacognostic standards for evaluating the *Curculigo orchioides* Gaertn rhizome. In addition, HPTLC is also performed to detect the presence of β- sitosterol as the marker compound. The anatomical markers observed were bundles of acicular calcium oxalate crystals, lysigenous cavities, starch grains and vessel with spiral thickening. Preliminary phytochemical analysis showed presence of glycosides, mucilage, tannins, steroids, flavonoids, saponins and essential oils. Phytochemical analysis using HPTLC showed presence of arbutin, bitter principles, cardiac glycosides, coumarins, essential oils, lignans, pungent – tasting principles, saponins, triterpenes and valepotraites.

**KEY WORDS;** ***Curculigo orchioides*** **Gaertn., Pharmacognosy, HPTLC, β - sitosterol**

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# INTRODUCTION

Herbal drugs have been in use by different civilizations in different parts of the world for centuries to fight a large number of diseases. Many of these are in common use even today. However as herbal drugs are derived from heterogeneous sources leading to variations, which makes the standardization more important, as erroneous results can cause variations in phytochemical and pharmacological studies. The pharmacognostic characters, physicochemical values and results from phytochemical analysis could be used as a diagnostic tool for the standardization of medicinal plants used in herbal medicines.

The drug Kali or Shyah- Musali, of Ayurvedic system of medicine is derived from the bitter mucilaginous rhizomes of *Curculigo orchioides* Gaertn(Family- Hypoxidaceae). It is one of the important Rasayana drugs of Ayurvedic Materia Medica for vigor and vitality and also reputed for its various medicinal properties [1]. It has tonic, aphrodisiac, demulcent, diuretic properties and used in asthma, impotency, jaundice, skin, urinary and venereal diseases [2]. It is used in many Ayurvedic and Unani compound formulations as an important ingredient [3, 4].

It was first introduced in 'Charak Samhita' of 'Agnivesha', the epic treatise of the medicine school of thought of the Hindu system of medicine and narrated as an ingredient of a cigar to alleviate cough. Talamuli (Shyah-Musali) has been used in the indigenous system of medicine for a long period. According to Bhavaprakash, the drug is sweet, bitter, acts as an aphrodisiac. In Raj Nighantu, it has been described as sweet, cooling, mucilaginous, incrases Kapha and reduces Pitta daha (burning sensation), acts as stimulant and gives strength. Musali prepared as a paste with goat's milk or honey and applied locally over the face, brightens the complexion of the face. Moving in to the modern period it is extensively used by the Ayurvedic practitioners, particularly an ingredient of aphrodisiac preparations [5, 6, 7, 8, 9]. Pharmacognostic parameters serve to identify the crude plant material and to ensure its quality. Some pharmacognostic parameters of *Curculigo orchioides* Gaertn. rhizomes were already reported in Ayurvedic Pharmacopoeia of India (API). However, the present study was carried out to re-evaluate some reported pharmacognostic parameters for comparative study and to report some other important parameters of *C. orchioides* Gaertn. rhizome; with an aim to enrich the existing pharmacognostic data which may serve as a measure of authentication and quality control for commercial samples of the crude drug. HPTLC fingerprint has been developed; as the chemical fingerprint obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines. Qualitative densitometric HPTLC analysis was also performed to detect the presence of β- sitosterol as the marker compound in methanolic extract *of C. orchioides* Gaertn. rhizome.

# MATERIALS AND METHODS

### *Collection and authentication of plant material*

The rhizomes of *Curculigo orchioides Gaertn*. were collected from Badlapur, Maharashtra, India. Herbarium of *Curculigo orchioides Gaertn*. was prepared and authenticated from Blatter Herbarium, St. Xavier's College, Mumbai. The rhizomes collected were washed under running tap water and were blotted dry. The rhizomes were then cut into small pieces and kept for drying in oven at temperature 40 ± 2º C. The dried rhizomes were ground into powder, stored in airtight container and used for further studies.

### *Pharmacognostic Evaluation*

Pharmacognostic studies were carried out according to methods described by Khandelwal (2008), Kokate *et al*.(2008), Anonymous (1996) and Mukherjee (2005) [10,11,12,13].

### *Macroscopic characteristics*

The rhizomes were macroscopically examined for shape, size, color, odour, taste, etc. [10].

***Microscopic characteristics***

Free hand sections were taken, cleared with chloral hydrate and then stained with safranine. Sections and powder diagnostic characters were observed under compound microscope and photographed.

***Physicochemical Evaluation***

Physicochemical parameters were determined using the method described by Khandelwal (2008), Kokate *et al*.(2008), Anonymous (1996) and Mukherjee (2005) [10,11,12,13].

In physicochemical evaluation, moisture content, ash values *viz.* total ash, acid insoluble ash and water soluble ash were determined. Considering the diversity of chemical nature and properties of contents of drug, five different solvents were used for determination of extractives *viz.* benzene, petroleum ether, alcohol, methanol and water. The determinations were performed in triplicate and results are expressed as mean ± SD. The percentage (w/w) values were calculated with reference to the air-dried drug.

***Preliminary Phytochemical Screening***

Powdered rhizome was extracted with benzene, petroleum ether, alcohol, methanol and water. The extracts were filtered and subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedure [10, 11].

Phytochemical analysis was also carried out using HPTLC as per methods described by Wagner and Bladt (1996) [14].

***Histochemical analysis***

The histochemical colour reactions on the fresh rhizome were performed for the identification of major cell constituents. Different histochemical reagents were applied on the section and the specific colours produced due to presence/absence of putative constituents were observed under the compound microscope. The presence/absence of different chemical constituents in different tissues was recorded [15].

***Fluorescence analysis:***

Fluorescence analysis was carried out as per the method described by Kokashi (1958) and Chase and Pratt [1949] [16, 17]. The reaction of certain drugs either in powdered form or on their smooth sectioned surfaces with filtered ultra violet light is of importance in several cases. They help in determining adulterants [18].

***Fluorescence analysis of powdered rhizome extracts with different solvents*:**

The fluorescence analysis of powdered rhizome extracts was carried out in different solvents (benzene, petroleum ether, alcohol, methanol and water) and observed in visible light, UV short (254nm) and long wavelength (366nm) regions [19].

***High Performance Thin Layer Chromatography***

A qualitative densitometric HPTLC analysis was performed with methanolic extract for the development of characteristic fingerprint profile which may be used for quality evaluation and standardization of the drug. 10 µl of extract was spotted on precoated silica gel G60 $F_{254}$ HPTLC plates (Merck) using CAMAG Linomat V applicator. The plate was developed in glass twin trough chamber (20 cm x 10 cm) pre-saturated with mobile phase (Toluene: Ethyl acetate: Methanol: Glacial Acetic acid in the ratio 7.5: 1.5: 0.6: 0.2 v/v). The plate was derivatized using methanolic $H_2SO_4$ and scanned using TLC Scanner III (CAMAG) at 254 nm, 366 nm and 540 nm.

Qualitative densitometric HPTLC analysis was also performed to detect the presence of marker compound β- sitosterol. Dried powder of rhizome was extracted with 20 ml of methanol under reflux for 30 minutes. 10 µl of extract and β- sitosterol were spotted on pre-coated silica gel G60 $F_{254}$HPTLC plates (Merck) using CAMAG Linomat V applicator. The plate was developed in glass twin trough chamber (20 cm x 10 cm) pre-saturated with mobile phase (Toluene: Methanol: Glacial

Acetic acid in the ratio 9: 1: 0.2 v/v). The plate was derivatized using Anisaldehyde sulphuric acid and scanned using TLC Scanner III (CAMAG) at 540 nm.

# RESULTS AND DISCUSSION

### *Macroscopic characteristics*

The rhizome of *C. orchioides* is the medicinally valuable part, which is stout, more or less cylindrical, 8.2 – 18.5 cm (Avg. 14.40 ± 0.13) long and 2.1 – 3.9 cm (Avg. 3.0 ± 0.61) in diameter. The upper portion of the root stock remains clothed with the withered leaf bases and with copious lateral roots which are long, almost whitish or yellowish grey and shrinked. Externally the rhizome is brownish black, fractured, starchy, odour indistinct, taste bland and mucilaginous (Table no. 1 Plate No. 1- A, B).

### *Microscopic characteristics*

Transverse section of rhizome shows an outer layer of cork consisting of 4-6 rows of rectangular cells. Cork surrounds broad cortex, composed of parenchymatous cells. Parenchymatous cells contain abundant starch grains, bundles of acicular calcium oxalate crystals and lysigenous cavities. The ground tissue of the stele is formed of parenchymatous cells similar in shape and size. Vascular bundles are close. The peripheral bundles are arranged in a ring while the inner ones appear scattered. The xylem elements partly or completely surround the phloem i.e. lepto-centric (Plate No. 1- C, D, E).

The microscopy of powdered rhizome showed presence of starch grains, bundles of acicular calcium oxalate crystals, lysigenous cavities and vessels with spiral thickening (Plate No. 1- F, G, H,I).

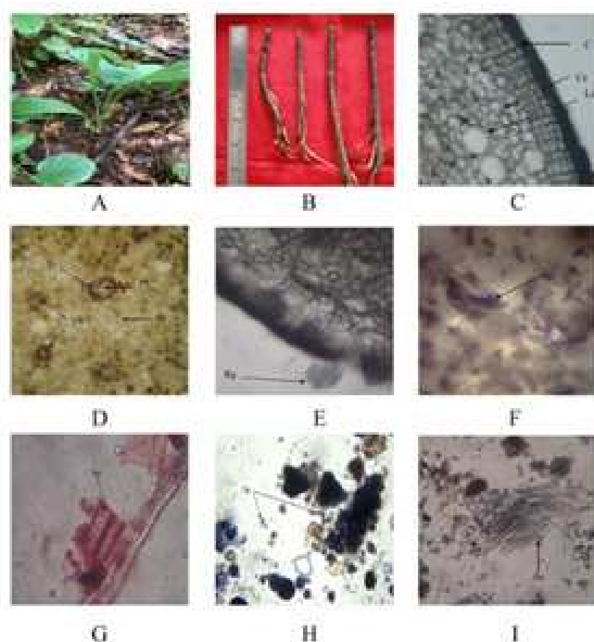



Plate no.1 : A- Flowering Plant of *Curculigo orchioides* Gaertn., B- Rhizomes, C & D- Transverse section of rhizome, E- Bundles of raphids (Rp), F- Parenchymatous cells showing starch grains, G - Powder showing vessels with spiral thickening (Spr), H- Powder showing starch grains (Sg), I- Powder showing of acicular calcium oxalate crystals (Ac)

Keywords: C- Cork, Cr- Cortex, Lc- Lysigenous cavity, VB- Vascular bundles

**Table no. 1**
***Macroscopic characteristics of Curculigo orchioides Gaertn. Rhizome***

| | |
|---|---|
| **Shape** | Cylindrical |
| **Colour** | Blackish brown |
| **Taste** | Sweet |
| **Odour** | Sweet |
| **Length** | 8.2 – 18.5 cm (Avg. 14.40 ± 0.13) |
| **Diameter** | 2.1 – 3.9 cm (Avg. 3.0 ± 0.61) |

## Physicochemical Evaluation

The physicochemical evaluation of any drug is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of rhizome was found to be 74.40%. Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash and water soluble ash were determined. The results are shown in Table no. 2. Total ash and acid insoluble ash values were found to be within the API limits.

Extractive values are primarily useful for the determination of exhausted or adulterated drugs. Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain. The variation in extractable matter in various solvents is suggestive of the fact that the formation of the bioactive principle of the medicinal plant is influenced by number of intrinsic and extrinsic factors. High alcohol soluble and water soluble extractive values reveal the presence of polar substances like phenols, tannins and glycosides, as also reported by Sharma *et al,* 2009 [20]. Extractive values using various solvents are tabulated in Table no. 2. Water and alcohol soluble extractive values were found to comply with the API limit.

**Table no. 2**
***Physicochemical Analysis of Curculigo orchioides Gaertn. rhizome powder***

| Sr. No. | Physicochemical Constant | Observation (%) |
|---|---|---|
| 1. | **Ash Values** | |
| a) | Total ash | 8.00 |
| b) | Acid insoluble ash | 0.89 |
| c) | Water soluble ash | 3.56 |
| 2. | **Extractive Values** | |
| a) | Benzene | 6.13 |
| b) | Petroleum ether | 5.60 |
| c) | Alcohol | 13.60 |
| d) | Methanol | 28.80 |
| e) | Water | 21.86 |

## *Phytochemical Screening*

The results of preliminary phytochemical screening are presented in Table no. 3. Preliminary phytochemical analysis of *C. orchioides* Gaertn. rhizome showed presence of aleurone grains, amino acids, proteins, carbohydrates, starch, fats and fixed oils, glycosides, mucilage, tannins, steroids,

phenols, flavonoids, saponins, essential oils and resins. These secondary plant metabolites are known to possess various pharmacological effects and might be responsible for the various actions exerted by C. orchioides Gaertn rhizome.

**Table . 3**
***Preliminary Phytochemical analysis of various extracts of Curculigo orchioides Gaertn. Rhizomes***

| Sr.No | Phytoconstituent | AE | EE | ME | BE | PEE |
|---|---|---|---|---|---|---|
| 1. | Acid compounds | - | - | - | - | - |
| 2. | Aleurone grains | + | + | + | - | - |
| 3. | Alkaloids | - | - | - | - | - |
| 4. | Amino acid | + | + | + | - | - |
| 5. | Proteins | + | + | + | - | - |
| 6. | Carbohydrates | + | + | + | - | - |
| 7. | Starch | + | - | + | + | + |
| 8. | Fats and fix oils | - | - | + | + | + |
| 9. | Glycosides | + | + | + | - | - |
| 10. | Mucilage | + | + | + | - | - |
| 11. | Tannins | + | + | + | - | - |
| 12. | Steroids | - | + | + | + | + |
| 13. | Phenols | + | + | + | - | - |
| 14. | Flavonoids | + | + | + | - | - |
| 15. | Saponins | + | + | + | - | - |
| 16. | Essential Oils | - | + | + | - | - |
| 17. | Resins | + | - | - | - | - |
| 18. | Anthraquinone | - | - | - | - | - |

***Key Words : + = Present; - = Absent; AE= Aqueous extract; EE= Ethanolic extract; ME= Methanolic extract; BE=Benzene extract; PEE= Petroleum ether extract***

A number of phytoconstituents were also separated using TLC and their respective R*f* values have been reported in Table no 4.
Medicinal plant material is obtained from different heterogeneous sources which may lead to variation in therapeutic values and variation in phytochemistry. Thus the developed chromatogram for *C. orchioides* rhizome will be specific with selected solvent system and R*f* values, and serve as better tool for standardization of the drug. Chemical compounds, some of which are having therapeutic activities, are species specific and vary from species to species. These compounds can be visualized by developing chromatograms.

**Table no. 4**
***Phytochemical evaluation of Curculigo orchioides Gaertn. rhizome by HPTLC***

| Sr. No. | Phytoconstituents | No. of Spots | R*f* values |
|---|---|---|---|
| **1.** | Anthracene derivatives | 8 | 0.11, 0.12, 0.22, 0.34, 0.38, 0.58, 0.59, 0.81 |
| **2.** | Arbutin derivatives | 6 | 0.11, 0.35, 0.55, 0.61, 0.80, 0.97 |
| **3.** | Cardiac glycoside drugs | 8 | 0.10, 0.14, 0.25, 0.37, 0.48, 0.59, 0.65, 0.73 |
| **4.** | Bitter drugs | 8 | 0.24, 0.34, 0.44, 0.48, 0.57, 0.65, 0.85, 0.90 |
| **5.** | Coumarin derivatives | 4 | 0.10, 0.36, 0.73, 0.89 |
| **6.** | Essential Oils | 10 | 0.06, 0.16, 0.21, 0.35, 0.69, 0.74, 0.76, 0.83, 0.88, 0.90 |

| | | | |
|---|---|---|---|
| **7.** | Lignans | 8 | 0.01, 0.10, 0.17, 0.220.25, 0.43, 0.56,0.77 |
| **8.** | Pungent-Tasting principles | 13 | 0.12, 0.14, 0.20, 0.25, 0.31, 0.35, 0.46, 0.50, 0.60, 0.70, 0.83, 0.95, 0.99 |
| **9.** | Saponins | 7 | 0.05, 0.12, 0.22, 0.40, 0.45, 0.54, 0.69 |
| **10.** | Triterpenes | 9 | 0.06, 0.11, 0.21, 0.29, 0.38, 0.46, 0.80, 0.87, 0.90 |
| **11.** | Valepotraites | 10 | 0.04, 0.10, 0.13, 0.23, 0.30, 0.34, 0.47, 0.66, 0.72, 0.83 |

***Histochemical analysis:***

The histochemical colour reactions on the rhizome were performed for the first time. The changes in the histochemical zones were observed under microscope and the results are shown in Table no. 5.

**Table no. 5**
***Histochemical Tests of Curculigo orchioides Gaertn. rhizome***

| Sr. No. | Test for | Colour | Histological Zones |
|---|---|---|---|
| **1.** | Starch | + | Cortex |
| **2.** | Tannins | - | - |
| **3.** | Lignin | + | Vascular bundles |
| **4.** | Ca oxalate crystals | + | Cortical cells near epidermis |
| **5.** | Mucilage | + | Cortex |
| **6.** | Oil globules | - | - |
| **7.** | Aleurone grains | - | - |
| **8.** | Stone cells | - | - |
| **9.** | Alkaloids | - | - |
| **10.** | Steroids | - | - |
| **11.** | Cellulose | - | - |

***Key Words : + = Present; - = Absent***

***Fluorescence analysis**:*

Fluorescent characteristics of powdered crude drug with different chemical reagents, under ordinary light and on exposure to UV light (254 nm and 366 nm) are reported for identification and authentication of the crude drug for the first time. This information may be useful as diagnostic parameters. Fluorescence analysis of rhizome powder was studied and observations were shown in Table no. 6.

**Table no. 6**
***Fluroscence Analysis of Curculigo orchioides Gaertn. rhizome powder***

| Sr. no. | Tests | Visible light | 254nm | 366nm |
|---|---|---|---|---|
| 1. | Powder as such | Buff Brown | White | Brown |
| 2. | Powder + nitrocellulose | Buff Brown | White | Brown |
| 3. | Powder + 1N NaOH in methanol | Dark Brown | Green | Grey |
| 4. | Powder + 1N NaOH in methanol+ nitrocellulose in amyl acetate | Dark Brown | Black | Grey |
| 5. | Powder + 1N HCl | Dark Brown | Brown | Black |
| 6. | Powder + 1N HCl + nitrocellulose in amyl acetate | Dark Brown | Dark Brown | Black |
| 7. | Powder + 1N NaOH | Dark Brown | Dark Brown | Dark Brown |
| 8. | Powder + 1N NaOH + Nitrocellulose in amyl acetate | Black | Dark Brown | Dark Brown |
| 9. | Powder + $HNO_3$ (1:1) | Dark Brown | Brown | Dark Brown |
| 10. | Powder + $H_2SO_4$ (1:1) | Black | Dark Brown | Dark Brown |

***Fluorescence analysis of drug extracts in different solvents***

The fluorescence analysis of drug extracts carried out in different solvents and observed in visible light and also in UV short and long wavelength regions shown corresponding colours in the solvent as described in the Table no. 7.

**Table no. 7**
***Fluorescence analysis of Curculigo orchioides Gaertn. rhizome extracts with different solvents.***

| Sr. No. | Extraction Solvent | Visible Light | UV Light | |
|---|---|---|---|---|
| | | | 254 nm | 366 nm |
| 1. | Benzene | - | White | Bluish White |
| 2. | Petroleum Ether | - | White | Bluish White |
| 3. | Ethanol | Golden Yellow | Golden Yellow | Greenish Yellow |
| 4. | Methanol | Golden Brown | Golden Brown | Yellowish Green |
| 5. | Water | Brown | Brown | Brownish Green |

***HPTLC fingerprint***

Organoleptic parameters are not enough in establishing the standards of herbal drugs. Instrumental analysis of herbal drugs, which gives a more concrete picture regarding the qualitative and quantitative aspects of bioactive molecules, is widely accepted in the quality assessment of herbal drugs [19]. However, such work related to traditional herbal medicines is lacking or in preliminary stage. In the present study, HPTLC finger print has been carried out for the first time for *C. orchioides* Gaertn. rhizome and the results provide referential information for standardization. R*f* values of the separated phytoconstituents are recorded in Table no. 8; Plate no. 3.

**Table no. 8**
***Rf values of the separated phytoconstituents by HPTLC fingerprint of Curculigo orchioidesGaertn. rhizomes***

| Sr. No. | Wavelength | | |
|---|---|---|---|
| | 254 nm | 366 nm | 540 nm |
| | R*f* | R*f* | R*f* |
| 1. | 0.22 | 0.02 | 0.01 |
| 2. | 0.42 | 0.07 | 0.05 |
| 3. | 0.53 | 0.16 | 0.20 |
| 4. | 0.66 | 0.21 | 0.27 |
| 5. | 0.73 | 0.42 | 0.41 |
| 6. | 0.76 | 0.70 | 0.43 |
| 7. | - | 0.76 | 0.60 |
| 8. | - | - | 0.67 |
| 9. | - | - | 0.79 |
| 10. | - | - | 0.83 |

A qualitative densitometric HPTLC analysis showed the presence of characteristic blue colour band of marker compound β- sitosterol at R*f* 0.34, which was detected in *C. orchioides* Gaertn. rhizome extract (Plate no.2).

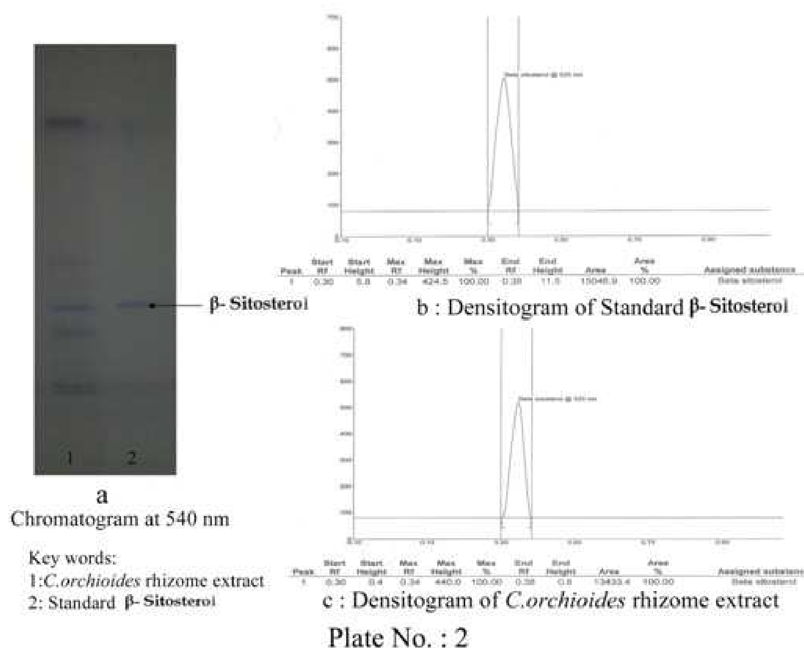
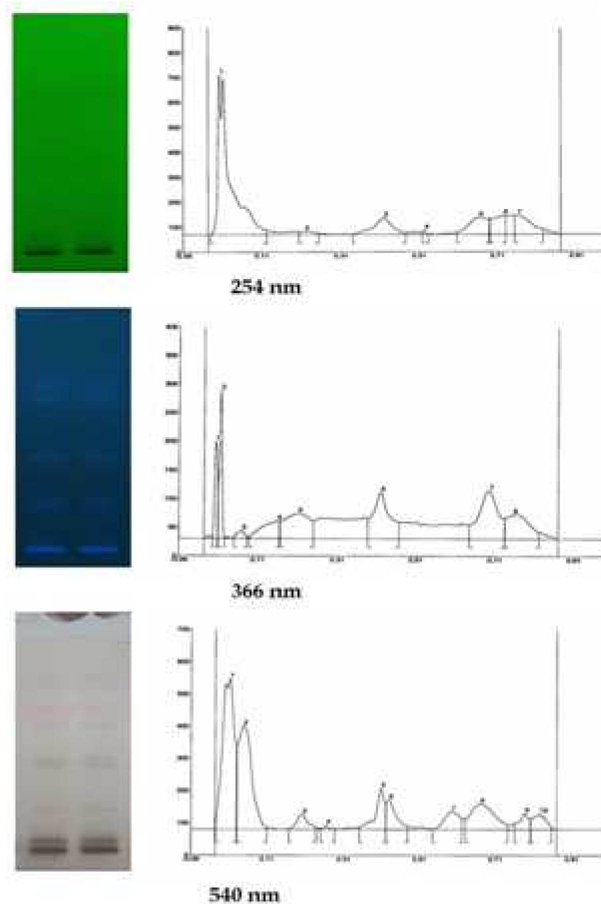


Plate No. : 2




**540nm Plate No.3: HPTLC fingerprint of Curculigo orchioides Gaerth.Thizomes**

## CONCLUSION

Morphology, as well as various pharmacognostic aspects of rhizome of *C. orchioides* Gaertn were studied and described along with phytochemical, physicochemical, TLC and HPTLC studies, which will help in authentication and quality control of crude drug. The data generated from the present studies would help in the authentication of rhizome when available both in fresh and powder form. The different spots observed in HPTLC finger print profile would be definitely useful in deciding the purity and quality of the drug, particularly of different batches and for market acceptability and competency of commercial formulation.

## ACKNOWLEDGEMENT

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## REFERENCES

1. Saba I, Singh J, Jain SP and Khanuja SPS, Curcligo orchioides Gaertn. (Kali Musali) An endangered medicinal plant of commercial value. Natural Product Radiance, 5(5): 369-372(2006).
2. Kurup PNV, Ram Das VNK and Joshi P, Handbook of medicinal plants, Central Council of Research in Ayurveda and Siddha, New Delhi, p. 211 (1979).

3. Singh RS, Vanaaushadhi Nidarshika (Ayurvedia Pharmacopoeia), 2ndEdn, Uttar Pradesh Hindi Sansthan, Lucknow, p. 312(1973).
4. Kumar S, Singh J, Shah NC and Ranjan V, Indian Medicinal and Aromatic Plants Facing Genetic Erosion, Central Institute of Medicinal and Aromatic Plants, Lucknow, p. 89-91 (1997).
5. Raghunathan K and Mitra R, Pharmacognosy of Indigenous drugs, Vol. II, Central council for research in Ayurveda and Siddha, New Delhi, 667-670 (2001).
6. Anonymous. The Ayurvedic Pharmacopoeia of India,1st Edition, Vol. IV, Department of Indian system of medicine and homoeopathy, New Delhi, 122-124 (2004).
7. Anonymous. Wealth of India, First Supplement Series, Vol. II, CSIR, New Delhi, 90-93 (2004).
8. Nadkarni KM, The Indian Materia Medica, 2nd Edition, Vol. I, Bombay Popular Prakashan, Mumbai, 410-413(2002).
9. Kirtikar KR and Basu BD, Indian Medicinal Plants, 2nd Edition Vol. IV, 2468-2470 (2002).
10. Khandelwal KR, Practical pharmacognosy, 19th ed., Pune, India: Nirali Prakashan, (2008).
11. Kokate CK, Purohit AP and Gokhale SB, Pharmacognosy 42nd ed., Pune, India: Nirali Prakashan, (2008).
12. Anonymous, The Ayurvedic Pharmacopoeia of India New Delhi: Govt. of India Publication, (1996).
13. Mukherjee PK, Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. India: Business Horizones, (2005).
14. Wagner H and Bladt S, Plant Drug Analysis: A Thin Layer Chromatography Atlas. Berlin: Springer (1996).
15. Panchal HH, Shukla SH and Lad BN, Pharmacognostic Studies on Leaves of Flacourtia ramontchi L.'Herit., Phcog J., 2(13):530-535 (2010).
16. Kokoski J, Kokoski R and Salma FJ, Fluorescence of powdered vegetable drugs under ultraviolet radiation. J. Am. Pharm. Ass., 47(10): 715-717 (1958).
17. Chase CR and Pratt RJ, Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J. Am. Pharm. Ass., 38: 324-333 (1949).
18. Muzumdar KP, Pharmaceutical Science in Homeopathy and Pharmacodynamics : Published by B. Jain Publishers (P) Ltd., (2001).
19. Rasheed NMA, Shareef MA, Mushtaq A, Gupta VC, Shamsul A and Shamshad AK, HPTLC Finger Print Profile of Dried Fruit of Physalis alkekengi Linn. Phcog J., 2(12):464-469 (2010).
20. Sharma A, Rao CV, Tiwari RK, Tyagi LK, Kori ML, Singh V, Gaur K and Shankar K, Role of Aloe barbadensis Linn in the removal of toxic heavy metal of kukkutandatwak (shell of hen's egg): A drug used in Indian system of medicine (Ayurveda). Adv. Biol. Res., 3: 79–83 (2009).