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PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL SCREENING OF THE ROOT TUBERS OF *CURCULIGO ORCHIOIDES* GAERTN.

Richa Tiwari ^{*1,2} and Pankaj Gupta ¹

School of Medical and Allied Sciences ¹, K. R. Mangalam University, Sohna Road, Gurgaon - 122103, Haryana, India.

IIMT College of Pharmacy ², Knowledge Park III, Plot No. 20A, Greater Noida - 201308, Uttar Pradesh, India.

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Correspondence to Author:

Richa Tiwari

PhD Scholar,
School of Medical and
Allied Sciences, K. R. Mangalam
University, Sohna Road, Gurgaon -
122103, Haryana, India.

E-mail: richapharma123@gmail.com

ABSTRACT: India is well known as the emporium of medicinal plants harboring many plants of medicinal importance, of which *Curculios orchioides* is one that has been reported to possess medicinal importance for the treatment of various diseases traditionally as well as scientifically. An attempt was made herein to undertake detailed pharmacognostical and preliminary phytochemical investigation of the root tubers of *Curculigo orchioides* Gaertn. for the first time. The pharmacognostical study comprises of the taxonomical characters of the root tubers of *Curculigo orchioides*, histochemical tests and diagnostic characters. The phytochemical analysis consists of determination of physicochemical constants, fluorescence analysis, and preliminary organic analysis. The diagnostic characters of the plant showed that the leaves are radical with parallel venation while flowers are solitary, trimerous and ovary inferior. These parameters help in the standardization of the crude drug and in formulating Pharmacopoeial standards for the crude drug. Preliminary phytochemical screening of the crude drug showed the presence of carbohydrates and glycosides, phytosterols, fixed oils and fats, phenolic compounds and tannins, saponins, flavonoids, gums and mucilage.

INTRODUCTION: India is well known as the 'Emporium of Medicinal Plants'. The use of plants to treat various diseases in India dates back to the times of Rigveda (3500 to 1800 B.C.). Later, the monumental Ayurvedic works like Charaksamhita ¹ and Sushrutasamhita ² followed by other Ayurveda and Siddha treatises have incorporated nearly 700 plant drugs entering into several medicinal preparations used in the management of health care. In fact these systems have been in practice even in remote areas of our country for centuries ³.

India has a well established indigenous system of medicine with rich materia medica which includes herbal, animal and mineral products. The use of herbs in India can be traced back to Vedic times; Rig veda (200-1000 B.C.) mentions the name of some medicinal herbs while the Atharva veda provides a more detailed account of herbs and the treatment of diseases. The most important and the authentic works on Indian medicinal plants is found in the classics of Ayurveda *i.e.*, Charaksamhita ¹, Susrutasamhita ² and Astanga hridaya ⁴ which are believed to have been written in the pre-Buddhist period, *i.e.*, before 600 B.C.

These works incorporate 700 - 800 drugs of medicinal value used in several preparations for the treatment of various diseases. They also serve as the basis for the medicinal plant research in India and other countries.

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Plants have contributed significantly to allopathic medicinal armory. Some of the drugs used today (e.g., aspirin, codeine, morphine, vinblastine, vincristine, pilocarpine, cocaine, atropine, emetine and ephedrine) have originated from medicinal plants⁵. It has been estimated that approximately one fourth of the prescriptions dispensed from community pharmacies in United States contain one or more ingredients derived from plant origin⁶. Around 80% of all contemporary pharmaceuticals are derived directly or indirectly from plant sources⁷. According to World Health Organization as much as 80% of population in developing countries are dependent on plants for primary health care⁸. Statistical data reveals that as many as 3226 out of 4752 communities in India are dependent on traditional medicine derived from plants⁹.

A status report on Ethnobiology in India undertaken by Ministry of Environment and forests has indicated that tribal communities use over 7,500 species of plants for medicinal purposes¹⁰. In India the total herbal products market, including sale of crude drugs, has been estimated at Rs. 2500 crore, of which 50% is contributed by Ayurvedic classical preparations and their modernized versions. This is a small percentage of the global trade estimated at \$ 2000 crore. Thus, opportunities for developing modern phytomedicines based on leads from Ayurvedic drugs are indeed vast¹¹.

Curculigo orchioides known as Musali or Talamuli in Ayurveda is used in diseases traditionally as well as scientifically and root tuber is used for the treatment of diabetes, leukoderma, pain and aphrodisiac¹². The diagnostic characters of the plant showed that the leaves are radical with parallel venation while flowers are solitary, trimerous and ovary inferior. It is found in sub-tropical Himalayas from Kumaon estwards, Khasi Hills, Manipur, Bihar, West Bengal, Western Ghats, Konkan and Nilgiri Hills¹³. An attempt was made herein to undertake detailed pharmacognostical and preliminary phytochemical investigations of the root tubers of *Curculigo orchioides* for the first time^{14, 15}.

MATERIALS AND METHODS: The root tubers of *Curculigo orchioides* was collected from the Botanical garden of the University of Agricultural Sciences, GKVK Campus, Bangalore. The plant

was authenticated at herbarium of the Regional Research Institute, Bangalore and authenticated by Dr. Yoganarasimhan and a voucher specimen HS1704990020 has been retained at the School of Medical and Allied Sciences, K. R. Mangalam University, Gurgaon. The root tubers were washed with water and dried in the laboratory at room temperature (30-40 °C). The roots were ground to pass a sieve of 1 mm and further subjected to analytical parameters like ash value and extractive value.

Pharmacognostical Studies: A small quantity of the fresh root tuber was collected and preserved in tubes containing 70% alcohol for pharmacognostical work. The macroscopical observations were carried out following reported procedures¹⁶. The microscopical investigations, histochemical tests were carried out following the given procedures¹⁷. For microscopical studies free hand sections were taken using a sharp razor. The sections were cleared by warming with a few drops of chloral hydrate, stained with phloroglucinol: HCl (1:1), treated with iodine solution and safranin. The sections were mounted temporarily in glycerine for microscopical observations. The measurement of tissues was recorded using stage and ocular micrometers, (Erma-Japan).

Physicochemical Constants and Preliminary Phytochemical Screening: Physical constants, phytochemical tests, fluorescence studies were carried using reported procedures^{18, 19, 20, 21, 22, 23}. The successive extracts obtained were subjected to qualitative tests for the identification of various plant constituents. For fluorescence studies, drug was sieved through 60 mesh and observations were made following the reported procedures^{21, 22, 23}.

RESULTS:

Microscopical Characters: A transverse section of root tuber is circular in outline and shows the following characters **Fig. 1**. Outer peripheral cortical portion having a starchy lustre and a central stellar region (3 mm in diameter) wherein most of the vascular bundles are located. The outermost tissue is the cork consisting of 5- 8 rows of cells with light brown coloured cell walls. In young tubers the cells of this tissue are cubical to rectangular in shape but in older tubers the cells are tangentially elongated.

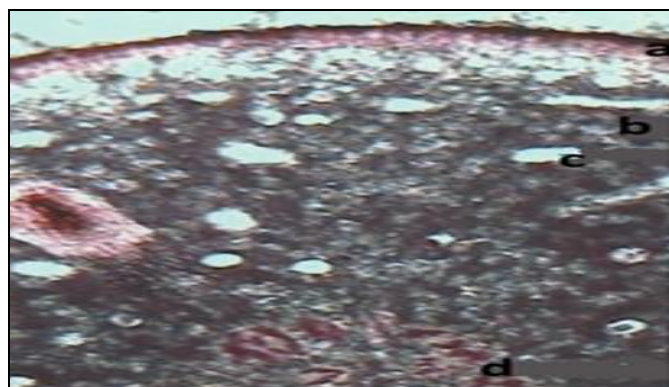


FIG. 1: TRANSVERSE SECTION OF ROOT TUBERS OF *CURCULIGO ORCHIOIDES*; a- CORK, b- CORTEX, c-LYSIGENOUS CAVITY, d- STELE

Next to the cork is the cortex containing 30 to 40 rows of large collenchymatous cells with many intercellular spaces. These cells are abundantly packed with starch grains. They are either single or in groups of 2 to 3. Some of the cortical cells are larger, rounded or oblong and contain bundles of raphides of calcium oxalate crystals. The raphides are acicular in shape. Within the cortex are found rounded to tangentially elongated lysigenous cavities. Small globules of mucilage are found within these cavities. In mature tubers the cortical region is differentiated into a narrow outer zone (0.5-1 mm in thickness) wherein starch grains are absent and an inner wider starchy zone. The cells of the outer zone resemble those of the inner in shape

TABLE 2: TREATMENT OF SECTIONS WITH DIFFERENT REAGENTS

Drug	Reagent	Test for	Reaction	Result
1. Section	Iodine solution	Starch	Blue colour	+
2. Section	Ferric chloride	Tannin	Black colour	+
3. Section	Sudan 3 solution	Oil globule	-	-
4. Section	Conc. HCl	Crystals	Effervescence	+
5. Section	Pinch of Phloroglucinol + dil. HCl	Lignin	Magenta colour	+

+ = present; - = absent

Phytochemical Analysis: The organic and inorganic constituents present in a drug or plant play a significant role in the identification of crude drug. The physical constants like ash and extractive values help in establishing the Pharmacopoeial standards of the drug.

The fluorescence analysis help to identify the drug in powder form and the organic analysis help to understand the organic constituents of the drug.

Physical Constants: The results of moisture content, ash and extractive values are presented in **Tables 3, 4, 5** respectively.

but their walls have nearly double the thickness of the latter and are slightly lignified. A few scattered vascular bundles are also observed in cortex sometimes. Endodermis and pericycle are indistinct but the stele is clearly demarcated from the cortex in having ground tissue made up of parenchymatous cells. The stele is crescent shaped and consists of numerous closed, collateral vascular bundles. The outer bundles are arranged in a ring while the inner are found scattered in ground tissue. The vascular bundles vary in size and shape and range from collateral to the amphivasal type. The xylem elements partly or completely surround the phloem. The cells of phloem tissue are very small and are polygonal; sieve tubes and companion cells are indistinct. A parenchymatous pith is present in the centre of the stele. The measurement of various cells are provided in **Table 1**.

TABLE 1: MEASUREMENT OF CELLS OF DIFFERENT TISSUE (IN μm)

Cells (T. S.)	Minimum	Average	Maximum
Cork cells	24	40.2	49
Raphides	50	89	130
Lysigenous cavity	105	121	140
Xylem	7	15.9	24
Phloem	4	8	11

Histochemical Tests: The sections were treated with different reagents and results are provided in **Table 2**.

TABLE 3: MOISTURE CONTENT OF THE ROOT TUBER OF *CURCULIGO ORCHIOIDES* GAERTN.

Fresh weight (g)	Dry Weight (g)	Loss on drying (g)	Moisture Content (%)
2.04	1.89	0.15	7.3

TABLE 4: ASH VALUES OF THE ROOT TUBER OF *CURCULIGO ORCHIOIDES* GAERTN.

Total ash %	Acid insoluble ash %	Water soluble ash %
8.52	4.13	2.82

TABLE 5: EXTRACTIVE VALUES OF THE ROOT TUBER OF *C. ORCHIOIDES* GAERTN.

S. no.	Extractives	Extractive values (% w/w)
1	Alcohol soluble	12.5
2	Water soluble	16.2

Fluorescence Analysis: Fluorescence provided by a drug is one of the several methods used for analyzing crude drugs. Fluorescence is a type of

luminescence in which the molecule emits visible radiation passing from a higher to lower electronic state. The results are presented in **Table 6**.

TABLE 6: FLUORESCENCE ANALYSIS OF THE ROOT TUBER OF *CURCULIGO ORCHIOIDES* GAERTN.

Treatment of powder	Visible rays	U.V. light	
		Short wave (254 nm)	Long wave (365 nm)
Powder as such	Light Brown	Light brown	Light brown
In methanol	Dark brown	Light green	Dark brown
In methanolic NaOH	Green	Dark green	Green
In ethanol	Dark brown	Light green	Light green
In ethanolic NaOH	Green	Dark Green	Dark green
In dil. HCl	Light brown	Dark green	Dark green

Successive Solvent Extraction: The results of Successive solvent extraction were presented in **Table 7**.

Preliminary Organic Analysis: The results of Preliminary organic analysis were presented in **Table 8**.

TABLE 7: SUCCESSIVE SOLVENT EXTRACTIVE VALUES AND NATURE OF EXTRACTS OF *CURCULIGO ORCHIOIDES* GAERTN.

S. no.	Solvent	Colour	Consistency	Extractive value(%w/w)
1	Petroleum ether (60-80 °C)	Pale yellow	Lumpy mass	2.161
2	Benzene	Creamy brown	Semi-solid	0.312
3	Chloroform	Mud colour	Semi-solid	0.275
4	Acetone	Reddish brown	Semi-solid	2.981
5	Ethanol	Brownish black	Semi-solid	9.506
6	Water	Black	Semi-solid	13.145

TABLE 8: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THE ROOT TUBER OF *CURCULIGO ORCHIOIDES* GAERTN.

Tests	Pet. ether extract	Benzene extract	Chloroform extract	Acetone extract	Ethanol extract	Water extract
Alkaloids	-	-	-	-	-	-
Carbohydrates & Glycosides	-	-	-	+	+	+
Phytosterols	+	+	-	-	-	-
Fixed oil and Fats	+	+	-	-	-	-
Phenolic compound & Tannins	-	-	-	+	+	-
Saponins	-	-	-	+	+	+
Flavonoids	-	-	-	-	+	-
Proteins & Amino acids	-	-	-	-	-	-
Gums and Mucilage	-	-	-	-	-	+
Volatile oils	-	-	-	-	-	-

+ = present; - = absent

DISCUSSION: The root tuber which is the part used can be identified by the following macro and microscopical characters - tuberous root with starchy cortex; presence of bundles of raphides made of acicular crystals; presence of lysigenous cavity in the ground tissue; crescent shaped vascular bundles; presence of pith.

Histochemical tests show the presence of starch, tannin, crystals and lignin. The physicochemical constants of the root tuber of *Curculigo orchioides* were determined. It was found that water soluble extractive (13.145% w/w) was more than alcohol

soluble extractive value (9.506% w/w). These help in formulating pharmacopoeial standards for the drug.

Fluorescence analysis helps in distinguishing the drug in powder form. In case of successive solvent extraction the quantity of extract was found to be maximum in case of water extract and minimum in case of chloroform extract. The extract obtained by successive solvent extraction were subjected to preliminary phytochemical analysis, which revealed the presence of carbohydrates and glycosides, phytosterols, fixed oils and fats,

phenolic compounds and tannins, saponins, flavonoids gums and mucilage. The preliminary organic analysis of drug helps to undertake further studies on the isolation and identification of specific chemical constituents.

CONCLUSION: *Curculigo orchioides* Gaertn. (Family Hypoxidaceae) commonly known as Musali or Talamuli in Ayurveda is an important drug of Ayurveda and Siddha system of medicine. The root tuber is used in disease like diabetes, pain, leukoderma, skin troubles, demulcent and useful in diarrhoea. In the present study pharmacognostical, phytochemical investigations on the root tuber of *C. orchioides* were carried out. It is concluded that scientific parameters based on taxonomical, pharmacognostical and phytochemical studies are essential to identify the plant.

These parameters not only help in the standardization of these drugs but also help in formulating pharmacopoeial standards for drugs. The exomorphic characters have been found to be useful tools to identify the species taxonomically. The phytochemical analysis helps to identify the drug in powder form on the basis of physical constants and fluorescence analysis.

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CONFLICT OF INTEREST: Nil

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