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GOLDEN EYE GRASS - A MAGICAL REMEDY BY NATURE

Nidhi Soni*[1], V. K. Lal [2], Shikha Agrawal [3] and Hemlata Verma [4]

School of Pharmacy, Suresh Gyan Vihar University [1], Jaipur, Rajasthan, India
Sagar Institute of Technology and Management [2], Barabanki, Uttar Pradesh, India
Swami Vivekanand College of Pharmacy [3], Indore, Madhya Pradesh, India
Azad Institute of Pharmacy and Research [4], Lucknow, Uttar Pradesh, India

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Correspondence to Author:

Nidhi Soni

Sr. Lecturer, Suresh Gyan Vihar University, Jaipur, Rajasthan, India

Email id: researchnidheee@yahoo.co.in

ABSTRACT

From the dawn of civilization, medicinal plants are known to be part of human society to combat diseases. In recent times, focus on plant research has increased all over the world and various evidences have been collected to show immense potential of medicinal plants used in various traditional systems. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine. This paper aims to provide a comprehensive review on the phytochemical and pharmacological aspects of *Curculigo orchioides*, Amaryllidaceae also known as "Golden eye grass". It possesses a vast ethnomedical history and represents a phytochemical reservoir of heuristic medicinal value. It is one of the oldest oriental medicines mentioned in Ayurveda as potential remedy for various ailments. The rhizome is rich in Curculigoside, other glycosides, steroids, flavonoids and also contains various polyphenolic compounds. Many pharmacological studies have demonstrated the ability of the rhizome shows antioxidant, anti inflammatory, spermatogenic, aphrodisiac, immunostimulant, hepatoprotective, antiasthamatic, supporting its traditional uses. In this review article, we have focused our interest on phytochemistry, traditional uses, tissue culture study and its reported pharmacological properties.

INTRODUCTION: Medicinal plants are wealth of mankind. India with its mega-biodiversity and knowledge of rich ancient traditional system of medicine (Ayuveda, Sidhdha, Unani, local health traditional) provide a strong base for utilization of a large number of plants in alleviation of common ailments of the people [1]. With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems, evaluation of the rich heritage of traditional medicine is essential.

In this regard, we have choosen Golden eye grass (*Curculigo orchioides*) for establishing its medicinal richness. *Curculigo orchioides* is a key member of the dasapushpa and a highly useful plant in the indigenous system of medicine. It is small, geophilous herb used as a rejuvenating and aphrodisiac drug.

Synonyms:

Sanskrit: Bhumitila, arshoghni, Assamese: Talmuli, Tailmuli, Bengali: Talamuli, Tallur English: Black musale, Gujrati: Kali musali, Hindi: Syahmusali, Muslikand,

Kannada: Neltal, Nelatale, Malayalam: Nilappenea, Marathi: Kali musali, Bhuimaddi, Oriya: Talamuli, Punjabi: Syah musali, Musali safed,, Tamil: Nilappanai, Telugu: Nel tadigadda, Urdu: Musali Siyah, Kali Musali [2, 3].

Taxonomical hierarchy:

Kingdom-Plantae

Division- Magnoliophyta

Class- Monocotyledon

Order- Liliales

Family- Amaryllidacae

Genus- Curculigo

Species- orchioides [4]

FIGURE 1: CURCULIGO PLANT

FIGURE 2: DRIED RHIZOME OF CURCULIGO

Morphology: It is a perennial herb about 30 cm in height with a short or elongated root stock bearing several fleshy and lateral root which are blackish brown externally and cream internally.

- **Rhizome:** Drug occurs in transversely cut pieces of 2.5-5cm long, cylindrical, straight to slightly curved, cut surface 1.0 to 4.5 cm in diameter; external surface blackish-brown, cut surface cream colored; surface with numerous shallow wrinkles and transverse cracks; With a few rootlets and root scars; nodes and internodes prominent; taste, mucilaginous and slightly bitter.

- **Leaf:** Leaves are simple 15-45 cm long crowded on the short stem, sessile or short petiolate with sheathing leaf base and often produce adventitious buds at the tip when in contact with soil.

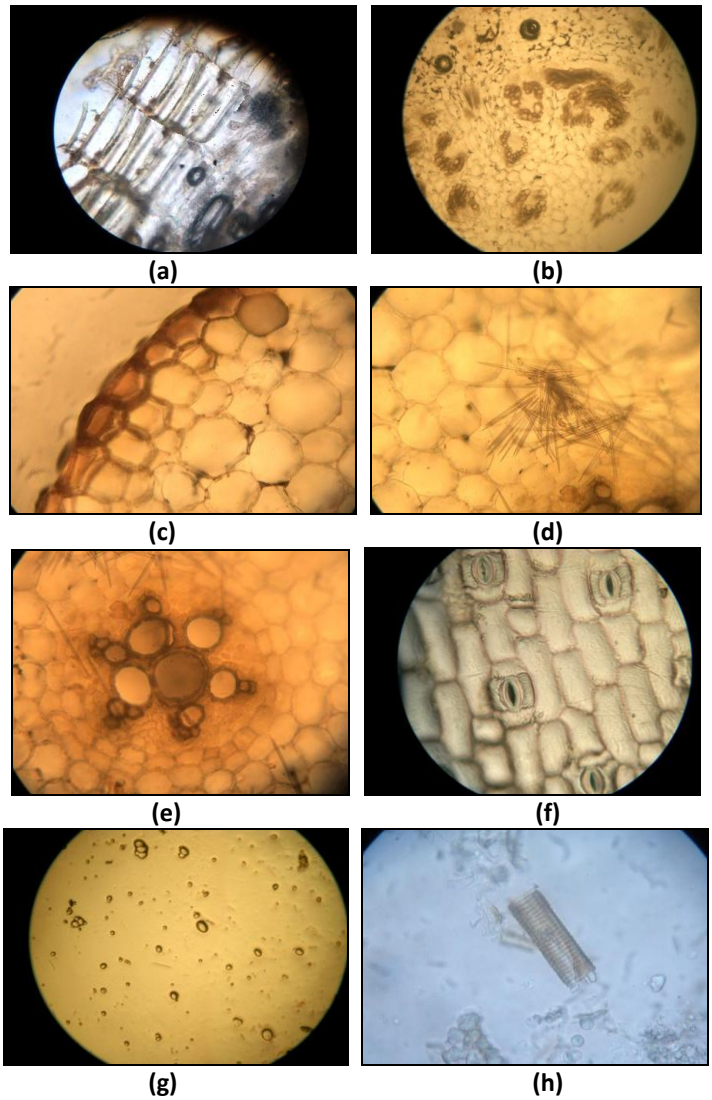
- **Flower:** Flowers are bright yellow distichously the upper few are male flower which are smaller in size, while the lower once are bigger and may be hermaphrodite or female. Inflorescences umbel-like racemes, 4–6-flowered, Pedicel 2 mm, Perianth yellow; segments oblong-lanceolate, 8–12 × 2.5–3 mm, outer ones sometimes abaxially laxly pilose. Stamens ca. 1/2 as long as perianth segments; filament 1.5–2.5 mm; anther 2–4 mm. Ovary narrowly oblong, to 7.5 mm, pilose. Stigma lobes longer than style. Berry subfusiform, 1.2–1.5 × ca. 0.6 cm; beak ca. 2.5 mm Fl. and fr. Apr–Sep.

- **Fruit:** Fruit is capsule, oblong glabrescent with a slender beak ans spongy septa, 1.5-2cm long 8mm broad; Seeds 8, globose, 1-2mm, black, beaked, deeply grooved in wavy lines [1,4, 5].

Microscopic Characters: Curculigo shows a narrow strip of cork, consisting of 5 to 7 rows of light brown cubical to rectangular cells; secondary cortex consists of thin-walled, parenchymatous cells, densely filled with starch grains and acicular crystals of calcium oxalate, either isolated or in bundles, in a few cells; a few small, round to tangentially elongated, lysigenous cavities also found scattered in this region; a few vascular bundles found embedded in cortical region with phloem towards outer side, and consisting of a few xylem elements; ground tissue consists of parenchymatous cells, some of which contain acicular crystals of calcium oxalate; numerous fibro-vascular

bundles found scattered throughout the region, mostly towards peripheral region having phloem, almost encircled by xylem vessels having annular and spiral thickenings; starch grains simple, rounded to oval and also compound of 2 to 4 components, measuring 4 to 21 µ in dia., present in cortical and central region, a number of deep red, resin canals found throughout the region, mucilage in the form of colorless mass found in a few cortical parenchymatous cells [2].

Powder Microscopy: It is Grayish; vessels with annular and spiral thickenings; simple, round to oval, starch grains measuring 4 to 21 µ in dia., and compound starch grains having 2 to 4 components and a few acicular crystals of calcium oxalate; mucilage in the form of colorless mass found in a few cortical parenchymatous cells. The quantity and kind of starch grains, the type of bundles of calcium oxalate crystals and of mucilage cavities in the periphery of the rootstock are very characteristic [2].



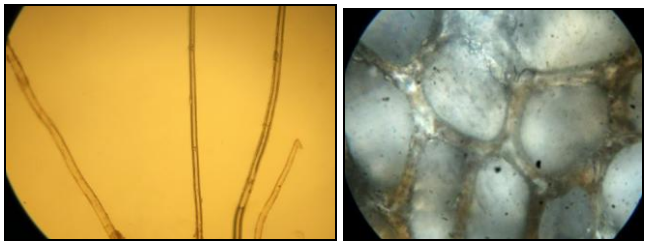


FIGURE (a-j): TRANSVERSE SECTIONS OF ROOT, RHIZOME, LEAF AND POWDER MICROSCOPIC CHARACTERS OF *CURCULIGO ORCHIOIDES* GAERTN.

(a) Cork cells of rhizome; (b) Scattered vascular bundle in rhizome; (c) Single layered epidermis of root; (d) Acicular crystal; (e) Vascular bundles; (f) Closed and Open stomata; (g)Simple and compound starch grains; (h)Spiral xylem vessels; (i)Fibres; (j) Polygonal parenchymatous cells

Distribution: It is believed to have originated in the shady forests of Asia. The plant is distributed in plains and shows prostrate growth on moist fertile soil. It is found in all parts of India from near sea level to 2300m altitude, especially in rock crevices and laterite soil. It has been recorded to occur in the subtropical Himalayas from Kumaon eastwards ascending to 1800m, the Khasia hills, Bengal, Assam, Konkan, Kanara, the western peninsula and Tamil Nadu extending south as far as Cape Comerin. It is also distributed in Sri Lanka, Japan, Malaysia and Australia.

The demand of the raw materials and derivatives of the plant for the indigenous drug industries is satisfied mainly from the wild source, depleting the natural population. In the CAMP (Conservation Assessment Management Planning) workshop at IIFM (June 1999) *Curculigo orchioides* was included in the IUCN(The International Union for Conservation of Nature and Natural Resources) category of "LOWER RISK near threatened" [7].

Cultivation and Propagation:

Propagation Material: Tuber segments of 1.5-2 cm size, containing the apical bud, are collected during February-March and used for propagation.

Agro-Technique:

Nursery technique:

1. **Raising propagule:** No stock is raised in the nursery. Tuber segments of size 1.5 cm × 2 cm, obtained from mother plants, are planted

directly in the main field at the onset of south-west monsoon, which breaks over South India in May-June. The tuber segments are planted at an optimum spacing of 10 cm × 10 cm. About 70%-80% sprouting is obtained after two months of planting in humid tropical regions like Kerala.

2. **Propagule rate and pretreatment:** The propagule rate is 600–750 kg of root segments per hectare. The tuber segments require no pretreatment before sowing.

Planting in the field:

1. **Land preparation and fertilizer application:** *Curculigo orchioides* grows well in moist and humus-rich soils. The land is ploughed well with the onset of monsoon. Organic manure is mixed before planting and raised beds are prepared to prevent water logging. FYM (farmyard manure) at the rate of 20 tonnes/ hectare is applied at the time of land preparation. Alternatively, FYM at the rate of 15 tons/hectare may be applied at the time of land preparation and NPK (nitrogen, phosphorus, potassium) at the rate of 25:15:10 kg/hectare can be applied as top dressing during October-November. If available, well-decomposed poultry manure at the rate of 2.7 tons/ hectare, instead of FYM, mixed well with the soil at the time of land preparation gives better yield.

2. **Planting and optimum spacing:** The tuber segments are directly planted in the field in rows. About 70%-80% germination/sprouting of tubers takes place after two months, when planted in humid tropical areas like Kerala. An optimum crop stand of 0.6-0.65 million is desirable for a pure crop with an optimum spacing of 10 m × 10 cm or 10 cm × 15 cm, while intercropping with a coconut gives a crop stand of approximately 0.2 million with a spacing of 20 cm × 25 cm.

3. **Intercropping system:** The crop grows well in the shade of irrigated coconut orchards. If it is to be raised as a pure crop, artificial shade has to be provided using shade nets of 25% density.

4. **Interculture and maintenance practices**: No additional manure is required for crop management. Manual weeding is usually adopted. Weeding twice at two and four months after planting is necessary to keep the crop weed-free. No special maintenance practices are required except for regular weeding and watering during dry spells.

5. **Irrigation practices:** The crop is grown in rain-fed area during the monsoon period. After the monsoon ceases, it is to be irrigated with 5 cm flooding fortnightly.

Disease and pest control: Seedling rot is observed during the rainy season and can be controlled by spraying and drenching the soil with 1% Bordeaux mixture. Black rot disease is also observed and can be controlled by spraying 0.05% tridemorph. Rhizomes are often eaten by rodents and hence standard control measures may be taken for their control.

Harvest management:

1. **Crop maturity and harvesting**: The plant starts flowering one month after planting and maximum number of flowers are noted during second and third months of planting. Flowering takes place throughout the year. However, fruits and seeds are not used as drug. Roots mature in the field in seven to eight months and may be harvested by digging.

2. **Post-harvest management**: Remnants of the shoot and rootlets are removed from tubers. The tubers are cleaned of the soil particles, dried well in the shade, and stored in gunny bags.

3. **Yield and cost of cultivation**: A dried tuber yield of 1000-1700 kg/hectare is obtained. The estimated cost of cultivation is Rs 28 000/hectare, which does not include the cost of planting material [5, 8].

Traditional Uses: *Curculigo orchioides* has been used in the indigenous system of medicine for long periods. It has been prescribed in various combinations and dozes by tribals and traditional vaidyas for a number of ailments and disorders.

1. 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.

2. According to Bhavaprakash the drug is sweet, bitter, acts as an aphrodisiac.

3. In Raj Nighantu it has been described as sweet, cooling, mucilaginous, incrases Kapha and reduces Pitta daha (burning sensation), acts as stimulant, gives strength.

4. Musali prepared as a paste with goat's milk or honey and applied locally over the face, brightens the complexion of the face.

5. Roots are prescribed usually combined with bitters and aromatics in the form of electuary, the dose being one teaspoonful twice a day.

6. The drug is given with warm milk and sugar in dises of two drachms in gonorrhea, dysuria, menorrhagia, leucorrhoea and menstrual derangements [9, 10, 11].

7. Juice of plant is applied on cuts and wounds (like tincture of iodine) and is considered as an effective anti-infective and healing agent [12].

8. In most Ayurvedic formulations the plant is used as a substitute to "safed musli" [13].

9. Rhizomes are prescribed in treatment of piles, jaundice, asthma, diarrhoea, and gonorrhea. The plant also holds the reputation of being a demulcent, diuretic, tonic and aphrodisiac [4, 14].

10. *Curculigo orchioides* Gaertn. is named "Xianmao" in Pharmacopoeia of the People's Republic of China and described as a tonic [4].

11. In the Unani system of medicine (originating from the Persian traditional healing system of medicine), the root is considered as bitter, sweet, carminative, tonic, aphrodisiac, antipyretic, useful in bronchitis, ophthalmia, indigestion, vomiting, diarrhoea, dyspnoea, gonorrhoea, gleet, hydrophobia, pains in the joints, etc. The rhizome is prescribed for asthma, piles, jaundice, diarrohea and gonorrhoea. Leaves of C. orchioides have been shown to possess anticancer property [15].

12. A decoction of the pounded rhizome along with the crushed ajwain (fruits of *Trachyspermum ammi*, Fam. Umbelliferae) is reportedly given to children in order to gain consciousness. Rhizomes have been claimed for the antidiabetic properties in various studies [16].

Pharmacological activity:

1. **Oxytocic activity:** Sharma *et al*., observed an oxytocic activity of a flavone glycoside of *Curculigo orchioides* [17].

2. **Hepatoprotective activity:** Rao *et al*., suggested the anti-inflammatory and hepatoprotective activities of *Curculigo orchioides* [18]. Rao *et al*., showed a hepatoprotective activity against rifampicin-induced Hepatotoxicities [19]. Rao *et al*., isolated curculignin A and curculigol and screened for their anti-hepatotoxic activity against thioacetamide and galactosomine-induced hepatoxic [20]. Venukumar *et al*., also showed Hepatoprotective effect of methanolic extract of Curculigo using different marker enzymes [21].

3. **Antioxidant activity:** Venukumar *et al*., showed anti-oxidant activity of methanol extract *Curculigo orchioides* in CCl_4-induced hepatopathy in rat [22]. Methanol extract was found to be extremely effective in scavenging superoxide radical (IC_{50} 29.28µg/ml) whereas activity was moderate in scavenging DPPH radical (IC_{50} 105.94 µg/ml), nitric oxide radical (IC_{50} 90.96 µg/ml) and in inhibition of lipid peroxidation (IC_{50} 94.78 µg/ml). Methanol extract showed different levels of antioxidant activities in tested models [23].

4. **Hearing Loss:** Noise exposure is one of the most common causes of hearing loss. Noise-induced hearing loss (NIHL) is thought to primarily involve damage to the sensory hair cells of the cochlea via mechanical and metabolic mechanisms. This study focused on examining the therapeutic effects of *Curculigo orchioides* on NIHL in a mouse model. Oral treatment with the extract of

Curculigo orchioides began 24 h following an examination that determined a shift in hearing threshold induced by noise exposure. Central auditory function was evaluated using auditory middle latency responses, and cochlear function was determined based on transient-evoked otoacoustic emissions. *Curculigo orchioides* reduced hearing threshold shifts, central auditory function damage, and cochlear function deficits [24].

5. **Immunomodulatory activity:** Lakshmi *et al*., [25] isolated two phenolic glycosides and a purified glycoside fraction and observed significant immuno-stimulant activity in purified glycoside-rich fraction isolated from the ethyl acetate extract. Bafna *et al*., [26] showed that methanol extract when studied on humoral and cell-mediated immunity in normal, as well as cyclophosphamide-induced immunosuppressed mice produced an increase in humoral antibody titre, delayed-type hypersensitivity and levels of white blood cells in a dose dependent manner.

6. **Aphrodisiac activity:** The ethanol extract of *Curculigo orchioides* rhizome at a dose of 100 mg/kg improved sexual behavior in male rats. Extract significantly changed the sexual performance as assessed by determining parameters such as penile erection, mating performance, mount frequency and mount latency. It also increased spermatogenesis and orientation behavior in male rats. The lyophilized aqueous extract of the plant showed significant improvement in sexual activity at a dose of 200 mg/kg body weight [27].

7. **Spermatogenic activity:** In the present study ethanolic extract of rhizomes was evaluated for its effect on orientation behavior and spermatogenesis in albino rats. A change in orientation behavior was assessed by orientation towards female, towards environment, towards self and type of mobility. Administration of 100 mg/Kg b. w. of ethanolic extract had pronounced effect on orientation of male towards the female rats. The increased spermatogenesis in treated group was confirmed by change in histoarchitecture as evidenced by increase in number of spermatocyte and spermatids [28].

8. **Antidiabetic activity:** In the present study, ethanolic and aqueous extracts also possess antihyperglycemic activity in normal, glucose-loaded and alloxan-induced diabetic rats. The extract exhibited significant hypoglycemic activity when compared with the control. The activity was also comparable to that of the effect produced by a standard antidiabetic agent glimeperide, 500µg/kg (p.o.). Dose-dependent antihyperglycemic effect was observed after treatment with extract [29, 30].

9. **Estrogenic activity:** Ethanolic extract of *Curculigo orchioides* rhizome possesses estrogenic activity as it showed a significant increase in percentage of vaginal cornification, uterine wet weight (P<0.01), uterine glycogen content (P<0.01) and a proliferative changes in uterine endometrium as compared with the control [31].

10. **Antiosteoporotic activity:** *Curculigo orchioides* ethanolic extract showed potential anti-osteoporosis activity as it prevented bone loss in the trabecular bone of the tibia in ovariectomized rats without affecting the weights of the body and the uterus, and increased serum phosphorus, calcium, and osteoprotegerin levels, decreased serum deoxypyridinoline crosslinks to creatinine ratio, tartrate-resistant acid phosphatase, adrenocorticotropic hormone, and corticosterone levels, but did not alter serum tumor necrosis factor-α, interleukin-6, and alkaline phosphate levels in ovariectomized rats. The ethanol extract of Curculigo also exhibited stimulatory effect on both osteoblast proliferation and ALP (alkalinephosphatase) activity [32, 33].

11. **Antiasthmatic activity:** Ethanol extract of *Curculigo orchioides* showed antiasthmatic activity as it was effective against histamine-induced contraction. In isolated goat tracheal chain preparation and isolated guinea pig ileum preparation, the extract exhibited maximum relaxant effect (P<0.01) against histamine at concentrations of 100 g/mL and 25 g/mL respectively [34].

12. **Antibacterial activity:** The root oil of *Curculigo orchioides* showed significant antimicrobial activity against various bacteria strains such as *Bacillus anthracis, Bacillus subtilis, Salmonella pyllorum and Staphyllococcus aureus* and fungi stains such as *Fusarium monili forme*, *F. solani, Aspergillus flavus and Cladosporium* [35].

13. **Analgesic activity:** The aqueous and alcoholic extracts of the roots of *Curculigo orchioides* were evaluated for analgesic activity using Eddy's Hot Plate method and Heat conduction method on Swiss albino mice. The aqueous extract showed significant analgesic activity. The findings support the use of this drug, *Curculigo orchioides* in the treatment of pain. Both the extracts were not toxic up to 3000 mg/kg body weight [36].

14. **Anticonvulsant activity:** The experiments showed that ethanolic extract of *Curculigo orchioides* had adaptive effects, such as enhancing tolerance towards high temperature and hypoxia. It also had sedative, anticonvulsant and androgen-like effect [37].

15. **Antihistaminic activity:** The stabilization potential of the alcoholic extract of *Curculigo orchioides* (COR) (100-400mg/kg) against mast cell degranulation was studied on isolated mice peritoneal mast cells. The antihistaminic activity was performed by determining the mortality rate of mice upon exposure to compound 48/80 and effect on inhibition of histamine release upon degranulation. The raised number of intact mast cells intimates that the COR stabilized the mast cell degranulation (60.96+/-1.96%) and percentage antihistaminic potential of the extract (63.58+/-1.8 inhibition at dose of 400mg/kg). This finding provides evidence that COR inhibits mast cell-derived immediate-type allergic reactions and mast cell degranulation [38].

16. **Antitumor activity:** The roots of *Curculigo orchioides* were fractionated with different solvents and screened for their antimicrobial and antitumor activity. Antifungal activity was screened using agar plate method, and antibacterial activity of the extracts was determined by disk diffusion method. Antitumor activity was screened against a human breast cancer cell line (MCF-7). Methanolic extract showed maximum activity due to the saponins present [39].

17. **Inhibitory activity:** The dried rhizomes of *Curculigo orchioides* yielded two phenolic glycosides, curculigoside, orcinol-beta-D-glucoside, and two cycloartane saponins, curculigosaponin G, curculigosaponin I. The structures were determined using spectroscopic methods. Among these isolates, compound 1 exhibited potent inhibitory activity against matrix metalloproteinase-1 in cultured human skin fibroblasts. In addition, it increased the level of Bcl-2 protein expression and decreased the level of Bax protein expression [40].

18. **Wound healing activity:** The methanolic extract obtained from root tubers of plant *Curculigo orchioides* was evaluated on excision wound model. The effect of methanolic extract (200mg/kg and 400mg/kg) was studied in Adult Wister albino rats. The results indicate that methanolic extract, at the dose of 200mg/kg & 400mg/kg was showed statically significant wound healing response when compared with the control group. Nitrofurazone ointment (0.2% w/w) was used as standard drug [41].

19. **Anti-inflammatory activity:** The methanolic extract obtained from root tubers of plant *Curculigo orchioides* at a dose of 200mg/kg and 400mg/kg was found to have statically significant anti-inflammatory activity as compare with control. The percentage inhibition of inflammation was found higher at the dose of 400mg/kg body weight at 3rd hr as compare to 200mg/kg. The activity was compared with that of the standard drug, Diclofenac sodium (15mg/kg) [42].

    In another study, the gels of *Curculigo orchioides* were formulated using the different concentration of gelling agent i.e. Carbomer 940 and Sodium CMC polymer. The sodium CMC (FS) gel formulation of rhizomes of *Curculigo orchioides* showed significant anti inflammatory activity in carrageenan induced rat paw edema [43].

In another study the effects of hydroalcoholic extract (HE) of *Curculigo orchioides* Gaertn. Rhizome and its alkaloidal and non-alkaloidal fractions (AF) and (NAF) were evaluated in carrageenan-induced paw edema experimental models of inflammation with indomethacin as a standard drug. The percentage of inhibition of inflammation of all extracts was dose dependent. The crude HE showed 22.45%, 35.62% and 39.03% inhibition; AF showed 31.68%, 36.89% and 41.17% inhibition; and NAF showed 28.34%, 34.49% and 37.43% inhibition of induced hind paw edema in rats at doses of 100 mg/kg, 300 mg/kg and 500 mg/kg, respectively, while indomethacin inhibited 48.66% of the edema [44].

20. **Chronic fatigue syndrome:** Rhizomes from Curculigo have also been used for years as a treatment for "chronic fatigue syndrome" in Chinese medicine [45].

21. **Cardio vascular activity:** A major chemical constituent Curculigoside from *Curculigo orchioides* can protect endothelial cells against oxidative injury induced by H_2O_2, suggesting that this compound may constitute a promising intervention against cardiovascular disorders [46].

22. **Antialgal activity:** Yang *et al*., reported that *Curculigo orchioides* shows antialgal inhibitory effect against *Microcystis aeruginosa* and their inhibitory rate is SZ-1,024 45.1±3.5[47].

23. **Antidiarrhoel activity:** Das studied that *Curculigo orchioides* also used for the treatment of diarrhoea [48].

Chemical Constituents:

1. *Curculigo orchioides* root tubers were investigated for its metal mineral content by using Energy Dispersive X-Ray Spectroscopy (EDX). The elemental analysis was performed to estimate eleven numbers of elements (C, O, Mg, Al, Si, Cl, K, Ca, Fe, Cu & Zn). The analysis of EDX showed that root tubers possess only seven types of essential elements. The root tuber was found deficient in Mg, Al, Si and Fe [49].

2. The dried rhizomes of *Curculigo orchioides* yielded two phenolic glycosides, curculigoside, orcinol-beta-D-glucoside, and two cycloartane saponins, curculigosaponin G, curculigosaponin I. The structures were determined using spectroscopic methods [40].

3. An extract from *in vitro* cultures of *Curculigo orchioides* grown as bulbils in shake flasks, afforded two new glucosides of substituted benzylbenzoate - curculigoside C and curculigoside D - together with two known compounds - curculigoside A and curculigoside B. Their structures were elucidated on the basis of spectral evidence, in particular by using 2D NMR methods [50].

4. A new orcinol glucoside, orcinol-1-O-beta-D-apiofuranosyl-(1-->6)-beta-D-glucopyranoside (3), was isolated from the rhizomes of *Curculigo orchioides* G., together with seven known compounds: orcinol glucoside, orcinol-1-O-beta-D-glucopyranosyl-(1-->6)-beta-D-glucopyranoside, curculigoside, curculigoside B, curculigoside C, 2,6-dimethoxyl benzoic acid, and syringic acid. The structures of these compounds were elucidated using spectroscopic methods [51].

5. Rao *et al*., reported the presence of mucilaginous component in the rhizomes of *Curculigo orchioides*. The composition of the mucilage was found to be mannose, glucose, glucuronic acid in the molecular ratio of 6: 9: 10. The total amount of the mucilage was found to be 8%-9% [52].

6. Tiwari *et al*., isolated new glycoside 5, 7 dimethoxymyricetin 3-O-α-L xylopyranosyl 4-O-β-D glucopyranoside from the rhizomes of *Curculigo orchioides* [53].

7. Curculigo's Rhizomes also contain β-sitosterol, sapogenin and alkaloid lycorine [54].

8. Kubo *et al*., isolated new phenolic glucoside named curculigoside and its structure was elucidated as 5-hydroxy-2-O-β-D-glucopyrano-sylbenzyl 2, 6-dimethoxybenzoate [55].

9. Two phenolic glycosides, curculigoside E and orchioside D were isolated and characterized from the rootstock of *Curculigo orchioides* and compounds were elucidated by means of spectroscopic methods such as one-dimensional (1D), two-dimensional (2D) nuclear magnetic resonance (NMR) and mass spectrometry [56].

10. The various aliphatic compounds isolated from alcoholic extract were named as 21-hydroxytetracontane-20-one, 4-methylhepta decanoic acid, 27-hydroxytriacontan-6-one and 23-hydroxytriacontane-2-one [57, 58].

11. Rhizomes yielded hentriacontanol, sitosterol, stigmasterol, cycloartenol, sucrose and new phenolic glycoside, named corchioside A (orchinol-3-beta-D-xylopyrnosyl-(1→6)-beta-D-glucopyranoside) [59].

12. The rhizome also contains curculigol, a cycloartane triterpene alcohol [60].

13. Xu *et al*., reported that several new cycloartane glycosides were isolated. One new triterpenoid sapogenin named curculigenin A, which was common to all the saponins, was identified as 3β, 11α, 16β-trihydroxycyloartane-24-one by mass spectrometry, 2D NMR analysis and chemical evidence. On the basis of chemical evidence and spectral data, the structure of curculigosaponins A-F was elucidated and also four new cycloartane-type triterpene glycosides named curculigosaponins G, H, I and J were isolated.

    Two new triterpenoid sapogenins named curculigenins B and C, which are formulated as (24S)-3β, 11α, 16β, 24-tetrahydroxycycloartane and 3β,11α,16β-trihydroxycycloartane-24(25)-en, respectively by 1H, 2C NMR, 2D NMR analysis and chemical evidence, and one new phenyl glycoside and two new chlorophenyl glycosides were isolated. The structure of curculigoside B, curculigines B and C was elucidated to be 2-β-D-glucopyranosyloxy-5-hydroxybenzyl-2′-methoxy-6′-hydroxybenzoate, 2,4-dichloro-3-methyl-5-methoxy-phenol-O-β-D-apiofuranosyl (1-6)-β-D-glucopyanoside and 2,4,6-trichloro-3-methyl-5-methoxyphenol-O-β-D-xylopyranosyl(1-6)-β-D-glucopyranoside respectively [61-64].

14. Yamasaki *et al*., determined the curculigoside by measuring the content of 2, 6-dimethoxybenzoic acid by high-performance liquid chromatography (HPLC) [65].

15. Lu *et al*., determined curculigoside by HPLC using Intersil ODS-3 chromatographic column, mobile phase of methanol-water-ice-acetic acid (45: 80:1) and detect wavelength was set of UV 283 nm [66].

16. Gupta *et al*., isolated two phenolic glucosides named orchiosides A and B [67].

17. Ethanolic extract of the roots of *Curculigo orchioides* also yielded phenolic glycoside orcinosides A, B and C [68].

18. Four esters namely n-decan-3-olyl pent-3′-en-1′-oate, and n-hexadec-9, 11-dienyl cinnamate, n-tridecanyl-hex-2′, 4′-dien-1′-oate, n-heneitria-cont-13-en-5, 10-diol hex-2′-en-1′-oate, were isolated from the rhizomes of *Curculigo orchioides* and characterized by the combination of chemical reactions and spectral data analysis [69].

19. A preparative high-speed counter-current chromatography (HSCCC) was used to isolate and separate curculigoside and curculigoside B from herb *Curculigo orchioides*. The recoveries of the two compounds were 91.6% and 92.5%, respectively [70].

20. High-performance thin-layer chromatography using toluene: ethyl acetate: glacial acetic acid (12.5, 7.5, 0.5 solvent ratio) was used to estimate gallic acid in crude drug [71].

Tissue Culture Study: This plant species have now become endangered due to reduction in the natural habitat that supports vegetation. Among the contributory factors, the following are the major ones:

a) Extensive denudation of the forest floor, caused by cattle grazing and collection of leaf litter;

b) Removal from the wilderness for tuberous roots which are highly priced in the market for its metabolic-enhancing principles and aphrodisiac formulations;

c) Poor seed setting and germination;

d) High incidence of viral and bacterial diseases affecting rhizomes;

e) Use of the rhizome as edible flour by many tribal people

f) Use of the plant as a substitute for safed musli.

1. In present times, tissue culture methods have become a powerful tool to develop micro-propagation method for such plants. Bulbils formation has gained considerable attention as a novel method for micro-propagation due to easy in transportation, better survivability of germinated bulbils. Large scale propagation through direct bulbils formation from leaf explants in shake flask culture is a method to overcome the problem of population depletion [72].

2. It is an endangered plant species of medicinal importance. Multiple shoots were obtained from the meristem tip culture on Murashige and Skoog (MS) medium supplemented with 6-benzyladenine (BA) (2.21 µmol/L). The shoots were rooted either on half strength of MS basal medium or on the one supplemented with 1-naphthaleneacetic acid (0.53 µmol/L). *In vitro* plantlets were transferred to pots containing a mixture of vermiculite and soil (1:1) for acclimation for a period of two to three weeks. At the end of a three-month period, averages of 125 plants were obtained from a single meristem [73].

3. Nema *et al*., suggested that Morphactin and cytokinin promotes high frequency bulbil formation from leaf explant of *Curculigo orchioides* grown in shake flask culture [74].

4. Suri *et al*., reported that by using a method developed for rapid multiplication through direct organogenesis and bulbil formation in vitro leaf and underground stem explants produced maximum number of shoots on B5 medium supplemented with 4.4 µmol/L benzyl-aminopurine [75].

5. Prajapati *et al*., showed in vitro regeneration of *Curculigo orchioides* Leaf explants inoculated in MS medium augmented with different concentrations of 2, 4-dichlorophenoxyacetic acid (2, 4- D) ranging from 0.5 to 2.5 mg/l showed differentiation of multiple shoots. The number of leaf explants showing differentiation of multiple shoots increased with increasing concentration of 2, 4-D [76].

6. Micropropogation of leaf explants of *Curculigo orchioides* cultured on a MS medium without cytokinins produced a limited number of plantlets that originated directly from the cut end of the midrib. BA (0.44-5.66 mol/L) was needed to produce plantlets from rhizome explants. A higher concentration of BA (2.22-4.44 mol/L) resulted in nodular callus that when transferred to cytokinin-free medium formed shoots [77].

7. An efficient protocol was developed for in vitro clonal propagation of *Curculigo orchioides* Gaertn. through apical meristem culture. Multiple shoots were induced from apical meristems grown on MS basal medium supplemented with 1.5 mg/L BA, 100 mg/L adenine sulfate (Ads) and 3% sucrose. Inclusion of indole-3-butyric acid (IBA) or indole-3-acetic acid in the culture medium improved the formation of multiple shoots. The highest frequency of multiplication was obtained on MS medium supplemented with 1.5 mg/L BA, 100 mg/L Ads, 0.25 mg/L IBA and 3% sucrose. Rooting was achieved upon transferring the microshoots to half-strength MS medium containing 0.25 mg/L IBA and 2% sucrose. Micropropagated plantlets were hardened in the greenhouse and successfully established in soil [78].

8. Sharma *et al*., reported that the effect of three arbuscular mycorrhizal (AM) fungal inocula on posttransplanting performance of in vitro raised *Curculigo orchioides* plantlets. The three

AM fungal inocula consisted of two monospecific cultures of *Glomus geosporum* and *G. microcarpum* and one crude consortium of AM fungal spores isolated from rhizosphere soil of *Curculigo orchioides* growing in natural habitat. Complete plantlets of *Curculigo orchioides* were raised by direct organogenesis of leaf explants on half strength MS medium devoid of any growth hormone. *Curculigo orchioides* plantlets responded significantly differently to all three mycorrhizal treatments. The study suggests use of mixed consortium of AM fungi over monospecific cultures for the sustainable cultivation and conservation of endangered medicinal plant: *Curculigo orchioides* [79].

9. Direct inoculation of leaf pieces on MS medium supplemented with various concentrations of benzylaminopurine (BAP) (2-8µmol/L) or thidiazuron (TDZ) (2-8µmol/L) alone or in combination with naphthalenacetic acid (NAA) (0.5 and 1.0µmol/L) produced low shoot induction both in terms of percentage of response and number of shoots per explant. Hence, leaf explants were pretreated with 15, 25 or 50µmol/L TDZ, for 6, 24 or 48 h with the aim of improving shoot regeneration from cultured explants. The pretreatment of explants with 15µmol/L TDZ for 24 h significantly promoted the formation of adventitious shoots and the maximum response was observed on MS medium supplemented with 6µmol/L TDZ [80].

10. Use of different elicitors' viz., methyl jasmonic acid, salicylic acid and ethephon influenced the production of curculigosides contents of leaves in in-vitro plantlets culture maintained on MS medium containing BA and IBA 0.1 mg/L each. Elicitation resulted in increased flux of phenolics and some new derivatives were produced. This involved changes in accumulation, transport and synthesis [81].

11. Suri *et al*., suggested a method for large-scale multiplication of *Curculigo orchioides* through bulbil formation of leaf explant in shake flask culture. Shake flask culture produces 2737 bulbils per litre medium where as static culture produces only 624 bulbils per litre medium at 6 weeks. This clearly indicates the superiority of shake flask culture overstatic flask culture in producing high number of bulbils by accommodation of higher number of explants per litre of the medium [82].

12. Nagesh described successful comparision for multiple shoot induction of *Curculigo orchioides* using shoot tip and rhizome disc and found that proximal rhizome discs are optimal for high frequency shoot bud formation than shoot tip and distal rhizome disc. They observed a synergistic effect between 6-benzyl aminopurine (BAP) and kinetin (KN) (each at 1 mg/L) on the regeneration of shoot buds from proximal rhizome disc than shoot tip explants [83].

13. Shreshta *et al*., monitored phenology, population structure, and regeneration strategies of *Curculigo orchioides,* a threatened medicinal herb of tropical to subtropical Asia, for 1 year at five sites in the inner Terai, Central Nepal. Only 20 - 26 % of mature individuals were in the reproductive phase during the phenologically most active months (June - July), and about 55 % of flowering individuals developed fruits. Soil moisture, stored reserves, and biotic pressure appeared to govern phenological patterns.

    Fruiting frequency was high under conditions of a partially open canopy and a thin litter layer. Seeds showed physiological dormancy and germinated 10 - 12 months after dispersal in natural habitats. Clonal propagation from leaves was induced by mild mechanical damage, high soil moisture, and humidity.

    Low regenerative potential through sexual reproduction and high vulnerability to habitat disturbance appear to be the major constraints to maintaining natural populations of *Curculigo orchioides* [84].

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