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Tissue culture and over exploitation of anti-cancer herb *Curculigo orchioides* Garten

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Abstract: Medicinal plants have been utilized to heal a variety of ailments throughout human civilization. Over-exploitation of these therapeutic plants has resulted in their extinction. *Curculigo orchioides* Gaertn. (Hypoxidaceae) often known as black muesli in India, is an endemic herbaceous plant that must be protected. The plant's rhizome and tuberous roots have been widely employed in traditional systems of medicine in India, for the treatment of numerous ailments such as jaundice, asthma, and diarthrosis. The rhizome juice has also been used as a tonic to treat impotency, prevent bone loss, and for anti-diabetic, anti-tumour, and antibacterial activities. The article presents a review on basic biology, in-vitro propagation techniques, medicinal properties, petrochemical constituents and toxicology of this important plant.

Keywords: Curculigo orchioides; Tissue Culture; Immunomodulator; Anticancer; Neuroprotective; Phytochemicals

1. Introduction

Plants have been employed as a primary natural source of alternative medicines and have played an essential part in human lives from ancient times. The ancient practise of using therapeutic plants has evolved into a very valuable industry in the worldwide market, resulting in the release of a vast number of herbal medicines Tsay, Shyur, Agrawal, Wu, and Wang (2016).Curculigo orchioides Gaertn. belonging to Hypoxidaceae family, often known as black musli, is a significant monotypic taxon of India.It is a major Rasayana drug in Ayurvedic system. The ayurvedic medicament is extracted from the mucilaginous tuberous roots of Curculigo orchioides Singh, Jain, and &khanuja (2006). It is referred by numerous vernacular names, Talmuli,Talusain West Bengal, while in Tamil Nadu it is known as Nilappanai.In Karnataka, Kerela and Andhra Pradesh it is known as Nelatigade, Nelappana and Nelatadi respectively Thakur, Puri, and Husain (1989). The herb C.orchioides is known for its aphrodisiac quality thus is used in traditional Chinese medicinal drugs to cure impotence Wong, Rabie, Bendeus, and &hägg (2007). Many different properties are also found like diuretic, antiulcer and tonic properties. Diseases like asthma, jaundice, venereal and urinary are also cured with the help of this drug Kurup, Ramadas, and Joshi (1979). Antioxidant, hepatoprotective, immunomodulatory and anticancer properties have also been found in this plant and thus has been intensively researched Dhawan, Dubey, Mehrotra, and Tandon (1980); Dhawan, Dubey, Mehrotra, and Tandon (1999). This perennial herb is found all over India, notably in subtropical climate of Western ghats and Himalayan region, up to 2250 m altitudes. The plant has also been reported in Assam, West Bengal, Bihar, Uttar Pradesh, Himachal Pradesh, Gujarat, Andhra Pradesh, Karnataka, Kerela and Tamil Nadu Kumar (1997). The availability of *C. Orchioides* in India is shown in Figure 1. The drugs are extensively used to study petrochemicals and pharmacological activities Kubo et al. (1983); Kubo et al. (2004); Kubo et al. (2002a). *C. Orchioides* roots are widely used in pharmaceutical enterprises to make medications, and a significant number of roots are harvested from woods by local residents and tribal people. It commands a high market price. The natural populations of this plant have been regarded low due to merciless economic exploitation and habitat loss, as

well as poor seed laying and germination Joy and &savithri (2004). The plant is not grown on a wide scale in the nation, although there are some occasional cultivation and tissue culture techniques available for in vitro culture and fast multiplication which have been discussed ahead.

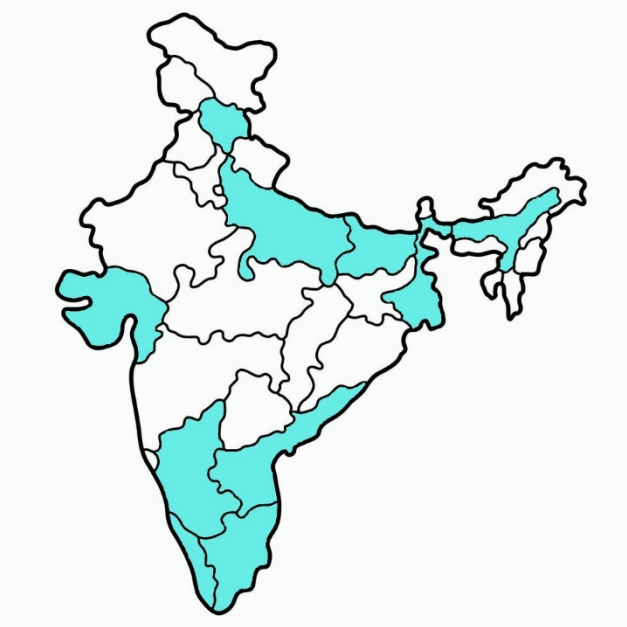

Diagram 1. Picture showing the occurrence of *Curculigo orchioides* in shaded areas.

1.1. Basic biology of Curculigo orchioides

C. orchioidesis an ever-lasting herb with a height of 30 cm. The rhizomes are erect, thick with creeping slender stolon. The leaves are often 4–7 cm long; the petiole is 30–80 cm long; the berries are white, sub globose, and beak less, and the seeds are black with irregular stripes.The plant originated in Asia's remote woods. On sodden rich soil, the plant is circulated in fields. It demonstrates prostate growth. Appearing in portions of India from close to the ocean level of about up to 2100 m height, especially in the rock holes and laterite soil Asif (2012). Flower blossoms as the year progresses, which is light yellow in colour, sexually open, sessile, customary, 1.2 cm. Perianthhassix lobes, which is yellow in colour [0.5-1 x 0.20-0.30 cm], stamens are 6 in number, filaments 2mm, stringy, anthers 2.0 mm, 3 celled ovaries, elongated to 4.0 mm. There are various ovules per cell, style is 2.0 mm, shame 3.0, projections prolong Soni, Lal, Agrawal, and Verma (2012). Fruit is capsulated, elongated glabrescent with a slim mouth and springy 'septa', 1.50-2.0cm in length 8.0mm broad; Seeds are 8 in number, 'globose', 1.0-2.0mm, dark, angled, profoundly scored in wavy lines Chauhan, Sharma, Thakur, and Dixit (2010); Chauhan, Sharma, Thakur, and Dixit (2012). The plant of *C. orchioidesis* represented in Figure 2.

1.2. Phyto-chemistry

The chemical constituents of *C. orchioides* have also been investigated. This summary allows an understanding of the general chemical information and the bioactive constituents that have been discovered. The compounds include phenols and phenolic glycosides Asif (2012); Asif (2010); Asif (1980); Asif (2004); Asif (1983); Asif (1997); Asif (1979); Asif (2004); Asif (1999); Asif (2013); Asif (2012); Asif (2006); Asif (2012); Asif (1989); Asif (2016); Asif (2002a); Asif (2007), lignans

Figure 1. Plant of *Curculigo orchioides*

and lignan glycosides C. X. Chen, Ni, and Mei (1999); C. X. Chen, Ni, and Mei (1989b); C. X. Chen, Ni, and Mei (2009), triterpenes and triterpenoid glycosides Fu, Lei, Cheng, Chen, and Zhou (2004); Fu, Lei, Cheng, Chen, and Zhou (2005); Fu, Lei, Cheng, Chen, and Zhou (2005); Fu, Lei, Cheng, Chen, and Zhou (1997); Fu, Lei, Cheng, Chen, and Zhou (1983); Fu, Lei, Cheng, Chen, and Zhou (1990); Fu, Lei, Cheng, Chen, and Zhou (1984); Fu, Lei, Cheng, Chen, and Zhou (1990); Fu, Lei, Cheng, Chen, and Zhou (1984); Fu, Lei, Cheng, Chen, and Zhou (1988); Fu, Lei, Cheng, Chen, and Zhou (1978); Fu, Lei, Cheng, Chen, and Zhou (1976); Fu, Lei, Cheng, Chen, and Zhou (2006); Fu, Lei, Cheng, Chen, and Zhou (2005); Fu, Lei, Cheng, Chen, and Zhou (1992); Fu, Lei, Cheng, Chen, and Zhou (1992); Fu, Lei, Cheng, Chen, and Zhou (2010); Fu, Lei, Cheng, Chen, and Zhou (2010); Fu, Lei, Cheng, Chen, and Zhou (2010); Fu, Lei, Cheng, Chen, and Zhou (2012); Fu, Lei, Cheng, Chen, and Zhou (2010), flavones Ansari (1993); Ansari (2000), alkaloids Odum and Barrett (1971); Odum and Barrett (1991); Odum and Barrett (2000); Odum and Barrett (2003), aliphatic compounds Augustine (1998); Augustine (2008); Augustine (2016); Augustine (2010); Augustine (2008); Augustine (2007), polysaccharides Bafna and Mishra (2006); Bafna and Mishra (1989a) and other types of compounds Cao et al. (2008). COPb-1 and COPf-1 are two water-soluble polysaccharides from *Curculigo orchioides* Gaertn and their molecular weights are 2.6×10^6 Da and 2.2×10^6 Da, respectively. COPb-1 is composed of glucose, fructose and xylose, and COPf-1 consists of stachyose, glucuronic acid and galacturonic acid Nie et al. (2013). The compounds that have isolated from the plant have been documented and listed in the Table 1.

Table 1. Phytochemicals extracted from *C. orchioides*.

No.	Chemical Constituents	References
A. Phenol and Phenolic Glycosides		
1	Curculigoside	18
2	Curculigoside B	33
3	Curculigoside C	21
4	Orchioside A	22
5	Orcinol glucoside	22
6	3-hydroxy-5-methylphenol-1-O-[β-D-glucopyranosyl-[1-6]-β-Dglucopyranoside]	39,40
7	Orcinol-1-O-β-D-apiofuranosyl-[1-6]-β-D-glucopyranoside	34,39
8	Corchioside A	22
9	Curculigine A	19
10	Curculigine B	35
11	Curculigine C	35
12	Curculigine D	35
13	Curculogoside E	20
14	4-hydroxy-3,5-dimethoxybenzoic acid	34
15	Orcinoside A	40
16	Orcinoside B	40
17	Orcinoside C	40
B. Lignans and Lignan Glycosides		
18	[1S,2R]orchioside D	20
19	Orchioside B	22
20	3,3',5,5'-tetramethoxy-7,9':7',9-diepoxyligan-4,4'-di-O-β-D-glucopyranoside	22
C. Triterpenes and Triterpenoid glycosides		
21	Curculigenin A	36
22	Curculigosaponin A	36,37
23	Curculigosaponin B	36,37
24	Curculigosaponin C	36,37
25	Curculigosaponin D	36,37
26	Curculigosaponin E	36,37
27	Curculigosaponin F	36,37
28	3β,11α,16β-trihydroxycycloartane-24-one-3-O-[β-D-glucopyranosyl [1-3]-β-D-glucopyranosyl[1-2]-β-D-glucopyranosyl]-16-O-α-Larabinopyranosid	41
29	Curculigosaponin G	36,38
30	Curculigosaponin H	36,38
31	Curculigosaponin I	36,38
32	Curculigosaponin J	36,38
33	Curculigenin B	36
34	Curculigosaponin K	36,38
35	Curculigosaponin L	36,38

Table 1 Cont...

C. Triterpenes and Triterpenoid glycosides		
36	[24S]-3β,11α,16β,24-tetrahydroxycycloartane-3-O-[β-D-glucopyranosyl [1-3]-β-D-glucopyranosyl[1-2]-β-D-glucopyranosyl]-24-O-β-Dglucopyranoside	41
37	Curculigosaponin M	18,38
38	Curculigenin C	36
41	24-methylcycloart-7-en-3β,20-diol	29
D. Flavones		
42	5,7-dimethoxmyricetin-3-O-α-L-xylopyranosyl-[4-1]-β-D-glucopyranoside	32
43	3',4',5'-trimthoxy-6,7-methylene dioxyflavone	32
E. Alkaloids		
44	1,3,7-trimethylxanthine	36
45	Methylacety[hydroxy]carbamate	30
46	Methyl-5-acetyl-1,2,3,5,6-oxatetrazinane-3-carboxylate	30
47	N1, N1, N4, N4 - tetramethylsuccinamine	30
48	Lycorine	31
F. Aliphatic Compounds		
49	3-[2-methoxypropyl]-4-methylnonacosan-2-one	26
50	4-acetyl-2-methoxy-5-methyltriacontane	25
51	27-hydroxytriacontan-6-one	27
52	23-hydroxytriacontan-2-one	27
53	21-hydroxytetracontan-20-one	28
54	4-methylheptadecanolc acid	28
G. Polysaccharides		
55	COPb-1	23
56	COPf-1	23
H. Other Type of Compounds		
57	2,3,4,7-tetramethoxyxanthone	24

2. Tissue culture techniques for cultivation

The genetic diversity of medicinal plants across the world is being threatened at an alarming rate due to destructive harvesting techniques and over-harvesting for medicine manufacture. Solid procedures for the mass propagation of endangered species are extremely appealing for meeting the needs of professional plantings and plant merchants while also re-establishing the plants to their natural environment Rout et al. (2000). *C.orchioides* is in high demand due to its numerous applications; nevertheless, its availability is unpredictable and insufficient. Ansari (1993). This plant species is presently threatened owing to habitat reduction in natural habitats that sustain vegetation. The following are the major contributing factors:

(i) Cattle ranching and leaf litter amassing have resulted in massive denudation of the forest floor.

(ii) Extraction from the forest for tuberous roots, which are highly valued in the market for their metabolic boosting principles and aphrodisiac compositions.

(iii) A lack of seed establishment and germination

(iv) A significant prevalence of viral and bacterial rhizome infections

(v) Many tribal people consume the rhizome as edible flour.

(vi) Usage of the plant as a safe substitute for Safed Muesli Wala and Jasrai (2003)

C.orchioides has also been included to the list of endangered plants by the Department of Biotechnology, Ministry of Science and Technology, New Delhi Swarup and Arora (2000). The progressive loss of this species' population necessitates the introduction of conservation initia-

tives to assure continuous and abundant supply by maintaining a balanced cycle of harvest and rejuvenation Odum and Barrett (1971). Keeping in mind the aforementioned facts, namely the progressive reduction of this endangered species, plant tissue culture has evolved into an effective technique for developing micro propagation systems for such plants Ramawat et al. (1991). Thus, we present an overview of previous researchers' in vitro propagation protocols for mass multiplication and conservation of this multi potential medicinal plant: *C. orchioides*, using different explants on different types of media, at different concentrations and combinations of growth regulators.

2.1. Shoot tip Culture

Through apical meristem culture an effective approach for in vitro clonal growth of *C.orchioides* Gaertn was devised. Murashige and Skoog [MS] basal media supplemented with 1.5 mg/l6-benzyladenine [BA], 100 mg/l adenine sulphate [Ads], and 3% sucrose was used to produce multiple shoots from apical meristems.The addition of indole-3-butyric acid [IBA] or indole-3-acetic acid [IAA] to the culture media aided in the production of numerous shoots Francis, Senapati, and Rout (2007).

2.2. Callus induction

The responses of rhizome discs from proximal to distal end were evaluated on MS basal media supplemented with varied quantities and combinations of auxins and cytokinins for callus induction; luxuriant callus was induced from the proximal end of the shoot axis rather than the distal end. Based on colour and texture, two types of callus were identified. Type I was induced on MS media which was supplemented with 2,4-D or NAA alone or NAA in combination with cytokinins. While Type II callus was grown on media with BAP or Kn in combination with 2,4-D Nagesh et al. (2010).

2.3. Leaf culture

Various experiments have been performed for rapid propagation of the plant through leaf culture. C. orchioides leaf explants cultivated on an MS medium lacking cytokinins generated a limited number of plantlets that arose straight from the cut end of the midrib.Plantlets from rhizome explants required BA Augustine (1998). Direct inoculation of leaf pieces on MS medium supplemented with benzylaminopurine [BAP] [2-8 M /L] or thidiazuron [TDZ] [2- 8 M /L] alone or in combination with naphthaleneacetic acid [NAA] [0.5 and 1.0 M /L] produced low shoot induction in terms of percentage of response and number of shoots per explant.As a result, leaf explants were pre-treated with 15, 25, or 50 M/L TDZ for 6, 24, or 48 hours in order to improve shoot regeneration from cultured explants. Pre-treatment of explants with 15 mol/L TDZ for 24 h greatly increased the production of adventitious shoots, with the greatest response seen on MS medium supplemented with 6 M /L TDZ Thomas (2007).

2.4. Root induction

A number of studies accomplished and reported rooting by moving micro shoots to half-strength MS medium with varying quantities of auxins.The shoots were rooted in media supplemented with either 0.54–5.37 M of 1-naphthaleneacetic acid [NAA], 0.57–5.71 M of indole-3-acetic acid [IAA], or 0.49–4.90 M of indole-3-butyric acid [IBA] Augustine et al. (2008).

2.5. Rhizome disc

Proximal rhizome discs outperform shoot tip and distal rhizome discs for high frequency shoot bud development. It was discovered that 6-benzylaminopurine [BAP] and kinetin [Kn] [both at 1 mg/L] had a synergistic impact on the regeneration of shoot buds from the proximal rhizome disc Nagesh and &shanthamma (2016).

Table 2. issue culture techniques ofC. orchioides

No.	Type	Media + Growth Regulators	Results	
1	Shoot Tip Culture	MS media + BA, Ads & Sucrose	Multiple shoot formation	48
2	Callus Induction	MS media + 2,4-D and BAP/NAA, Kn	A luxuriant callus growth	49
3	Leaf Culture	MS media + BAP, TDZ, Kinetin & 2,4-D	Good growth in explant with improved shoot regeneration.	50,51
4	Root Induction	MS media + NAA, IBA & GA3	Optimum root induction	52
5	Rhizome disc	MS media + BAP	Multiple shoot formation	53
6	AM Fungal inocula	Glomus microcarpum + Glomus geosporum	Sustainable cultivation	54

3. Arbuscular mycorrhizal [AM] fungal inocula

The influence of three arbuscular mycorrhizal [AM] fungal inocula on post-transplantation performance of in vitro grown *C. orchioides* plantlets was observed. The three AM fungal inocula comprised of two mono-specific cultures of *Glomus geosporum* and *G. microcarpum* and one crude consortium of AM fungal spores isolated from *C. orchioides* rhizosphere soil growing in natural environment.For the sustainable production and protection of this rare medicinal plant, the study advises to use a mixed consortium of AM fungus rather than monospecific cultures Sharma et al. (2008). The discussed tissue culture techniques have been listed in the Table 2.

4. Medicinal properties of *Curculigo orchioides*

Curculigo orchioides ethanol extracts have been proven to improve adaptive effects. These extracts may improve tolerance to extreme temperatures and hypoxia. They had sedative, anticonvulsant, androgen-like properties, as well as enhanced immunological activity in mice Q. S. Chen et al. (1989a). Methanol extracts of *Curculigo orchioides* rhizomes have also been shown to raise white blood cell counts, humeral antibody [HA] titer, and delayed type hypersensitivity [DTH] reaction in cyclophosphamide-treated immunocompromised mice. These findings suggest that methanol *Curculigo orchioides* extracts stimulate the immune system through mediating cells and humeral antibodies Bafna and Mishra (2006).

In both in vivo and in vitro experiments, *Curculigo orchioides* rhizome extracts demonstrated antiosteoporotic efficacy. In ovariectomized rats, an ethanol extract of *Curculigo orchioides* rhizomes reduced bone loss in the trabecular bone of the tibia through regulating osteoprotegerin, the ratio of deoxypyridinoline crosslinks to creatinine, and tartrate-resistant acid phosphatase [TRAP] activity Cao et al. (2008). *Curculigo orchioides* rhizome methanol extract was shown to be significantly efficient in scavenging super-oxide radicals and moderately effective in scavenging DPPH radicals, nitric oxide radicals, and inhibiting lipid peroxidation Bafna and Mishra (2005). In rats treated with carbon tetrachloride, the methanol extract of *Curculigo orchioides* rhizomes increased food consumption and weight gain, decreased serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma glutamyl transpeptidase, reduced serum levels of total protein and liver, and restored serum levels of total lipid, triglyceride, showcasing that the rhizome has hepatoprotective activity Venukumar and &latha (2002b).

In isolated mouse peritoneal mast cells and mice exposed to compound 48/80-induced systemic anaphylaxis, an ethanol extract of the *Curculigo orchioides* rhizome significantly reduced

mast cell degranulation Venkatesh et al. (2009). *Curculigo orchioides* ethanol extract was reported to have relaxing action in isolated goat tracheal chain preparation and isolated guinea pig ileum preparation. Further research revealed that the extract had a strong protective effect against guinea pig bronchoconstriction, rat passive paw anaphylaxis, and haloperidol-induced catalepsy in mice. These findings suggest that *Curculigo orchioides* ethanol extract may be beneficial in treating asthma Pandit, Singh, Bafna, Kadam, and Patil (2008).

Curculigoside dramatically decreased N-methyl-D-aspartate-induced neuronal cell loss, the amount of apoptotic and necrotic cells, excitotoxicity, and intracellular reactive oxygen species [ROS] generation in cultured cortical neurons.Curculigoside's neuroprotective effects may be mediated via lowering apoptotic protein levels and decreasing intracellular ROS generation in cultured cortical neurons Tian et al. (2012).Recent research has linked nuclear factor-κB [NFκB] and high-mobility group box 1 [HMGB1] to the pathogenesis of cerebral ischemia.Curculigoside treatment of SH-SY5Y cells reduced oxygen-glucose deprivation-induced cytotoxicity and apoptosis, prevented TNF-α-induced NF-κB and IκB-α phosphorylation and also lowered HMGB1 expression Jiang, Fu, Tian, Zhu, and Hou (2011). *Curculigo orchioides* rhizome oil demonstrated significant antimicrobial activity against *Bacillus anthracis, Bacillus subtilis, Salmonella pullorum, Salmonella newport,* and *Staphylococcus aureus*, as well as fungal strains *Fusarium moniliforme*, *Fusarium solani, Aspergillus flavus*, and *Cladosporium* spp. Jaiswal, Batra, and Mehta (1984).

Curculigo orchioides rhizome gel formulations exhibited considerable anti-inflammatory effect against carrageen-induced rat paw edema Dode, Wani, Deshmukh, and Patil (2009). In the Philippines, the rhizomes are used as a tonic, pectoral, diuretic, and aphrodisiac alone or in conjunction with carminative medications, and are put into a salve for itchy skin and other skin diseases Burkill (1966). A study indicates that *Curculigo orchioides* Gaertn fractions and compounds efficiently suppressed H2O2 induced oxidative stress by boosting antioxidant enzyme content and have cytotoxic potential on cancer cell lines HepG2, HeLa, and MCF-7 Hejazi et al. (2018). The various medicinal properties of *Curculigo orchioideshas* been listed in the Table 3.

4.1. Toxicology

As per Chinese Pharmacopoeia records, *Curculigo orchioidesis* poisonous and the clinical dosage suggested for adults is 3-9 g daily Pharmacopoeia (2010). *Curculigo orchioides*' hepatotoxicity may be caused by a triterpenoid ketone, which reduced the viability of a human liver cell line. HL-7702. The dosages chosen for the toxicity trials of *Curculigo orchioides* [30 g/kg, 60 g/kg, and 120 g/kg] appear to be excessively high, and while no fatality was recorded, there were some adverse effects Jiao, Chen, Wang, Lu, and Shao (2013). As a result, toxicity studies at lower levels are required to produce physiologically significant results. In general, taking *Curculigo orchioides* at the recommended clinical daily dose does not induce any severe side effects in people. However, taking *Curculigo orchioides* in high quantities over an extended length of time might produce cold sweats and numbness in the extremities. As a result, measures should be made to ensure the safe use of *Curculigo orchioides*; usage warnings have arisen in the medical literature. This is especially critical for the liver, kidneys, and reproductive systems Nie et al. (2013).

4.2. Future prospects of Curculigo orchioides

Direct comparison of inhibitory potency and IC50 profiles of compounds demonstrated that chemicals from the plant [capsaicin and piperine] show stronger binding affinity with the provided antioxidant enzymes than the rest of the compounds. Thus, these chemicals may be more effective antioxidants in reducing oxidative stress by boosting the antioxidant enzyme defense system, which in turn activates anticancer enzymes. Nevertheless, additional research on the antioxidant and anticancer properties of the specific chemicals is required. Furthermore, additional research may be conducted on the pathways involved at the cellular level, which can provide a concise overview of the active cell machinery engaged in programmed cell death. Understanding the molecular mechanisms that drive apoptosis in response to anticancer medicines, as well as how cancer cells avoid apoptotic death, opens up new avenues for a more balanced

Table 3. edicinal properties ofC. orchioides

Medicinal Properties	Tested substance	Model	
Adaptive activity	Ethanol extract	Normal Mice	55
Immunostimula-tory effect	Methanol extract	Cyclophosphamide-induced immunosuppressed mice	56
Antiosteoporotic activity	Ethanol extract	Ovariectomized rats	57
Antioxidant activity	Ethanolic extract	Scavenging DPPH radical	58
Hepatoprotec-tive activity	Methanolic extract	A carbon tetrachloride [CCl4]- induced liver injury in rats	59
Mast cell stabilization,	Ethanol extract	Compound 48/80-induced systemic anaphylaxis in the male Swiss albino mice	60
	Ethanol extract	Histamine induced bronchoconstriction in guinea pigs	61
	Ethanol extract	Haloperidol-induced catalepsy in Swiss mice	61
	Ethanol extract	Passive paw anaphylaxis in Wistar rats	61
Neuroprotective effect	Curculigo-side	NMDA-induced cell loss in cultured cortical neurons	62
	Curculigo-side	Human neuroblastoma [SH-SY5Y] cells	63
Antibacterial activity	Rhizome oil	Human pathogenic bacteria and phytopathogenic fungi	64
Anti-inflammatory activity	Ethanol extract	Carrageenan induced rat paw edema	65

approach to developing molecular-targeted cancer treatments Hejazi et al. (2018). *Curculigo orchioides* was also shown to lower hearing threshold shifts, central auditory function damage, and cochlear function deficits, indicating that it might be used as a possible therapeutic natural product for noise-induced hearing loss in mice Hong, You, and Kang (2011).

5. Conclusions

We reviewed the known traditional usage of medicinal plants: *Curculigo orchioides* as well as studies on their phytochemistry, pharmacology, toxicity, and in-vitro propagation techniques in this study. It is on the verge of extinction and hence requires both ex-situ and in-situ conservation. Its cultivation is also necessary to suit industry needs. Furthermore, it is critical to discover whether pharmacological research on this plant is accessible to validate their traditional usage. Modern pharmacological investigations have investigated the traditional medicinal applications of *Curculigo orchioides* in the traditional medicine system. Indigenous, limited distribution, small population, accessibility in specific season, poor seed setting and germination have resulted in a fall in natural population as well as a big problem for exploitation/extraction. To address these issues, in vitro cultures might well be employed as an alternate approach for drug extraction

and preservation.

Abbreviations

MS media Murashige and skoog media; **BA** Benzyl adenine; **Ads** Adenine sulphate; **NAA** Naphthaleneacetic acid; **IAA** Indole-3-acetic acid; **IBA** Indole-3-butyric acid; **BAP** Benzylaminopurine; **Kn** Kinetin; **2,4-D** 2,4-Dichlorophenoxyacetic acid; **TDZ** Thidiazuron; **AM** Arbuscular mycorrhizal; **DTH** Delayed type hypersensitivity; **HA** Humeral antibody; **TRAP** Tartrate-resistant acid phosphatase; **ROS** Reactive oxygen species; **NFκB** Nuclear factor-κB; **HMGB1** High-mobility group box 1

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