2. REVIEW OF LITERATURE

Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. In 19th century, chemical analyst and scientists start to extract and modify the active constituents from plants. Later, chemists began making their own version of plant compounds. Nowadays almost one fourth of pharmaceutical drugs are derived from botanicals. Recently, World Health Organization estimated that 80% of people going towards the use of herbal medicines in different parts of the world, due to side effects of synthetic medicine.

Medicinal plants have played an essential role in the development of human culture. Plants have formed the basis of sophisticated traditional medicine (TM) practices that have been used for thousands of years by people in China, India, and many other countries. One of the main areas of interest in Chinese and Aurvedic medicine was search for plants having aphrodisiac activity or Vajikaran herb. Vajikaran herbs have been used in traditional herbal medicine in India and other parts of the world for the treatment of male reproductive disorders like erectile dysfunction, low libido, premature ejaculation and infertility, and also for the treatment of mental ailments directly affecting the above-mentioned problems. Curculigo orchioides known as Xianmao in vernacular is popularly used as Vajikan Rasayana. One such ancient effort led to study the pharmacognostical, pharmacological and phytochemical aspects of this wonder herb, Curculigo orchioides.

2.1 PHARMACOGNOSTICAL PLANT PROFILE - CURCULIGO ORCHIOIDES GAERTN

Genus – Curculigo

Curculigo chiefly a paleotropical and subtropical genus of over twenty species, was established by Gaertner in 1788. Seven species of the genus have previously been recorded in China, of which, however two Hainan species, Curculigo fuziwarae...
(Yamamoto) and *Curculigo senporeiensis* (Yamamoto), are considered by writer to be identical with *Curculigo capitulate* and *Curculigo glabrescens* respectively, since Yamamoto’s description agree very well with the two latter species, of which many specimens collected from the island of Hainan have been carefully examined. Two new species and one new record are detected, thus the genus now is represented in China by seven species belonging to two sections (Singh, 1966). Seven species occurs in China, among them, *C. orchioides* and *C. capitulata* were recorded as tonic traditional Chinese medicine, and the other species were also used as folk medicinal herbs by local people (Wang *et al.*, 2009).

The medicinal plants of genus *Curculigo* have emerged as a good source of the traditional medicines. Some uses of these plants in the traditional medicines have been validated by pharmacological investigation. Phytochemical investigation of all species of the genus *Curculigo* has resulted in identification of more than 110 compounds. The medicinal plants of this genus have showed a wide spectrum pharmacological activities, including adaptive, immunostimulatory, taste-modifying and sweet-tasting, antioxidant, mast cell stabilization, antihistaminic and antiasthmatic, hepatoprotective and neuroprotective activity (Nie *et al.*, 2013). Traditionally used *Curculigo* species are as under:

- *Curculigo capitulate*
- *Curculigo orchioides*
- *Curculigo breviscapa*
- *Curculigo glabrescens*
- *Curculigo crassifolia*
- *Curculigo sinensis*
Table 2.1: Different *Curculigo* species

<table>
<thead>
<tr>
<th>SERIAL NO.</th>
<th>SPECIES</th>
<th>CHEMICAL CONSTITUENTS</th>
<th>BIOLOGICAL ACTIVITY</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Curculigo capitulata</em></td>
<td>Capitulatin B</td>
<td>Dysmenorrheal and rheumatism</td>
<td>(Ning <em>et al</em>., 2005a)</td>
</tr>
<tr>
<td>2.</td>
<td><em>Curculigo breviscapa</em></td>
<td>Breviscapin, Breviscasid</td>
<td>Antioxidant and free radical scavenging activity</td>
<td>(Zhu, 2010; Zhu <em>et al</em>., 2010a)</td>
</tr>
<tr>
<td>3.</td>
<td><em>Curculigo crassifolia</em></td>
<td>Crassifoside A-E and Isocrassifoside</td>
<td>Antioxidant, pneumonitis and free radical scavenging activity</td>
<td>(Ning <em>et al</em>., 2005b; Wang and Li, 2007)</td>
</tr>
<tr>
<td>5.</td>
<td><em>Curculigo orchioides</em></td>
<td>Curculigoside A-D</td>
<td>Aphrodisiac, spermatogenic, hepatoprotective</td>
<td>Chauhan <em>et al</em>., 2010</td>
</tr>
</tbody>
</table>

Out of all above species *Curculigo orchioides* is a very reputed drugs in all medicinal system. *Curculigo orchioides* species is common in the tropics of Asia. In present study, an effort was made to explore this powerful aphrodisiac drug for pharmacognostical, phytochemical and pharmacological actions. This would be useful for researchers to understand the medicinal benefits of this plant.
Pharmacognostical, Phytochemical and Pharmacological investigations of *Curculigo orchioides* Gaertn, 2013

**Figure 2.1: Plant: Curculigo orchioides**

**Synonym:** (Anonymous, 1999; Kirtikar and Basu, 1988)

- Hindi: Syahmusali, Muslikand
- English: Black musale
- Sanskrit: Bhumitila, arshoghni
- Urdu: Musali Siyah, Kali Musali
- Bengali: Talamuli, Tallur
- Gujarati: Kali musali
- Kannada: Neltal, Nelatala
- Tamil: Nilappanai
- Malayalam: Nilappenea
- Assamese: Talmuli, Tailmuli
- Oriya: Talamuli
- Punjabi: Syah musali, Musali safed,
- Telugu: Nel tadigadda
- Marathi: Kali musali, Bhuimaddi
**Taxonomical Classification:** (Chauhan et al., 2010)

- **Kingdom:** Plantae
- **Subkingdom:** Tracheobionta
- **Superdivision:** Spermatophyta
- **Division:** Magnoliophyta
- **Class:** Monocotyledon
- **Subclass:** Liliidae
- **Order:** Liliales
- **Family:** Amaryllidaceae
- **Genus:** Curculigo
- **Species:** orchoides

**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 to 5 cm long and 1.0 to 4.5 cm in diameter; external surface is blackish brown, with wrinkles, rootlet, root scars, nodes, internodes and transverse cracks, cylindrical in shape, straight to slightly curved, internal surface is cream colored; taste is 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 in colour and the upper few are male flower which are smaller in size, while the lower once are bigger and may be female. Inflorescences umbel-like racemes, 4–6-flowered. Anther 2–3 mm; ovary narrowly oblong in shape and of 7 mm, pilose; stigma lobes longer than style.
Fruit

Fruit is capsule, oblong glabrescent with a slender beak and spongy septa, 1.5-2cm long and 8mm broad. It contain 8 seeds which are globose, size 1-2mm, black, deeply grooved in wavy lines. (Anonymous, 1999; Xian, 2000; Irshad et al., 2006)

Distribution:

Curculigo orchioides grow in the shady forests area of Asia. This small herb is widely distributed in plains and shows prostate growth in moist and humus rich soil. The optimum spacing between two tubers are 10X10cm. It has been cultivated and distributed in the subtropical Himalayas regions, Kumaon eastwards, Bengal, Assam, Konkan, Khasi hills, Kanara, the western peninsula and Tamil Nadu extending south as far as Cape Comerin. It is also grow in Japan, Malaysia, Sri Lanka, and Australia. The demand of the raw materials and derivatives of the Curculigo for the herbal drug industries is satisfied mainly from the wild source. In the CAMP workshop at IIFM (June 1999) Curculigo orchioides was included in the IUCN category of “LOWER RISK, near threatened” (Joy et al., 2004).

Cultivation and Propagation:

PROPAGATION MATERIAL

For propagation root tuber of Curculigo are collected during March-April. Size of tuber taken for propagation was 2-2.5cm.

AGRO-TECHNIQUE

Plantation techniques

Raising propagule

In nursery technique, root tuber obtained from parent plant are planted directly in the field. Spacing between the tuber should be 10x10cm. Best season for growing of plant is monsoon. About 60-70% sprouting is obtained after two month. No pretreatment require for tuber segments before sowing.
**Pretreatment and Propagule rate** - 600–750 kg is the propagule rate for root segments per hectare.

**Planting in the soil area**

**Fertilizer application and land preparation**

In southern part of India (Kerla, Tamilnadu, Karnataka, etc.) Kali musali or talamuli grow better in humus-rich & moist soils. With the onset of monsoon, the land is ploughed properly. Organic manure is merged prior to planting and to prevent waterlogging, raised beds are prepared. At the time of land preparation, farmyard manure (FYM) at the rate of 20 tonnes/hectare is applied. Other way, FYM at the rate of 15 tonnes/hectare may be applied and nitrogen, phosphorus & potassium (NPK) can be applied at the rate of 25:15:10 kg/hectare as top dressing during October–November. Instead of FYM, Well-decomposed poultry manure, if available, at the rate of 2.7 tonnes/ hectare can be applied, mix nicely with the soil at land preparation stage which gives better yield.

**Intercropping methods**

In the shade of coconut orchards, the crops grown good. Artificial shade has to be provided using shade nets of 25% density if it is to be raised as a pure crop.

**Planting and most advantageous spacing**

In the field in rows, the tuber segments are directly planted and about 70%–80% sprouting/germination of tubers develop after a couple of months.

**Irrigation techniques**

The crop is grown generally in during the monsoon period in rain-fed area. It is to be irrigated with 5 cm flooding bi monthly, after the monsoon ceases.

**Disease and pest control**

Deseases can be prohibited by spraying and drenching the soil with 1% bordeaux mixture. A black-rot disease, may also be observed, can be controlled by...
putting/sparying 0.05 percentage tridemorph. Rodents usually eat rhizomes hence stipulated control measures may be followed.

**Maintenance and Interculture process**

For crop management, no additional manure is required. Generally adopted weeding is manual. Weeding two times at 2 and 4 months after planting, is important to stay crop weed-free. During dry spells, except for routine weed and water, no expertise maintenance systems are necessary.

**Harvesting techniques**

**Crop harvest**

One month post planting, the crop begins flowering and highest numbers of flowers are observed during second and third and sometimes forth months of planting. Seeds & fruits are not consider/ used as medicine yet. Roots mature in soil by seventh or eight months and can be harvested from diging soil.

**Post-harvest techniques**

Rootlets and remnants of the shoot are taken out from tubers. The tubers are washed well of the soil particles, dried carefully in the shade, and then kept in gunny bags.

**Cultivation costs & Yield**

Dry tuber yield of 1000–1700 kg/hectare is arranged. Rs 28 000/hectare is considered to be the estimated cost of cultivation excluding cost of material for planting. (Irshad et al., 2006; Anonymous, 2008)

**Traditional uses:**

Raghunathan and Mitra (2001); Nadkarni (2002); Anonymous (2004) reported that various vaidhyas and traditional tribals prescribed *Curculigo orchioides* in the various system of medicine specially in Indian system, for long periods. Herb properties in various doses and combinations for the treatment of a number of diseases have been described. Some of them are as under:
The epic treatise of the medicine and school of thought reveals that the Hindu system of medicine described first *Curculigo* herb in ‘Charak Samhita’ of ‘Agnivesha’, and described as an imminent part of cigar to alleviate cough.

The drug is bitter and sweet as per Bhavaprakash which acts as a powerful aphrodisiac.

*Curculigo* herb described as a sweet, mucilaginous & cooling in Raj Nighantu, which increases *Kapha* and reduces *Pitta daha* (burning sensation) acts as stimulant, gives power/strengths.

To brightens face complexion, *Curculigo* is prepared paste with goat’s milk or honey and used over face.

Rhizome of *Curculigo* are prescribed usually in cobination with bitters and aromatics in the form of electuary, the dose being one teaspoonful twice a day.

The drug is given with warm milk and sugar for treatment of menorrhagia, leucorrhoea, gonorrhoea, dysuria and menstrual derangements.

*Curculigo* juice is applied on cuts and wounds and considered as an effective anti-infective and healing agent (Atal and Kapoor, 1977).

The herb is used as an alternate to *Chlorophytum borivillianum* (safed musli) in most of the Ayurvedic formulations (Bhattacharjee, 1998).

For treatment of jaundice, asthma piles, gonorrhea and diarrhea, this plat is used in most Ayurvedic formulations. *Curculigo* also held the creditibility of being a diuretic, tonic, demulcent, and aphrodisiac. *Curculigo orchioides* Gaertn. is also named “Xianmao” in Pharmacopoeia of the People’s Republic of China and mentioned as a tonic (Chopra, 1956; Chauhan et al., 2010).

The plant root is carminative, tonic, aphrodisiac, bitter, sweet, antipyretic, useful in ophthalmia, indigestion, bronchitis, dyspnoea, gonorrhoea, gleet, vomiting,
diarrhoea, hydrophobia, pains in the joints, etc., as per Unani system of medicine (originating from the Persian traditional healing system of medicine). The rhizome is advised for diarrhea, asthma, piles, jaundice, and gonorrhoea. Leaves have been shown to have anticancer property (Agrawal, 1997).

- A decoction of the powdered rhizome with the crushed ajowain (fruits of *Trachyspermum ammi*) is effective in kids to gain consciousness. Rhizomes have been contested for the antidiabetic properties in many research studies (Parrotta, 2001).

- Rhizomes from *Curculigo* have also been used for years as a treatment for “chronic fatigue syndrome” in Chinese medicine (Chen et al., 2010).

- Dash and Padhy (2006) reported that *Curculigo orchioides* can also be used for the treatment of diarrhoea.

**Ayurvedic properties:**

Vipaka : Madhura  
Rasa : Madhura, Tikta  
Virya : Usna  
Karma : Vrsya, Rasayana  
Guna : Guru, Picchila (Anonymous, 1999)

**Important Marketed Formulations:**

Gandharvahastadi Kvatha Churna, Gandanadi Churna, Dasapushpa, Heezon, Gandharvahastad kasayam, Rejuvin, Dermoguard, On and On Alday, Kohinoor Gold, Vidaryadighrta, Vidaryadi lehya, Marmagulika, Musalyadi churna (Anonymous, 1999). Some of the commercial formulations containing *Curculigo orchioides* are, Volumizer (herbopharm), Vigorous capsule (Tampcol), Potency Plus (Chinese), Strong-nite capsule (Medimix), Spermomax (Pills male pvt ltd) Braincare 2000 (Chinese), Kama sutra capsule (Alma Health Care), Sharmiotone Syrup (Sharmila), Vaipani kamon (Vaipani) and Meno-Peace Capsule (D’arcy Natural)(Joy et al., 2004).
2.2 Phytochemical work reported on *Curculigo orchioides* Gaertn:

- Rao and Beri (1951) observed that *Curculigo orchioides* rhizome contain some mucilaginous component. The total amount of the mucilage was found to be 8%-9%. The composition of the mucilage was found to be glucose, mannose and glucuronic acid in the ratio of 9:6:10. β-sitosterol, sapogenin and alkaloid lycorine are also found in *Curculigo’s* rhizomes (Rao et al., 1978).


- Agrahari *et al* (2010) investigated for eleven elements (C, O, Mg, Al, Si, Cl, K, Ca, Fe, Cu & Zn) from the rootstock of *Curculigo’s* rootstock by using Energy Dispersive X-Ray Spectroscopy (EDX). Out of eleven elements *Curculigo* only contains seven type (C, O, Cl, K, Cu, Ca & Zn) of essential elements.

- Wu *et al* (2005) isolated eight compounds from the rhizomes of *Curculigo orchioides* in which, a new orcinol glucoside named as orcinol-1-O-beta-D-apiofuranosyl-(1-->6)-beta-D-glucopyranoside, and other seven known compounds: Curculigoside A, B, C, orcinol glucoside, 2,6-dimethoxybenzoic
acid, orcinol-1-O-beta-D-glucopyranosyl-(1-->6)-beta-D-glucopyranoside and syringic acid. The structures of all these compounds were elucidated using different spectroscopic methods. Tiwari and Misra (1976) also isolated a new glycoside named as 5, 7 dimethoxymyricetin 3-O-α-L xylopyranosyl 4-O-β-D glucopyranoside from the rhizome of *Curculigo orchioides*. Yokosuka et al (2010) isolated Cycloartane glycosides (1–9) from *Curculigo* rhizome.

- Mishra *et al* (1984a) and (1984b) isolated different aliphatic compounds from the alcoholic extract of *Curculigo orchioides*. Their structure determined by different spectroscopic methods. Their named as 21-hydroxytetracontane-20-one, 4-methylheptadecanoic acid, 27-hydroxytriacontan-6-one and 23-hydroxytriacontane-2-one.

- Garg *et al* (1989) isolated different constituents. These constituents named as stigmasterol, cycloartenol, hentriacontanol, sucrose, sitosterol and new phenolic glycoside, named corchioside A. Mishra *et al*, (1990) isolated Curculigol and a cycloartane triterpene alcohol from the rhizome of *Curculigo orchioides*.


- Daffodil *et al* (2012) investigated ethanol extract of *Curculigo orchioides*. they carried out GC-MS analysis of extract and found six compounds. The compounds
were Hexadecane, 5-butyl, Benzoic acid, 4-ethoxy-ethyl ester, Ethyl isoallocholate and Dodecane 2,6,11-trimethyl.

- Jiao et al (2012) investigated a new cycloartane-type triterpenoid ketone together with four known components by using different spectroscopic methods, including 1D and 2D NMR, and single-crystal X-ray diffraction analysis.

**Chemical composition of *Curculigo orchioides***

Phytoconstituents present in rhizomes of *Curculigo orchioides* are given below.

1. **Saponins**- curculigenin A, B and C; curculigosaponins A-F; curculigosaponins G, H, I and J; Yuccagenin.
2. **Glycosides (Phenol and Flavonoids)**- curculigoside A, B, C and curculigoside D; orcinol-beta-D-glucoside; orcinol glucoside, orcinol-1-O-beta-D-apiofuranosyl-(1-->6)-beta-D-glucopyranoside; 2,6-dimethoxy benzoic acid; syringic acid; 5, 7 dimethoxy myricetin 3-O-α-L xylopyranosyl 4-O-β-D glucopyranoside; 5-hydroxy-2-O-β-D-glucopyranosylbenzyl2,6 dimethoxybenzoate; curculigoside E; corchioside A; orchiosides A and B, C and D.
3. **Aliphatic compounds**- 21-hydroxytetracontane-20-one, 4-methylheptadecanoic acid, 27-hydroxytriacontan-6-one and 23-hydroxytriacontane-2-one.
4. **Fatty Acids**- Palmitic, oleic, linoleic, arachidic and behenic acid.
5. **Alkaloid**- Lycorine.
6. **Steroids**- Hentriacontanol, sitosterol, stigmasterol, cycloartenol, β-sitosterol.
7. **Triterpene alcohol**- Curculigol.
8. **Elements**- C, O, Cl, K, Cu, Ca & Zn.
9. **Mucilage**- Mannose, glucose, glucuronic acid.
10. **Esters**- n-decan-3-olyl pent-3′-en-1′-oate; n-hexadec-9, 11-dienyl cinnamate; n-tridecanyl-hex-2′,4′-dien-1′-oate; n-heneitriacont-13-en-5,10-diol hex-2′-en-1′-oate; Hexadecane, 5-butyl, Benzoic acid, 4-ethoxy-ethyl ester, Ethyl isoallocholate and Dodecane 2,6,11-trimethyl.
11. **Others**- tannin, starch, resin and hemicellulose etc.
Figure 2.2: Structure of different glycosides and phenolic compounds isolated from *Curculigo orchioides*
Figure 2.3: Structure of different glycosides and phenolic compounds isolated from *Curculigo orchioides*
Figure 2.4: Structure of different glycosides and phenolic compounds isolated from Curculigo orchioides
<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curculigosaponin A</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Curculigosaponin B</td>
<td>Glc</td>
<td>H</td>
</tr>
<tr>
<td>Curculigosaponin C</td>
<td>H</td>
<td>Ava(p)</td>
</tr>
<tr>
<td>Curculigosaponin D</td>
<td>Glc₋₁₋₁Glc</td>
<td>H</td>
</tr>
<tr>
<td>Curculigosaponin E</td>
<td>Glc₋₁₋₁Glc</td>
<td>Ava(p)</td>
</tr>
<tr>
<td>Curculigosaponin F</td>
<td>Glc₋₁₋₁Glc₋₁₋₁Glc</td>
<td>H</td>
</tr>
</tbody>
</table>

Figure 2.5: Structure of different saponins isolated from *Curculigo orchioides*
2.3 Pharmacological activities of Curculigo orchioides:

- **Oxytocic activity**
  Sharma *et al* (1975) observed an oxytocic activity of a flavone glycoside of *Curculigo orchioides*.

- **Hepatoprotective activity**
  Rao and Mishra (1996a, 1996b) suggested the anti-inflammatory and hepatoprotective activities of *Curculigo orchioides*. They observed hepatoprotective activity against rifampicin-induced hepatotoxicities and also isolated curculignin A and *Curculigol* and screened for their anti-hepatotoxic activity against thioacetamide and galactosamine-induced hepatoxic. Venukumar and Latha (2002a) observed hepatoprotective effect of methanolic extract of *Curculigo* using different marker enzymes.

- **Antioxidant activity**
  Venukumar and Latha (2002b) observed anti-oxidant activity of *Curculigo orchioides* in carbon tetrachloride-induced hepatopathy in rat. Alcoholic extract was found to be extremely effective in scavenging superoxide radical whereas activity was moderate in scavenging DPPH radical, nitric oxide radical and in inhibition of lipid peroxidation.

- **Hearing Loss**
  Noise exposure is one of the most common causes of hearing loss. Noise-induced hearing loss (NIHL) damage sensory hair cells of the cochlea via mechanical and metabolic mechanisms. Hong *et al*, (2011) examined 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.
➢ **Immunomodulatory activity**

Lakshmi *et al* (2003) observed immuno-stimulant activity in purified glycoside-rich fraction isolated from the ethyl acetate extract of *Curculigo orchioides*. Bafna and Mishra (2006) also observed that methanol extract produced an increase in humoral antibody titre, delayed-type hypersensitivity when studied on humoral and cell-mediated immunity in normal, as well as cyclophosphamide-induced immunosuppressed mice.

➢ **Aphrodisiac activity**

Chauhan *et al* (2007b) studied sexual behavior in male rats. They used ethanol extract of *Curculigo orchioides* rhizome at a dose of 100 mg/kg. Extract significantly changed the sexual performance as assessed by determining different parameters such as mating performance, mount frequency and latency, penile erection. 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.

➢ **Spermatogenic activity**

Chauhan *et al* (2008) evaluated that ethanolic extract of rhizome of *Curculigo* for its effect on orientation behavior and spermatogenesis in albino rats. They observed a change in orientation behavior towards female, environment and self. Administration of 100 mg/Kg b. w. of ethanolic extract had pronounced effect on orientation of male towards the female rats.

➢ **Antidiabetic activity**

Chauhan and Dixit (2007a) and Madhavan (2007a) studied that both ethanolic and aqueous extract of *Curculigo* showed antihyperglycemic activity in normal, glucose-loaded and alloxan-induced diabetic rats. The extract exhibited significant hypoglycemic activity in all animal models when compared with the control. In another study, Thakur *et al* (2012) evaluated the aqueous extract of the herb for its effectiveness against streptozotocin-induced hyperglycemic stress and subsequent sexual dysfunction due to hyperglycemia in male rats. In this study, the body and organ weight of the animals were recorded. Behavioral
analysis of rats was undertaken to observe the effect on mount, ejaculation and intromission (latencies and frequencies) and hesitation time. Blood glucose and serum testosterone levels were determined 28 days past treatment with carbon-monooxide (CO) at 100 and 200mg/kg doses. Glibenclamide and sildenafil citrate were used as positive controls. They observed that *Curculigo orchioides* treatment was helpful in ameliorating the damage caused by sustained hyperglycemia evidenced in the principle parameters viz. male sexual behavior, sperm count, penile erection index and seminal fructose content.

- **Estrogenic activity**

  Vijayanarayana *et al* (2007) evaluated that ethanolic extract of *Curculigo orchioides* rhizome possesses estrogenic activity as it showed a significant increase in percentage of vaginal cornification, uterine glycogen content, uterine weight, and a proliferative changes in uterine endometrium as compared with the control.

- **Antiosteoporotic activity**

  Cao *et al* (2008) and Jiao *et al* (2009) showed that *Curculigo orchioides* ethanolic extract possess potential antiosteoporosis activity. *Curculigo* prevented bone loss in the trabecular bone of the tibia in ovariectomized rats without affecting the weights of the body and the uterus. It also increased calcium, serum phosphorus level and osteoprotegerin levels, decreased serum deoxypyridinoline crosslinks to creatinine ratio, tartrate-resistant acid phosphatase, adrenocorticotropic hormone and corticosterone levels. Ethanol extract 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 the osteoblast proliferation and the ALP (alkalinephosphatase) activity. In another study, Wang *et al* (2012) investigated the protective effects of *Curculigo orchioides* against oxidative stress in calvarial osteoblasts and discussed the related mechanisms. It was found that osteoblast viability decreased significantly after 48-h exposure to 400 μM of H₂O₂, compared with vehicle-treated cells, and the cytotoxic effect of H₂O₂ was reversed significantly when pretreated with 0.1-10 μM of *Curculigo orchioides*.
(P<0.05). In addition, H₂O₂ induced reduction of differentiation markers such as alkaline phosphatase, calcium deposition, and Runx2 level was significantly recovered in the presence of *Curculigo orchioides*.

- **Antiasthmatic activity**
  Pandit *et al* (2008) observed that ethanol extract of rhizome of *Curculigo orchioides* showed antiasthmatic activity against histamine-induced contraction when used with isolated goat tracheal chain preparation and isolated guinea pig ileum preparation.

- **Antibacterial activity**
  Nagesh and Shanthamma (2009) reported that root oil of *Curculigo orchioides* showed significant antimicrobial activity against various bacteria strains such as *Bacillus anthracis, Bacillus subtilis, Salmonella pylorum* and *Staphylococcus aureus* and fungi stains such as *Fusarium monili forme, F. solani, Aspergillus flavus* and *Cladosporium*.

- **Analgesic activity**
  Madhavan (2007b) evaluated the aqueous and alcholic extracts of *Curculigo orchioides* for analgesic activity using Eddy’s Hot plate method and Heat conduction method on swiss albino mice. In another study, ethanolic extract of *Curculigo orchioides* were investigated for antinociceptive activity using modified Eddy’s hot plate method in albino wistar rats. Asprin (100mg/kg) was used as a standard. It showed antinociceptive effect significantly increased the reaction time in hot-plate test at a dose of 500 mg/kg (Asif *et al.*, 2010). Pandit *et al* (2011) evaluate antipyretic and analgesic activity of methanolic extract. Antipyretic activity was evaluated using yeast induced pyrexia. The results obtained revealed significant (p<0.05) antipyretic activity at 400mg/kg dose tested. Analgesic activity was evaluated using Eddy’s hot plate method; acetic acid induced writhing method and heat conduction method. The analgesic activity was observed maximum at 750 mg/kg (significance p<0.001). The results demonstrate that methanolic extract of the rhizomes possess significant antipyretic and analgesic activity.
➢ **Anticonvulsant activity**
Chen *et al* (1989) reported that ethanolic extract of *Curculigo orchioides* showed adaptive effects, such as enhancing tolerance towards high temperature and hypoxia. Ethanol extract also showed sedative, anticonvulsant and androgen-like effect.

➢ **Antihistaminic activity**
Venkatesh *et al* (2009) investigated about the stabilization potential of the alcoholic extract of *Curculigo orchioides* (100-400mg/kg) against Mast cell degranulation 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 CO inhibits mast cell-derived immediate-type allergic reactions and mast cell degranulation.

➢ **Antitumor activity**
Singh and Gupta (2008) screened *Curculigo orchioides* with different solvents for their antimicrobial and antitumor activity. Antitumor activity was screened against a human breast cancer cell line (MCF-7). Antifungal activity was determined by agar plate method, and antibacterial activity was determined by disk diffusion method. Methanolic extract of *Curculigo* showed maximum antibacterial activity. But it does not show any antitumor activity.

➢ **Inhibitory activity**
Lee *et al* (2009) yielded two phenolic glycosides, Curculigoside, orcinol-beta-D-glucoside, and two cycloartane saponins, *Curculigosaponin G, Curculigosaponin I* from the dried rhizomes of *Curculigo orchioides*. Among these isolates, Curculigoside 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.

- **Wound healing activity**
  Agrahari *et al* (2010a) evaluated the methanolic extract obtained from root tubers of plant *Curculigo orchioides* using wound excision model. The effect of methanolic extract at a dose of 200 and 400mg/kg was studied in adult wister albino rats. The results indicated that methanolic extract, at a dose of 200mg/kg & 400mg/kg showed statically significant wound healing response when compared with the control group. The percentage of protection was found higher at the dose of 400mg/kg body weight compare to 200mg/kg. The activity was compared with that of the standard drug, Nitrofurazone ointment (0.2% w/w) was used as a standard drug.

- **Anti-inflammatory activity**
  Mohammad and Kumar (2010) reported that 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 compared to control. The percentage inhibition of inflammation was found higher at the dose of 400mg/kg body weight at 3rd hr as compared to 200mg/kg. The activity was compared with that of the standard drug, Diclofenac sodium (15mg/kg). In another study Agrahari *et al* (2010a) formulated the gels of *Curculigo orchioides* 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* G. showed significant anti-inflammatory activity in carrageenan induced rat paw edema. 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 (Dode et al., 2009).

- **Cardiovascular activity**
  Wang et al (2010) reported that a major chemical constituent curculigoside, present in *Curculigo orchioides* can protect endothelial cells against oxidative injury induced by H₂O₂, suggesting that this compound may constitute a promising intervention against cardiovascular disorders.

- **Antialgal activity**
  Yi et al (2012) reported that *Curculigo orchioides* shows antialgal inhibitory effect against *Microcystis aeruginosa* and their inhibitory rate is SZ-1,024 45.1±3.5.

- **Central nervous system activity**
  Jiang et al (2011) reported that curculigoside, a major bioactive component of *Curculigo orchioides* protects brain against ischemia and reperfusion injury with a favorable therapeutic time-window by alleviating cerebral ischemia and reperfusion injury and attenuating blood brain barrier breakdown and its protective effect may involve HMGB1 and NFκB signalling pathway.

- **Ameliorative activity**
  Wu et al (2012) observed the ameliorating effects of Curculigoside through animal behaviour studies by using Y maze test and step down test. They observed that curculigoside can improve cognitive function in aged animals, possibly by decreasing the activity of AchE in the cerebra and inhibiting the expression of BACE1 in the hippocampus.

2.4. **Tissue culture studies on Curculigo orchioides Gaertn:**

Many plants have been described in various authoritative texts of Indian system of medicine for their valuable medicinal activities. Over the years and centuries, they
have been procured only from wild sources. Unfortunately, due to lack of application of scientific measures for their systemic cultivation, as well as unscientific over exploitation from nature, few plants are now-a-days on the verge of becoming extinct. Some of them have been duly identified by Govt. of India an endanger plants. Govt. of India also include this plant species as a endangered plant due to over exploitation and reduction in the natural habitat that supports vegetation. Among the contributory factors, the following are the major ones:

(a) Extensive denudation of the forest area, caused by cattle grazing.
(b) Many tribal people use the rhizome as edible flour.
(c) Various viral and bacterial infections affecting rhizomes;
(d) Musli is used as a substitute for safed musli in many places.
(e) Root stocks are highly priced in the market for its aphrodisiac formulations and metabolic-enhancing principles.

Therefore cultivation of this plant as well as micropropagation through tissue culture is required for sustained supply of this valuable plant drug. Tissue culture studies carried out on *Curculigo orchioides* are so under:

- Suri and Arora (1998) reported that bulbils formation has gained considerable attention as a novel method for micropropagation 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. Nema et al (2008) suggested that Morphactin and cytokinin promotes high frequency bulbil formation from leaf explant of *Curculigo orchioides* grown in shake flask culture. Suri et al (1999) 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 benzylaminopurine. Suri et al (2000) 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 over static flask culture in producing high number of bulbils by accommodation of higher number of explants per litre of the medium.

- Wala and Jasrai (2003) obtained multiple shoots from the meristem tip culture on Murashige and Skoog (MS) medium supplemented with 6-benzyladenine (BA). The shoots of *Curculigo* were rooted on half strength of MS basal medium. Then they transferred plantlets to pots containing a mixture of vermiculite and soil (1:1) for acclimation for a period of two to three weeks. After three-month period, they found averages of 125 plants from a single meristem.

- Prajapati *et al* (2003) 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.

- Augustine and Souza (1997) studied the 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.

- Francis *et al* (2007) developed a protocol for in vitro clonal propagation of *Curculigo orchioides* through apical meristem culture. The highest frequency of shoots multiplication from apical meristem was obtained on MS medium supplemented with 1.5 mg/L butyric acid, 0.25 mg/L indole-3-butyric and 3% sucrose.

- Sharma *et al* (2008) observed the post transplanting performance of in vitro raised *Curculigo orchioides* plantlets through fungal (*Arbuscular mycorrhizal*) effect. They showed that complete plantlets of *Curculigo orchioides* were
raised by direct organogenesis of leaf explants on half strength MS medium devoid of any growth hormone.

- Thomas (2007) observed that the MS medium supplemented with various concentrations of benzylaminopurine (BAP) or thidiazuron (TDZ) alone or in combination with naphthalenacetic acid (NAA), inoculated with leaf pieces produced low shoot induction.

- Nema et al (2009) observed the effect of different elicitors’ like, salicylic acid and methyl jasmonic acid on the production of curculigosides contents of leaves in in-vitro plantlets culture maintained on MS medium containing butyric acid and indole -3- butyric acid.

- Nagesh (2008) 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-benzylaminopurine (BAP) and kinetin (KN) (each at 1 mg/L) on the regeneration of shoot buds from proximal rhizome disc than shoot tip explant.

- Adiyecha and Jasrai (2012) cultured, In vitro derived leaf explants of Curculigo orchioides Gaertn. on MS media containing halfstrength nitrogen salts and 0.44 μM BA. The histological events leading to de novo shoot formation were evaluated. The studies clearly demonstrated initiation of the shoot buds from the phloem parenchyma cells on 9th day of culture initiation. The work focused on the sequence of events leading to the shoot organogenesis of Curculigo orchioides.
REFERENCES


Pharmacognostical, Phytochemical and Pharmacological investigations of *Curculigo orchioides* Gaertn, 2013


