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Phytochemical and Callus induction studies of C*urculigo orchioides* Gaertn. (Kali Musili).

Miss S. Mariammal** and **Dr. M. Selvi***

*Assistant professor, ** Research scholar,
PG& Research Department of Botany,
Sri Parasakthi College for Women, Courtallam. -627802

ABSTRACT

The present study reveals that the following such as, *Curculigo orchioides* contains variety of phytochemicals like Alkaloids, Tannins, glycosides, sterols, saponins, proteins Aminoacids, lipids carbohydrates, starch, sugar aromatic acids ,and chlorophyll. Absence of gummy substances were found in all the three (Leaf, tuber and root stock) samples. Though the plant has more significant in both medicinally and economically, the availability resource is very poor in Western ghats especially in Courtallam hills. Hence the regeneration of plant through tissue culture is essential. The callus induction was observed in the present experiment, within seven days in M.S medium under *in vitro* using leaf, tuber and root stock.. Among the three, tuber showed callus formation at three days intervals. Clones of leaf, tuber, and root stock were used to raise the plants under green house condition. Tuber and root stock of *C. orchioides* showed fast growth than leaf. Single callus induction was observed under MS- Medium but multiples of Callus obtained with addition of enriched BAP and NAA.

Key words: Callus induction, BAP, NAA and Regeneration.

Introduction

Plants have been used as healers and health rejuvenators since time immemorial. Even now, WHO recognizes that medicinal plants plays an important role in the health care of about 80 percent of world population in developing countries and depend largely on traditional medicines, of which herbal medicines constitutes the most prominent pert (Farnsworth *et al*. 1988) . The rest of the 20 percent also depend substantially on the plant-based medicines. Many people have defined medicinal plants in many ways, out of which the accepted definition given by the Agricultural and Natural Resource Development is, Plants that are recognized by people to have reliable and effective medicinal values, are commonly used in treating and preventing specific ailments and diseases, and play an essential role in health care. The number of medicinal plants in India, both indigenous and introduced has been estimated to be between 3,000 to 3500 species of higher plants. The glossary of Indian Medicinal plants has listed around 3,000 plants (Chopra *et al.*, 1956, 1974). Two thousand five hundred plants have been reported to be used in ethno-medicine (Gadgil,1996). Introduction……..

The occurrence of these medicinal plants and availability of raw material from them is as follows; a)Plants occurring wild in forests, grasslands, aquatic and desert ecosystems, associated with other forms of natural vegetation. b) Plants growing as weed. c) Plants cultivated as ornamental or as cereal, fruit, vegetable, spice, oil seed, essential oil or other cash crop d) Plants cultivated as medicinal crop. e) Medicinal plants are used at the household level by women to improve the health of the family members. f) At the village level by medicine men or tribals. g) By the practitioners of classical traditional systems of medicine such as Ayurveda, Chinese medicine, or the Japanese Kampo system.) Medicinal plants are gaining importance in the fields of research, especially in the field of genetics and biotechnology. Recent years have witnessed a resurgence of interest in traditional medicines and plant derived drugs and a return to "Nature cure" all over the world. Many adverse and undesirable side-effects, some times toxic, and the high cost of modern drugs are prompting public health workers, governmental and non-governmental organization to study, promote and market plant based health foods, functional foods, drugs and neutraceuticals. Modern drugs are inadequate to treat diabetes, amoebic dysentery, rheumatoid arthritis and hepatitis for which effective treatments are available in ayurveda. More over, medicinal plants assume special significance in preventive medical practices especially against aging, obesity, hypertension and depression.

Materials and methods

1.Collection of Plant material:

Young plant of *C.orchioides* from one month old plant were used as explants and were collected from the M.S University campus, Abishekapatti, Tirunelveli , during the months of November and December. Pot culture maintained under green house condition at Sri Parasakthi College for women, Courtallam.

2. Morphological studies:

The sample identified by following taxonomic descriptions of Hooker (1986); Fischer (1956); Hooker (1892); Gamble (1967); Hutchinson (1973); and Nair and Nair, (1961) .

3. Phytochemical (Qualitative) analysis: (Harbourne,1936)

- Preparation of plant extracts: Hot water extraction &Solvent extraction
- **S**tandard methods for Alkaloids,Tannins,Glycosides, Sterols,Saponins, Proteins, Aminoacids, Lipids, Carbohydrates, Starch, Sugar, Chlorophyll, Aromatic acids, flavonoids, terpenoids and Gum.

4.In vitro **culture studies:** Murashighe and Skoog (1962).

Sterilization of Explants & Preparation of culture:

The leaves and rhizome of *C.orchioides* were thoroughly washed with running tap water to remove all the dust particles adhere, followed by three rinses with sterile double distilled water. To eliminate other contamination explants were rinsed with 70% alcohol for 1 minute followed by distilled water washing twice. The explants were then treated with 0.1% (w/v) mercuric chloride for 3 minutes under aseptic conditions. After this explants were then thoroughly washed 4-5 times with sterilized double distilled water to remove the traces of mercuric chloride. (Bhojwani & Razdan, 1996).

Culture Conditions

All cultures were maintained in culture room under light intensity of 40 µmol/m2/s and 16 h photoperiod providedby cool-white fluorescent lamp at 25˚C ± 2˚C. Cultures for callus induction were kept in dark room at25˚C ± 2˚C.

NAA

Chemistry:

1-Naphthaleneacetic acid (NAA) is an organic compound with the formula$C_{10}H_7CH_2CO_2H$. This colorless solid is soluble in organic solvents. It features a carboxylmethyl group (CH_2CO_2H) linked to the "1- position" of naphthalene.

Uses: NAA is a synthetic plant hormone in the auxin family and is an ingredient in many commercial plant rooting horticultural products; it is a rooting agent and used for the vegetative propagation of plants from stem and leaf cutting. It is also used for plant tissue culture.

BAP -6-Benzylaminopurine *(N*-(Phenylmethyl)-7*H*-purin-6-amine)

Chemistry:

6-Benzylaminopurine is a organic compound with the formula $C_{12}H_{11}N_5$, . This colorless solid is soluble in organic solvents and White to off-white powder in appearance. It was first synthesized and tested in the laboratories of plant physiologist Folke K. Skoog. **6-Benzylaminopurine**, **benzyl adenine** or **BAP** is a first-generation synthetic cytokinin.

Uses:BAP is elicits plant growth and development responses, setting blossoms and stimulating fruit richness by stimulating cell division.

Results

Classification According to Bentham and Hooker's (1862-1883)

system of classification Curculigo orchioides is classified as follows:

Kingdom **:** **Plantae**

Sub-kingdom **:** **Angiosperm**

Class : **Monocotyledons**

Series **:** **Epigynae**

Family **:** **Hypoxidaceae**

Genus **:** ***Curculigo***

Species: ***orchioides***

Scientific Name : *Curculigo orchioides* Gaertn.1788

Tamil name : நிலப்பனை

Distribution

It is believed to have originated in the shady forests of Asia. It is found in all parts of India from near sea level to 2300 m altitude, especially in rock crevices and laterite soil. It has been recorded to occur in the subtropical himalayas from Kumaon eastwards ascending to 1800 m, the Khasia hills, Bengal, Assam, Konkan, Kanara, the western peninsula and Tamil Nadu extending south as far as Cape Comerin (Agharkar, 1953; Joy et al, 1998; Gupta et al, 1994) It is also distributed in Srilanka, Japan, Malaysia and Australia (Pandey et al, 1983). It is a shade-loving plant and thrives well in areas that receive high rainfall.

Floral biology

Flowers are epigynous, bright yellow, sessile, bisexual or unisexual, with a lanceolate and membranous bract. Perianth is located at the top of a slender sterile long extension of the ovary by means of which it is exposed above the ground. Perianth is gamopetallus with six equal lobes of size 1.5 cm × 0.2 cm; outer lobes are hairy on the back, while the inner ones are sparsely hairy along nerves. Stamens 6, filaments filiform, anthers 2 mm, ovary 3-celled, oblong to 4 mm . Ovary is tricarpellary, syncarpous, and trilocular with a fairly long slender beak (stipe). Ovules numerous per cell, style 2 mm, stigma-3, lobes elongate. Fruit oblong, 1.5-2.0 cm long 8 mm broad; seeds 8, globose to 2 mm, black, beaked, deeply grooved in wavy lines (Anonymous, 1963; Bhaskaran and Padmanabhan, 1983; Dong and Zhang, 1998). Flowering and fruiting occur mostly from October to January, rarely throughout the year (Joy et al, 2004b).

Table-1 : Qualitative analysis of phytochemicals of *Curculigo orchioides*.

Sl.no	Name of the tests	Results		
		Leaf	Tuber	Root stock
1.	Alkaloides	+	+	_
2.	Tannins	+	+	+
3.	Glycosides	+	+	+
4.	Sterols	+	+	_
5.	Saponins	+	+	_
6.	Proteins	+	+	_
7.	Aminoacids	+	+	_
8.	Lipids	+	+	+
9.	Carbohydrates	+	+	_
10.	Starch	+	+	+
11.	Sugar	+	+	_
12.	Chlorophyll	+	_	_
13.	Aromatic acids	+	+	_
14.	Gum	_	_	_
"+" Presence ; "–" Absence of bio molecules				

Table-2. Callus induction studies (NAA mg·l−1) on *C.orchioides*

NAA (mg·l−1)	Callus induction (%) (Mean ± SE)	Color	Texture
0.0	±00	White-grey	Soft and spongy
0.5	±30	White-grey	Soft and spongy
1.0	±80	White-grey	Soft and spongy
1.5	±20	White-grey	Soft and spongy

Table-2. Callus induction studies (BAP mg·l−1) on *C.orchioides*

BAP (mg·l−1)	Callus induction (%) (Mean ± SE)	Color	Texture
0.0	±00	White-grey	Soft and spongy
0.5	±80	White-grey	Soft and spongy
1.0	±70	White-grey	Soft and spongy
1.5	±30	White-grey	Soft and spongy

The present investigation reveals the following results: the morphology of plants **(Plate-I**) and its young ones obtained through clone (bulbil) cultivation. The morphological identification done with standard taxonomic references described by Hooker (1986); Fischer (1956); Hooker (1892); Gamble (1967); and Hutchinson (1973)).The plant has polygamous, leaves long or short petioled or subsessile lanceolate membranous, plicate glabrous, scape very short subterranean, flowers subsessile, bracts lanceolate membranous ovary small amongst the leaf bases, stipes of the perianth long filiform, stigmas 3 erect separate, capsule oblong, beak slender, septa spongy, male flower with no ovary, style or stigma. **Plate I -** describes the regeneration plant through tubers/bulbils. New young leaves develop from meristamatic apices of tubers. It was maintained in green house of our college. It is necessary to cultivate for its medicinal importance which was supported by Nagesh *et.al.*, (2010).

Various types of bio molecules identified through qualitative method in leaf, tuber and root stock of *C.orchioides*. The extracts of leaf and tuber contains alkaloids, tannins, glycosides, sterols, saponins, proteins, amino acids ,lipids, carbohydrates, starch, sugar, aromatic acids, chlorophyll. Among the three sample, leaf and tuber have rich number of biomolecules compared to root stock. Absence of gummy substances observed in all the three parts **(Table-1**). Thess qualitative test enable to help to identify the active substances. These results inconformity with the previous reports of the rhizomes of *C.orchioides* yielded a phenolic glycoside (orchioide) characterized as orcinol-3-ß-D-xylopyranosyl-(1-6-ß-D-gluco-pyranoside). These structures were elucidated by spectroscopic methods and chemical trans formation (Garg *et.al.*,2001).The active substances of *C.orchioides* have various clinical significance such as tonic, aphrodisiac, immunomodulators, anti inflammatory, hepato protective and contol skin diseases. Clinical significance studied by many experts namely, Chauhan and Dixit (2007). The present results incontrast with the previous reports of Datta

and Chakrabarthy (1982) have evaluated all parts of *Clerodendron visosum*. The three days soaked leachate was found to be more toxic than the 7-day soaked one. Root, stem, and leaf leachate s caused greater inhibition during the monsoon than during other seasons (Hoveland and Fried man ,1964; Horowitz ,1971; and Bendall ,1975). Though *Curculigo orchioides* have variety of phytochemicals, would not be harm to the plant growth . Hence, the plant to be used as liquid fertilizer. Though the plant *C.orchioides* considered as economically and medicinally significant, it is an endangered plant in its natural habitat due to indiscriminate harvesting and deforestation. The rhizome and tuberous roots of the plant have been used extensively in Indian indigenous is constantly on the rise, however, the supply is rather erratic and inadequate. Therefore, the *invitro* propagation of this plant is crucial the present investigation callus initiation was found at 4 days intervals . At 7 days intervals the root stock and tuber shows multiple callus initiation **(Plate-I).** Absence of callus was observed in leaf explants. This is due to presence of root primodia has growing apices and it would develop mass of proliferated tissue. The callus induction study were done with the applications of different percentage of NAA and BAP, **(Table: 2 & 3)** where, the mean values of callus were measured at the range of ± 20 to ±80. The colour and texture of the callus observed as, white gray and soft and spongy.

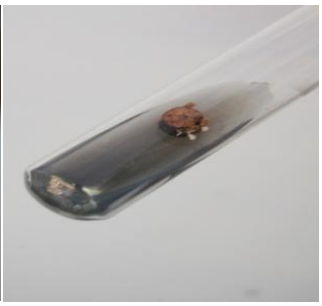
Similar reports were supported by Nagesh et.al.,(2010); Madhavan *et.al*., (1968); Misra *et.al* (1984); Xu *et.al*., (1992); Augustine and D'Souza (1997); Sanyal *et.al* (1998); Thomas and Jocob(2004) and Williams and Maheshwaran (1986) .Salema *et.al.,* (2007) reported that, an efficient protocol was developed for *invitro* clonal propagation of *Curculigo orchioides* Gaertn. through apical meristem culture. Multiple shoots were induced from apical meristem grown on Murashige and Skoog (MS) basal medium supplemented with 1.5 mg1-1 adenine sulfate(Ads) and 3% sucrose. Indole-3-acetic acid (IAA) in the culture medium improved the information of multiple shoots. The highest frequency of multiplication was obtained on M.S. medium supplemented with 1.5 mg1-1 BA, 100 mg1-1 ADS, 0.25 mg1-1 IBA and 3% sucrose. Rooting was achieved upon transferring micro shoots. Of half-strength ms medium containing 0.25 mg1-1 IBA and 2% sucrose. Micro propagated plantlets were hardened in the green house and successfully established in soil. Plant drugs have been in use for the treatment of disease since times immemorial. Due to over exploitation, destructive mode of collection, and under the threat of extinction. *Curculigo Orchioides* Gaertn commonly known as Kalimusali is an endangered medicinal plant and as such required to be conserved and domesticated. It is extensively utilized as

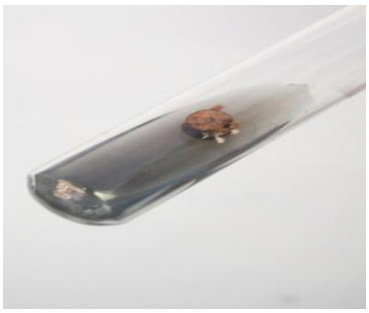
nutritive tonic for strength, vigour and vitality. In the recent times, many novel chemical constituents have been isolated and characterized and many pronounced biological activities such as anticancer antihepatotoxic and Immuomodulator activities have been reported from the plant. The present article highlights Medicinal values, chemical constituents and biological activities along with cultivation strategies of the species in India, (Saba Irshad *et.al.*, (2005)).Venukumar and Latha (2002) studied that, an antioxidant activity of Methanol extract of rhizomes of *Curculigo Orchioides* (MEC) was investigated using carbon tetrachloride(cc14)- intoxicated rat liver as the experimental model. The hepototoxic *rats* were administered MEC for 90 days (daily, orally at dose of 70 mg per Kg body weight). Lipid peroxidation (LPO) in cc14 – in toxicated rats was evidenced by a marked increment in the levels of thiobarbituric acid reactive substances (TBARS) and diene conjucates (CD) and also a distinct diminution in glutathione (GSH) content in the liver. In cc14+MEC-administered *rats* revealed the efficacy of MEC in combating oxidative stress due to hepatic damage. Elevated level of glutathione transferase (GTS) observed in hepatotoxic *rats* too showed signs of returning towards normally in MEC co-administered animals, thus corroborating the antioxidant efficacy of MEC. The findings provide radionale for further studies on isolation of active principles and its pharmacological evaluation.

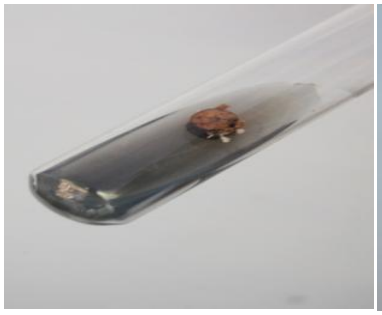
Plate-1

1.Habit-*C.orchioides*. **2. Clonal (rhizome) propagation Under green house condition**

3 4 5







6 7 8

3-explant (rhizome) inoculated. 4 & 7 -single callus initiation.

5, 6 & 8- multiples of callus formed in MS-medium supplemented with BAP.

Nagesh *et. al.*, (2010) reported that, *Curculigo orchioides* Gaertn. is now an endangered medicinal plant in its natural habitual due to indiscriminate harvesting and deforestation. The rhizome and tuberous roots of the plant have been used extensively in Indian indigenous medicine. The demand for *C.orchioides* is constantly on the rise however, the supply is rather erratic and inadequate. Therefore, the need for *in vitro* propagation of this plant in crucial. A successful protocol for effective plant regeneration through somatic embryogenesis has been described. Embryogenic callus was induced from rhizome explants,

and the maximum induction frequency(6.2%) was obtained on Murashige and Skoog (1962) basal medium(MS) containing 0.5-3 mg/L of 2.4-D and 0.5 mg/L BAP. Transfer of embryogenic calli to MS medium with 1-4 mg/L BAP resulted in somatic embryogenesis at high frequencies. With an average of 23=0.8 somatic embryos per gram embryogenic callus on MS medium with BAP(1mg/L). After transfer onto ½ ms medium without growth regulators, approximately 90%-of somatic embryos developed into complete plant lets. The rooted plant lets were successfully transferred to soil with 65-70%- survival rate. The plants showed normal morphological characters.

Conclusion

The present findings concludes as,

- The taxonomic identity of *C.orchioides* was done with standard references: Flowers are epigynous, bright yellow, sessile, bisexual or unisexual, with a lanceolate and membranous bract. Perianth is gamopetallus with six equal lobes of size 1.5 cm × 0.2 cm; . Stamens 6, filaments filiform, anthers 2 mm, ovary 3-celled, oblong to 4 mm . Ovary is tricarpellary, syncarpous, and trilocular with a fairly long slender beak (stipe). Ovules numerous per cell, style 2 mm, stigma-3, lobes elongate. Fruit oblong, 1.5-2.0 cm long 8 mm broad; seeds 8, globose to 2 mm, black, beaked, deeply grooved in wavy lines. Flowering and fruiting occur mostly from October to January, rarely throughout the year.
- *C.orchioides* contains variety of phytochemicals such as Alkaloids, Tannins, Glycosides, Sterols, Saponins, Proteins, Aminoacids, Chlorophyll. Absence of Gummy substances were found in all the three (Leaf, Tuber and Root stock) samples.
- Though the plant has more significant in both medicinally and economically, the availability of resources is very poor in western ghats, especially in Courtallam hills. Hence, the regeneration of plant through tissue culture is essential. The initiation of callus was observed from 3 days onwards in M.S medium under *invitro* conditions.
- The regeneration of callus induction was observed from 7-25days intervels in M.S medium but, multiples of calli were observed upto 7- 20 days intervels with the supplement of NAA and BAP at different concentrations.
- Among the three concentrations (0.5, 1.0, 1.5 mg/l^{-1}) of NAA and BAP, 0.5 mg/ l^{-1} was observed as optimum dose for callus induction of *C.orchioides*.

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