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# **Development of Micropropagation Protocol** for *Clerodendron infortunatum* Gaertn

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ABSTRACT: The present study was conducted at the Department of Botany, R. S. Bidkar College, Hinganghat dist Wardha (MS) during 2014 to 2016 to investigate the effect of different concentrations of BAP and NAA on virus free plant regeneration, shoot multiplication and different concentrations of cytokinin and an auxin on *invitro* root formation of Clerodendron infortunatum. For rapid in vitro multiplication, shoot tips from young suckers of 10-50 cm height were used as explants. From the selected suckers a cube of tissue of about 1-2 cm<sup>2</sup> containing the apical meristem was excised. This block of tissue was dipped in 70% ethanol for 60 seconds, surface sterilized in a 2% sodium hypochlorite solution, and after 20 min rinsed three times for 10 min in sterile water. Subsequently a shoot tip of about 1x3 mm, consisting of the apical dome covered with several leaves primordia and a thin layer of root tissue was aseptically dissected. The explants were placed directly on a multiplication inducing culture medium. For this MS-based media was adopted. Usually two types of growth regulators, a cytokinin and an auxin are added to the growth medium. Their concentration and ratio determines the growth and morphogenesis of the tissue. In the present study 2.0, 2,25, 3.0, 3.4, 4.0, 5.0, 7.0, 9.0, 11.0, 13.0, 15.0 mg/l benzyl amino purine (BAP) and 0.5 mg/l kinetin was added to the initiation medium. The formation of multiple shoots and bud was promoted by supplementing the medium with relatively high concentrations of cytokinin. MS medium containing 3, 4, 5, 7, 9 mg/l BAP and 0.5 mg/l kinetin was used. Clusters were separated, trimmed and repeatedly sub cultured at 4-6 week intervals. Individual shoot or shoot clumps were transferred to a nutrient medium which does not promote further shoot proliferation but stimulate root formation. Within 2 weeks, shoot tips develop into shoot. To initiate rhizogenesis, for the current study, IAA was used at concentration ranging between 0.5-2 mg/l. After rooting, plants were hardened in vitro for 2 extra weeks on the rootening medium prior to transplantation to soil.

**KEY WORDS**: *Clerodendron infortunatum*, regeneration, micropropagation, plantlet.

### I. INTRODUCTION

*Clerodendron infortunatum* Gaertn. (Verbenaceae) is a small perennial shrub growing in moist deciduous forests and occasionally in plains of peninsular India and the Western and Eastern Himalayas up to 1,400 feet above sea level. The leaf and root of this plant have great medicinal value. Root bark contains mainly sapogenins (Rangaswami and Sarangan 1969), while leaves contain flavonoids and phenolic acids (Rastogi 1999). The root has been traditionally used in Ayurveda and Siddha systems of medicine for treatment of chronic bronchial asthma and other respiratory diseases, different types of fevers and skin infections.

It is one of the ingredients of the ayurvedic drug, "Kasadamana," an effective expectorant and antitussive remedy (CSIR 2001). It also forms an ingredient of the Siddha drug, "Siruteku," with significantly high antibacterial properties (Narayanan *et al.* 2004). It is an excellent antihistaminic and antiallergic agent (Gupta and Gupta 1968; Gupta *et al.* 1967) as well as an immunomodulator (Juvekar *et al.* 2006) and also has antinociceptive and anti-inflammatory properties (Narayanan *et al.* 1999). Although *C. infortunatum* is recorded to occur in South Africa, Madagascar, South Asia and South East Asian countries, in India, this species has been regionally assessed as "vulnerable" in Northern India (WWF India and ZOO/CBSG India 1997) and "endangered" in Chhattisgarh and Madhya Pradesh region (Ved *et al.* 2003). Over collection and unsustainable extraction of wild plants for medicine and trade coupled with poor seed viability are the major factors for the continuous decline of its natural populations in



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India. Hence, development of an in vitro method for rapid clonal multiplication of *C. infortunatum*, is warranted for providing uniform raw material for medicinal uses as well as for its conservation. Presently, there is no published report on *in vitro* clonal propagation of *C. infortunatum*.

*C. infortunatum* is a medicinally very useful and wild plant commonly known as Khanduchuka. The most of the tribal people of Chandrapur and Gadchiroli District of Maharashtra State used the various parts of this plant from ancient period (Rajurkar, 1910). Due to traditional and very consistent use of this plant, common habitat required for proper growth of the plant get disturbs. It result into presently very few plants remain survive. It is very less researched as for as medicinal source is concerned. In India Khanduchuka is found in the forests of Tadoba-Andhari in the form of patches. Wild plants are diploid with vegetative mode of reproduction. The plants produce parthenocarpic fruits. The plants are usually produced from suckers of parent plants. The plant is perennial, under shrub, with broad leaves. Stem shows modified suckers, roots are tap root type as well as adventitious type, inflorescence is spike, and the flowers are showy, bluish white with large bracts. Generally flowers develop in the month of February and March (Rajurkar, 1910).

The Khanduchuka plants are medicinally very important. It is generally used by tribal people to cure wounds caused due to mechanical means. It is also useful for diabetic patients. The plant part especially leaves extracts gives to anemic patient to increase hemoglobin percentage in their blood. It is also used for the patients suffer from piles. Due to over exploitation of this valuable plant, it is now at the stage of extinction and its protection as well as its cultivation by using modern technique is very essential.

The use of *in vitro* plants obtained by in vitro budding is one of the main improvements. Among the wild plant, Khanduchuka is the in vitro propagated plant. In addition to the advantages of using little space and providing continuous availability, the plants produced by this technique have a very high sanitary quality. However, the cost of the in vitro plants are produced by in vitro budding remains high compared to conventional methods. This high cost is the most significant obstacle to its common use. Khanduchuka tissue culture plantlets have several advantages. They have higher survival rate than suckers, reduce the cost of disease and pest control, show uniform and vigorous growth, and have a shorter harvesting period. India still lags behind in the research and development, regarding micro propagation. A low cost protocol for commercial production *in vitro* plantlets is necessary. The present investigation therefore attempts to develop a micropropagation protocol for Khanduchuka with shoot meristem as the explants for *in vitro* multiplication. Therefore, considering the above facts, the present study was undertaken with the following objectives:

- i) To maintain required population of highly medicinal endangered plant like *Clerodendron infortunatum* (Khanduchuka) by regeneration.
- ii) To produce plantlets by using tissue culture technique and provide them for general cultivation.

### II. MATERIALS AND METHODS

*Initiation of shoot culture:* Shoot cultures of *Clerodendron infortunatum* are conventionally taken from any plant part that contains a shoot meristem, i. e. the parental stem, small suckers, peepers and lateral buds. The apex of the inflorescence and auxillary flower buds are also suitable explants for tissue culture initiation. Overall, it is important to select explants material from preferably mature individuals whose response to environmental factors is known and whose quality traits governed by genotypic and environmental effects have been identified.

For rapid *in vitro* multiplication of *Clerodendron infortunatum*, shoot tips from young suckers of 10-50 cm height were used as explants. From the selected suckers a cube of tissue of about  $1-2 \text{ cm}^2$  containing the apical meristem was excised. This block of tissue was dipped in 70% ethanol for 60 seconds, surface sterilized in a 2% sodium hypochlorite solution, and after 20 min rinsed three times for 10 min in sterile water. Subsequently a shoot tip of about 1x3 mm, consisting of the apical dome covered with several leaves primordia and a thin layer of root tissue was aseptically dissected.

The explants were placed directly on a multiplication inducing culture medium. For this MS-based media (Murashige and Skoog 1962) was adopted. The media was supplemented with sucrose as a carbon source at a concentration of 30 gm/l. The tissue culture often suffers from excessive blackening caused by oxidation of polyphenolic compounds released from wounded tissues. These undesirable exudates form a barrier around the tissue, preventing nutrient uptake and hindering growth. Therefore during the 4-6 weeks, fresh shoot tips were transferred to new medium every fifteen



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days. Antioxidants, such as ascorbic acid or citric acid in concentration 50 mg/l, were added to the growth medium to reduce blackening, or the explants were dipped in antioxidant solution (Cysteine 50 mg/l) prior to their transfer to culture medium.

Usually two types of growth regulators, a cytokinin and an auxin are added to the growth medium. Their concentration and ratio determines the growth and morphogenesis of the tissue. In the present study 2.0, 2,25, 3.0, 3.4, 4.0, 5.0, 7.0, 9.0, 11.0, 13.0, 15.0 mg/l benzyl amino purine (BAP) and 0.5 mg/l kinetin was added to the initiation medium. In *Clerodendron* micropropagation system, semisolid media are used. A geling agent agar (3 gm/l) was added to the culture medium but our preference was the gelrite (2 gm/l) because of its higher transparency, allowing much earlier detection of microbial contamination. However, higher cost limited its use.

*Cleodendron* shoot tip cultures were incubated at the optimal growth temperature of  $26 + 2^0$  C in a light cycle of 16 h with aphotosynthetic photon flux (PPF) of about 60  $\mu$ E/m<sup>2</sup>s<sup>1</sup>.

Category of element	Essential Elements	Concentration in medium mg/l
Macro Elements	NH <sub>4</sub> NO <sub>3</sub>	1650
	KNO <sub>3</sub>	1900
	CaCl <sub>2</sub> .2H <sub>2</sub> O	0440
	MgSO <sub>4</sub> •7H <sub>2</sub> O	0370
	$KH_2PO_4$	0170
Micro Elements	Kl	0.83
	$H_3BO_3$	6.2
	MnSO <sub>4</sub> .4H <sub>2</sub> 0	22.3
	ZnSO <sub>4</sub> .7H <sub>2</sub> O	8.6
	Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.25
	CuSO <sub>4</sub> .5H <sub>2</sub> 0	0.025
	CoCl <sub>2</sub> .6H <sub>2</sub> O	0.025
Iron source	FeSO <sub>4</sub> .7H <sub>2</sub> O	27.8
	Na <sub>2</sub> EDTA.2H <sub>2</sub> 0	37.3
Organic supplements	Myoinositol	100
	Nicotinic acid	0.5
	Pyridoxine HCl	0.5
	Thiamine HCl	0.5
	Glycine	2
Carbon source	Sucrose	30000

Table 1 : Composition of Murashige and Skoog (MS) Basal tissue culture medium

*Multiplication of shoot tip cultures:* The formation of multiple shoots and bud was promoted by supplementing the medium with relatively high concentrations of cytokinin. In *Clerodendron*, BA is the preferred cytokinin and is usually added in a concentration of 0.1-20 mg/l. for the multiplication of propagules, MS medium containing 3, 4, 5, 7, 9 mg/l BAP and 0.5 mg/l kinetin was used. Clusters were separated, trimmed and repeatedly sub cultured at 4-6 week intervals.

**Regeneration of plants:** Individual shoot or shoot clumps were transferred to a nutrient medium which does not promote further shoot proliferation but stimulate root formation. The cytokinin in the regeneration medium is greatly reduced or even completely omitted. Within 2 weeks, shoot tips develop into shoot. To initiate rhizogenesis IAA, NAA (a-naphthalene acetic acid) or IBA (Indole-3-butyric acid) are commonly included in the medium between 0.1 and 2 mg/l. however, for the current study, IAA was used at concentration ranging between 0.5-2 mg/l. activated charcoal (0.1%) was added to the rooting medium to enhance rooting. After rooting, plants were hardened in vitro for 2 extra weeks on the rootening medium prior to transplantation to soil.

Acclimatization in the nursery: After removing the plantlets from the culture vessel, the clusters were washed to remove the medium, separated into individual plants and sorted according to size (large: more than 5 cm high, Medium: 3 to 5 cm and Small: less than 3 cm). They were then transplanted into soil boxes. Planting density was high, with 126



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planted in a box measuring 58 x 36 x 12.5 cm. After planting, the box was covered with a perforated transparent plastic cover for seven to ten days, to conserve moisture.

The plastic was then removed, and two or three weeks later, the rooted plantlets were removed from the box and transferred to the field shed house. Here the plantlets were transplanted into plastic pots 9 cm high and 10 cm in diameter. They were kept in the shed house for six to eight weeks until they reach a height of around 100 cm. At this stage, they were released for planting out in the field.

### III. OBSERVATIONS AND RESULTS

The *Clerodendron infortunatum* selected for the present investigation is popularly found in the dense forests of Tadoba National park. To initiate micropropagation of this plant, hardy mother plants that were able to tolerate high summer temperatures were selected. Suckers from such mother plants that were at least 10 month olds were removed for experimentation. The response of such suckers on establishment media was investigated.





Fig.1: Plant of *Clerodendron infortunatum* Gaertn. Fig.2 : Bud break resulting into shoot formation on nutrient agar

#### medium

The positive correlation was observed between sucker size and response on establishment medium. In general, too young or older suckers had lower response as compared to suckers of medium size.

Length of Sucke	r 0.5 mg/l BAP	1 mg/l BAP	1.5 mg/l BAP	2 mg/l BAP
10-20cm	No response	84%	95%	89%
21-35cm	38%	65%	97%	91%
36-45	23%	48%	76%	78%
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Table 2 : Response of *Clerodendron infortunatum* in establishment medium

The suckers within the range 21-35 cm showed highest percentage of establishment 97%, as compared to the other two sizes range i.e. 95% in 10-20 and 76% in 36-45 cm fifteen days after inoculation. (Table-2)

An overall positive correlation was observed between BAP concentration and rate of establishment of suckers. The establishment rate was seen to increase with increase in BAP concentration. Maximum rates were observed at 1.5 mg/l BAP for all sucker sizes. Concentration of BAP above or lower than that had relatively less success rate of establishment. (Table-2)



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Fig. 3 : Proliferation of shoots in a medium Fig. 4: Rooting in isolated shoots in half-

strength MS medium

Induction of multiple shoots: The established shoot tips were sub cultured on the 22nd day of establishment, on MS medium with 3% sucrose and 0.3% agar supplemented with 0.5 mg/l kinetine and a range of BAP from 2.00 to 15.00 mg/l for induction of multiple shoots. Multiple shoots primordial and buds started appearing on 8th day after sub culture. In all the BAP concentration tried, multiple shoots appeared within 8 to 12 days after subculture. The multiple shoots were counted on the 18th day after sub culturing. Only shoots 2-3 cm length, that could be easily dissected and sub cultured were counted.

A positive correlation was seen on the induction of multiple shoots with increase in concentration of BAP from 2.00 to 7.00 mg/l. there was an overall decline in induction of multiple shoots beyond 7.00 up to 15.00 mg/l. a maximum of 7.64 multiple shoots were inducing in 7.00 mg/l BAP (Table-3).

The inclusion of kinetin in the medium was essential for proper growth of the induced shoots. In a medium devoid of kinetin, there was proper growth of induced multiple shoots.

BAP	concentration	Kinetin concentration	Number of
	mg/l	mg/l	shoots
	2.0	0.5	2.46
	2.25	0.5	4.78
	2.50	0.5	4.95
	3.0	0.5	5.36
	3.5	0.5	5.50
	4.0	0.5	5.46
	5.0	0.5	7.25
	7.0	0.5	7.64
	9.0	0.5	5.30
	11.0	0.5	3.15
	13.0	0.5	3.60
	15.0	0.5	3.38

Table 3 : Induction of multiple shoots in *Clerodendron infortunatum* 

Shoot proliferation in secondary sub culture: Shoot proliferation was rapid in secondary sub cultures on the all BAP concentrations tried. Multiple shoot buds were visible within 7 days of sub culture and were ready for transfer (2-3 cm) on 15th day. Here also, maximum shoots (13-20) were induced in 7.00 mg/l BAP. (Table-4)



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BAP	concentration	Kinetine	concentration	Number	of
mg/l		mg/l		shoots	
3.0		0.5		7.36	
4.0		0.5		8.50	
5.0		0.5		11.05	
7.0		0.5		13.20	
9.0		0.5		10.25	

Table 4 : Response of *Clerodendron infortunatum* in shoot proliferation medium

*Effect of gelling agent Agar and Gelrite on Shoot proliferation:* In order to select a suitable gelling agent, two geling agents, viz. gelrite and agar were tried. There was no significant difference on the induction and proliferation of multiple shoots when different gelling agents were used. However, gelrite was superior as it was easy to remove or place the explants in this medium. Agar was more compact and hence difficult to manipulate. Secondly the transparency of gelrite is more as compared to agar and hence infections can be easily detected. Gelrite was also superior for root induction and washing of the media during transplantation.

*Induction of roots:* For induction of roots, well grown shoots (6-9 cm) of 3rd or 4th sub culture were selected. Shoots were placed on half strength MS media with 3% sucrose and different concentrations of IAA with or without charcoal. The results indicated that maximum of 4.78 roots were induced per shoot in 1.5 mg/l IAA, within 18 days of induction.

Presence of activated charcoal increases the number of roots to 5.25 per explants. The time of induction was also found to be reduced by 3 days. (Table 05)

Half strength MS+IAA mg/l	No. of Roots	Days to induction	Success %
0.5	1	20	85
1.0	3.72	18	93
1.5	4.78	17	95
2.0	3.75	19	94
1.5 mg/l IAA +0. 1% Charcoal	5.28	14	96

Table 5 : Induction of roots

### IV. DISCUSSION AND CONCLUSION

The present research was started with the aim to protect Ethnobotanical medicinally important plants like *Clerodendron infortunatum* (Khanduchuka) and thus to find out use of this plant as new remedies on the various ailments. It was proposed to study biological knowledge of Khanduchuka from tribal people and its practical use in medicine, agriculture, health, industry, maintaining required population by using tissue culture and provide them for general cultivation.

To fulfill the above said objectives, observations on different aspects were undertaken for two years i.e. 2014-16. The observations on initiation of shoot culture, multiplication of shoot tip cultures, regeneration of plants, acclimatization in the nursery, induction of multiple shoots, shoot proliferation in secondary sub culture, effect of gelling agent Agar and Gelrite on Shoot proliferation and induction of roots.

In India Khanduchuka is found in the forests of Tadoba-Andhari in the form of patches (Rajurkar, 1910). Wild plants are diploid with vegetative mode of reproduction. The plants produce parthenocarpic fruits. The plants are usually produced from suckers of parent plants. The plant is perennial, under shrub, with broad leaves. Stem shows modified suckers, roots are tap root type as well as adventitious type, inflorescence is spike, and the flowers are showy, bluish white with large bracts. Generally flowers develop in the month of February and March (Rajurkar, 1910).

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The leaves and root of the plant are employed externally for tumours and certain skin diseases, and internally as tonics (Maisch, 1885). Pharmacological investigations of Khanduchuka were studied (Rajurkar, 1911). The tissue culture plantlets have several advantages. They have higher survival rate than suckers, reduce the cost of disease and pest control, show uniform and vigorous growth, and have a shorter harvesting period. India still lags behind in the research and development, regarding micro propagation. A low cost protocol for commercial production in vitro plantlets is necessary.

The present investigation therefore attempts to develop a micro propagation protocol for Khanduchuka with shoot meristem as the explants for in vitro multiplication.

*Establishment of Excised Explants (Shoot tips):* Hardy mother plants that were overall a good plant type, and could withstand high summer temperatures  $45-48^{\circ}$ C in the field, were selected. Suckers from such plants of different sizes were used to dissect out the shoot tips.

It was found that shoot tips from too young or too old suckers that are of size range 10-20 cms or 35-45 cms and above did not respond well to the establishment medium containing 1.5 mg/l BAP, the best response with 97% success rate was achieved with sucker size between 21-35 cms range. In general, increase in BAP concentration increased the percentage of shoot tip establishment, from 0.5 mg BAP to 1.5 mg BAP. However, beyond that the percentage of establishment decreased. The percentage of establishment was calculated on the 15th day after inoculation on different BAP concentrations. Since explants taking longer days to establish in a particular concentration cannot be selected as it would not be commercially viable.

*Induction of multiple shoots*: Maximum and rapid induction of multiple shoot in the established tips is the primary aim of any micropropagation protocol. This is dependent upon the hormonal status of the shoot tip. In order to achieve axillary bud proliferation, apical dominance must be broken. To find out suitable hormonal concentration for multiple shoot in *Clerodendron*, different concentration of BAP were tried. It was observed that maximum multiple shoots per explants were induced by 7 mg/l BAP+0.5 mg/l kinetin.

The induction was rapid and visible within 8 days of subculture (Bhaskar *et al.* 1992). In general, the number of multiple shoots per explants in primary culture was found to increase with increase in concentration of BAP from 2-7 mg/l. However, at concentration beyond 7 mg/l, there was a decline in multiple shoot induction. Inclusion of kinetin 0.5 mg/l was essential for proper growth of the induced multiple shoots. Absence of kinetin resulted in poor growth of the induced multiple shoots. More or less similar results have been reported in *Clerodendrum colebrookianum* by Mao *et al.* (1995).

Furthermore, shoots produced from inoculum comprising a group of 10 shoots during successive subcultures exhibited the problem of vitrification probably due to crowding of shoots. However, no or very little proliferation was obtained when single shoot was cultured in the same treatment. For induction and multiplication of shoots from various explants of woody species, superiority of BAP over other cytokinins has already been established (Murashige 1974; Chaturvedi *et al.* 2004).

The usefulness of AdS for improved multiplication of shoots is well documented by many workers (Rao and Bapat 1978; Reuveni *et al*; 1990). Although IPA is a mild auxin, its effectiveness in rooting has been reported by Shanker Rai and Jagadish Chandra (1987) and Cheetam *et al.* (1992). Contrary to this, in case of other members of the family Verbenaceae, like *Vitex negundo* - a woody shrub (Sahoo and Chand, 1998) and *Tectona grandis* (a large tree), poor rooting was observed in medium supplemented with IPA (Devi *et al.* 1994; Shirin *et al.* 2005).

Shoot proliferation in secondary subculture: There was induction of multiple shoots in secondary subcultures. On an average, more number of multiple shoots was induced per explants in secondary subculture in all the BAP concentrations. A maximum of 13.2 shoots per explants were observed in 7 mg/l BAP and 0.5 mg/l kinetin. The time of proliferation was also rapid, since induced shoots were ready for subculture within 15 days of first sub culture. A



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positive correlation between increase in shoot multiplication and BAP concentration was also reported by Murashige; (1974) and Chaturvedi *et al.* (2004).

*Induction of Roots:* In order to establish successfully on culture medium, proper and profuse root growth is essential. A critical concentration of auxin in the medium determines root morphogenesis. In the present investigation, IAA was used to induce development of roots in well grown shoots (Ahsan Habib *et al.* 1998).

Best response to rooting with 4.78 roots/shoots and highest success rates i.e. 95% was achieved 17 days after subculture of shoots in rooting medium containing 1.5 mg/l IAA. Addition of charcoal to this medium further induced higher root proliferation 5.28 roots explants and induction was more rapid within 14 days of sub culture. Arinaitwe *et al.* 2000 reported such rapid and profuse root induction and proliferation in excised shoots.

*Effect of gelling agent on shoot proliferation:* The most widely and gelling agent is agar. However, gelrite is also becoming popular now a day. Gelrite has a selective advantage over agar as it is more transparent and softer than agar. In a present investigation there was no significant difference in the proliferation of shoot in secondary sub culture between agar and gelrite (Acuna *et al.* 1993)

*Survival percentage:* Well grown plantlets with a mean shoot height of 6 cm and root length of 8 cm were removed from the culture tube or bottle, wash and transfer to sand, soil and cow dung mixture in the ratio of 1:1:1. The plantlets were hardened in the culture room under conditions of high humidity for 15 days and then transfer to green house. The survival percentage was 82%. The present investigation provides a complete in vitro process, which is simple, reproducible and efficient for rapid clonal multiplication of an important rare and threatened medicinal shrub, *Clerodendron infortunatum*.

### REFERENCES

- [1]. Acuna, P.I. Micropropagation of Banana from vegetative shoot tips. Corbana Revsta, 17(39):9-12, 1993.
- [2]. Ahsan, Habib., Jahan, M.A.A. and Hossain, M.T., Accelaration of the process of *In vitro* root development in *Musa sapientum* var. *Sagar. Bangladesh J. Scientific Industrial Res.* 33(3):341-344, 1998.
- [3]. Arinaitw, G., Rubaihayo, P.R., and Magambo, M.J.S. Proliferation rate effects of cytokinins on Banana (*Musa Spp*) Cultivars. *Scienetia Horticulturea*, 2000, 86(1):13-21, 2000.
- [4]. Chaturvedi, H.C., Sharma, A.K., Agha, B.Q., Jain, M., Sharma, M., Production of cloned trees of *Populus deltoides* through in vitro regeneration of shoots from leaf, stem and root explants and their field cultivation. *Indian J Biotechnol* 2:203–208, 2004.
- [5]. Cheetam, R.D., Mikloiche, C., Glubiak, M., Weathers, P., Micropropagation of a recalcitrant male Asparagus clone (MD 22-8). Plant Cell Tissue Organ Cult 31:15–19, 1992.
- [6]. CSIR The wealth of India: raw materials, vol 2. Publications and Information Directorate, CSIR, New Delhi, p 69, 2001.
- [7]. Devi, Y.S., Mukherjee, B.B., Gupta, S. Rapid cloning of elite teak (*Tectona grandis* Linn.) by *in vitro* multiple shoot production. *Indian J Exp Biol* 32:668–671, 1994.
- [8]. Gupta, S.S., Gupta, N.K. Development of antihistamine and antiallergic activity after prolonged administration of a plant saponin from *Clerodendrum serratum. J Pharm Pharmacol* 20:801–802, 1968.
- [9]. Gupta, S.S., Rai, M., Gupta, N.K. Histamine releasing effects of a few Indian medicinal plants used in bronchial asthma. *Curr Sci* 36:42–43, 1967.
- [10]. Juvekar, A.R., Nachankar, R.S., Hole, R.C., Wakade, A.S., Kulkarni, M.P., Ambaye, R.Y., In vitro and in vivo immunomodulatory activity of aqueous extract of Clerodendrum serratum L. roots. Planta Med 72. doi:10.1055/s-949887, 2006.
- [11]. Mao, A.A., Wetten, A., Fay, M., Caligari, P.D.S., *In vitro* propagation of *Clerodendrum colebrookianum* Walp, a potential natural antihypertension medicinal plant. *Plant Cell Rep* 14:493-496, 1995.
- [12]. Maisch, J.M. On some useful plants of the natural order of Verbenaceae. American Journal of Pharmacy 57, Botanical medicine monographs and sundry, 1885.
- [13]. Murashige, T. Plant propagation through tissue culture Annual Review plant Physiology 25:135-166, 1974.
- [14]. Murashige, T., Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473-497, 1962.
- [15] Narayanan, N., Thirugnanasambantham, P., Viswanathan, S., Vijayasekaran, V., Sukumar, E., Antinociceptive, anti-inflammatory and antipyretic effects of ethanol extract of *Clerodendrum serratum* roots in experimental animal. *J Ethnopharmacol* 65:237–241, 1999.
- [16]. Narayanan, N., Thirugnanasambantham, P., Viswanathan, S., Rajarajan, S., Sukumar, E., Comparative antibacterial activities of *Clerodendrum* serratum and *Premna herbacea*. Indian J Pharm Sci 66:453, 2004.
- [17]. Rajurkar, B.M. Morphological study and medicinal importance of *Clerodendron infortunatum* Gaertn (Verbaenaceae) found in Tadoba National Park India *JPRHC*2(2) 216-220, 2010.
- [18]. Rajurkar, B.M., Phyto pharmacological investigations of Clerodendron infortunatum Gartn IRJP 2(11)130-132, 2011.
- [19]. Rajurkar, B.M. A Comparative study on antimicrobial activity of *Clerodendron infortunatum*, *Simarouba glauca* and *Psoralea corylifolia IJRRPAS* 1(4):278-282, 2011.



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- [20]. Rangaswami, S., Sarangan, S., Sapogenins of Clerodendrum serratum constitution of a new pentacyclic triterpene acid, serratagenic acid. Tetrahedron 25:3701-3705, 1969.
- [21]. Rao, P.S., Bapat, V.A. Vegetative propagation of sandalwood plants through tissue culture. Can J Bot 56:1153–1156, 1978.
- [22]. Rastogi, R.P. Compendium of Indian medicinal plants, vol I, CDRI, Lucknow and NISC, New Delhi, p 113, 1999.
- [23]. Reuveni, D., Shlecinger, D/R/, Lavi, U., In vitro clonal propagation of dioecious Carica papaya. Plant Cell Tissue Organ Cult 20:41-46, 1990.
- [24]. Sahoo, Y., Chand, P.K., Micropropagation of Vitex negundo L., a woody aromatic medicinal shrub, through high-frequency axillary shoot proliferation. *Plant Cell Rep* 18:301-307, 1998. [25]. Shirin, F., Rana, P.K., Mandal, A.K., *In vitro* clonal propagation of mature *Tectona grandis* through axillary bud proliferation. *J For Res*
- 10:465-469, 2005.
- [26]. Ved, D.K., Kinhal, G.A., Ravikumar, K., Karnat, M., Vijaysankar, R., Indresha, J.H., (eds) Report of the threat assessment and management prioritisation (TAMP) for the medicinal plants of Chhattisgarh and Madhya Pradesh, TAMP Workshop, Bhopal, 23-26 July 2003, 2003.
- [27]. WWF India, and ZOO/CBSG India North Indian Medicinal Plants categorized according to new IUCN Red List Criteria, under the Biodiversity Conservation Prioritization Project, by CAMP Workshop Process. U.P. Forest Department, Kukrail Park, Lucknow, 1997.