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Research Article

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In Vitro Effect of Various Plant Growth Promoters on *Clerodendrum serratum*

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ABSTRACT

Background and Aim: *In-vitro effect of various growth regulators on propagation of Clerodendrum serratum, a medicinally herbs of India was observed in this study. C. serratum is an important medicinal plant and used in the various ancient traditional system of medicine.*

Materials and Method: *Healthy nodal segments of the herb was used as explants with basic MS medium for shoot initiation and multiplication containing various combinations of different growth regulators. MS full and $^{1/2}$MS were used for rooting of plantlets with 25 to 200 mg/l Activated Charcoal.*

Result: *Maximum mean number of proliferated plantlets 1.4 ± 0.84 with mean length 3.5 ± 2.01 were found in MS medium treated with lowest concentration of BA, while maximum mean number of multiplied plantlets 22.8 ± 1.75 with mean length 5.3 ± 1.15 were found in MS medium treated with 6.66 µM. Maximum mean number of roods 11.6 ± 1.17 with Mean length 9.01 ± 0.57 was observed on $^{1/2}$MS medium with 100 mg/l activated charcoal. The rooted plantlets were successfully hardened in 1:1:1 ratio of sand: soil: vermicompost and successfully established in soil.*

Key words: *Clerodendrum serratum, Micropropagation, Medicinal Plants, Plant Tissue Culture.*

INTRODUCTION

Clerodendrum serratum L. is one of the important medicinal plants of India belong to the Lamiaceae family[10]. Few important commen names of this plant is Moon, Bharangi, Phelang Riho, Bamun Hatee, Baman hatee, Bhuijam, Bharangee etc. This plant is distributed in the deciduous forests of the Western Ghats of India[6]. The leaf and root of *C. serratum* is a good source of drugs for diseases like "asthma, bodyache, bronchitis, cholera, dropsy, eye diseases, fever, inflammations, malaria, ophthalmia, rheumatism, snakebite, tuberculosis, ulcers and wounds"[1,2,3,4]. This plant is also used for the treatment of various diseases such as typhoid, cancer, jaundice and hypertension[5] various activities like anti-inflammatory, analgesic, antipyretic activity[14] and hepatoprotective activity[12].

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Propagation of *C. serratum* by seeds is very poor and the percentage seed germination is very low. Due to that the natural population of the plant species is decreasing[13]. In vitro plant propagation is the most widly used technology for conservation of rare and endangered plants and mass production of economically important plants. A few reports for in vitro rapid clonal propagation of *C. serratum* employing nodal stem segments are available[7,11,13]. In the present study we have used different plant regulators for standardization of an efficient micropropagation technique for mass production of *C. serratum.*

MATERIAL AND METHOD

Collection of Explant

Branches of *C. serratum* were collected from healthy growing plants from medicinal garden of Prof. T.S. Murthi Science and Technology Station Obaidullaganj, Raisen (M.P.)

Sterilization of Explant

Nodal explants were cut and washed in running tap water to remove the superficial dust particles and mud adhering to its surface. Explants were washed with sevelon (3-4 drops/100ml) in a vial by gentle agitating conditions. The explants were thoroughly rinsed with distilled water for several times. Again these explants were dipped in to the 1% fungicide (Bavistin) treatment was given for 15 minutes and then washed with distilled water. For surface sterilization, Explants were transferred to sterile empty flasks under aseptic conditions and given a quick dip in 70% alcohol and subsequently they were washed in distilled water. After that, the explants were surface sterilized with different concentration of sterile ($HgCl_2$) for different duration as per the treatment to find out the best treatment for sterilization of explants. To remove the traces of sterile explants were washed in sterilized distilled water at least 5-6 times. The procedure was carried out in the inoculation chamber under laminar air flow hood.

Preparation of MS Medium

Culture media was prepared as per described method of Murashig and Skoog (1962) and different growth regulator was added as per requirement. For the initiation of ex-plant various concentration of BA (0.44 to 2.22 µM) alone and with IA (0.57 to 1.71 µM) and NA (0.54 to 1.61 µM) were used, while concentration of BA (2.22 to 11.1 µM) alone and IA (0.57 to 1.71 µM) and NA (0.54 to 1.61 µM) with BA (2.22 to 6. 66 µM) were used for multiplication. MS full and $^{1/2}$MS were used for rooting of plantlets with 25 to 200 mg/l Activated Charcoal (AC) combination adding 30 g/l sucrose and 5.7% agar. The hormones used for experiment were taken from stock solutions, which were previously prepared and kept under cold condition in refrigerator. The pH of the medium was adjusted to 5.7 with 0.1 NaOH before autoclaving at 15 lbs and 121°C for 18 min

Aseptic Inoculation of Explant

Nodal segments about 0.5-0.8 cm were prepared aseptically and were implanted vertically on Surface disinfected nodal explants were inoculated onto full strength MS medium fortified with specific concentrations of growth regulators. The cultures were incubated at a constant temperature of 26±2°C with 16±1 h photoperiod (3000 lux).

RESULT AND DISCUSSION

Surface Sterilization and Induction of Axillary Shoots

Treatment of explants with 0.1% $HgCl_2$ for 3 minutes resulted 100% contamination-free viable cultures. Final observation after 3-4 weeks showed that MS media supplemented with 0.88 µM mg/l of BA proved to be most capability in shoot induction. On this medium an average of 1.4 ± 0.84 shoots with mean shoot length 3.5 ± 2.01 cm were obtained (table 1, figure 1A and 2). Sharma *et al*[8]., showed bud-break within 15 days of culture on modified Murashige and Skoog (MS) medium supplemented with 0.25 mg/l each of 6-benzylaminopurine and indole-3-acetic acid along with 15 mg/l adenine sulphate (AdS).

Shoot Multiplication

Shoot multiplication is depending on different concentration of plant growth regulators. Sometimes BA increasing is best for shoot or just opposite. Activated auxiliary shoots from the nodal explants and transfer to fresh medium containing BA alone and combitation of BA with NA and IA to establish a stock of shoots used for *in vitro* multiplication. When we look Results in the present study showed the essential of plant growth regulators for *in vitro* multiplication, as the shoots cultured on basal medium did not multiply and become dead. BA at a concentration of 6.66 µM just gave an average of 22.8 ± 1.75 shoots with mean shoot length 5.3 ± 1.15 cm after 3-4 weeks of culture (table 2, figure 1B and 3). Increasing the concentration of BA to 6.66 µM, a decrease in shoot multiplication rate was observed. However, comparative number, length and health of shoots on media with BA + IA/NA were not good. Sharma *et al*[8]., found shoots could be further multiplied on the same agarified morphogenetic medium in presence of 0.5 mg/l 2-chloroethyltrimethyl ammonium chloride with increased concentration of AdS, i.e., 30 mg/l. Upadhyay and Koche[10], observed BA at lower concentration of 0.5 mg/L gave best shoot bud induction and multiplication.

***In vitro* Rooting**

After 20 Days of growth, rooting growth is rarely increased day by day in best culture. The multiple-shoot clumps produced on this medium were transferred to solidified MS growth-regulator-free medium for shoot elongation and rooting. On opposite, shoots were also observed for rooting on full or half-strength MS medium with Activated Charcol ensuing excellent response for root induction. Maximum rooting was recorded in medium containing 125 mg/l Activated Charcol Supplemented with ½ MS medium (figure 1C). On this medium an average of 11.6 ± 1.17 roots with average root length 9.01 ± 0.57 cm was observed after 3-4 weeks (table 3, figure 4). Sharma *et al*[8]., were rooted the multiplied shoots in half-strength MS medium having 1 mg/l indole-3-propionic acid. Upadhyay and Koche[10], observed that the higher concentration root development declines in all hormones whereas at lower concentration of 0.5 mg/L, NAA gave best root development.

Swamy *et al*[9]., developed a protocol for the induction of callus and shoot proliferation from leaf and nodal explants of *C. serretum* L. they observed Green compact callus on MS medium supplemented with 2.0mg/lBAP +0.5mg/l2,4-D.Where as an optimum shoot proliferation was recorded from nodal explants on MS medium supplemented with 3.0mg/lBAP+1.0mg/L-Glutamic acid and shoots were rooted on ½ strength MS medium with 3.0mg/lIBA. Vidya et al[13], confirms maximum number of shoots with maximum length were from stem derived callus on LM media fortified with 1.5 mg/l BAP and 0.3 mg/l NAA. In their experiment nodal explants showed direct organogenesis on LM media containing BAP (0.5 mg/l) alone. The regenerated shoots were successfully rooted by Vidya *et al*[13]., with maximum frequency (100%) on half strength LM media supplemented with 0.5 mg/l NAA.

Table 1: Effect of plant growth regulators on *in vitro* axillary shoot induction in *Clerodendrum serratum

SL	MS + PGR (µM)			Observations after 25 days	
	BA	IA	NA	Mean shoot number	Mean shoot length (cm)
Control	0	0	0	0.2 ± 0.42	0.2 ± 0.421637
1	0.44 µM	0	0	1.3 ± 0.48	2 ± 0.6666667
2	0.88 µM	0	0	1.4 ± 0.84	3.5 ± 2.013841
3	1.33 µM	0	0	1.2 ± 0.78	3.4 ± 1.95
4	1.77 µM	0	0	1 ± 0.94	2.3 ± 2.05
5	2.22 µM	0	0	0.8 ± 0.91	1.7 ± 1.82
6	0.44 µM	0.57 µM	0	0.8 ± 0.63	2.9 ± 2.13
7	0.88 µM	1.14 µM	0	1.1 ± 0.87	2.1 ± 1.59
8	1.33 µM	1.71 µM	0	1.3 ± 0.67	2.3 ± 0.94
9	0.44 µM	0	0.54 µM	1.4 ± 0.51	5 ± 0.81
10	0.88 µM	0	1.07 µM	0.8 ± 0.78	1.7 ± 1.56
11	1.33 µM	0	1.61 µM	1.5 ± 0.52	3.7 ± 0.82

Table 2: Effect of plant growth regulators on in vitro axillary shoot multiplication in *Clerodendrum serratum

S.no	MS + PGR (µM)			Observations after 25 days	
	BA	IA	NA	Mean shoot number	Mean shoot length (cm)
Control	0	0	0	4.9 ± 1.87	3.6 ± 1.26
1	2.22 µM	0	0	8.3 ± 1.15	3.9 ± 0.87
2	4.44 µM	0	0	21.8 ± 1.87	4.6 ± 0.96
3	6.66 µM	0	0	22.8 ± 1.75	5.3 ± 1.15
4	8.88 µM	0	0	18.6 ± 1.50	3.6 ± 1.17
5	11.1 µM	0	0	14.1 ± 1.10	3.4 ± 1.07
6	2.22 µM	0.57 µM	0	13.1 ± 1.10	4.9 ± 0.87
7	4.44 µM	1.14 µM	0	11.4 ± 1.17	3.9 ± 0.87
8	6.66 µM	1.71 µM	0	11.2 ± 1.93	3 ± 0.94
9	2.22 µM	0	0.54 µM	13.3 ± 1.49	4.4 ± 1.17
10	4.44 µM	0	1.07 µM	8.1 ± 1.52	3.2 ± 0.78
11	6.66 µM	0	1.61 µM	7.9 ± 0.73	2.9 ± 0.87

Table 3: Effect of auxins on in vitro rooting in *Clerodendrum serratum

S. No.	Medium	Activated charcoal (mg/l)	No. of roots/ Plantlets (Mean ± SE)	Root length (Mean ± SE)
1.	Control	0	5.7 ±0.82327	5.79 ±0.62619
2.	MS Full	0	6.1 ±1.1005	6.02 ±0.42635
3.	MS Full	0	6.7 ±0.94868	6.54 ±0.76041
4.	MS Full	25	7.3 ±1.1595	6.92 ±0.79833
5.	MS Full	50	9.2 ±0.78881	7.73 ±0.76891
6.	MS Full	100	11.4 ±1.07497	8.48 ±0.97045
7.	MS Full	125	11.6 ±1.173788	9.01 ±0.572422
8.	MS Full	150	7.2 ±1.54919	6.33 ±1.59168
9.	MS Full	200	6.9 ±1.1005	6.39 ±1.20596

***Note: Each treatment consisted of 10 replications. Data (Mean ±SE) were recorded after 20 days of culture**

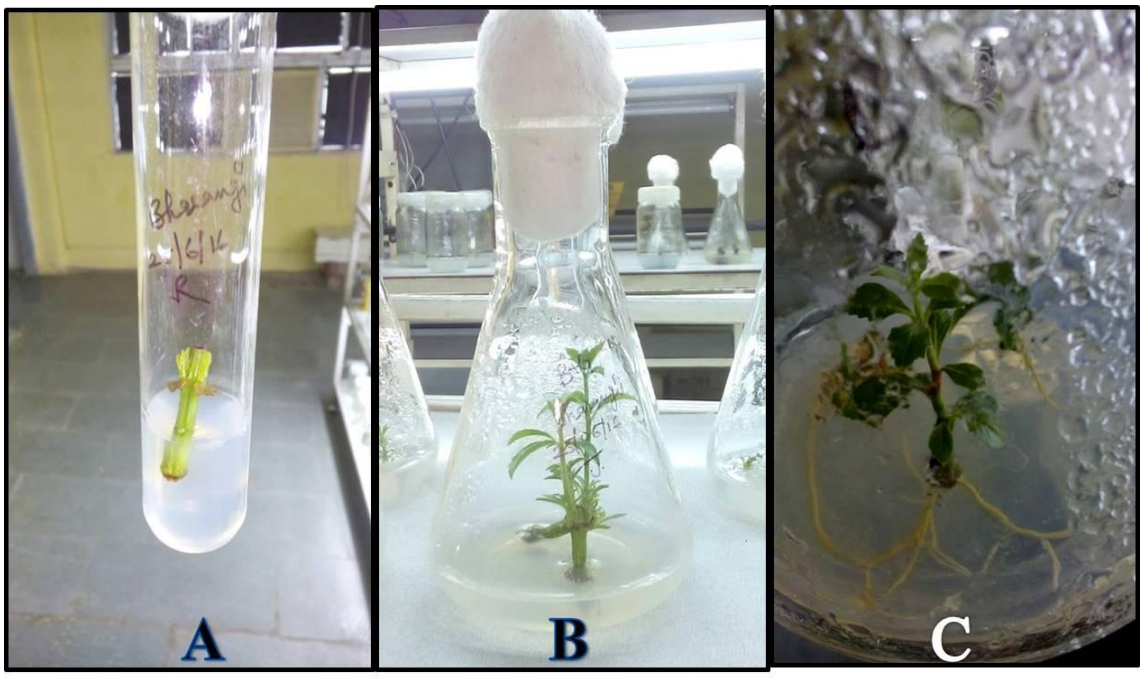

Fig. 1: Micropropagation of *Clerodendrum serratu. (*A) Initiation (B) Multiplication (C) Rooting

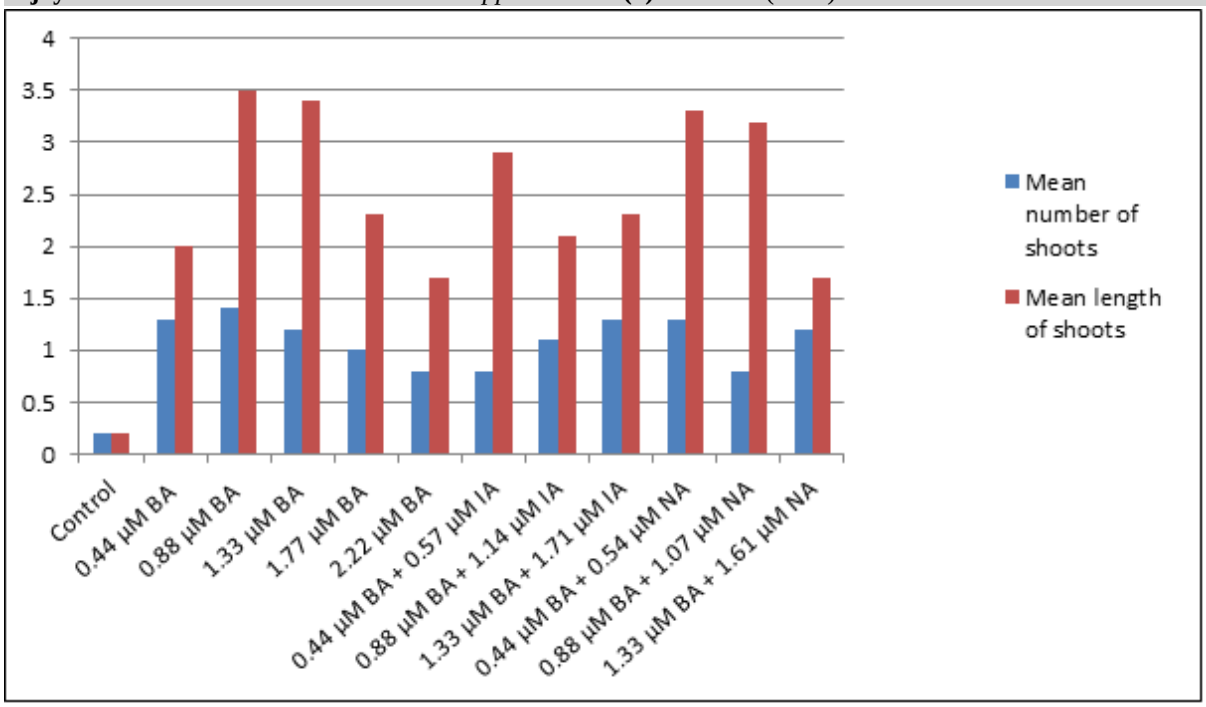

Fig. 2: Effect of plant growth regulators on in vitro axillary shoot induction in *Clerodendrum serratum*

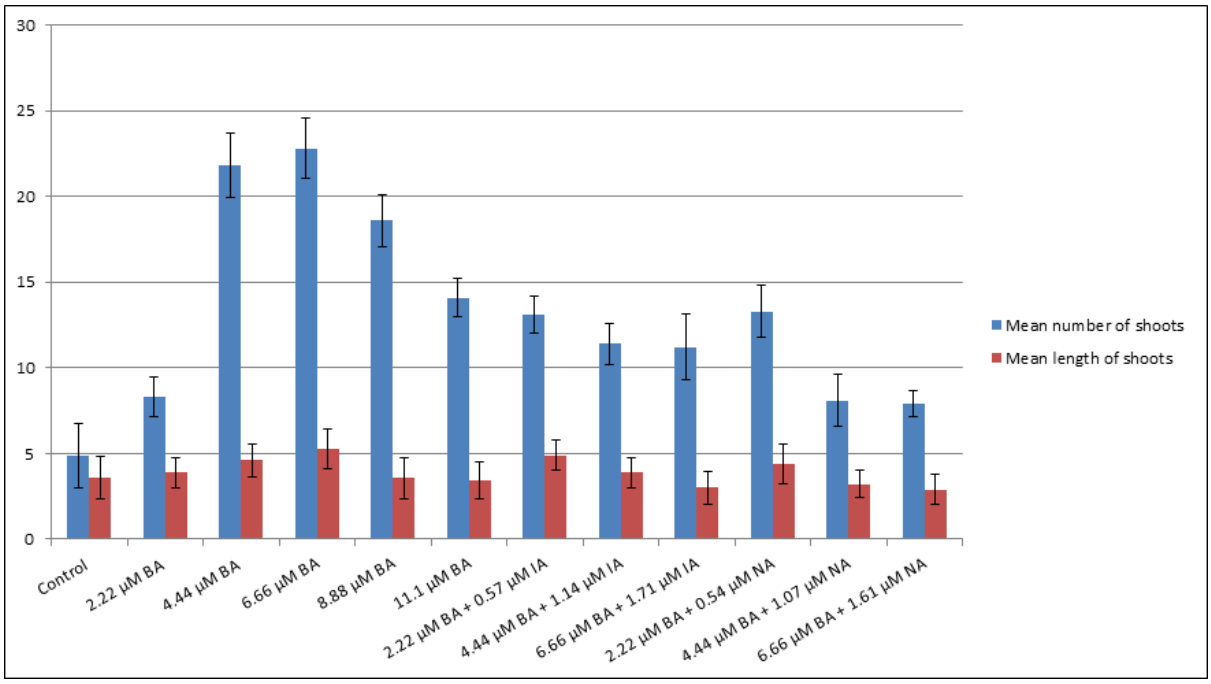

Fig. 3: Effect of plant growth regulators on in vitro axillary shoot multiplication in *Clerodendrum serratum*

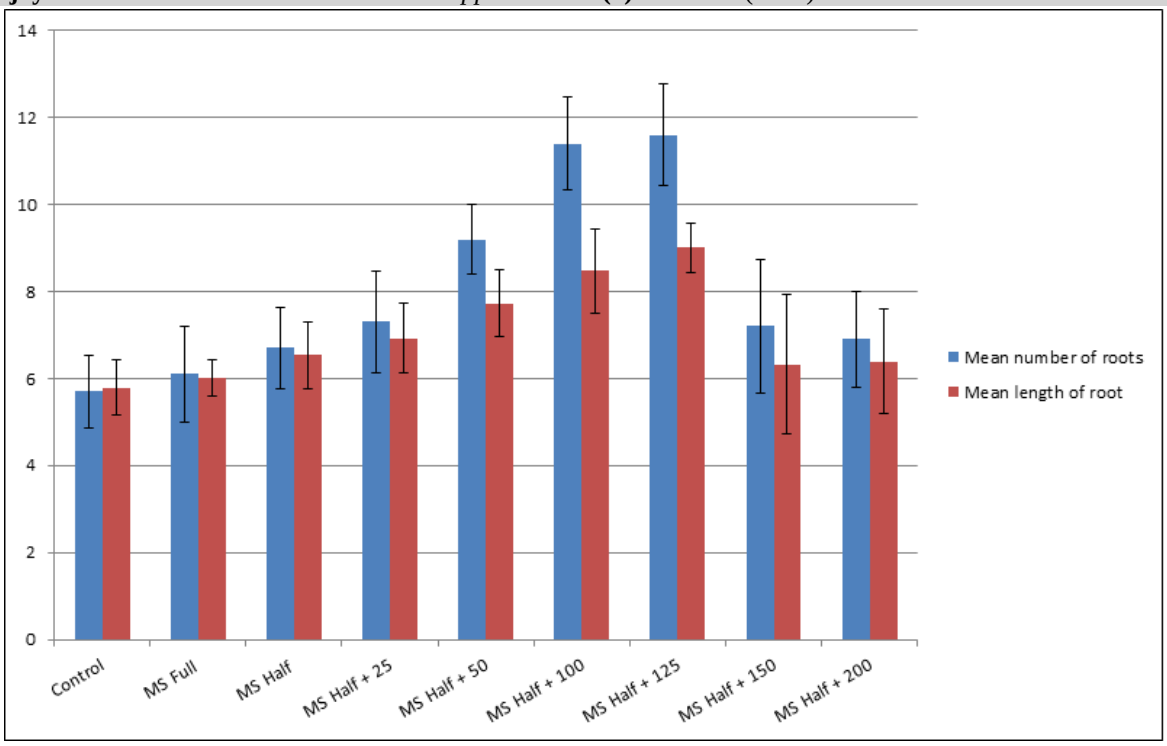

Fig. 4: Effect of auxins on in vitro rooting in *Clerodendrum serratum*

CONCLUSION

C. serratum has always been a topic of interest of researchers. From tissue culture point of view several studies have been performed to propagate the plant *in vitro.* The highlights of the study are Use of low concentration of plant growth regulators and minimization of time required for field transfer of tissue culture raised plantlets. Free plants produced open the scope for utilization of plant material for antimicrobial testing and suitable pharmaceutical preparations. Apart from this in vitro propagation of *C. serratum* showed a highest rate of multiplication which cannot be seen in naturally found species of *C. serratum.* The Malkangani research will give a new insight of research in medicinal components of plants through various advance techniques.

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REFERENCES

1. Babenko, N.A. and Shakhonp, E.G., Effects of Flavonoids on Sphingolipid Turnover in the Toxin-Damaged Liver and Liver Cells. *J Lipids Health Dis*., **7:** 1-11 (2008).
2. Fuchs, J. and Milbradt, R., Skin Anti-inflammatory Activity of Apigenin-7-Glucoside in Rats. *J Arzneimittelforschung*., **3:** 370-372 (1993).
3. Keshavamurthy, K.R., Medicinal Plants of Karnataka, 92, Karnataka Forest Department, Bangalore, India. (1994).
4. Krishna, V., Vidya, S.M., Manjunatha, B.K., Ahmed, M. and Singh, J.S.D., Evaluation of hepatoprotective activity of *Clerodendrum serratum*. *Indian Journal Experimental Biology*, **45:** 538-542 (2007).
5. Mukesh, K.R., Gaurav, K., Shiv, K.I., Gotmi, S. and Tripathi, D.K., *Clerodendrum serratum*: A clinical approach. *Journal of Applied Pharmaceutical Science*, **2(2):** 11-15 (2012).
6. Saldanha, C.J. and Nicolson, D.H., Flora of Hassan District, Karnataka. Amerind Publishing Co., New Delhi. 231 (1976).
7. Sharma, M., Rai, S.K., Purshottam, D.K., Jain, M., Chakrabarty, D., Awasthi, A., Nair, K.N. and Ashok Kumar Sharma, In

vitro clonal propagation of *Clerodendrum serratum* (Linn.) Moon (barangi) : A rare and threatened medicinal plant, Springer. *Acta Physiol Plant*., DOI 10.1007/s11738-008-0245-4 (2008).

8. Sharma, M., Rai, S.K., Purshottam, D.K., Jain, M., Chakrabarty. D., Awasthi, A., Nair, K.N. and Sharma, A.K., In vitro clonal propagation of *Clerodendrum serratum* (Linn.) Moon (barangi): a rare and threatened medicinal plant. *Acta Physiologiae Plantarum*., **31(2):** 379–383 (2009).
9. Swamy, T.N., Prasad, B., Ghani, M.O., Suresh, V. and Md. Mustafa, Callus Induction From Leaf and Shoot Proliferation From Nodal Explants of *Clerodendrum Serretum* L,a Thretened Medicinal Plant . *International Journal of Recent Scientific Research*, **6(4):** 3332-3335 (2015).
10. Upadhyay, S. and Koche, V., Comparison of Different Medium and Establishment of an Efficient Micropropagation Technique of *Clerodendrum Serratum* L. An Endangered Medicinal Plant. *Journal of Environmental Science, Toxicology and Food Technology*., **1(2):** 27-35 (2012).
11. Vidya, S.M., Krishna, V. and Manjunatha, B.K., Micropropagation of *Clerodendrum serratum* L. from leaf explants. *J Non-Timber For Prod*., **12:** 57–60 (2005).
12. Vidya, S.M., Krishna, V., Manjunatha, B.K., Mankani, K.L., Manzoor, A. and Jegadeesh singh, S.D., Evaluation of hepatoprotective activity of *Clerodendrum serratum* Linn. *Indian J. Exp. Biol*., **45:** 538-542 (2007).
13. Vidya, S.M., Krishna, V., Manjunatha, B.K. and Pradeepa, Micropropagation of *Clerodendrum serratum* L. through direct and indirect organogenesis. *Plant Tissue Cult Biotechnol*., **22:** 179–185 (2012).
14. Vishwanathan, S., Narayanan, N., Thirugnanasambantham, P., Vijayasekaran, V. and Sukumar, E., Antinociceptive, anti-inflammatory and antipyretic effects of ethanol extract of *Clerodendrum serratum* in Experimental animals. *J. Ethanopharmacology*., **65:** 237-241 (1996).