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MICROPROPAGATION PRACTICES OF *CELASTRUS PANICULATUS* WILLD. FOR CONSERVATION: AN OVERVIEW

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Abstract

Celastrus paniculatus Willd. is a member of the Celastraceae family and is a large, woody climber (called a climbing shrub), with a yellow, corky bark. The plants exhibit varying degrees of therapeutic values, some of which are its use in the treatment of cognitive dysfunction, epilepsy, insomnia, rheumatism, gout, dyspepsia. Oil from the seeds (called as Jyothismati oil), which contains the alkaloids celastrine and paniculatin in varying amounts, is the most commonly used plant part. It looks promising as a source for the development of drug for treatment of Polycystic Ovary Syndrome and this use of medicinal plant will also give boon to the future researchers. Many more researches are still going on and the potential health benefits of *C. paniculatus*. Hence, *C. paniculatus* is highly vulnerable and it is highly endangered plant of Western and Eastern Ghats. The article is review of medicinal and pharmacological properties of *C. paniculatus*.

Key words: *Celastrus paniculatus* Willd., Jyothismati, Polycystic Ovary Syndrome, Endangered plant, Pharmacological

Introduction

This chapter deals with the literature survey of effects on various studies and analysis with an aim to understand the use of *in vivo* and *In vitro* samples (nodal, leaves and callus) in respective aspects to improve the medicinal plant through Micropropagation, synthetic seed formation and molecular biology in *Celastrus paniculatus* Willd. It includes the extensive research work done by different workers in improving the crop plants through *In vitro* technique.

Biological research has been transformed from a collection of single discipline endeavors into an interactive science with bridges between a numbers of traditional disciplines. This synergism has made biology the "sunrise field" of the new millennium. In the last decade, bioactive compounds had became a subject of dramatically increasing interest relevant to their significant practical implication for medicinal, nutritive and cosmetic purposes, as well as to their indisputable importance in plant stress physiology (Wang *et al*., 2015). Plant organisms being devoid of motility and immune system, have elaborated alternative defense strategies, involving the variety of secondary metabolites as tools to overcome stress constraints, adapt to the changing environment and survive. The large diversity of chemical types and interactions displayed by the secondary metabolites can underlie the impressive multiplicity of protective functions ranging from toxicity and light/UV shielding to signal transduction (Yang *et al*., 1997; Grassmann *et al*., 2002; Hadacek, 2002; Vasconsuelo and Boland, 2007).

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Medicinal plants tissue culture

Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in Ayurveda. The Rigveda (5000 BC) has recorded 67 medicinal plants, Yajurveda 81 species, Atharvaveda (4500-2500 BC) 290 species, Charak Samhita (700 BC) and Sushrut Samhita (200 BC) had described properties and uses of 1100 and 1270 species respectively, in compounding of drugs and these are still used in the classical formulations, in the Ayurvedic system of medicine. Unfortunately, much of the ancient knowledge and many valuable plants are being lost at an alarming rate. In India, forest cover is disappearing at an annual

rate 1.5 ha/year. With such rapid depletion of forests, impairing the availability of raw drugs, Ayurveda and other systems of herbal medicines has reached a very critical phase. About 50% of the tropical forests, the treasure house of plant and animal diversity have already been destroyed.

Plant tissue culture

Plant tissue culture has brought a revolutionary break through because of its several advantages in micropropagation and production of secondary metabolites. Plant tissue culture has facilitated in producing and raising the yield of commercially important biosynthetic compounds (Dougall, 1981; Heble, 1985; Hay *et al*., 1988; Parr, 1989; Phillipson, 1990). The role of plant tissue culture system in the production of secondary metabolites and as a future source of generation of natural products has been discussed (Speak *et al*., 1964; Butenko, 1985; Murashige and Skoog, 1962; Abou and Ahmed, 1977; Bhatnagar and Jaggi, 1981; Furuya, 1982; Berlin, 1986; Anderson *et al*., 1985; Misawa, 1985; Staba, 1985; Bourgaud *et al*., 2001; Rao and Ravishankar, 2002; Gueven, 2012, Filová, 2014).

Micropropagation

One of the important aspects of Plant Tissue Culture is regeneration of plant through micropropagation and induction of callus along with its differentiation which has been achieved using various plant growth regulators which has been proved to be of significant value as supported by various workers (Bhojwani and Razdan, 1983; Heble, 1985; Gautheret, 1985; Thorpe, 1990; Gharyal and Maheshwari, 1990; John Britto *et al*., 2001; Yokoya and Handro, 2002; Nishikoshta and Bansal, 2002; Anand and Jeyachandran, 2004; Rao *et al*., 2005; Agrawal and Sardar, 2006; Sujatha *et al*., 2007; Rout *et al*., 2007; Kumar *et al*., 2008; Naghmouchi *et al*., 2008; Rai *et al*., 2009; Jahan and Anis, 2009; Badoni *et al*., 2010; Senapati *et al*., 2013; Filova, 2014; Anusha *et al*., 2017).

A plant consists of different organs, each being composed of different tissues, which in turn are made up of individual cells. There are many different building materials within a plant, which can be cultured *In vitro*, such as embryos, organs, single cells and even protoplasts. According to the studies of Sharada *et al*., (2003), Rao and Purohit (2006) and Gerald *et al*., (2006) nodal segments, meristems, hypocotyls, epicotyls and cotyledons can be used as explants for some Celastraceae species.

There are number of past researches done on family Celasteraceae (Matu *et al*., 2006, Arya *et al*., 2001, Mederos, 2002, Barghchi and Anderson, 1996, Bojwani *et al*., 1996 and Rao and Purohit, 2006). Matu *et al*., (2006) achieved optimal shoot induction of *Maytenus senegalensis* in low levels of BA (0.5-1.0 mg/l) in their study and shoot multiplication was achieved in MS medium supplemented with 0.5 mg/l BA alone.

Micropropagation of *Celastrus paniculatus*

The cost for regenerating plantlets *In vitro* varies with species and depends upon the technique used. In general, it is believed to be more costly than conventional nursery propagation methods. Therefore, *In vitro* techniques are only profitable in commercial scale for species, which cannot be easily multiplied by conventional methods, where a high financial return is expected. Thus it would be useful to reproduce plants of *Celastrus paniculatus In vitro* for large-scale commercial production for sustainable utilization and conservation, since this is threatened plant and also has a very low rate of propagation by germination of seeds of *C. paniculatus*.

There are records on induction of multiple shoots and complete plantlets from tissue cultures of many rare endangered aromatic and medicinal plants (Bojwani and Razdan, 1996 and Wawrosch *et al*., 1999). Sharada *et al*., (2003) described plantlet regeneration through adventitious shoot formation via embryo derived callus in *C. paniculatus*. They used MS medium as the basal medium. The cotyledonary leaves from mature embryo callused on MS medium supplemented with 5.0 uM NAA and 5.0 uM Kn with doubling time of 16.2 days. They could obtain well developed multiple shoots of 5.6 cm via organogenesis within 28 days on MS medium supplemented with 0.2 mg/l BAP. Rooting of 85% of shoots was achieved on WPM containing 5.0 mg/l IBA. Acclimatization of rooted plants was successful in pots containing sand, garden soil and vermiculite mixture. In this study embryos from mature seeds were used as explant. Though this protocol was successful there is a limitation of using it due to lack of availability of seeds.

Nair and Seeni (2001) reported the establishment of an efficient protocol for *In vitro* shoot proliferation from nodal explants of *Celastrus paniculatus* on semi-solid medium with 1.0 mg/l BAP after 6 weeks. Rooting of 3-7 cm shoot cuttings was induced in half-strength MS liquid medium containing IAA (1.0 mg/l) with formation of 7.25 roots of 2.41 cm length within 6 weeks. Rooted plants were established at 84-96% rate in community pots without hardening, the least value (84%) being obtained with NAA- induced thick and calloid rooted plants.

Arya *et al*., (2001) described about micropropagation of *C. paniculatus* through nodal segments. In this study

four to five shoots were obtained from the nodal region within 15 - 20 days on MS medium containing 1.5 mg/l BAP, 0.1 mg/l NAA and 50.0 mg/l ascorbic acid, 25 mg/l each of adinine sulfate, arginine and citric acid as additives. Number of multiple shoots were by 10 - 15 folds on subsequent sub culturing on MS medium containing 0.8 mg/l BAP and 0.1 mg/l NAA. Ex vitro rooting was obtained by treating the shoots with a solution of 100.0 mg/l each of IBA and NAA. Ex vitro rooted plants were hardened and transferred to poly bags / earthen pots containing black soil, fine sand, farm yard manure and soil rite in the ratio of 4:2:1:1 (v/v).

Arya *et al.* (2002) established an efficient and rapid micropropagation technique from nodal segment of *C. paniculatus*. Four to five shoots differentiated from nodal region within 15-20 days on Murashige and Skoog's medium containing 1.5 mg/l 6-benzylamino purine (BAP) + 0.1 mg/l naphthalene acetic acid (NAA) + additives (50 mg/l ascorbic acid and 25 mg/l each of adenine sulfate, arginine and citric acid) at 28 ± 2°C temperature and 36 µmol $m^{-2}8^{-1}$ photon flux density, 10 hour/day photoperiod. *In vitro* produced shoots were further multiplied by subculturing on modified Murashige and Skoog's medium containing 0.8 mg/l BAP + 0.1 mg/l NAA + additives. Five to seven shoots regenerated from each node of sub-cultured shoots; on subsequent sub-culturing rates of multiplication were increased to 10-15 folds.

Rao and Purohit (2006) discovered that multiple shoots could be induced directly from internode explants of *C. paniculatus* inoculated in MS medium containing different growth regulators. In this study best results were obtained when 4.44 uM BAP was incorporated in to the medium. Adventitious shoot buds were multiplied and elongated on MS medium containing 2.22 uM BAP and rooting of shoots (80 %) was obtained when bases of *In vitro* produced shoots were dipped in pre autoclaved IBA solution (2.45 uM) for 10 min followed by their implantation on medium containing 1/4 MS salts, 1.0 % sucrose and 0.6 % agar.

Gerald *et al.,* (2006) also have developed a protocol for micropropagation of *C. paniculatus* through nodal explants. An average of five shoots per explant were produced in MS medium supplemented with 1.5 mg/l BA and 0.1 mg/l NAA after two subculture cycles with a 30 day interval. There were several problems encountered in this study such as reduction of number of multiple shoots (2 or 3 shoots), vitrification of shoots and callus formation because of continuous subculture in the same medium. MS medium supplemented with lower concentrations of BA (0.05 mg/l) and NAA (0.01 mg/l) overcome the vitrification. Qualitative chemical similarity of the tissue culture regenerates of *C. paniculatus* with the mother plant was confirmed using high performance thin layer chromatography (HPTLC).

Martin *et al.,* (2006) developed micropropagation protocol for *Celastrus paniculatus*, a vulnerable medicinal plant. Cultures were initiated from nodal explants collected from young shoots of a 12 years old plant in MS medium. An average of 5 shoots were produced in MS medium supplemented with 1.5mg/l benzyl adenine (BA) and 0.1mg/l naphthalene acetic acid (NAA) after two subculture cycles with a 30 days interval. Continuous subculture in the same medium for three more cycles resulted in reduction of the number of multiple shoots (2 or 3 shoots), vitrification of the shoots, and callus formation. Vitrification of cultures could be overcome by the use of MS medium supplemented with lower concentrations of BA (0.05mg/l) and NAA (0.01mg/l). Regenerated and elongated shoots were rooted on half strength MS medium fortified with IBA.

Raju and Prasad, 2007 are obtained high frequency shoot multiplication through *In vitro* seed germination. Seed were subjected to three different treatments – room temp., cold (4^0C) and water soaking treatments when cultured for 3- weeks on MS medium with a combination of BA and KN was sufficient to induce maximal shoot response (81.2%) and gave the highest number of shoots per explant (4.4 shoots). The excision of the node and shoot tip from *In vitro* grown seedlings and their subsequent culture on MS medium supplemented with 2.2 uM BA and a combination of MS + 4.4 uM BA + uM KN facilited enhanced axillary bud proliferation (66.6%). Best rooting was observed on MS medium fortified with 10.7 uM NAA.

De Silva and Senarath (2009) established a protocol for multiple shoot induction *via* the culture of nodes of *C. paniculatus*, through callogenesis and organogenesis. Explants were cultured on Murashige and Skoog's medium supplemented with different concentrations and combinations of BAP, 24-D and NAA for shoot induction. Callus sub-cultured on MS basal medium supplemented with higher concentration of BAP (5.0 µM BAP) and lower concentration of IAA (0.5 µM) showed maximum rate of shoot multiplication.

De Silva and Senarath (2009) developed a successful protocol for *In vitro* mass propagation of *C. paniculatus* and also studied the growth and physiology of greenhouse established tissue cultured plants. Shoot tips and nodal segment explants produced multiple shoots in the MS medium in the presence of 10.0 µM BAP and 14.0 µM IAA. Multiple shoots were induced in the MS media with 5.0 µM BAP and 0.5 µM IAA. Elongated shoots developed

roots and the highest rooting percentage (73.3%) was obtained in the MS medium supplemented with 5.6 µM IAA and 9.6 µM Indole-3-butyric acid (IBA).

Lal and Singh (2010) reported high frequency of plant regeneration from axillary meristems. The maximum number of shoots (8.9 ± 0.5) along with hundred per cent bud break was noticed on the MS medium supplemented with 1.0 mg/l BAP. The *In vitro* regenerated shoots were excised aseptically and implanted on full and half strength MS medium without or with growth regulators (IAA, NAA and IBA) at the concentrations of 0.5 and 1.0 mg l^{-1} for rooting. MS half strength medium supplemented with 0.5 mg/l NAA proved best with hundred per cent rooting.

An efficient and reproducible *In vitro* protocol for large scale multiplication of *Celastrus paniculatus* employing shoot tip explants has been developed by Yadav *et al.,* 2011. Season of collection of explants showed direct influence on bud break. Highest percentage of bud break (90%) with multiple shoot formation (4.3) was obtained on MS medium containing BAP (1.0 mg/l).The regenerated shoots were further elongated on same medium. MS half strength medium supplemented with 0.5 mg/l NAA proved to be best with hundred per cent root formation.

Rao *et al*. (2012) reported direct shoot regeneration in *Celastrus paniculatus*. The regeneration protocol was standardized by using different explants like petiolated leaf explants and non- petiolated leaf explants leave on Murashige and Skoog (MS) medium with different concentrations of sucrose, plant growth regulators BAP and Kn. Shoot bud differentiation was much better on BAP media as compared to Kn. BAP alone at its 2.0mgL concentration produced a maximum of 11 shoots buds on petiolated leaf explants while more than 13 shoot buds per explants could be obtained when both BAP and Kn at 0.5 mgL concentration each were incorporated in the medium.

Phulwaria *et al*. (2012) noticed that shoot multiplication was achieved maximum when repeated transfer of mother explants sand subculturing of *In vitro* produced shoot clumps on MS medium supplemented with various concentrations of 6-benzylaminopurine (BAP) alone or in combination with auxin indole-3-acetic acid (IAA) or a-naphthalene acetic acid (NAA). The maximum number of shoots (47.75+2.58) was observed on MS medium supplemented with BAP (0.5 mg/l) and IAA (0.1 mg/l). *In vitro* raised shoots were rooted underex vitro conditions after treating them with indole-3-butyric acid (300 mg/l) for 3 min.

Gowdru *et al*., (2013) developed a protocol for plant regeneration with an intervening callus phase for *C. paniculatus* using immature inflorescence segments and flower buds. The floral explants are proliferate into callus mass on Linsmaier and Skoog (LS) medium augmented with 3% fructose as the carbon source and the plant growth regulators Kn and IBA in range of 6-8 mg L^{-1}andd 0.25 mg/l, respectively. Lower concentration of Kn and IBA induced the calli to differentiate into shoot buds and produced average 9 shoots per callus. These micro-shoots developed a good root system on half strength LS medium fortified with 0.3 mg/l IBA.

Rahane, 2013 was found nodal segment as best explant for micropropagation of Malkangni which gave better results than shoot tip explants. Maximum percentage of callus induction was observed on MS + 1.0 mg/l BAP + 0.1 mg/l IAA i.e. 53.7 % and 46.9 % for nodal segment and shoot tip respectively. Among the ten different media tried for shoot initiation, multiplication and number of shoots per plant, MS + 2.5 mg/l BAP + 0.5mg/l TDZ medium was found best treatment not only for nodal segment but for shoot tip also. It took 10.6 and 13.3 days for shoot initiation from nodal segment and shoot tip, respectively. Maximum number of shoots per explant was observed on MS + 2.5 mg/l BAP + 0.5mg/l TDZ media which gave 5 and 4 shoots from nodal segment and shoot tip, respectively. For rooting initiation and percentage of rooting ½ MS + 1.5 mg/l + 1.0 mg/l IBA was found best treatment. It gave earliest rooting in 14.3 days and 46.9 % of roots from *In vitro* shoots.

Senapati *et al*., (2013) observed that the Murashige and Skoog (MS) basal medium supplemented with 0.5 mg/l 6- benzyl amino purine (BAP) and 0.1 mg/l naphthalene acetic acid (NAA) showed maximum percentage of shoot multiplication (83.4%) with 8.2 shoots/explants.

Sentiya*et al*., 2016 developed an efficient protocol for high frequency shoot regeneration through nodal segments in *C. paniculatus*. The Nodal segments of *C. paniculatus* inoculated into Murashige and Skoog Medium supplemented with different concentrations and combination of BAP, Kn, TDZ, and NAA. Shoot multiplication was achieved by repeated transfer of mother explants and sub-culturing of *In vitro* produced shoot clumps on Murashige and Skoog's medium supplemented with various concentrations of BAP alone or in combination with auxin NAA. The maximum number of shoots was observed on MS medium supplemented with BAP (1 mgL^{-1}) and NAA (0.5 mg/l) kept under red light treatment.

Vijay *et al*. (2016) established a rapid micro-propagation protocol for rare medicinal plant *C. paniculatus* through shoots explants. Maximum mean number of initiated plantlets 1.8 ± 0.42 with mean length 4.6 ± 1.34 were found in MS medium treated with lowest concentration of BA, while maximum mean number of multiplied plantlets 21.7 ± 1.25 with mean length 6.8 ± 0.91 were found in MS medium treated with 4.44 µM. Maximum mean number of roods16.2 ± 0.78 with Mean length 9.19 ± 0.68 was observed on 1/2MS medium with 100 mg/l activated charcoal.

Billore *et al* (2016) established a protocol of homogenous cell suspension culture of *Celastrus paniculatus* and multifold production of alkaloids and obtained total phenols under the influence of monochromatic lights. One month old leaf derived friable callus of *C. paniculatus* was used to raise homogenous suspension culture and kept on rotary shakers in cabinets illuminated with different monochromatic LED lights (Blue, Yellow and Red). Maximum production and enhancement in alkaloids and phenols (98 and 44.7%, respectively) over control was obtained from cell mass grown under yellow light treatment, followed by blue (64 and 23.7%) and red light (50 and 26%) treatments.

Anusha *et al.,* 2016 established a protocol for callus production and phytochemical analysis of *Celastrus paniculatus*. The callus was induced in MS medium fortified with various concentrations of 2, 4-D (0.5 - 3.0 mg/l) alone and a combination with optimum concentration of 2, 4-D (1.5 mg/l) with varying concentrations of NAA (0.5 - 2.5 mg/l). The powdered samples of callus extract were screened for phytochemical constituents.

Anusha *et al*. (2017) developed protocol was successfully employed for the large scale production of this endangered species from nodal cuttings, shoot tips and midrib of the leaves explant. The maximum number of shoots was induced in MS medium with 1.5 mg L of BAP through nodal explants, it was 4.67±1.58 shoots. Subsequent culture on medium with 1.0 mgl of BAP facilitated rapid multiplication and a mean of 30.52±2.64 shoots were developed. The developed shoots were healthy and a height of approximately 8 cm.

Tejavathi and Raja Niranjan, 2017 developed an effective protocol for micropropagation through nodal cultures. Maximum number of 15.43±0.17 shoots was regenerated directly from the nodal explants on MS medium supplemented with BAP+NAA+GA3. Histological studies have confirmed the direct regeneration of shoots from the cultures. Thus, obtained shoots were sub-cultured to rooting medium containing auxins for root induction.

Sharma and Sharma, 2018 have been established an efficient and reproducible *In vitro* protocol for large scale callus cultures of *Celastrus paniculatus* by nodal explants on MS medium containing different combination like BAP+NAA (1.0+ 0.5 mg/l), BAP+NAA (1.0+0.2 mg/l), 2, 4-D+Kn (0.5+1.0 mg/l) and 2, 4D + BAP (0.5+0.5mg/l). Best response in terms of callus cultures was obtained in IAA+Kn (1+0.5mg/l) under controlled condition of 16 h of photoperiod and 8 h dark period at a temperature of 25 ± 2°C. Further synthetic seed production was also investigated using the sodium alginate (NaAlg) encapsulation technique.

In conclusion, the use of plant tissue in conservation of endangered plant species helped in conservation of natural resources and protection against natural disasters that can lead to loss of the species, resulting in reduction of biodiversity and affecting the ecosystem.

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References

Ahmad, F., R.A. Khan and S. Rasheed (1994). Preliminary screening of methanolic extracts of *Celastrus paniculatus* Willd. for analgesic and anti-inflammatory activities. *J. Ethnopharm*. **42(3)**: 193-198.

Anusha, T.S., M.V. Joseph and K.K. Elyas (2016). Callus Induction and Elicitation of Total Phenolics in Callus Cell Suspension Culture of *Celastrus paniculatus* – willd, an Endangered Medicinal Plant in India. *Pharmacogn. J.,* **8(5):**471-475.

Anusha, T., K. Sasidharan, M. Joseph and K.E. Kothanam (2017). Development of an Efficient Technology for Rapid Clonal Multiplication of *Celastrus paniculatus*–Willd, an Endangered Medicinal Plant. *Asian J. Plant Sci.,* **16(4)**: 184-192.

Arora, N. and P. Rai (2012). Miraculous cognitive & other therapeutic properties of an endangered Indian medicinal plant- *Celastrus paniculatus* Willd. *Int. J. of Pharma & Biosciences,* **3(3)**:290-30.

Arora and, Rai (2012). Isolation of secondary metabolites from *Celastrus paniculatus Willd. Int. J. of Pharma & Biosciences,* **3(3)**:412-413.

Arya, V., R.P. Singh and N.S. Shekhawat (2002). A micropropagation protocol for mass multiplication and off-site conservation of *Celastrus paniculatus* – a vulnerable medicinal plant of India. *J. Sustain Forest,* **14**: 107–120.

Bhojwani, S.S. and M.K. Razdan (1996). Plant Tissue Culture Theory and practice, Elsevier, *Amsterdam,* 483-536.

Billore, V., L. Khatediya and M. Jain (2016). Sink - Source System

of *In vitro* Suspension Culture of Celastruspaniculatus under Regulation of Monochromatic Lights. Plant *Tissue Cult. & Biotech.*, **26(1)**: 175-185, 2016.

Britto, S.J., B. Mani, S. Thomas and S. Prabhu (2017). A new subspecies of *Celastrus* (Celastraceae) from the Palni hills of South India. *Taiwania,* **62(3)**: 311?314.

CSIR. Wealth India-raw material (1992); Council for Scientific and Industrial Research.

De Silva, M.A.N. and W.T.P.S.K. Senarath (2009). Development of a Successful Protocol for *In vitro* Mass Propagation of *Celastrus paniculatus* Willd. – A Valuable Medicinal Plant. *Trop. Agri. Res.*, **21(1)**: 21-29.

Gattu, M., J.R. Pauly, K.L. Boss, J.B. Summers and J.J. Buccafusco (1997). Cognitive impairment inspontaneously hypertensive rats: Role of central nicotinic receptors. *Brain research,* **771(1)***: 89-103*

Geetha, S.P., A.V. Raghu, G. Martin, S. George and I. Balachandran (2009). *In vitro* propagation of two tuberous medicinal plants: Holostemma ada-kodien and Ipomoea mauritiana. *Methods Mol. Biol.*, **547**: 81-92.

George, L., M. Karthik and A.S. Irudhaya (2010). Effect of *Celastrus paniculatus Willd.* seed oil (Jyothismati oil) on acute and chronic immobilization stress induced in albino mice. 130-138.

Gerald, M.S., P. Geetha, S.R. Sudhakar, A.V. Raghu, B. Indira and N. Ravindran (2006). An efficient micropropagation system for *Celastrus paniculatus* willd. a vulnerable medicinal plant, *Journal of Forest Research,* **11(6):** 461 – 465.

Grassmann, J., S. Hippeli and E.F. Elstner (2002). Plant's defense and its benefits for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. *Plant Physiol. Biochem.*, **40**: 471-478.

Gueven, A. and D. Knorr (2011). Isoflavonoid production by soy plant callus suspension culture. *Journal of Food Engineering,* **103**: 237–243.

Kinnersley, A.M. and D.K. Dougall (1980). Increase in anthocyanin yield from wild-carrot cell cultures. *Planta,* **149**: 200-204

Lal, D. and N. Singh (2010). Mass multiplication of *Celastrus paniculatus* Wild: An important medicinal plant under *In vitro* conditions via nodal segments. *Int. J. Biodiv. Conserv.*, **2**: 140–145.

Mathur, N.T., M. Varma and V.P. Dixit (1993). Hypolipidaemic and antiatherosclerotic effect of *Celastrus paniculatus* Willd. seed extract in cholesterol fed rabbit. *Indian Drugs,* **30**: 76-82.

Mederos, S.M. (2002). *In vitro* propagation of *Maytenus canariensis* (Loes.) from *Apical Meristem Culture,* **12**:2, 99-108

Mulabagal, V., C.Y. Lee, S.F. Lo, S.M. Nalawade, C.Y. Lin and H.S. Tsay (2004). Studies on the production of some important secondary metabolites from the medicinal plants by tissue cultures. *Bot. Bull. Acad. Sin.*, 45.

Nair, L.G. and S. Seeni (2001). Rapid *In vitro* multiplication and restoration of *Celastrus paniculatus* Willd. sub sp. *paniculatus* (Celastraceae), a medicinal woody climber." *Indian J. Exp. Biol.,* **39(7)**:697-704.

Patel, R.P. and B.M. Trivedi (1962). The *In vitro* antibacterial activity of some medicinal oils. *Indian J. Med. Res.,* **50**:218-222.

Pandya, K.K., R.B. Patel and B.K. Chakravarthy (1990). Antibacterial activity of some Indian medicinal plants. *Indian Drugs,* **27**:4115-4117.

Pavanandt, K., W.H. Kyle, K. Yongvanitchit, A. Kun-anake, T. Dechatiwonse, W. Nutakul and J. Bansiddhi (1989). Schizontocidal activity of *Celastrus paniculatus* Willd. against *Plasmodium falciparum In vitro*. *Phytother Res.,* **3(4)**: 136-139.

Phulwaria, M., M.K. Rai, A.K. Patel, V. Kataria and N.S. Shekhawat (2013) A genetically stable rooting protocol for propagating a threatened medicinal plant—*Celastrus paniculatus* Willd. *AoB PLANTS 5: pls054; doi:10.1093/aobpla/pls054*.

Phulwaria, M., M.K. Rai, A.K. Patel, V. Kataria and N.S. Shekhawat (2013) "Roots of *Celastrus paniculatus* Willd. used in treatment of cancerous tumours" *AoB PLANTS 5: pls054; doi:10.1093/aobpla/pls054*.

Prathanturarug, S., N. Soonthornchareonnon, W. Chuakul, Y. Phaidee and P. Saralamp (2004). High frequency shoots multiplication in *Curcuma longa* L. using thidiazuron, *Plant Cell Reports,* **21(11)**, 1054-1059.

Rahane, V.B. (2013). Micropropagation Studies In Malkangni (*Celastrus paniculatus* Wild). MPKV, University Library Rahuri thesis.

Rao, S.M. and G.A. Ravishankar (2002). Plant cell cultures: chemical factories of secondary metabolities. *Biotechnol. Adv.,* **20**: 101–153

Rao, M.S. and S.D. Purohit (2006). *In vitro* shoot bud differentiation and plantlet regeneration in *Celastrus paniculatus* WilId. *Journal of Biologia Plantarum,* Springer Netherlands, **50**:4 501-506.

Rao, M.S., N. Rakhi, S. Poonam and D.P. Sunil (2012). Shoot bud differentiation and plantlet regeneration from *In vitro* derived leaf explants of *Celastruspaniculatus*Willd. *International Journal of Recent Scientific Research,* **3(11)**: 932 -935.

Rastogi, S. (2001). Building bridges between Ayurveda and modern science. *Int. J. Ayurveda Res.*, **1(1)**: 41-46

Russo, A., A.A. Izzo, V. Cardile, F. Borrelli and A. Vanella (2001). Indian medicinal plants as antiradicals and DNA cleavage protector. *Phytomed.*, **8(2)**: 125-132.

Sharma, V. and A. Sharma (2018). Synthesis of indigenous callus culture and artificial seeds from *Celastrus paniculatus* Willd.via nodal segments. *International Journal of Advanced Scientific Research and Management,* **Special**

Issue I, Jan 2018.

Sharada, M., A. Ashok and M.K. Kaul (2003) Regeneration of plantlets via callus culturesin *Celastrus paniculatus* Wilid. - A rare Endangered Medicinal Plant. *Journal of Biochemistry and Biotechnology*, **12**: 65 -69.

Senapati, S.K., S. Aparajita and G.R. Rout (2013). Micropropagation and assessment of genetic stability in *Celastrus paniculatus*: An endangered medicinal plant. *Biologia*, **68(4)**: 627– 632.

Sentiya, P., T. Sharma and R. Pragya (2016). Efficient protocol for high frequency shoot regeneration *via* Nodal segment. *International journal of pharmacy and pharmaceutical research*, **6(2)**: 362-370.

Tapsell, L.C., L. Hemphill, L. Cobiac, C.S. Patch, D.R. Sullivan, M. Fenech, S. Roodenrys, J.B. Keogh, P.M. Clifton, P.G. Williams, V.A. Fazio and K.E. Inge (2006). Health benefits of herbs and spices particularly when compared with standards applied for assessing pharmaceutical agent. *Med J Aust.*, **185(4suppl)**:S4-24.

Yang, H., K.R. Landis-Piwower, D. Lu, P. Yuan, L. Li, G.P. Reddy, X. Yuan and Q.P. Dou (2008). Pristimerin Induces Apoptosis by Targeting the Proteasome in Prostate Cancer Cells. *Journal of Cell Biochemistry*, **103(1)**:234–244.

Wangoo, D. and P.P. Bidwai (1988). Anti-spermatogenic effect of *Celastrus paniculatus* Willd. seed extract on the testis of albino rats. *Fitoterapia*, **59**:377-382.