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Sink - Source System of *In vitro* Suspension Culture of *Celastrus paniculatus* under Regulation of Monochromatic Lights

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Key words: Cell suspension culture, Secondary metabolites, *Celastrus paniculatus*

Abstract

Plants are wonderful resource of bioproducts encompassing significant value to medicines and drug development. The plant cell suspension cultures bear immense potential for production of high-value secondary metabolites and are chosen as alternative sources of raw material for industrial use. In the present study, homogenous cell suspension culture of *Celastrus paniculatus* a medicinally important plant was established and multifold production of alkaloids and total phenols was obtained 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). The monochromatic lights proved to be a strong abiotic elicitor in driving the production of secondary metabolites so much so that the metabolites were released extracellularly and the medium served as sink or spacious pool for leaked out metabolites from the cell mass. 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. Further scale up of secondary metabolite production was hence performed under yellow light conditions, starting from 2.5 gm cell mass suspended in 250 ml of media extended up to 1000 ml culture media for one month. The continuous culture system exhibited remarkable potential of this plant cell system as multifold yield of total alkaloids

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(91.69 μg/1750 ml) and phenols (70.59 μg/1750 ml) was obtained during 30 days of culture under yellow light conditions. The production was remarkably enhanced by 100-folds (5.29 to 48.21 μg; Fig. 2) for alkaloids and 70-folds (1.73 to 46.33 μg; Fig. 3) for phenols, from zero days to 30-day-old culture phase. Hence, strategic implementation of monochromatic lights holds great promises for controlled commercial production of myriads of valuable secondary metabolites from plant cell cultures of important medicinal plants. Present work contributes to the first reports where continuous, enhanced, multifold yield of important secondary metabolites were obtained from cell suspension culture of *Celasrus paniculatus* an endangered medicinal plant.

Introduction

Celastrus paniculatus is a woody plant commonly known as climbing staff tree, black oil plant and intellect tree. It belongs to family Celastraceae. It is a rare and endangered medicinal plant of India found in tropical forest and subtropical Himalaya region (Prakash and Singh 2001). The phytochemicals present in *Celastrus paniculatus* are alkaloids celastrine, celapanine, celapanigine, celapagine, (Basu and Pabrai 2006) polyalcohols (malangunin, malkanginnol, malkanguniol and paniculatusdiol) (Patel et al. 1995). It also contain tri terpenoid pristimerin (Yasu et al. 2006) and sterols (β-amyrin and β-sitosterol), sesquiter peniodpolyol esters (Khatekhaye et al. 2011).

Different parts of *C. paniculatus* have been evaluated for various pharmacological activities. Leaves of the plant show wound healing activity (Harish et al. 2008). Seed possess hypolipidemic (Mathur et al. 1993) and antioxidant activity (Godkar et al. 2006, Kumar et al. 2002). Seed oil of the plant has been found to reverse the scopolamine induced deficits in navigational memory performance (Gattu et al. 1997). Seeds also show potent relaxant effect on ileum (Borrelli et al. 2009) and significant effects in adjuvant induced arthritis in rats (Patil and Suryavanshi 2007). The extract of whole plant shows anti-epileptic activity (Atigari et al. 2012, Ahmad et al. 1994).

The production of secondary metabolites *in vitro* is made possible through plant cell culture (Barz and Ellis 1981, Dues and Zenk 1965) and cell cultures prove to be a promising potential alternative source for the production of high-value secondary metabolites of industrial importance. It is now a well-known fact that light as an important physical factor influence the growth and formation of primary and secondary metabolites (Yu et al. 2005, Krewzaler and Hahlbrock 1973, Zhong et al. 1991, Liu et al. 2012, Luczkiewicz et al. 2002, Mosaleeyanon et al. 2005, Shohael et al. 2006, Mulder-Krieger et al. 1988). Hence, by optimizing the light conditions, secondary metabolites production can be manipulated

effectively and good quality raw material for pharmaceutical industry can be achieved in *in vitro* cultures of medicinally important plants.

Present work was performed with the aim to obtain enhanced levels of important secondary metabolites mainly alkaloids and phenols from cell cultures of *Celastrus paniculatus* an important medicinal plant directed by light quality; a strong abiotic factor. Whole experiment was performed in three phases. In first phase impact of monochromatic light was studied on production of high yield of callus derived from leaf segments of *Celastrus paniculatus,* followed by second phase; where suspension culture was established from the callus and production of secondary metabolites was obtained under the influence of monochromatic lights and lastly the third phase; where scale up was performed for extensive production of alkaloids and phenols under the light conditions which greatly influenced the secondary metabolite pathway in suspension culture of *Celastrus paniculatus*.

Materials and Methods

Overnight soaked seeds of *Celastrus paniculatus* were decoated and surface sterilized under aseptic conditions with freshly prepared 0.1% (w/v) mercuric chloride solution for 5 - 6 min, rinsed in 70% alcohol for 30 second followed by washing with distilled water. The surface sterilized seeds were inoculated in MS. Leaf explants were collected from 5 days old seedling and inoculated onto fresh agar medium fortified with 2-4, D (1 mg/l) gellified with 0.6% agar for callogenesis. Cultures were placed in culture room under the chambers illuminated with monochromatic lights emitted by Light Emitting Diodes Blue, Yellow and Red (Fig. 1) with various intensities (Table 1) compared with white light (control) for 16/8 hrs photoperiod at 25ºC.

Cell suspension cultures of *Celastrus paniculatus* were initiated from one month old friable callus tissue. One gram of friable callus mass was transferred to 250 ml flasks containing 100 ml liquid MS, fortified with 2, 4-D (1 mg/l). The flasks were agitated at 150 rpm on rotary shakers kept under cabinets illuminated with Light Emitting Diodes Blue, Yellow and Red compared with White light (control) for 16/8 hrs photoperiod at 25ºC. Subculture was performed at every ten days interval of culture phase.

The secondary metabolites released from the cells into the medium were extracted with methanol and estimated for the presence of alkaloids, phenols and flavonoids using standard phytochemical procedures.

Estimation of total alkaloid was performed by the protocol proposed by Devanaboyina et al (2013). Methanolic extract containing secondary metabolites

was added to phosphate buffer of pH 4.7 in presence of BCG solution in a test tube, and was shaken vigorously with chloroform. The absorbance was determined at 470 nm and estimated by standard plot prepared with various concentrations of atropine.

Estimation of total phenolics was performed by Devanaboyina et al. (2013). Methanolic extract containing secondary metabolites was added to Folin-Ciocalteure agent followed by addition of 7.5% Sodium carbonate. The absorbance was determined at 750 nm and estimated by standard plot prepared with various concentrations of gallic acid.

Scale up of the continuous production of secondary metabolites was established at large scale by taking 2.5 gm of friable callus initially in 250 ml of MS fortified with 2,4-D. The cultures were kept on rotary shaker at 150 rpm under yellow light treatment (based on the above experiment results) with 16/8 hrs of photoperiod at 25°C. On tenth day the cells were scale up by removing the exhausted media and supplementation of 500 ml fresh MS fortified with 2,4-D. The exhausted medium was used for extraction and quantification of secondary metabolites. The procedure was repeated on 20th day of culture and this time the cells were scale up to volume of 1000 ml fresh MS fortified with 2,4-D. On 30th day the experiment was terminated and whole medium leaving the cells was extracted and estimated for the presence of secondary metabolites secreted extracelluarly.

All the experiments were performed in triplicates. The data values (mean ± error) were statistically calculated by SPSS (7.1 version) and were assessed for significant difference at $p \leq 0.005$.

The samples drawn from the scale up were analyzed through HPLC. The analysis was performed according to the method developed by Sarma et al. (2002). Chromatographic analysis was carried out using a C-8 column at 25ºC. The running conditions included : injection volume 20 µl; with mobile phase: methanol: water (60 : 40), flow rate of 0.500 ml/min; and detection at 205 nm. Samples were filtered through an ultra membrane filter (pore size 0.45 µm) prior to injection in the sample loop.

Results and Discussion

Light plays a vital role in production of secondary metabolites in plant cell cultures. Many researchers have acclaimed light as an important physical factor for production of secondary metabolites in cell suspension cultures (Anasori et al. 2008). Based on this comprehension, the effect of light and its individual

components referred as monochromatic lights like, blue, yellow and red were examined for production of alkaloids and phenols in cultures of *C. paniculatus.*

At the initial phase within ten days of culture, callogenesis from the cut ends of the leaf was observed which rapidly multiplied and covered whole leaf surface. At this stage crucial effect of light was observed and the cell multiplication capacity was found to be significantly affected by each light quality. The fresh weight of the cell mass was found to be significantly affected with each light treatment and maximum fresh weight was observed in callus raised under yellow light treatments (Table 2). The callus compactness and texture also varied with each light quality. Friable cell mass suited for suspension culture was obtained under yellow and red light conditions, whereas in blue light and white light as a whole, the callus was compact as compared to the callus obtained from higher wavelengths monochromatic light treatments.

Table 1. Light Intensities of experimental setup.

Light	Intensity (μmol/m^2/s)
White	17.7
Blue	22.5
Yellow	24.6
Red	15.6

Table 2. Effect of monochromatic light on fresh weight of suspension culture (gm) (Values are the mean ± standard error from three replicates).

Treatments	Fresh weight
White light	1.004 ± 0.0476
Blue light	1.667 ± 0.317
Yellow light	2.854 ± 0.274
Red light	2.065 ± 0.192

Equal weight (one gram) of one month old friable cell mass was selected and homogenous suspension culture was established. Within fifth day of suspension culture phase, significant impact of light was observed with an increased amount of coloration in media due to the secretion of secondary metabolites extracellularly, from the homogenous cell mass. Initially in this phase, the liquid medium appeared light green in color and up to tenth day of culture phase it turned brown which indicated the level of secretion of metabolites through the cells. At each subculture similar pattern of coloration was observed till one month of production phase. The cells followed a sigmoid growth curve where the biomass (Fig. 1) declined after second subculture but secondary metabolite production increased. This can be correlated with the cells entering the stationary

phase begin synthesizing secondary metabolites which is usually followed generally in cell suspension culture system.

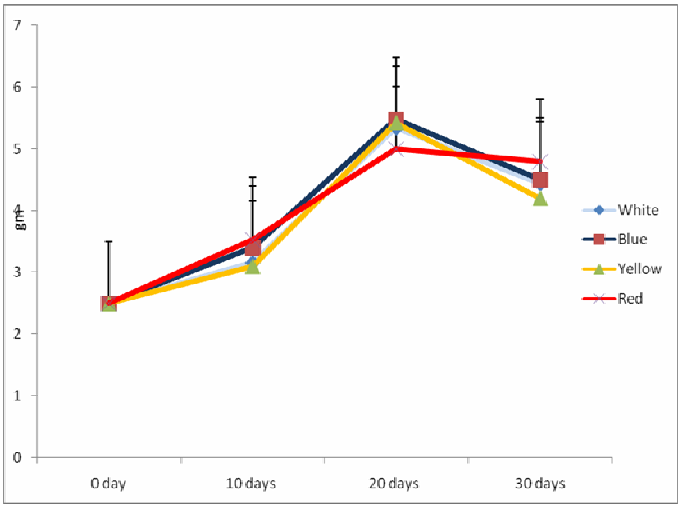

Fig. 1. Effect of monochromatic lights on biomass obtained from suspension cultures of *C. paniculatus* in 10, 20 and 30 days of growth period (values are the mean ± standard error from three replicates).

In this study, role of monochromatic lights was found to play a significant role, as each light quality affected the production of secondary metabolites and influenced the extracellular secretion of secondary metabolites where the medium served as sink for these metabolites of medicinal value. However, the colour intensity of the medium varied with each light treatment. This was further confirmed with the spectrophotometric estimation of the secondary metabolites where yellow light treatment exhibited maximum production and enhancement in alkaloids and phenols by 98 and 44.7%, respectively, followed by blue (64 and 23.7%) and red light (50 and 26%) treatments as compared to natural plant system (Tables 3 and 4). Similar type of studies and stimulatory effect of monochromatic lights on secondary metabolite production have been reported in *Panax ginseng* (Yu et al. 2005), *Peteroselinum hortense (*Krewzaler and Halhlbrock 1973), *Perilla frutiescnens* (Zhong et al. 1991), *Artimisia annua* (Liu et al. 2012), *Rudbeckia hirata* (Luczkiewicz et al. 2002) and *Hypericum perfaratum* (Mosaleeyanon et al. 2005). These studies and our study too go in accordance where different monochromatic lights significantly affected the production of secondary metabolites with variations as per the plant system. *Celastrus paniculatus* in this context was found to be an interesting plant system, which responded to different wavelengths and exhibited a significant enhanced production of secondary metabolites released extracellularly specifically under

yellow light conditions. The effect of yellow light can be attributed to some photo sensors specific to wavelengths which show spectral overlaps switches between green, orange and red spectral regions and which can be selectively toggled to control plant growth, physiology, and biochemistry. However this concept is further needed to be investigated with different radiation band widths for production of important bioproducts in suspension culture system.

Table 3. Total yield of alkaloid obtained from 30 days of suspension culture of *C. paniculatus* under different light treatments (values are the mean ± standard error from three replicates).

Total alkaloids				
Treatments	0 day (μg/100 mg tissue)	10 days	20 days	30 days
Plant	0.145		(μg/100 ml)	
White light	0.148 ± 0.0022	1.82 ± 0.0002	5.26 ± 0.3939	8.87 ± 0.0815
Blue light	0.196 ± 0.0045	1.66 ± 0.0002	9.27 ± 0.5132	9.56 ± 0.1815
Yellow light	0.200 ± 0.0033	3.08 ± 0.0013	9.54 ± 0.3212	14.43 ± 0.3023
Red light	0.163 ± 0.0029	2.67 ± 0.0004	3.65 ± 0.1532	7.51 ± 0.2029

Table 4. Total yield of Phenols obtained from 45 days of suspension culture of *C. paniculatus* under different light treatments (values are the mean ± standard error from three replicates).

Total Phenols				
Treatments	0 day (μg/100 mg tissue)	10 days	20 days	30 days
Plant	0.200		(μg/100 ml)	
White light	0.187 ± 0.0089	1.42 ± 0.0284	4.53 ± 0.1540	7.82 ± 0.179
Blue light	0.312 ± 0.0106	1.81 ± 0.0141	5.35 ± 0.0522	4.94 ± 0.059
Yellow light	0.322 ± 0.0051	4.03 ± 0.0218	9.24 ± 0.0455	9.15 ± 0.137
Red light	0.115 ± 0.0064	3.40 ± 0.1192	4.04 ± 0.0946	5.53 ± 0.059

Based on the above results, a scale up with continuous cell culture system was established and continued yield of metabolites was obtained from cell inoculum till one month of growth period. With each subculture, the exhausted medium was replaced with an increased volume of fresh medium (starting from 250 to 1000 ml) and production of secondary metabolites was estimated in each subculture. Since the suspension culture worked as continuous culture system, multifold yield of total alkaloids (91.69 μg/1750 ml) and phenols (70.59 μg/1750 ml) was obtained during 30 days of culture period under yellow light conditions. The production of alkaloids and phenols was remarkably enhanced by 100 folds

(5.29 to 48.21 µg; Fig. 2) and 70-folds (1.73 to 46.33 µg; Fig. 3), respectively from zero to 30 days old culture phase. Since alkaloids are major produce, their presence was evaluated by HPLC studies (Fig. 4). The chromatogram exhibited distinct peaks of alkaloids and its types which were comparable to the standard of plant alkaloids.

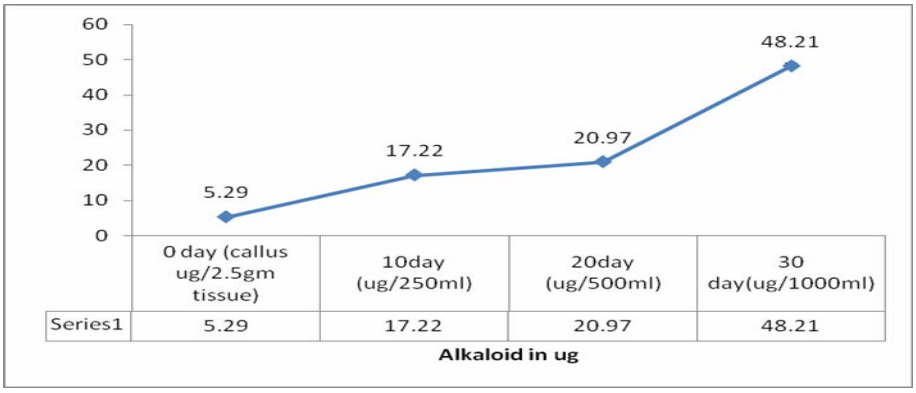

Fig. 2. Effect of yellow light treatment on total alkaloid of of *Celastrus paniculatus* (values are the mean ± standard error from three replicates)

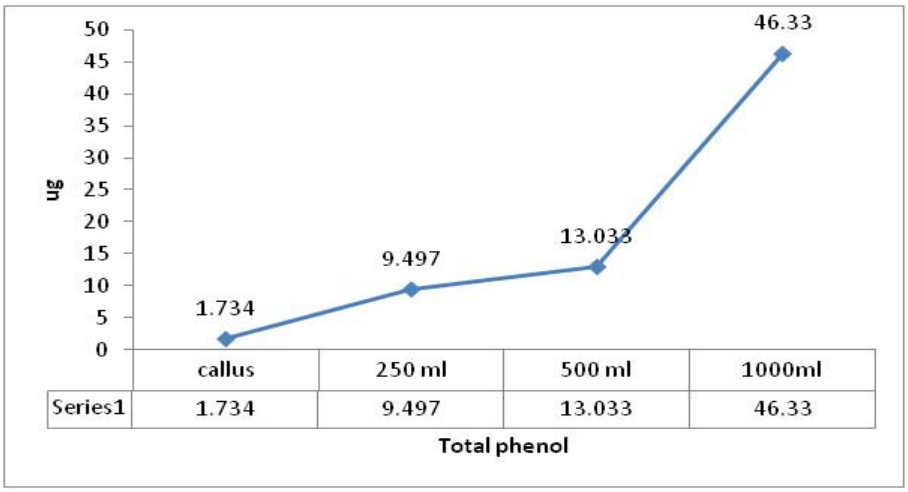

Fig. 3. Effect of yellow light treatment on total phenol of *Celastrus paniculatus* (values are the mean ± standard error from three replicates).

Multifold production of alkaloids and phenols in present study indicate the potential use of cell suspension culture system for continuous production of secondary metabolites from *C. paniculatus* for commercial use, where the cell culture medium served as a source initially by providing essential nutrients and ions for the growth of the cells and which later served as sink for the secondary

metabolites too. The study provides an effective alternative for elevated production of quality raw material for medicinal use compared to the plants growing in natural habitat by exploiting the impact of monochromatic radiation in production of principal active component of medicinal plant species.

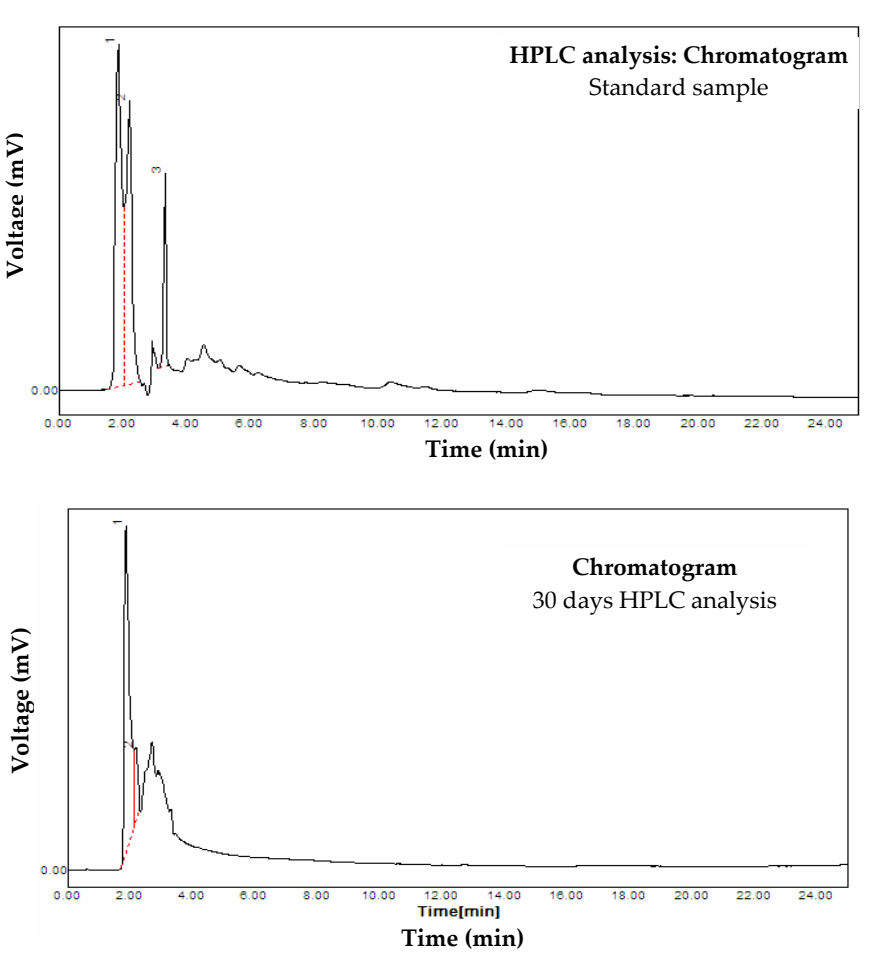

Fig. 4. HPLC chromatogram: (A) Standard alkaloid and (B) 30th day total alkaloids yield.

In our studies monochromatic light proved to be a strong abiotic elicitor in driving the primary metabolites towards secondary metabolite production pathway, where the medium served as an external sink as compared to the vacuole which is a smaller intracellular sink for these metabolites. The culture medium served as spacious pool for leaked out metabolites from cell mass under the influence of monochromatic lights. Strategic monochromatic light treatments thus hold great promise for controlled commercial production of myriads of valuable secondary metabolites *in vitro*.

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References

Ahmad F, Khan RA and **Rasheed S** (1994) Preliminary screening of methanolic extracts of *Celastrus paniculatus* and *Tecomella undulata* for analgesic and anti-inflammatory activities. J. Ethnopharmacol. **42**: 193-8.

Anasori P and **Asghari G** (2008) Effects of light and differentiation on gingerol and zingiberene production in callus culture of *Zingiber officinale* Rosc. Res. Pharm. Sci. **3**: 59-63.

Atigari DV, Gundamaraju R, Sabbithi S, Chaitanya KB and **Ramesh C** (2012) Evaluation of antiepileptic activity of methanolic extract of *Celastrus paniculatus* Willd. whole plant in rodents. Int. J. Pharm. Phytopharmacol. Res. **2**: 20-25.

Barz W and **Ellis B (1981)** Potential of plant cell cultures forms pharmaceutical production. *In:* Beal JL, Reinhard E (eds.) Natural Products as Medicinal Agents. Stuttgart, Hippokrates. pp. 471-507.

Basu NK and **Pabrai PR** (2006) Chemical investigation of *Celastrus paniculatus* Willd. J. Am. Pharm. Assoc. **35**: 272-273.

Borrelli F, Borboneb N, Capassoa R, Montesanoc D, Marinob S and **Aviello G** (2009) Potent relaxant effect of a *Celastrus paniculatus* extract in the rat and human ileum. J. Ethnopharmacol. **122**: 434-438.

Deus B and **Zenk MH** (1982) Exploitation of plant cells for the production of natural compounds. Biotechnol. Bioeng. **24**: 1965-1974.

Gattu M, Boss KL, Terry AV and **Buccafusco JJ** (1997) Reversal of scopolamine-induced deficits in navigational memory performance by the seed oil of *Celastrus paniculatus*. Pharmacol. Biochem. Beha. **57**: 793-799.

Godkar PB, Gordon RK, Ravindran A and **Doctor BP** (2006) *Celastrus paniculatus* seed oil and organic extracts attenuate hydrogen peroxide- and glutamate-induced injury in embryonic rat forebrain neuronal cells. Phytomedicine **13**: 29-36.

Harish BG, Krishna V, Santosh Kumar HS, Khadeer Ahamed BM, Sharath R and **Kumara Swamy HM** (2008) Wound healing activity and docking of glycogen-synthase-kinase-3-beta-protein with isolated triterpenoid lupeol in rats. Phytomedicine **15**: 763-767.

Katekhaye S, Duggal S and **Singh AP** (2011) An inside preview of nutritional and pharmacological profile of *Celastrus paniculatus*. Int. J. Recent Adv. Pharm. Res. **1**: 19-24.

Krewzaler F and **Hahlbrock K** (1973) Flavonoid glycosides from illuminated cell suspension cultures of *Petroselinum hortense*. Phytochem. **12**: 1149-1152.

Kumar MH and Gupta YK (2002) Antioxidant property of *Celastrus paniculatus* Willd: A possible mechanism in enhancing cognition. Phytomedicine **9**: 302-311.

Liu CZ, Guo C, Wang YC and **Ouynag F** (2012) Effect of light irradiation on hairy root growth and artemisinin biosynthesis of *Artemisia annua* L. Biochem. J. **38**: 581-585.

Luczkiewicz M, Zarate R, Migas WD, Migas P and **Verpoorte R** (2002) Production of pulchelin E in hairy roots, callus and suspension cultures of *Rudbeckia hirta* L. Plant Sci. **163**: 91-100.

Mathur NT, Varma M and **Dixit VP** (1993) Hypolipidaemic and antiatherosclerotic effect of *Celastrus paniculatus* seed extract in cholesterol fed rabbits. Indian Drugs **30**: 76-82.

Mosaleeyanon K, Zobayed MA, Afreen F and **Kozai T** (2005) Relationships between net photosynthetic rate and secondary metabolite contents in St. Johns wort. Plant Sci. **169**: 523-531.

Mulder-Krieger T, Verpoorte R, Svendse A and **Scheffer J** (1988) Production of essential oils and flavours in plant cell and tissue cultures. Plant Cell Tissue Org. Cult. **13**: 85-114.

Patel DK, Amin KS and **Nanavati DD** (1995) Chemistry and pharmacology of *Celastrus paniculatus* Willd. Indian Drugs **32**: 566-573.

Patil KS and **Suryavanshi J** (2007) Effect of *Celastrus paniculatus*, Willd. seed on adjuvant induced arthritis in rats. Pharmacogn. Mag. **3**: 177-1781.

Prakash A and **Singh KK** (2001) Observation on some threatened plants and their conservation in Rajaji National Park, Uttaranchal, India. J. Econ. Tax Bot. **25. 2**: 363-366.

Sarma BK, Singh UP and **Sing KP** (2002) Variability in Indian isolates of S. Mycologia **94**(6): 105-110.

Shohael AM, Ali MB, Yu KW, Hahn EJ and **Islam Paek KY** (2006) Effect of light on xidative stress, secondary metabolites and induction of antioxidant enzymes in *Eleutherococcus enticosus* somatic embryos in bioreactor. Process Biochem. **41**: 1179-1185.

Yasu LU, Yang S, Zou Z, Chen H, Zhen X and **Zhongemei Z** (2006) Evoninoate sesquiterpene alkaloids from the stem of *Celastrus paniculatus*. Heterocycl. **68**: 1241-1247.

Yu KW, Murthy HN, Hahn EJ and **Paek KY** (2005) Ginsenoside production by hairy root culture of *Panax ginseng*: Influence of temperature and light quality. Biochem. Engin. **23**: 53-56.

Zhong JJ, Seki T, Kinoshita S and **Yoshida T** (1991) Effect of light irradiation on anthocyanin production by suspended cultures of *Perilla frutescens*. Biotechnol. Bioeng. **38**: 653-658