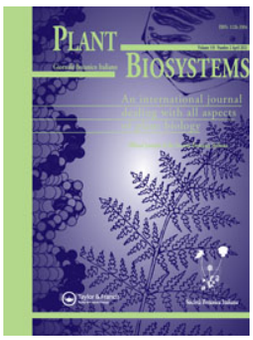

Taylor & Francis
Taylor & Francis Group

Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology

Official Journal of the Societa Botanica Italiana

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tplb20

Cadmium chloride elicitation of *Abutilon indicum* cell suspension cultures for enhanced stigmasterol production

Kiranmayee Rao, Bhuvaneswari Chodisetti, Suryakala Gandi, Archana Giri & P. B. Kavi Kishor

To cite this article: Kiranmayee Rao, Bhuvaneswari Chodisetti, Suryakala Gandi, Archana Giri & P. B. Kavi Kishor (2021): Cadmium chloride elicitation of *Abutilon indicum* cell suspension cultures for enhanced stigmasterol production, Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology, DOI: 10.1080/11263504.2021.1891151

To link to this article: https://doi.org/10.1080/11263504.2021.1891151

PLANT BIOSYSTEMS - AN INTERNATIONAL JOURNAL DEALING WITH ALL ASPECTS OF PLANT BIOLOGY
https://doi.org/10.1080/11263504.2021.1891151

Cadmium chloride elicitation of *Abutilon indicum* cell suspension cultures for enhanced stigmasterol production

Kiranmayee Rao[a], Bhuvaneswari Chodisetti[a], Suryakala Gandi[b], Archana Giri[b] and P. B. Kavi Kishor[c]

[a]Department of Genetics, Osmania University, Hyderabad, India; [b]Centre for Biotechnology, Jawaharlal Nehru Technological University Hyderabad, Hyderabad, India; [c]Department of Biotechnology, Vignan's Foundation for Science, Technology & Research Vadlamudi, Guntur, India

ABSTRACT

Abutilon indicum (Malvaceae), a therapeutically valuable shrub can act as a continuous source of stigmasterol, accredited with pharmacological significance. In the present study, the content of stigmasterol when analyzed in both *in vivo* and *in vitro* plants was found to be 13.89 ± 1.43 and 20.50 ± 2.34 µg/gFW, respectively. The callus obtained from the *in vitro* plants of *A. indicum* was found to contain 10.78 ± 0.19 µg/gFW of stigmasterol and was used for initiation of suspension cultures. In comparison to the calli, suspension cultures of *A. indicum* accumulated considerable amounts of stigmasterol (16.08 ± 1.92 µg/gFW) on the 12th day, i.e., end of log phase. The suspensions on further elicitation with Cadmium Chloride have shown a significant increase (2.59-fold) in the amount of stigmasterol compared to the initial calli, reaching 41.73 ± 3.77 µg/gFW. Thus, cell suspensions of *A. indicum* offer a unique advantage for large-scale production of stigmasterol under *in vitro* conditions, by retaining its natural essence and safety in human consumption.

ARTICLE HISTORY
Received 24 January 2020
Accepted 9 February 2021

KEYWORDS
A. indicum; stigmasterol; suspensions; elicitation; cadmium chloride

1. Introduction

Stigmasterol ($C_{29}H_{48}O$), a phytosterol classified under the triterpene category is produced in plants *via* the isoprenoid pathway (Piironen et al. 2000). Stigmasterol is attributed with many pharmaceutical applications *viz*. antiinflammatory (Kangsamaksin et al. 2017), antimicrobial (Panda et al. 2009), antioxidant (Edilu et al. 2015), antihyperlipidemic and cardiovascular health (Franca and Andrea 2010). In addition to the innumerable medicinal properties, stigmasterol also acts as a precursor during the biosynthesis of steroids *viz*. progesterone, androgens, estrogens, corticoids (Sundararaman and Djerassi 1977) and vitamin D_3 (Kametani and Furuyama 1987). Plant-based drug research has become more promising in recent years as an alternative for synthetic medicine and therapeutic usage (Vanwyk and Wink 2004). Harvesting wild plants for commercial phytochemical extraction might lead to loss of genetic diversity and habitat destruction (Canter et al. 2005), whereas, cell suspensions can offer an alternative for large-scale production of these bioactive compounds under *in vitro* conditions. Although studies on the use of suspensions for production of phytochemicals have been reported *viz*. taxol from *Taxus* species - *T. yunnanensis, T. chinensis and T. chinensis marv.* (Wu et al. 2001); anthraquinones from *Morinda citrifolia* (Zenk et al. 1975), only a few secondary metabolites were able to reach the market after commercial level production, e.g., paclitaxel (Phyton Biotech, Germany & Samyang Genex, South Korea); docetaxel (Phyton Biotech, Germany) from *Taxus* species and shikonin from *Lithospermum erythrorhizon* (Mitsui Petrochemical Industry Co. Ltd., Japan).

Abutilon indicum (L.) Sweet (Malvaceae), a perennial shrub, is widely distributed throughout the tropical and subtropical regions of India. The various parts of *A. indicum* such as roots, leaves and seeds are documented to possess various medicinal properties in ethnobotanical surveys (Seetharam et al. 2002; Porchezhian and Ansari 2005; Roshan et al. 2008; Kashmiri et al. 2009). The main chemical constituents of *A. indicum* include β-sitosterol, stigmasterol, scoparone, scopoletin, abutilin–A and eugenol (Kuo et al. 2008). Keeping in view the immense value of stigmasterol and the biosafety of compounds extracted from natural sources, this study was undertaken in an attempt to make a breakthrough in terms of a cost-effective and sustainable production system for enhanced stigmasterol production from *in vitro* cultures of *A. indicum* using Cadmium chloride ($CdCl_2$) elicitation.

2. Materials and methods

2.1. In vitro cultures of A. indicum

Mature seeds of *A. indicum* collected from greenhouse - grown plants (Rajadhani Agrofarms, Ghatkesar, Hyderabad), were thoroughly washed and treated with concentrated sulphuric acid for 10 min to soften the tough seed coat. The seeds were then surface sterilized with 0.1% mercuric

CONTACT Kiranmayee Rao raorskiran@rediffmail.com or raorskiran@gmail.com Department of Genetics, Faculty of Science, Osmania University, Hyderabad 500 007, India

Supplemental data for this article is available online at https://doi.org/10.1080/11263504.2021.1891151.

Published online 15 Mar 2021

chloride for 5 min followed by thorough washing (8–10 rinses) with sterile distilled water. They were then left to imbibe (48 h) before transfer to semi-solid (0.8% agar) MS media (Murashige and Skoog 1962) supplemented with gibberellic acid (GA_3) at a concentration of 1 mg/L (Rao et al. 2016). The seeds were incubated under standard culture conditions [25 ± 2 °C following a photoperiod of 16/8 h (light/dark) with 40–50 µmol $m^{-2}s^{-1}$ light].

2.2. Regeneration of plantlets

The nodal explants from *in vitro* germinated seedlings of *A. indicum* were cultured on MS media supplemented with different concentrations (0.125 – 1.00 mg/L) of cytokinins, viz. kinetin (Kn), zeatin (Z), thidiazuron (TDZ), benzylaminopurine (BAP), for multiple shoot induction. The basal callus obtained was transferred to MS semi-solid media with a combination of cytokinins (Kn, BAP and TDZ) to initiate embryogenesis under standard culture conditions. The somatic embryo formation was recorded after a culture period of 6 – 8 weeks.

2.3. Callus induction

Explants obtained from leaves, stems (nodal and internodal sections) and roots of *in vitro* germinated seedlings were transferred to MS semi-solid media fortified with different combinations of auxins [2,4–dichlorophenoxyacetic acid (2,4–D), α–naphthaleneacetic acid (NAA)] and cytokinins (BAP; Kn) in the range of 0.25 – 1 mg/L, for callus initiation. The obtained callus was periodically sub-cultured for further proliferation under standard culture conditions.

2.4. Growth pattern of A. indicum suspensions

Friable callus (3 g) obtained from the leaf explants was transferred to conical flasks (100 mL volume) containing 30 mL of MS liquid media (sterilized by autoclaving at 121 °C under a pressure of 15 psi for 20 min) supplemented with 2,4–D, Kn and NAA at a concentration of 1 mg/L each; and incubated on an orbital shaker, under standard culture conditions (25 ± 2 °C temperature; 40–50 µmol $m^{-2}s^{-1}$ illumination;16/8 h (light/dark) photoperiod; 120 rpm aeration). The increase in biomass was assessed along with stigmasterol content by harvesting the suspensions at regular intervals, i.e., every 4 days for a period of 28 days, until the culture reached its decline phase. The biomass and stigmasterol content in the suspensions are expressed as gram fresh weight (gFW) and microgram per gram fresh weight (µg/gFW), respectively.

2.5. Elicitor treatment

An abiotic elicitor, cadmium chloride ($CdCl_2$), was used for eliciting stigmasterol production in the suspension cultures of *A. indicum*. The elicitor was prepared as a stock solution (1 M) in distilled water and filter sterilized (0.22 µm membrane filters). $CdCl_2$ was added aseptically to the suspensions in the range of 0.5–4 mM, on the 11th day of growth studies. Stigmasterol production in combination with biomass was monitored at an interval of 24 h for three consecutive days (24, 48 and 72 h).

2.6. Cell viability

The *A. indicum* cell suspensions were analysed for cell viability preceding the addition of elicitors by selective labelling of cells with 75 µg/mL fluorescein diacetate and the obtained results are expressed in terms of gram fresh weight (gFW). (Darzynkiewicz et al. 1994).

2.7. Stigmasterol quantification

The amount of stigmasterol was quantified in various samples i.e., non-elicited and $CdCl_2$ elicited samples, by a modified HPLC method (Gayathri and Archana 2012). The samples (1 g) were macerated in 1 mL of HPLC grade methanol and filtered (0.22 µm) before subjecting them to HPLC analysis. Stigmasterol was detected at 229 nm and the content expressed as micrograms per gram fresh weight (µg/gFW). HPLC was performed using a Shimadzu-LC-10 AT VP series equipped with a supelco column (Sigma-Aldrich, Bellefonte, Pennsylvania, USA) (250×4.6 mm, C 18 ODS with particle size of 5 µm) (Shimadzu, Kyoto, Japan). The stigmasterol present in the samples was quantified by comparing the obtained HPLC data with that of standard stigmasterol (95% purity; MP Biomedicals, California, USA).

2.8. Statistical analysis

Experiments were performed in triplicate and results are expressed in terms of means ± standard deviations. The least significant difference (LSD) of the test results in comparison to the control was determined by performing Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test (Graph pad Prism 6.0, USA). The results for which the "*p*" value is ≤ 0.05 in comparison to the control were considered to be statistically significant.

3. Results and discussion

A. indicum (Atibala) found in the tropical regions is an annual weed that germinates during the first showers of monsoon. The high therapeutic value according to the Indian traditional medicinal systems (Roshan et al. 2008; Mohite et al. 2012; Khan et al. 2015) has emphasized the potential of *A. indicum* as a template in the development of plant-based drugs. The continuous production of such pharmaceutically important phytochemicals requires a steady supply of healthy plant material, wherein tissue culture techniques offer the advantage of hand-picking elite clones for rapid multiplication by relinquishing seed dependency along with higher secondary metabolite content.

3.1. In vitro cultures

The *in vitro* cultures of *A. indicum* were established using the mature seeds collected from the greenhouse-grown

plants and cultured on MS media supplemented with Kn at a concentration of 0.5 mg/L (Rao et al. 2016). Further, *in vitro* propagation was carried out using the nodal explants causing axillary bud formation resulting in complete plantlet regeneration. Adventitious shoot proliferation from seedlings, hypocotyls and axillary buds; has been the preferred method of mass propagation (Mithilesh and Rakhi 2010); which in turn depends on the sensitivity of plant part and ability of the cytokinin to alter the endogenous hormone levels (von Aderkas and Bonga 2000; Jimenez 2005). The content of stigmasterol was analysed in both the *in vivo* (13.89 ± 1.43 µg/gFW) and *in vitro* (20.50 ± 2.34 µg/gFW) plants, which was found to be statistically significant ($p = 0.012$) (supporting information Figure S1). This gap in the secondary metabolite content of *in vivo* and *in vitro* aerial parts could be due to the timely availability of all the growth factors and no exposure to harsh climatic conditions.

Earlier, the somatic embryos obtained on MS media with TDZ and Kn (0.2 mg/L each) were analysed at various developmental stages for the presence of stigmasterol (Rao et al. 2016). The organogenic calli produced 19.48 ± 0.35 µg/gFW of stigmasterol, whereas, *in-vitro* plants regenerated *via* somatic embryogenesis were found to accumulate 24.36 ± 2.73 µg/gFW of stigmasterol. The plantlets regenerated *via* somatic embryogenesis were found to contain 1.18-fold higher content of stigmasterol in comparison to *in vitro* plants propagated by direct regeneration, which was not statistically significant ($p = 0.163$) (supporting information Figure S2). In addition to rapid regeneration, higher amounts of plant secondary metabolites were reported from somatic embryo derived plants (Lee et al. 2001). Somatic embryogenesis offers innumerable advantages as compared to organogenesis such as low chimera frequency, prolonged embryogenecity, somaclonal variations, bipolar morphology and scale-up potential (Raemakers et al. 1995; Shohael et al. 2013).

Though somatic embryo derived cultures have shown higher amount of stigmasterol, initiation of callus cultures from various explants of *in vitro* regenerated plants was preferred due to short doubling time. Among them, the leaf and stem explants gave positive response on MS semi solid media fortified with 2,4–D, Kn and NAA (1 mg/L each). Root explants were incapable of callus induction and in addition, they became necrotic in the presence of high phytohormone concentration and subsequent sub-cultures (supporting information Table S1). Rapid proliferation of callus was observed from the leaf explants of *A. indicum in vitro* plants (Figure 1), which was in accordance with the report given by Ramar and Ayyadurai (2015). The obtained friable callus was assessed for the presence of stigmasterol and found to contain 10.78 ± 0.19 µg/gFW. Earlier studies on production of stigmasterol from the callus cultures of various plant species has been reported *viz*. 0.4741% from *Tylophora indica* (Pratibha and Abhay 2014); 1.33 mg/gDW from *Cyperus rotundus* (Krishna and Renu 2015).

Leaf explants of *Abutilon indicum* from *in vitro* regenerated plants, excised and cultured on MS media supplemented with 2,4–D, Kn and NAA at a concentration of 1 mg/L each. The obtained callus was sub-cultured every fortnight on the same media combination for maintenance of cultures.

3.2. Suspension cultures

Friable callus (3 g) was transferred into 30 mL of MS liquid media supplemented with 2,4–D, Kn and NAA at a concentration of 1 mg/L, all the while maintaining an optimized inoculum percentage of 10% w/v, in order to facilitate uniform proliferation. The increase in biomass and stigmasterol content were analysed simultaneously every four days and found that maximum biomass was obtained on the 16th day (28.49 ± 3.27 gFW), while stigmasterol reached its peak on the 12th day (16.08 ± 1.92 µg/gFW) of culture (Figure 2). During biomass estimation, it was found that there was a slight increase from day 4 to day 8, which steadily increased from day 12 till it reached day 28, showing significant ($p < 0.001$) increase in comparison to day zero. Similarly, the stigmasterol content also has exhibited a significant ($p < 0.001$) increase till day 20, after which there was a steady decrease till day 28 and was not statistically significant ($p = 0.051$; $p > 0.99$) in comparison to day zero. The higher stigmasterol content observed in cell suspensions, could be due to the suspended nature of plant cells facilitating gaseous exchange along with efficient nutrient uptake caused by large surface area exposure.

The pattern of stigmasterol accummulation was studied along with the increase in biomass by harvesting the samples and subjecting the same to HPLC, every 4 days. Data given (mean±*SD*) is from three independent experiments. The significant difference in the content of stigmasterol as well as biomass has been determined by comparing with day zero, where *=$p < 0.05$; **=$p < 0.01$ (Dunnett's post-hoc test)

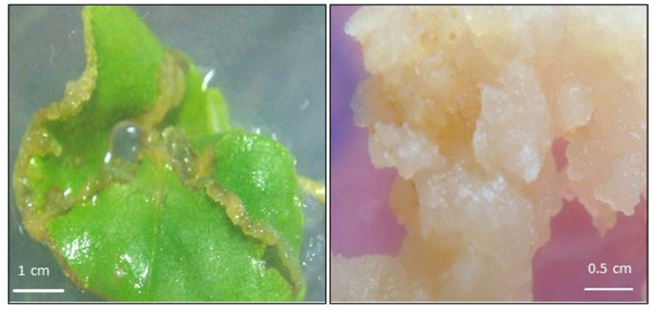

Figure 1. Callus initiation and proliferation from the leaf explants of *Abutilon indicum*.

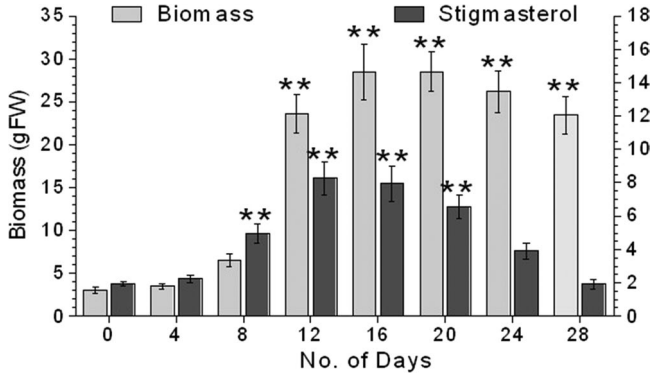

Figure 2. Estimation of stigmasterol in correlation to biomass increase in the suspension cultures of *Abutilon indicum*.

Table 1. Quantification of stigmasterol in *Abutilon indicum* cell suspensions at various time intervals and concentrations of $CdCl_2$.

$CdCl_2$ (mM)	Accumulation of stigmasterol at various time periods (µg/gFW)			Maximum fold increase in stigmasterol
	24 h (12th day)	48 h (13th day)	72 h (14th day)	
--	15.83 ± 1.67	16.08 ± 1.71	16.02 ± 1.70	--
0.5	24.18 ± 1.98**	21.65 ± 1.91**	17.37 ± 1.91	1.50
1	41.73 ± 3.77**	27.51 ± 2.24**	18.42 ± 2	2.59
2	17.42 ± 1.82	14.29 ± 1.21	8.68 ± 0.82**	1.08
4	05.88 ± 0.61**	03.15 ± 0.46**	--	--

Since sterols are the integral components of membrane lipid bilayer regulating the permeability and fluidity along with phospholipids (Mouritsen and Zuckermann 2004), their presence has been detected at every stage of plant development. But, in the present study, maximum stigmasterol production was observed towards the end of log phase (12th day) i.e., 4.23 fold higher ($p < 0.001$) in comparison to the initial value of 3.80 ± 0.29 µg/gFW. This could be due to the strengthening of cell walls in order to restrict the membrane permeability and nutrient efflux (Hodzic et al. 2008) caused by exhaustion of media components and accumulation of toxic metabolic wastes resulting from various physiological processes.

The two-stage culture system adopted by the cell suspensions offers an advantage by minimizing complex factors involved in the various organogenic processes (Kim et al. 1995; Choi et al. 2000). Pharmacologically important compounds *viz.* antioxidants, organic acids, amino acids, vitamins, secondary metabolites – phenols, flavonoids, terpenoids etc. are being produced on a large-scale using cell suspension cultures (Barbara et al. 2009). The amount of stigmasterol found in the *in vitro* and suspension cultures of *A. indicum* is far too low to be able to manufacture at a commercial level, hence an elicitation technique was tested for secondary metabolite enhancement.

3.3. Elicitation

The concentration of plant secondary metabolites during the log/exponential phase is normally very low, as the cellular machinery is directed towards biomass production with the available primary metabolite precursors. Hence, an effective strategy to maximize the biosynthesis of secondary metabolites would be elicitation, during the second phase of culture i.e., end of log phase (Malik et al. 2011). Inorganic metal salts and heavy metal ions have been used to elicit plant secondary metabolite production in a variety of plant culture systems like hairy roots and adventitious roots (Verpoorte et al. 2002). Among them, cadmium exhibits high affinity for sulfhydryl groups of proteins and thus inactivates SH-bearing, redox-regulating enzymes involved in cellular processes (Hall 2002). Cadmium, acting as an external stressor, has the ability to stimulate signal transduction pathways either by oxidative burst (Piqueras et al. 1999) or through Calcium as secondary messenger, which can rapidly transmit the signals to key transcription factors (Lin and Aarts 2012).

Based on the biomass and stigmasterol production, the abiotic elicitor – Cadmium (Cd^{2+}) was added to the suspensions in the form of $CdCl_2$, on the 11th day of inoculation. The elicitor was added to *A. indicum* suspensions at various concentrations and harvested every 24 h i.e., 12th (24h), 13th (48 h) and 14th (72 h) day; for quantification of stigmasterol. Both 0.5 and 1.0 mM $CdCl_2$ were able to significantly elicit higher amounts of stigmasterol on the 12th day (24 h, $p < 0.001$; $p < 0.001$) and 13th days (48 h, $p < 0.002$; $p < 0.001$). Among the elicited cultures, 2.59-fold (41.73 ± 3.77 µg/gFW) higher accummulation of stigmasterol was observed in comparison to the control at 1 mM $CdCl_2$ concentration within a short exposure time of 24 h (Table 1). Whereas on the 14th day, both 0.5 and 1.0 mM $CdCl_2$ concentrations were able to elicit reduced amount of stigmasterol, which was statistically not significant. The stigmasterol content obtained with 2 mM $CdCl_2$ on 12th day was found to be on par with the control, while on the 13th day, there was a significant reduction ($p < 0.001$). The higher concentration i.e., 4 mM $CdCl_2$ significantly ($p < 0.001$) lowered the content of stigmasterol irrespective of the harvest time.

The time-dependent response of *Abutilon indicum* suspension cultures exposed to different concentrations of $CdCl_2$ with respect to accumulation of stigmasterol. The given data is the result of three independent experimental repeats expressed as "mean±*SD*." The significant difference of treatments in comparison to the control (unelicited culture) was represented, where *=$p < 0.05$ and **=$p < 0.01$ (Dunnett's post-hoc test).

The assessment of plant cell viability after $CdCl_2$ addition is very important, in order to achieve promising results for large-scale production on a continuous basis, hence it was analyzed at every stage of elicitation (supporting information Figure S3). The elicitation of *A. indicum* suspensions with 0.5 and 1 mM $CdCl_2$ has not shown any significant influence over cell viability at all tested treatment periods i.e., 24, 48 and 72 h in comparison to the control/unelicited cultures. On the other hand, the elicitation with 2 mM $CdCl_2$ has caused a slight reduction in viability at 24 h ($p = 0.177$), which on further incubation (48 and 72 h) significantly lowered the viability ($p = 0.007$; $p = 0.017$). Similarly, elicitation with 4 mM $CdCl_2$ has significantly diminished the viability at varying treatment periods ($p < 0.001$). Hence, the lower concentrations of $CdCl_2$ i.e., 0.5 – 1 mM, were resourceful in eliciting higher accumulation of Stigmasterol within a short exposure time, i.e., 24 h, without adversely effecting the viability. Since the elicitor addition takes place at the end of log phase i.e., before the suspension reaches its full growth potential, the reduction in cell density is not observed but secondary metabolite concentration might be affected. The effect of elicitors on viability and cell growth could be due to the diversion of metabolic flux from primary metabolism towards secondary metabolism (Sivakumar and Paek 2005). In the present study, increased elicitor exposure duration and concentration has led to a drastic fall in stigmasterol content, which could be a result of elicitor toxicity towards plant cells.

A. indicum suspensions responded well towards lower concentrations of $CdCl_2$ (1 mM) with a prominent increase (2.59-fold) in the content of stigmasterol, when compared to untreated suspension cultures. This higher response of cell

suspensions towards $CdCl_2$, indicates its probable role in induction of cell signaling pathways. The role of Cd^{2+} in initiating a protein phosphorylation cascade by activating the specific signal transduction pathways involved in plant cell responses has been established in a study conducted by Olmos et al. (2003) in tobacco cell suspensions. $CdCl_2$ has been employed in various cell culture studies involving enhancement of secondary metabolites like gymnemic acid from *Gymnema sylvestre* (Ch et al. 2012) and sanguinarine from *Eschscholtzia californica* (Frantisek et al. 2013). The present study reporting elevated levels of stigmasterol from suspension cultures of *A. indicum* after exposure to relatively low concentrations of $CdCl_2$, could pave the way for large-scale production of this therapeutically important phytosterol by using bioreactors.

4. Conclusions

The naturally occurring phytosterol – stigmasterol considered as an equivalent of cholesterol in plants (Burg et al. 2013), can reduce cholesterol absorption thereby lowering the cholesterol levels by 8 – 10% (Franca and Andrea 2010). *A. indicum* suspension cultures proved to be a sustainable platform for production of stigmasterol, which has achieved a further 2.59-fold increase by employing the abiotic elicitor-$CdCl_2$. The suspensions hold promise in becoming a continuous source of the naturally occurring steroidal sapogenin - stigmasterol that can combat hyperlipidemia involved in obesity and cardiovascular health similar to diosgenin, alliin, glycyrrhizin, among others. Plant-based drugs like paclitaxel and docetaxel produced by pharma giants (Phyton and Samyang Genex) on a commercial scale, have become a vital part of modern medicine in the treatment of various cancers involving organs like breast, lung and esophagus (Hemant Sood 2020).

Disclosure statement

All the authors in the manuscript declare that they have no conflict of interest.

Funding details

We are grateful to the University Grants Commission (UGC) for funding the work, in the form of Dr. D.S. Kothari Post Doctoral Fellowship to Dr. Kiranmayee Rao under the grant number [No.F.4-2/2006(BSR)/13-738/2012(BSR)].

ORCID

Kiranmayee Rao https://orcid.org/0000-0002-1313-3043

References

Barbara R, Laura P, Alessandra B, Luisa P. 2009. Plant Cell Cultures: Bioreactors for industrial production. In: Maria TG, Giuseppina R, Bruno B, editors. Bio-Farms for nutraceuticals: Functional food and safety control by biosensors. New York: ©Landes Bioscience and Springer Science + Business Media.

Burg VK, Grimm HS, Rothhaar TL, Grosgen S, Hundsdorfer B, Haupenthal VJ, Zimmer VC, Mett J, Weingartner O, Laufs U, et al. 2013. Plant sterols the better cholesterol in Alzheimer's disease? A mechanistical study. J Neurosci. 33(41):16072–16087.

Canter HP, Thomas H, Ernst E. 2005. Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. Trends Biotechnol. 23(4):180–185.

Ch B, Rao K, Gandi S, Giri A. 2012. Abiotic elicitation of gymnemic acid in the suspension cultures of *Gymnema sylvestre*. World J Microbiol Biotechnol. 28(2):741–747.

Choi SM, Son SH, Yun SR, Kwon OW, Seon JH, Paek KY. 2000. Pilot-scale culture of adventitious roots of ginseng in a bioreactor system. Plant Cell Tiss Organ Cult. 62(3):187–193.

Darzynkiewicz Z, Robinson JP, Crissman HA. 1994. Flow cytometry. 2nd ed. In: Methods in cell biology. Vol 42. San Diego: Academic.

Edilu A, Adane L, Woyessa D. 2015. *In vitro* antibacterial activities of compounds isolated from roots of *Caylusea abyssinica*. Ann Clin Microbiol Antimicrob. 14:15

Franca M, Andrea P. 2010. Phytosterols and cardiovascular health. Pharmacol Res. 61(3):193–199.

Frantisek B, Andrea B, Andrea B, Ivana H. 2013. Effect of abiotic elicitation on the sanguinarine production and polyphenol oxidase activity in the suspension culture of *Eschscholtzia californica* CHAM. Ceska Slov Farm. 62(4):169–173.

Gayathri B, Archana G. 2012. Production of Stigmasterol and Hecogenin from *in vitro* cultures of *Chlorophytum borivilianum*. J Pharmacog. 3(2):101–103.

Hall JL. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot. 53(366):1–11.

Hemant Sood 2020. Production of medicinal compounds from endangered and commercially important medicinal plants through cell and tissue culture technology for herbal industry. In: Sharma K and Mishra K, editors. Bioactive compounds. London: Intechopen.

Hodzic A, Rappolt M, Amenitsch H, Laggner P, Pabst G. 2008. Differential modulation of membrane structure and fluctuations by plant sterols and cholesterol. Biophys J. 94(10):3935–3944.

Jimenez VM. 2005. Involvement of plant hormones and plant growth regulators on *in vitro* somatic embryogenesis. Plant Grow Reg. 47(2):91–110.

Kametani T, Furuyama H. 1987. Synthesis of vitamin D3 and related compounds. Med Res Rev. 7(2):147–171.

Kangsamaksin T, Chaithongyot S, Wootthichairangsan C, Hanchaina R, Tangshewinsirikul C, Svasti J. 2017. Lupeol and stigmasterol suppress tumor angiogenesis and inhibit cholangiocarcinoma growth in mice *via* downregulation of tumor necrosis factor-α. PLoS One. 12(12):e0189628

Kashmiri MA, Yasmin S, Ahmad M, Mohy-Ud-Din A. 2009. Characterization, compositional studies, antioxidant and antibacterial activities of seeds of *Abutilon indicum* and *Abutilon muticum* grown wild in Pakistan. Acta Chim Slov. 56:345–352.

Khan RS, Senthi M, Rao PC, Basha A, Alvala M, Tummuri D, Masubuti H, Fujimoto Y, Begum AS. 2015. Cytotoxic constituents of *Abutilon indicum* leaves against U87MG human glioblastoma cells. Nat Prod Res. 29(11):1069–1073.

Kim SG, Soh WY, Cho DY. 1995. Saikosaponin content in adventitious root formed from callus of *Bupleurum falcatum*. Korean J Plant Tissue Cult. 22:29–33.

Krishna S, Renu S. 2015. Quantitative estimation of phytosterols *in vivo* and *in vitro* from *Cyperus rotundus*. World J Pharm Pharmaceut Sci. 4(02):488–497.

Kuo PC, Yang ML, Wu PL, Shih HN, Thang TD, Dung NX, Wu TS. 2008. Chemical constituents from *Abutilon indicum*. J Asian Nat Prod Res. 10(7-8):699–703.

Lee YL, Sagare AP, Lee CY, Feng HT, Ko YC, Shaw JF, Tsay HS. 2001. Formation of protoberberine-type alkaloids by the tubers of somatic embryo-derived plants of *Corydalis yanhusuo*. Planta Med. 67(9):839–842.

Lin YF, Aarts MGM. 2012. The molecular mechanism of zinc and cadmium stress response in plants. Cell Mol Life Sci. 69(19):3187–3206.

Malik S, Cusido RM, Mirjalili MH, Moyano E, Palazon J, Bonfill M. 2011. Production of the anticancer drug taxol in *Taxus baccata* suspension cultures: A review. Process Biochem. 46(1):23–34.

Mithilesh S, Rakhi C. 2010. Improved clonal propagation of *Spilanthes acmella* Murr. for production of scopoletin. Plant Cell Tiss Organ Cult. 103(2):243–253.

Mohite MS, Shelar PA, Raje VN, Babar SJ, Sapkal RK. 2012. Review on Pharmacological Properties of *Abutilon indicum*. Asian J Pharmaceut Res. 2(4):156–160.

Mouritsen OG, Zuckermann MJ. 2004. What's so special about cholesterol?Lipids. 39(11):1101–1113.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 15(3):473–497.

Olmos E, Martinez-Solano JR, Piqueras A, Hellin E. 2003. Early steps in the oxidative burst induced by cadmium in cultured tobacco cells (BY-2 line). J Exp Bot. 54(381):291–301.

Panda S, Jafri M, Kar A, Meheta BK. 2009. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. Fitoterapia. 80(2):123–126.

Piironen V, Lindsay DG, Miettinen TA, Toivo J, Lampi AM. 2000. Plant sterols: biosynthesis, biological function and their importance to human nutrition. J Sci Food Agric. 80(7):939–966.

Piqueras A, Olmos E, Martínez-Solano JR, Hellín E. 1999. Cd-induced oxidative burst in tobacco BY2 cells: time course, subcellular location and antioxidant response. Free Radical Res. 31(sup1):33–38.

Porchezhian E, Ansari SH. 2005. Hepatoprotective activity of *Abutilon indicum* on experimental liver damage in rats. Phytomedicine. 12(1-2):62–64.

Pratibha C, Abhay C. 2014. Stigmasterol enhancement in *Tylophora indica* (*Asclepeadaceae*) callus. The J Bioproc Technol. 99:344–349.

Raemakers CJJM, Jacobsen E, Visser RGF. 1995. Secondary somatic embryogenesis and applications in plant breeding. Euphytica. 81(1):93–107.

Ramar K, Ayyadurai V. 2015. The present investigation deals with *in vitro* callus induction and plant regeneration of *Abutilon indicum* (L.). J Pharmacog Phytochem. 3(6):248–251.

Rao K, Chodisetti B, Gandi S, Giri A, Kishor PB. 2016. Regeneration-based quantification of coumarins (Scopoletin and Scoparone) in *Abutilon indicum in vitro* cultures. Appl Biochem Biotechnol. 180(4):766–779.

Roshan S, Ali S, Khan A, Tazneem B, Purohit MG. 2008. Wound healing activity of *Abutilon indicum*. Pharmacog Mag. 4(15):85–88.

Seetharam YN, Chalageri G, Setty SR. Bheemachar 2002. Hypoglycemic activity of *Abutilon indicum* leaf extracts in rats. Fitoterapia. 73(2):156–159.

Shohael AM, Khatun SM, Alam MF, Paek KY. 2013. Effects of Murashige and Skoog medium strength on germination and secondary metabolites production of *Eleutherococcus senticosus's* somatic embryos in bioreactor. Int J Biosci. 3(3):155–163.

Sivakumar G, Paek KY. 2005. Methyl jasmonate induce enhanced production of soluble biophenols in *Panax ginseng* adventitious roots from commercial scale bioreactors. Chem Nat Compd. 41(6):669–673.

Sundararaman P, Djerassi C. 1977. A convenient synthesis of progesterone from stigmasterol. J Org Chem. 42 (22):3633–3634.

Vanwyk BE, Wink M. 2004. Medicinal plants of the world. 1st ed.Pretoria, South Africa: Briza Publications.

Verpoorte R, Contin A, Memelink J. 2002. Biotechnology for the production of plant secondary metabolites. Phytochem Rev. 1(1):13–25.

von Aderkas P, Bonga JM. 2000. Influencing micropropagation and somatic embryogenesis in mature trees by manipulation of phase change, stress and culture environment. Tree Physiol. 20(14):921–928.

Wu J, Wang C, Mei X. 2001. Stimulation of taxol production and excretion in *Taxus* spp cell cultures by rare earth chemical lanthanum. J Biotech. 85(1):67–73.

Zenk MH, El-Shagi H, Schulte U. 1975. Anthraquinone production by cell suspension cultures of *Morinda citrifolia*. Planta Med. 28(S 01):79–101.