Sodium Nitroprusside Enhances Biomass And Gymnemic Acids Production In Cell Suspension of *Gymnema Sylvestre* (Retz.) R.Br. Ex. Sm.

Ganesan Mahendran
CSIR-CIMAP: Central Institute of Medicinal and Aromatic Plants CSIR
Deepak Kumar
CSIR-CIMAP: Central Institute of Medicinal and Aromatic Plants CSIR
Sanjeet Kumar Verma
CSIR-CIMAP: Central Institute of Medicinal and Aromatic Plants CSIR
Ashish Chandran
CSIR-CIMAP: Central Institute of Medicinal and Aromatic Plants CSIR
Zafar Iqbal Warsi
CSIR-CIMAP: Central Institute of Medicinal and Aromatic Plants CSIR
Zakir Hussain
CSIR-CIMAP: Central Institute of Medicinal and Aromatic Plants CSIR
Prasant Kumar Rout
CSIR-CIMAP: Central Institute of Medicinal and Aromatic Plants CSIR
Laiq Ur Rahman (l.rahman@cimap.res.in)
CIMAP

Original Article

Keywords: Suspension culture, Elicitation, Medicinal plant, Secondary metabolites

Posted Date: February 4th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-172209/v1

Version of Record: A version of this preprint was published at Plant Cell, Tissue and Organ Culture (PCTOC) on March 28th, 2021. See the published version at https://doi.org/10.1007/s11240-021-02058-7.

Abstract

Gymnema sylvestre (Retz.) R.Br. ex Sm. is widely used as an efficient Ayurvedic traditional medicine for the treatment of diabetes. Phytochemical investigations of this plant showed gymnemic acids (a group of triterpenoid saponins) as the main active components. The present study aims to investigate the effectiveness of sodium nitroprusside (SNP) treatment for enhancement of cell suspension culture biomass and to evaluate their deacylgymnemic acid, gymnemagenin, gymnemic acid IV and gymnemic acid XVII contents of *G. sylvestre*. Callus was obtained from *in vitro* derived leaves of *G. sylvestre* on MS medium fortified with 3.0 mg/L 2, 4-D (2, 4-dichlorophenoxyacetic acid) and 2.0 mg/L Kn (Kinetin), and the same were used further to produce cell suspension cultures. Cell suspensions were exposed to different concentrations of SNP (5, 10, 20 and 40 µM) and data were collected at 20, 30 and 40 days. Out of the tested concentrations, 20 µM SNP had the highest level of cell culture growth (398.94 ± 8.32 g/L FCW and 40.00 ± 0.75 g/L DCW) on 40-day as compared to control. High-performance liquid chromatography analysis showed that maximum accumulation of deacylgymnemic acid (5.51 mg/g DCW), gymnemagenin (2.80 mg/g DCW) and gymnemic acid XVII (2.08 mg/g DCW) in 20 µM SNP treatment which is (13.43, 13.86 and17.33 folds) higher than the respective control at 40 days exposure. This research suggests that *G. sylvestre* cell suspension culture with optimal SNP elicitation treatment could be used as a good strategy for the large-scale production of these secondary metabolites at the industrial level.

Introduction

Metabolic disorders have emerged as a major health problem around the world, as reported by the WHO. Diabetes and obesity are categories of metabolic disorders that are characterized by high blood sugar levels associated with carbohydrate and fat metabolism. Diabetes and obesity are a leading cause of mortality worldwide, with an estimated 422 million people worldwide and estimated to be seventh leading cause of death by 2030 (Hossain et al., 2020). WHO has estimated that diabetes deaths will rise by two-thirds between 2008 and 2030. Clinically, diabetes is related to the inability of beta cells to produce enough pancreatic hormone insulin. Research into effective antidiabetic agents to prevent this disease has fuelled extensive research worldwide on new therapeutic drug discovery strategies targeting notorious metabolic characteristics antidiabetic products (Pappahan et al., 2019; Rasouli et al., 2020). On this basis, many plant secondary metabolites and their semi-synthetic analogues have found significant roles for the management of diabetics because of their structural diversity and their ability to overcome multiple metabolic disorders (Tabatabaei-Malazy et al., 2015). In this post-genomic period, the use of plant-based natural active phytochemicals in the production of antidiabetic drug development phase have become more refined through a better understanding of their structure-based high-throughput metabolic targets (Vieira et al., 2019; Pappachan et al., 2019; Jugran et al., 2020; Artasensi et al., 2020).

The most practised antidiabetic drug development process chiefly remained engrossed in searching for novel drugs focus on glucose-lowering level by defining their mechanisms of action and site-specific binding behaviour amongst phytochemicals (Belete, 2020). Diverging research efforts around the world

have led to the successful launch of the hypoglycaemic agent such as insulin secretagogues, insulin sensitization and inhibitors of glucose-absorbing drugs in the gastrointestinal tract as potential antidiabetic drugs for the direct treatment of different types of diabetics (Pappachan et al., 2019; Belete, 2020). However, over the years, the accuracy of these anti-hyperglycemic drugs or metabolites in managing or preventing diabetes has remained difficult to achieve because of the trend in glucose metabolism (Belete, 2020). Whereas managing with these difficulties in treatment of diabetes, the restorative benefits of gymnemic acids, an antisweet and most promising molecule of naturally occurring triterpenoid saponin glycosides, have picked up noticeable quality through the disclosure of their inalienable gut glucosidase inhibition capacity connected to its generally remarkable blood-glucose-lowering effect and initiate insulin release from β-cells (Alkefai et al., 2019; Saha and Pal, 2020).

Gymnema sylvestre (Retz.) R.Br. ex Sm. belongs to the family Asclepiadaceae and has been used in Ayurveda medicine since antiquity and used to treat diabetes, malaria and snake bite (Anjum and Hasan, 2013; Khan et al., 2019). In Ayurveda, Ayurvedic practitioner prescribed this plant for the treatment of dyspepsia, jaundice, asthma, leucoderma and bronchitis (Anis et al., 2000; Laha and Paul, 2019; Khan et al., 2019). Kanetkar et al. (2007) and Singh et al. (2008) found multiple potential applications mentioned in Ayurveda, Siddha and Unani system of medicine in India for treating diverse human complaints, but only a few achieved scientific information on its secondary metabolites production. *G. sylvestre* contains many phytomolecules among them GAs and gymnemasaponins are major secondary metabolites and are classified as oleanane saponins (triterpenoid saponins) for clinical application (Laha and Paul, 2019; Khan et al., 2019).

It was found that 18 types of gymnemic acids (GAs) isolated and confirmed from G. sylvester leaves block the receptor site and prevent absorption of glucose into the intestine, thus reducing blood glucose levels (Khan et al., 2019; Laha and Paul, 2019; Tran et al., 2020). Published reports indicated that oral administration of GA-IV to 13.4 mg/kg body weight decreased blood sugar by 60.0% (Sugihara et al., 2000). GA-IV (150 mg/kg) was administered to high fat and fructose diabetic rats to restore antioxidant levels in the liver and kidneys (Karthikeyan et al., 2020). Recently, oral administration of GAs to diabetic rats at 5 mg/kg body weight has shown a significant decrease in blood sugar and increased levels of insulin in plasma and haemoglobin (Mahadeva Rao et al., 2020). Oral administration of 200 mg/kg deacyl gymnemic acid significantly improved the fast plasma glucose level and insulin resistance. Arylated gymnemic acids and olean-15-ene type gymnemic acids showed α-glucosidase inhibition (Alkefai et al., 2018 and 2019). GAs improve insulin release and lower blood lipid concentrations (Barbara et al., 2015). Literature suggests that GA XVII at 0.5 M led to block the sweet taste induced by 0.4 M sucrose and GA IV at A 1.0 mM conquest of sweetness induced by 0.4 mM sucrose (Kazujo et al., 1989; Yoshikawa et al., 1992). GA IV showed inhibitory activity against glucose uptake (Masuyuki et al., 1997).

Plant cell and tissue cultures ensured that cell suspension cultures have been explored to serve as an alternative source for large scale production of useful secondary metabolites (Thorat et al., 2017). Also, accumulation of secondary metabolite in *in vitro* cultures outperformed those seen in plants cultivated in the field (Das and Bandyopadhyay, 2020). Accumulation of secondary phytoconstituents in cell

suspension or callus culture is highly dependent on different types of elicitors which lead to activate biosynthetic pathway route key enzymes (Sharan et al., 2019; Pandey et al., 2020).

The recent studies showed that signalling molecules involved in the regulation of plant metabolite synthesis include reactive oxygen species, nitric oxide (NO), calcium ion, polyamines (PAs) salicylic acid and jasmonic acid (Ma et al., 2013). Sodium nitroprusside (SNP), highly reactive bioactive molecule that plays an important role in plant tissue and organ culture such as growth and development of plant (Kolbert et al., 2008), stimulates callogenesis regeneration response in *Albizzia lebbeck* (Kalra and Babbar, 2010), enhances callus and multiple shoot induction (Sarropoulou and Maloupa 2017; Subiramani et al., 2019; Pandey et al., 2020), promote root formation and callus induction (Hesami et al., 2020) and enhanced shoot regeneration and improved salinity stress in soybean (Karthik et al., 2019). Ötvös et al. (2005) reported that SNP in combination with 2,4-D significantly stimulated cell division and embryogenic cell growth in *Medicago sativa*. In *Hyoscyamus niger*, supplementation of SNP at 50 µM increased callus fresh weight (Samsampour et al., 2018). As a result, this led us to study the effect of sodium nitroprusside (SNP) in secondary metabolites production from suspension culture of *G. sylvestre* for enhancement of deacylgymnemic acid, gymnemagenin, gymnemic acid IV and gymnemic acid XVII metabolites.

Material And Methods

Resource of plant material and culture condition

In this study, *Gymnema sylvestre* immature follicles were obtained from the field of CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow. The immature follicles were sterilized by our previous experiment (Mahendran et al., 2021). The immature embryo was inoculated on MS (Murashige and Skoog, 1962) basal medium with sucrose (3.0%) and agar (0.8%) to initiate aseptic seedlings. The seedlings were maintained in cooling white fluorescent light (60 µmol m^{-2} s^{-1}) with light/dark (16/8 h) of photoperiod at 25 ± 2°C. *In vitro* derived young leaves were utilized as explants for the establishment of callus and cell suspension culture of *G. sylvestre*.

Chemicals

Murashige and Skoog (1962) medium, 2,4-Dichlorophenoxyacetic, (2,4-D), Kinetin (Kn), and Sodium nitroprusside (SNP) were purchased plant tissue culture grade from Hi-media (Mumbai, India). Deacylgymnemic acid, gymnemagenin, gymnemic acid IV and gymnemic acid XVII standards were supplied by Sigma-Aldrich (Münich, Germany). HPLC grade methanol and acetonitrile obtained from Merck (Mumbai, India) and ultra-pure water was prepared using a Milli-Q (Millipore, France) was used.

Cell suspension cultures

In the present study, cell suspension cultures initiated from 30 days old whitish friable callus (1 g) that was inoculated into 50 mL of MS liquid medium supplemented with 2,4-D (3 mg / L) and Kn (2.0 mg /L) combination. The cultures were incubated on a shaker (100 rpm) at 25 ± 2 °C for 16/8 h (light/dark).

Treatment of culture with Sodium nitroprusside

In the present investigation, SNP was dissolved in distilled sterile water and sterilized through syringe filters then used at different concentrations (5, 10, 20 and 40 µM). SNP supplemented on the same day of cell suspension initiated and the cells were harvested at 20, 30 and 40 days. Cell suspension culture was collected from the medium at 20, 30 and 40 days for determination of cell growth biomass accumulation (fresh and dry cell weight) and yield of GAs contents.

Analysis of cell suspension biomass

At the end of each culture intervals, the cell suspension was harvested from the culture medium for fresh cell weight and dry cell weight measurement. Harvested cells passed through a two-layer muslin cloth to remove the water from the cell and measured the fresh cell biomass weight. Further, cells biomass was placed on a Petri dish and dried at 50 °C in a hot air oven for 24 h and cell dry biomass measured.

Morphology of suspension cells

100 mg cells from suspension cell cultures were checked in 1 mL 0.005% (w/v) neutral red and acridine orange solution to analysis the morphological variations during growth phases. 30 µL of stained cells were transferred to the slide and observed under the light microscope (Olympus, India).

Sample and Standards Stock preparation

The dried biomass obtained from cell culture and wild plant leaves were grounded into powder. Then, 1 g of sample was extracted in 10 mL of petroleum ether for defatting at 50ºC for 6 h and dried at room temperature. The defatted sample was then extracted using 70:30 (Water: Methanol) at 50ºC for 6 h for complete extraction of gymnemic acids. The extract was dried using rotatory evaporator and kept at 4ºC until further use.

Deacylgymnemic acid, gymnemagenin, gymnemic acid IV and gymnemic acid XVII standard stock solutions were diluted separately at a concentration of 1 mg/mL in methanol. 10 mg of each of the above prepared extract was weighed accurately and dissolved in methanol (1 mL) for making final concentration of 10 mg/mL and kept 15 min for sonication and filtered on a 4 mm membrane filter. 20 µL of injection volume of sample was injected into the HPLC system.

Quantification of deacylgymnemic acid, gymnemagenin, gymnemic acid IV and gymnemic acid XVII

A newly modified gradient method was developed for the quantification of Bio-markers present in the sample. Waters HPLC system built with a binary pump, photodiode array detector (PDA) and Rheodyne injector. *SunFire C18* column (5 µm, 250 × 4.6 mm) and Empower Pro software (Waters, USA) was used for analysis. The detection was achieved at 205 nm, column temperature kept at 30ºC and the flow rate was 1 mL/min. The deacylgymnemic acid, gymnemagenin, gymnemic acid IV and gymnemic acid XVII contents were quantified by according to Mahendran et al. (2021).

Statistical analysis

Each experiment was repeated thrice and data expressed in terms of mean ± standard deviation (SD). The significant difference between control and SNP treatment was analysed by Tukey's HSD (honestly significant difference) test using one-way ANOVA with IBM SPSS statistics (version 20.0, USA). Significant was considered when $P < 0.05$.

Results And Discussion

Initiation and growth of cell suspension cultures

Nitric oxide (NO) is a bioactive molecule which can be generated through the breakdown of NO donor sodium nitroprusside molecules (SNP). Recently, SNP has been reported as an elicitor involved in regulating several signalling responses (Siddiqui et al., 2017; Khezerluo et al., 2018; Hao et al., 2020; Kara et al., 2020). In previous studies, many researchers have reported that SNP treatments are very effective in shoot regeneration of *Chrysanthemum* cultivars (Arun et al., 2017), *Canscora diffusa* (Subiramani et al., 2019), *Antirrhinum majus* (Rezaei Zafarghandi and Rahmati Joneidabad 2020) *Valeriana jatamansi* (Pandey et al., 2020) and callus induction in *Dioscorea opposita* (Xu et al., 2009), *Hyoscyamus niger* (Samsampour et al., 2018) *Ficus religiosa* (Hesami et al., 2019) *Canscora diffusa* (Subiramani et al., 2019) and *Valeriana jatamansi* (Pandey et al., 2020), Furthermore, SNP play important roles in increasing metabolites accumulation (Khezerluo et al., 2018; Pradhan et al., 2020; Tsolmon et al., 2020).

A perusal of literature revealed that cell suspension culture has to be the most capable method used to increase the cell growth, biomass and secondary metabolite production *in vitro* cultures (Mahendran et al., 2018; Hu et al., 2019; Açıkgöz, 2020). In this study, white friable callus obtained from 3.0 mg/L (2, 4-D) + 1.0 mg/L (Kn) (Fig. 1A & B), and same combination was used to establish a culture of cell suspension in which there were no aggregations or clumps of cells noted. In the present study, suspension cell culture growth kinetic was monitored in terms of fresh cell weight (FCW) and dry cell weight (DCW) and results are shown in Fig. 2. Cell suspension growth was shown to be increased greatly with the increasing concentration of SNP. Among the different concentrations of SNP tested, the highest

accumulation of FCW and DCW were 71.21 ± 1.56 g/L and 7.05 ± 0.04g/L observed on 20th day with 20 µM SNP that were 1.43 and 1.41 times higher compared with control. The biomass accumulation in cell culture increased with increasing concentration of SNP up to an optimal level of 20 µM (Fig. 1). SNP has increased the growth of cell culture biomass at 5–20 µM concentrations tested and the entire exposure period of the study compared with the control. The maximum amount of biomass accumulation was achieved at 40th day with 20 µM SNP (398.94 ± 8.32 g/L FCW and 40.00 ± 0.75 g/L DCW) and was nearly 1.17 folds higher than control (Fig. 1C-F). Earlier studies demonstrated that elicitors are very effective for the enhancement of cell growth and increase metabolites accumulation. Furthermore, the type of elicitors, concentration and exposure period favours the biomass accumulation in cell suspension culture (Salma et al., 2018; Zare-Hassani et al., 2019; Açıkgöz, 2020).

Our study confirms the efficiency of SNP treatments, promoted cell growth and biomass accumulation. Similar effectiveness of SNP on cell growth has been reported in *Hypericum perforatum* (Xu et al., 2005). Furthermore, SNP in combination with 2,4-D significantly promoted embryogenic cell in *Medicago sativa* (Ötvös et al., 2005). Some studies have shown that SNP either alone or in combination treatments are very effective for callus induction (Samsampour et al., 2018; Subiramani et al., 2019; Pandey et al., 2020). Results of the present investigation in *G. sylvestre* cell suspension cultures suggested that SNP at 40 µM drastically diminished cell growth and biomass accumulation (225.39 ± 5.13 g/L FCW) compared with control (339.08 ± 1.76 g/L FCW) at 40 days. Similar inhibitory effects have been reported at higher concentrations of elicitors in *Psoralea corylifolia* (Gajula et al., 2018), *Leucas aspera* (Vijendra et al., 2020) and *Ocimum basilicum* (Açıkgöz, 2020).

Morphology of G. sylvestre cell suspension

The morphological differences at different growth times in the suspension cultures are shown in Fig. 1C-F. The cells were rapidly growing and individually or in cell masses of small groups of cells aggregated and settled at the bottom of the flask were detected in the suspension culture (Fig. 1C-F). The shape of the cells varied in size with round, oval shape or elongated shaped and health of cell visible clearly nuclei and cell components (Fig. 1G & J). In contrast, death cells showed cytoplasm and cell wall shrinkage (Fig. 1H, I and L).

Hplc Analysis Of Gymnemic Acids

To enhance the deacylgymnemic acid, gymnemagenin, gymnemic acid IV and gymnemic acid XVII content in suspension culture of *G. sylvestre*, different concentrations of SNP (5, 10, 20 and 40 µM) and days (20, 30 and 40) were investigated. The results displayed that SNP had significantly boosted the production of deacylgymnemic acid, gymnemagenin, gymnemic acid IV and gymnemic acid XVII at all tested concentration over control. However, SNP at 20 µM dose produced the highest accumulation of deacylgymnemic acid (1.80 mg/g DCW), gymnemagenin (1.91 mg/g DCW), gymnemic acid IV (0.74 mg/g DCW) and gymnemic acid XVII (1.14 mg/g DCW) contents for 20 days of exposure in cell suspension culture, which was represented 6.09, 17.36, 1.01 and 9.5 folds higher compared with the

control cultures (Fig. 3A-D). Similarly, Xu et al. (2005) demonstrated that cell cultures of *Catharanthus roseus* treated with 10 and 20 mM SNP enhanced formation of catharanthine, ajmalicine and total alkaloids. Khezerluo et al. (2018) found that maximum accumulation of hyoscyamine and scopolamine production (1.2-fold and 1.5-fold) in hairy root culture of *Hyoscyamus reticulatus* was detected at 50 and 100 µM SNP at 48 and 24h.

Interestingly, higher content of gymnemagenin (2.86 mg/g DCW) and gymnemic acid IV (1.24 mg/g DCW) was also observed at 5 µM of SNP treated suspension cell culture at 30 and 40 days of culture respectively (Fig. 4B and 5C). Likewise, the maximum 3.25 mg/g gymnemic acid XVII production was observed at 10 µM SNP at 40 days (Fig. D). Similarly, Wang et al. (2009) reported that SNP (10 µM) and cerebroside (30 µg/mL) combination in hairy root callus of *Artemisia annua* exhibited more effective for improving artemisinin production up to 2.3-fold over the control. In another study, researchers reported that lower concentrations of SNP (15 µM) have been more effective in enhancing catharanthine production in suspension cells culture of *C. roseus* (Xu and Dong, 2005). Likewise, the maximum 3.2-fold hypericin production in suspension culture was observed at 15.0 mmol/L SNP at 14 days (Xu et al., 2005).

In the present study, SNP at 20 µM treatment at 30 days resulted in the highest deacylgymnemic acid (2.32 mg/g DCW) Fig. 4A, whereas 40 µM SNP treatment at 30 days cell suspension culture period produced maximum of gymnemagenin (2.53 mg/g DCW), gymnemic acid IV (1.24 mg/g DCW) and gymnemic acid XVII (1.90 mg/g DCW) contents over control and 20 µM SNP (Fig. 4. B, C & D). Nevertheless 20 µM SNP treatment yielded higher 13.43 (5.51 mg/g DCW) fold deacylgymnemic acid (Fig. 5A), 13.86 fold (2.80 mg/g DCW) gymnemagenin (Fig. 5B) and 17.33 fold (2.08 mg/g DCW (Fig. 5D, Fig. 6A-C) gymnemic acid XVII production in *G. sylvestre* cell suspension cultures compared with respective control, however, this deacylgymnemic acid, gymnemagenin and gymnemic acid XVII yield was found to be 3.11, 1.78 and 1.18 fold lesser at SNP (40 µM) treated cultures compared with respective SNP 20 µM. In contrast to this study, Tsolmon et al. (2020) in their investigation of *Sophora alopecuroides* cell suspension culture, reported that accumulation of oxymatrine content was 18.6 µg/g higher in suspension culture treated at higher 200 µM Jasmonic acid (JA) and 50 µM SNP combination. The ginsenoside accumulation was recorded highest at 200 µM of SNP treatment (Rahimi et al., 2016).

Conclusion

In conclusion, this work suggested that the effects of SNP treatment approach improved cell culture growth (biomass) and yield of gymnemic acids (triterpenoid saponins) content in cell suspension cultures of *G. sylvestre*. 20 µM SNP treatment and exposure time of 40 days showed the highest rate of cell culture biomass accumulation (398.94 ± 8.32 g/L FCW and 40.00 ± 0.75g /L DCW) and the maximum production of deacylgymnemic acid (5.51 mg/g DCW), gymnemagenin (2.80 mg/g DCW) and gymnemic acid XVII (2.08 mg/g DCW) compared with the control culture. The present study has demonstrated that establishment of efficient cell suspension cultures of *G. sylvestre* for biomass and enhanced production of triterpenoid saponins (gymnemic acids) at industrial scale.

Declarations

Acknowledgements

First author gratefully acknowledges the financial support by SERB, New Delhi, India for awarding DST-SERB-National Post-Doctoral Fellowship (PDF/2017/000368). The authors are also thankful to the Director, CSIR-CIMAP for providing necessary facilities.

Author contributions: GM: designed the experiment, performed tissue culture experiments, contributed to writing and corrected manuscript. DK: designed the experiment, performed HPLC experiments and analysed data. SKV: performed tissue culture experiments, analysed data, reviewed and edited manuscript. AC, ZIW and ZH: performed tissue culture experiments. PKR: conceptualization, methodology, formal analysis and editing, LR: conceptualization, supervision, funding acquisition, review, editing and improved the manuscript.

Compliance with ethical standards

Conflict of interest: All authors declare that there is no conflict of interest.

References

Açıkgöz MA (2020) Establishment of cell suspension cultures of *Ocimum basilicum* L. and enhanced production of pharmaceutical active ingredients. Ind Crop Prod 148:112278

Alkefai NH, Ahamad J, Amin S, Mir SR (2018) Arylated gymnemic acids from *Gymnema sylvestre* R.Br. as potential α-glucosidase inhibitors. Phytochem Lett 25:196–202

Alkefai NH, Sharma M, Ahamad J, Amin S, Mir SR (2019) New olean-15-ene type gymnemic acids from Gymnema sylvestre (Retz.) R.Br. and their antihyperglycemic activity through α-glucosidase inhibition. Phytochem Lett 32:83–89

Anis M, Sharma MP, Iqbal M (2000) Herbal ethnomedicine of the Gwalior forest division in Madhya Pradesh, India. Pharm Biol 38(4):241–253

Anjum T, Hasan Z (2013) *Gymnema Sylvestre* plant used by peoples of Vidisha district for the treatment of diabetes. Int J Eng Sci Invent 2(6):98–102

Artasensi A, Pedretti A, Vistoli G, Fumagalli L (2020) Type 2 diabetes mellitus: a review of multi-targeted drugs. Molecules 25:1987

Arun M, Naing AH, Jeon SM, Ai TN, Aye T, Kim CK (2017) Sodium nitroprusside stimulates growth and shoot regeneration in *Chrysanthemum*. Hortic Environ Biotechnol 58:78–84

Barbara R, Festa C, Marino SD, Micco SD, D'Auria MV, Bifulco G, Fiorucci S, Zampella A (2015) Molecular decodification of gymnemic acids from *Gymnema Sylvestre*. discovery of a new class of liver X receptor antagonists. Steroids 96:121–131

Belete TM (2020) A recent achievement in the discovery and development of novel targets for the treatment of type-2 diabetes mellitus. J Exp Pharmacol 12:1–15

Das D, Bandyopadhyay M (2020) Novel approaches towards overproduction of andrographolide in vitro seedling cultures of *Andrographis paniculata*. S Afr J Bot 128:77–86

Gajula H, Kumar V, Vijendra PD, Rajashekar J, Sannabommaji T, Basappa G (2018) A combination of elicitor and precursor enhances psoralen production in *Psoralea corylifolia* Linn. suspension cultures. Ind Crop Prod 124:685–691

Hao YJ, An XL, Sun HD, Piao XC, Gao R, Lian ML (2020) Ginsenoside synthesis of adventitious roots in *Panax ginsengis* promoted by fungal suspension homogenate of *Alternaria panax* and regulated by several signalling molecules. Ind Crops Prod 150:112414

Hesami M, Tohidfar M, Alizadeh M, Daneshvar MH (2020) Effects of sodium nitroprusside on callus browning of *Ficusreligiosa*: an important medicinal plant. J For Res 31(3) :789–796

Hossain H, Das AK, Ghosh S, Sil PC (2020) An overview on the role of bioactive α-glucosidase inhibitors in ameliorating diabetic complications. Food Chem Toxicol 145:111738

Hu M, Hu Z, Du L, Du J, Luo Q, Xiong J (2019) Establishment of cell suspension culture of *Lonicera japonica* Thunb. and analysis its major secondary metabolites. Ind Crops Prod 137:98–104

Jugran AJ, RawatS, Devkota HP, Bhatt ID, Rawal, RS (2020) Diabetes and plant-derived natural products: From ethnopharmacological approaches to their potential for modern drug discovery and development. Phytother Res doi: 10.1002/ptr.6821.

Kalra C, Babbar SB (2010) Nitric oxide promotes *in vitro* organogenesis in *Linum usitatissimum* L. Plant Cell Tiss Organ Cult 103:353–359

Kanetkar P, Singhal R, Kamat M (2007) *Gymnema sylvestre* : a memoir. J Clin Biochem Nutr 41(2):77–81

Kara, Z., Yazar, K., Doğan, O, Vergili E (2020) Sodium nitroprusside and gibberellin effects on seed germination and seedling development of grapevine (*Vitis vinifera* L.) Cvs. Ekşi Kara and Gök Üzüm. *Erwerbs-Obstbau* 62:61–68

Karthik S, Pavan G, Krishnan V, Sathish S, Manickavasagam M (2019) Sodium nitroprusside enhances regeneration and alleviates salinity stress in soybean [*Glycine max* (L.) Merrill]. Biocat Agric Biotechnol 19:101173

Karthikeyan P, Chakrapani LN, Mohan T, Tamilarasan B, Kannan P, Periandavan K (2020) Gymnemic acid, a potent antidiabetic agent protects skeletal muscle from hyperglycemia mediated oxidative stress and apoptotice vents in high fat and high fructose diet fed adult rats. Int J Res Pharm Sci 11(2):1526–1538

Kazujo Y, KayokoA, Shigenobu A, Kouji M (1989) Gymenmic acids V, VI and VII from Gurma, the leaves of *Gymnema sylvestre* R. Br. Chem Pharm Bull 37:852–854

Khan F, Sarker MMR, Ming LC, Mohamed IN, Zhao C, Sheikh BY, Tsong HF, Rashid MA (2019) Comprehensive review on phytochemicals, pharmacological and clinical potentials of *Gymnema sylvestre*. Front Pharmacol 10:1223

Khezerluo M, Hosseini B, Amiri J (2018) Sodium nitroprusside stimulated production of tropane alkaloids and antioxidant enzymes activity in hairy root culture of *Hyoscyamus reticulatus* l. Acta Biol Hung 69(4):437–448

Kolbert Z, Bartha B, Erdei L (2008) Exogenous auxin-induced NO synthesis is nitrate reductase-associated in *Arabidopsis thaliana* root primordia. J Plant Physiol 165:967–975

Laha S, Paul S (2019) *Gymnema sylvestre* (Gurmar): A potent herb with anti-diabetic and antioxidant potential. Pharmacog J 11:201–206

Li M, Peebles CAM, Shanks JV, San K-Y (2011) Effect of sodium nitroprusside on growth and terpenoid indole alkaloid production in Catharanthus roseus hairy root cultures. Biotechnol Prog 27:625–630

Ma MM, Zhan YG, Wang XD, Sun ML, Fan GZ (2013) Interaction between putrescine and hydrogen peroxide on regulating triterpenoid production in *Betula platyphylla*. Chinese Tradit Herb Drugs 44:2916–2922

Mahadeva Rao US, Shanmuga Sundaram C, Srinivasan S (2020) Gymnemic acid mitigates hyperglycemia by attenuating the hepatic glucose metabolic enzymes in high fat diet fed-low dose streptozotocin-induced experimental rodents. *Research J* Pharm Tech 13(20):719–726

Mahendran D, Kavi Kishor PB, Sreeramanan S, Venkatachalam P (2018) Enhanced biosynthesis of colchicine and thiocolchicoside contents in cell suspension cultures of *Gloriosa superba* L. exposed to ethylene inhibitor and elicitors. Ind Crops Prod 120:123–130

Mahendran G, Iqbal Z, Kumar D, Verma SK, Rout PK, Rahman L (2021) Enhanced gymnemic acids production in cell suspension cultures of *Gymnema sylvestre* (Retz.) R.Br. ex Sm. through elicitation. Ind Crop Prod 162: 113234INDCRO-D-20-05229R1 INDCRO-D-20-05229R1

Masuyuki Y, Toshiyuki M, Hisashi M (1997) Medicinal foodstuffs. X. Structures of new triterpene glycosides, Gymnemosides-c, -d, -e, and -f, from the leaves of *Gymnema sylvestre* R. Br.: Influence of Gymnema glycosides on glucose uptake in rat small intestinal fragmentes. Chem Pharm Bull 45:2034–2038

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:173–197

Ötvos K, Pasternak TP, Miskolczi P, Domoki M, Dorjgotov D, Szucs A, Bottka S, Dudits D, Feher A (2005) Nitric oxide is required for, and promotes auxin-mediated activation of, cell division and embryogenic cell formation but does not influence cell cycle progression in *Alfalfa* cell cultures. Plant J 43:849–860

Pandey S, Sundararajan S, Ramalingam S, Pant B (2020) Effects of sodium nitroprusside and growth regulators on callus, multiple shoot induction and tissue browning in commercially important *Valeriana jatamansi* Jones. Plant Cell Tiss Organ Cult 142:653–660

Pappachan JM, Fernandeza CJ, Chacko EC (2019) Diabesity and antidiabetic drugs. Mol Asp Med 66:3–12

Pradhan N, Singh P, Dwivedi P, Pandey DK (2020) Evaluation of sodium nitroprusside and putrescine on polyethylene glycol induced drought stress in *Stevia rebaudiana* Bertoni under in vitro condition. Ind Crops Prod 154:112754

Rahimi S, Kim, YJ, Renuka Devi, BS, Oh, JY, Kim, SY, Kwon WS, Yang DC (2016) Sodium nitroprusside enhances the elicitation power of methyl jasmonate for ginsenoside production in Panax ginseng roots. Res Chem Intermed 42:2937–2951

Rasouli H, Yarani R, Pociotc F, Popović-Djordjević J (2020) Anti-diabetic potential of plant alkaloids: Revisiting current findings and future perspectives. Pharmacol Res 155:104723

Rezaei Zafarghandi MS, Rahmati-Joneidabad M (2020) Indirect shoot organogenesis and in vitro root formation of *Antirrhinum majus* L. by using of sodium nitroprusside. Adv Hortic Sci 34(1):105–111

Saha S, Pal D (2020) Gymnemic Acids: Sources, Properties, and Biotechnological Production. In Swamy MK (ed) Plant-derived Bioactives. Springer Nature, Singapore, pp 177–193

Salma U, Kundu S, Ali Md N, Nirma Mandal N (2018) Elicitor mediated enhancement of wedelolactone in cell suspension culture of *Eclipta alba* (L.) Hassk. Plant Cell Tiss Org Cult 134:409–421

Samsampour D, Sadeghi F, Asadi M, Ebrahimzadeh A (2018) Effect of nitric oxide (NO) on the induction of callus and antioxidant capacity of *Hyoscyamus niger* under in vitro salt stress. J Appl Bot Food Qual 91:24–32

Sarropoulou V, Maloupa E (2017) Effect of the NO donor sodium nitroprusside (SNP), the ethylene inhibitor cobalt chloride ($CoCl_2$) and the antioxidant vitamin E "α-tocopherol"on *in vitro* shoot proliferation of *Sideritis raeseri* Boiss & Heldr subsp. *Raeseri*. Plant Cell Tiss Organ Cult 128:619–629

Sharan S, Sarin NB, Mukhopadhyay K (2019) Elicitor-mediated enhanced accumulation of ursolic acid and eugenol in hairy root cultures of *Ocimum tenuiforum* L. is age, dose, and duration dependent. S Afr J

Bot 124:199–210

Siddiqui MH, Alamri SA, Al-Khaishany MYY, Al-Qutami MA, Ali HA, Khan MN (2017) Sodium nitroprusside and indole acetic acid improve the tolerance of tomato plants to heat stress by protecting against DNA damage. J Plant Interact 12(1):177–186

Singh VK, Umar S, Ansari SA, Iqbal M (2008) *Gymnema sylvestre* for diabetics. J Herbs Spices Med Plants14 (1–2):88–106

Subiramani S, Sundararajan S, Sivakumar HP, Rajendran V, Ramalingam S (2019) Sodium nitroprusside enhances callus induction and shoot regeneration in high value medicinal plant *Canscora diffusa*. Plant Cell Tiss Org Cult 139:65–75

Sugihara Y, Nojima H, Matsuda H, Murakami T, Yoshikawa M, Kimura I (2000) Antihyperglycemic effects of gymnemic acid IV, a compound derived from *Gymnema sylvestre* leaves in streptozotocin-diabetic mice. J Asian Nat Prod Res 2(4):321–327

Tabatabaei-Malazy O, Larijani B, Abdollahi M (2015) Targeting metabolic disorders by natural products. J Diabetes Metab Disord 14: 57

Thorat AS, Sonone NA, Choudhari VV, Devarumath RM, Babu KH (2017) Plant regeneration from cell suspension culture in *Saccharumofficinarum* L. and ascertaining of genetic fidelity through RAPD and ISSR markers. 3 Biotech 7:16

Tran N, Pham B, Le, L (2020) Bioactive compounds in anti-diabetic plants: from herbal medicine to modern drug discovery. Biology 9:252

Tsolmon M, Oyundari G, Yu O, Kalaiselvi S (2020) Establishment of callus and cell suspension culture of *Sophora alopecuroides* Linn. for the production of oxymatrine. J Phytol 12:035–039

Vieira R, Souto SB, Sanchez-Lopez E, Machado AL, Severino P, Jose S, Santini A, Fortuna A, Garcia ML, Silva AM, Souto EB (2019) Sugar-lowering drugs for type 2 diabetes mellitus and metabolic syndrome-review of classical and new compounds: Part-I. Pharmaceuticals 12(4):152

Vijendra PD, Jayannaa SG, Kumar V, Torankumar Sannabommaji T, Rajashekar J, Hari Gajula H (2020) Product enhancement of triterpenoid saponins in cell suspension cultures of *Leucas aspera* Spreng. Ind Crops Prod 156:112857

Wang JW, Zheng LP, Zhang B, Zou T (2009) Stimulation of artemisinin synthesis by combined cerebroside and nitric oxide elicitation in *Artemisia annua* hairy roots. Appl Microbiol Biotechnol 85:285–292

Xu J, Yin H, Wang W, Mi Q, Liu X (2009) Effects of sodium nitroprusside on callus induction and shoot regeneration in micropropagated *Dioscorea opposita*. Plant Growth Regul 59:279–285

Xu M, Dong J (2005) Elicitor-induced nitric oxide burst is essential for triggering catharanthine synthesis in *Catharanthus roseus* suspension cells. Appl Microbiol Biotechnol 67:40–44

Xu M, Dong J, Zhang G (2005) Enhancement of hypericin production and cell growth of *Hypericum perforatum* L. suspension culture by nitric oxide. Chin J Biotechnol 21:66–70

Yoshikawa K, Nakagawa M, Yamamoto R, Arihara S, Matsuura K (1992) Antisweet natural products. V. Structures of gymnemic acids VIII-XII from *Gymnema sylvestre* R. Br. Chem Pharm Bull 40:1779–1782

Zare-Hassani E, Motafakkerazad R, Razeghi J, Kosari-Nasab M (2019) The effects of methyl jasmonate and salicylic acid on the production of secondary metabolites in organ culture of *Ziziphora persica*. Plant Cell Tiss Org Cult 138:437–444

Figures

Fig. 1

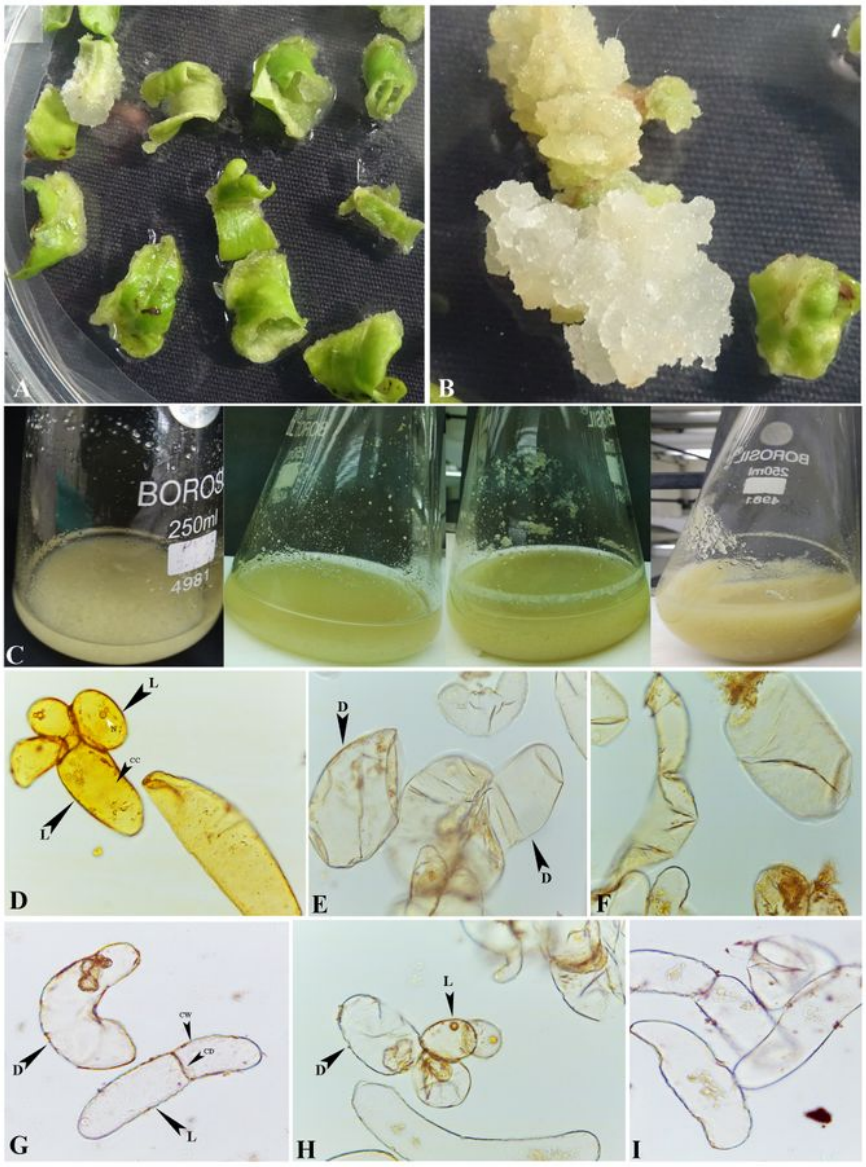
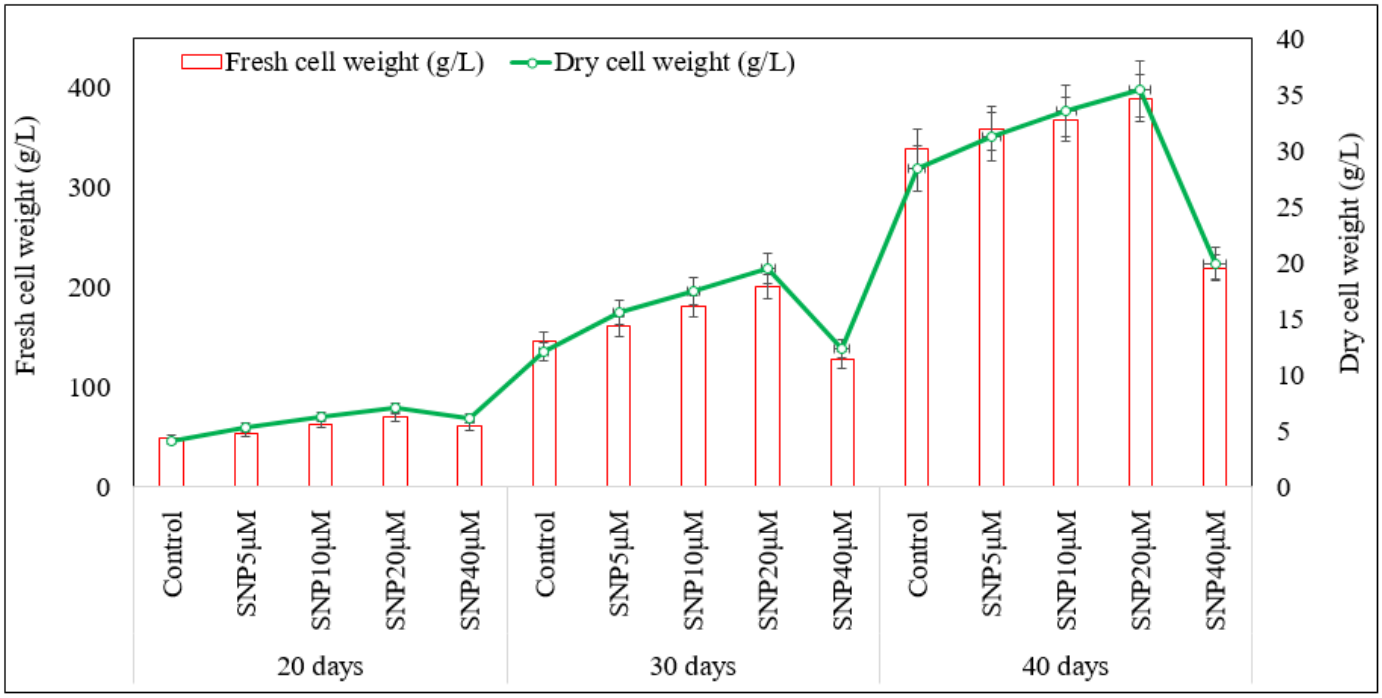


Figure 1

Callus induction and cell suspension of G. sylvestre A. Induction of callus on MS medium supplemented with 3.0 mg/L (2,4-D) + 1.0 mg/L (Kin). B. Induced optimal callus. C. Different growth stage of cell suspension culture 0. 20, 30 and 40 days. D. Microscopic view of cell aggregates showing round and elongated cells (10x). E. Shrinkage of cells F. Shrinkage and death cells G. Cell division H. Live cell I.

Cytoplasm shrinkage viable/live (L); non-viable/dead cell (D); cell wall (CW); cytoplasmic contents (CC); nucleus (N); Cell division (CD)



Values represent mean ±standard deviation (SD) of five replicates

Figure 2

Growth index of G. sylvestre cell suspension culture indicating the fresh and dry biomass of cells and time intervals (20th, 30th and 40th day).

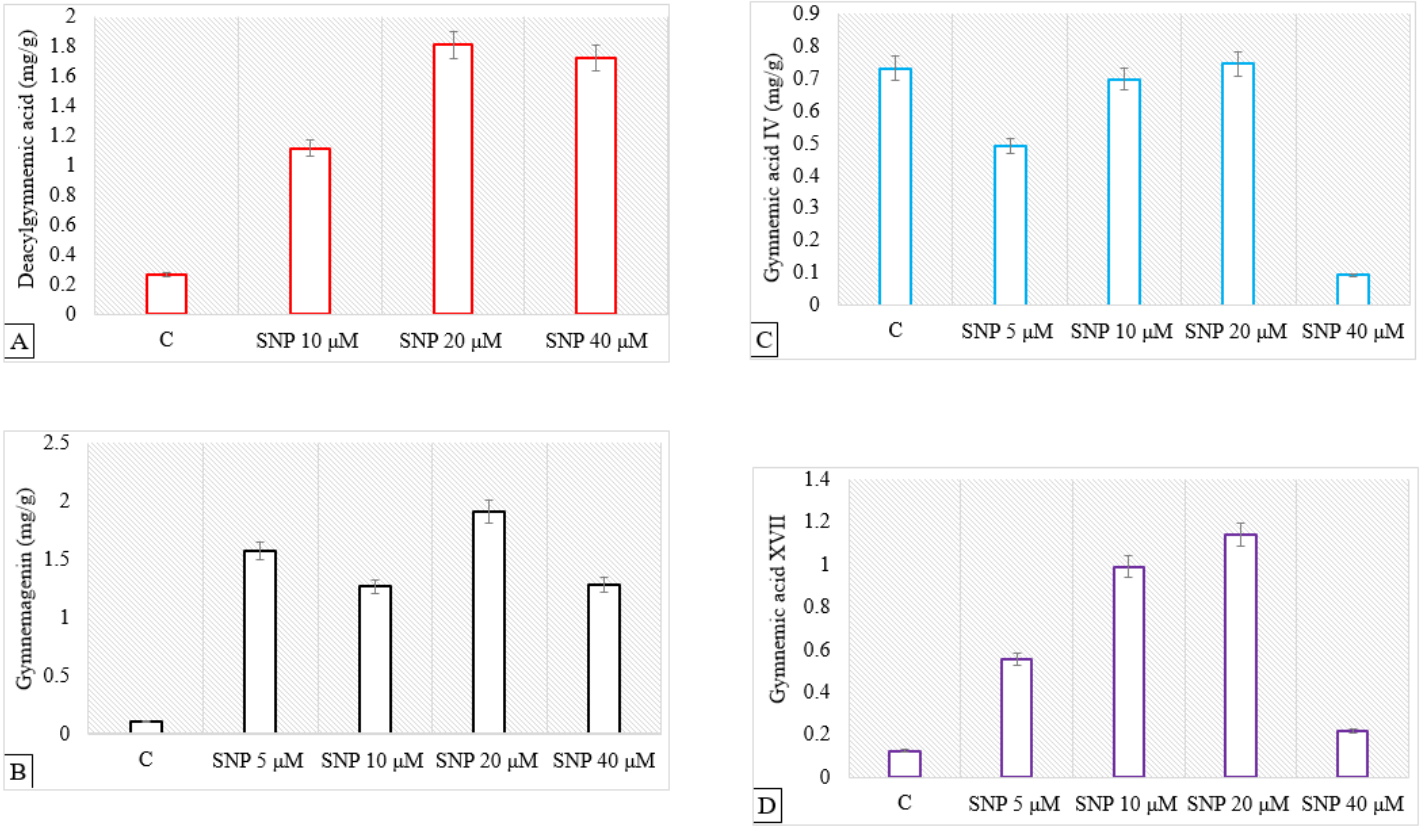


Figure 3

Effect of different concentrations of SNP on deacylgymnemic acid (A), gymnemagenin (B), gymnemic acid IV (C) and gymnemic acid XVII (D) content in cell suspension cultures of G. sylvestre at 20 days

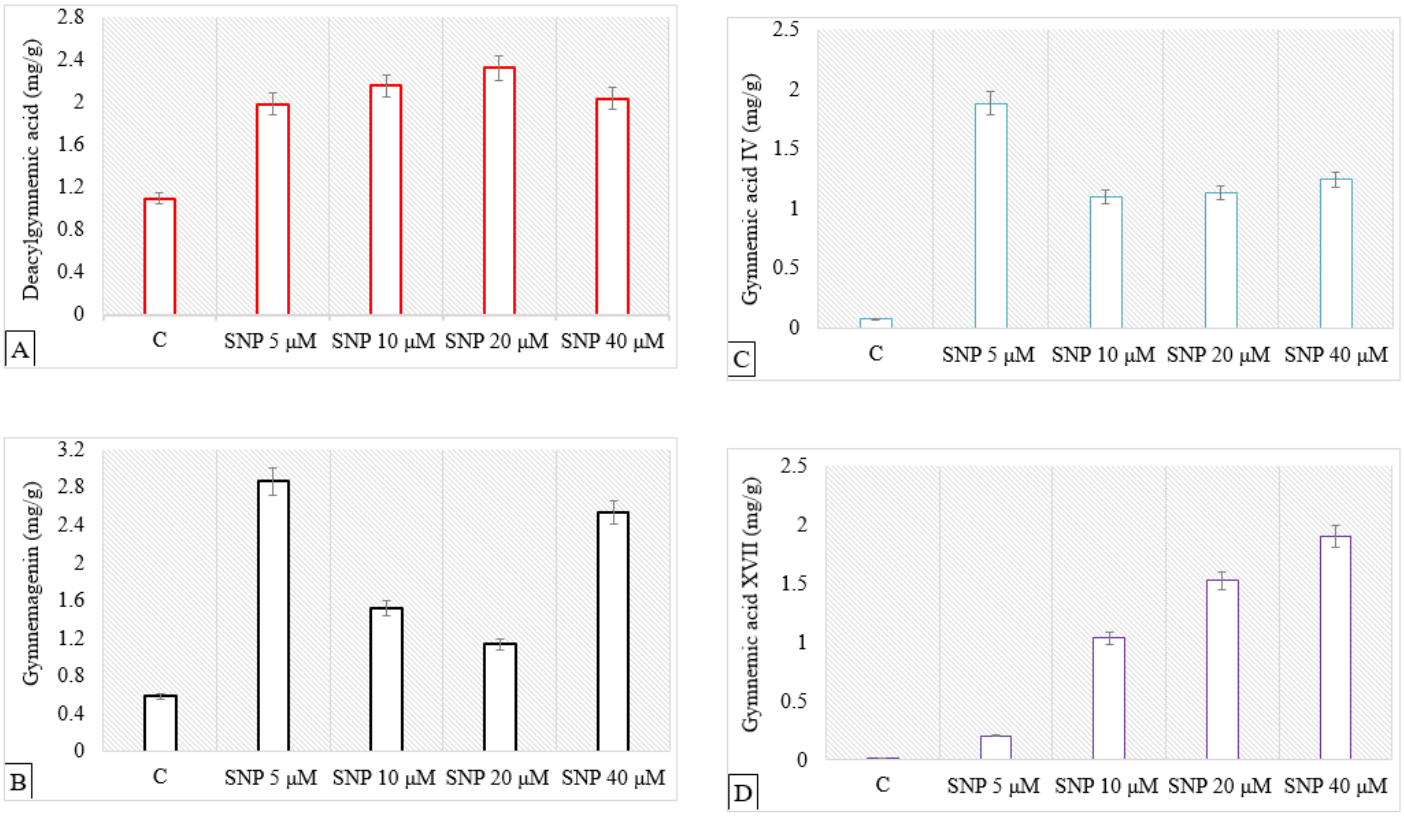


Figure 4

Effect of different concentrations of SNP on deacylgymnemic acid (A), gymnemagenin (B), gymnemic acid IV (C) and gymnemic acid XVII (D) content in cell suspension cultures of G. sylvestre at 30 days

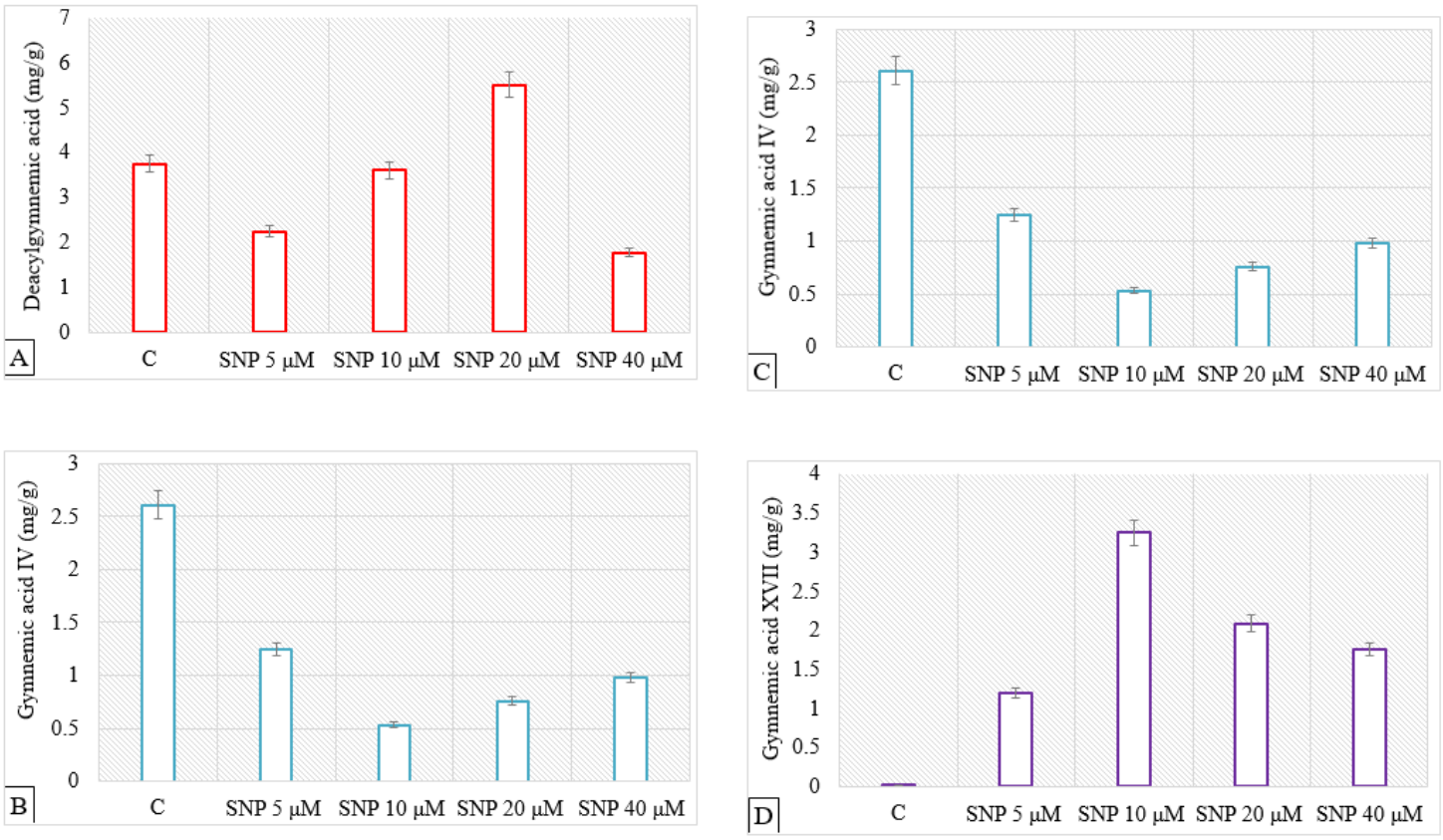


Figure 5

Effect of different concentrations of SNP on deacylgymnemic acid (A), gymnemagenin (B), gymnemic acid IV (C) and gymnemic acid XVII (D) content in cell suspension cultures of G. sylvestre at 40 days

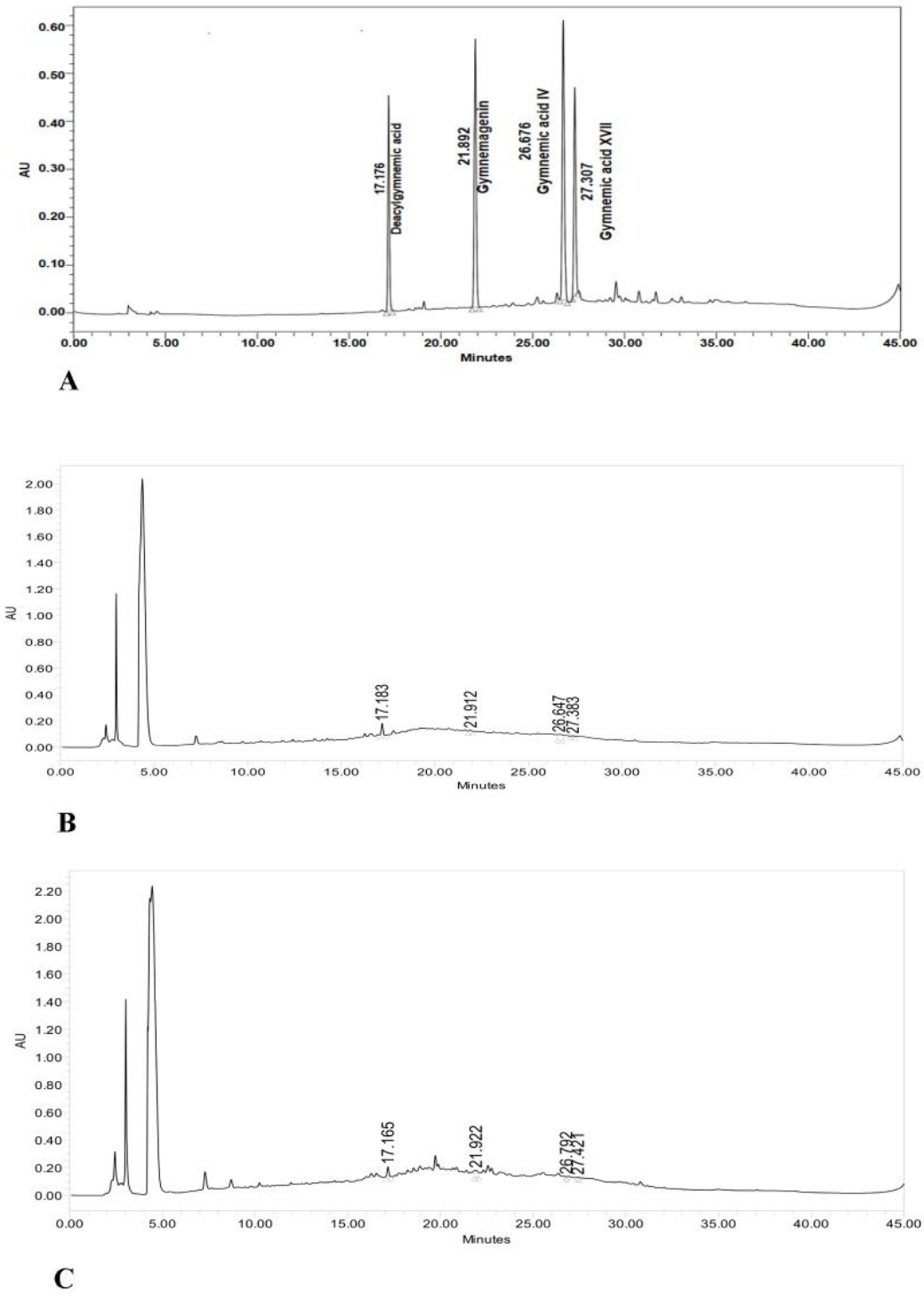


Figure 6

HPLC chromatogram of G. sylvestre suspension cells A. HPLC chromatogram of 4 standard mixed solutions. B. 20 μM SNP treatment at 30 days C. 40 μM SNP treatment at 30 days