

Enhancement of Bioactive Compounds in *Gymnema sylvestre* cell Suspension Cultures Using Green-synthesized Silver Nanoparticles

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Abstract

Gymnema sylvestre (Retz.) R.Br.ex Sm. is a medicinally important plant. It has a diverse array of pharmacologically important bioactive metabolites such as gymnemic acids, gymnemagenin, gymnemasaponins, phenolic compounds, etc. The increasing demand for these phytochemicals necessitates the development of sustainable production strategies independent of field cultivation. In the present study, phytosynthesized silver nanoparticles (AgNPs) are assessed as nano-elicitor to enhance the production of bioactive compounds in suspension culture of *G. sylvestre*. Silver nanoparticles were synthesized using *Pteris vittata* L. frond extract. Established cell suspension cultures were treated with 1, 3 and 5 ppm of AgNPs for 3, 5 and 7 days. Effects of different concentrations and treatment period on cell growth and accumulation of bioactive compounds were investigated. Among various concentrations 5ppm AgNP treatment for 3 days promoted maximum production of targeted bioactive compounds compared with untreated controls. LCMS analysis confirmed elevated levels of some gymnemic acids (viz., II, V, IX, XII, XV and XVII), gymnemanol, dihydroxy gymnemic triacetate, β -amyrin related glycoside and Gymnamine (8-Hydroxy gymnamine). These findings demonstrated that phytosynthesized silver nanoparticles serve as effective nano elicitors for enhancement of bioactive metabolite production. This study thereby highlights the potential for integration of green nanotechnology and plant tissue culture techniques for the commercial exploitation of medicinal plants and the sustainable production of pharmaceutically important metabolites.

1. Introduction

Medicinal plants represent a rich reservoir of structurally diverse bioactive compounds that have long been utilized in traditional and modern healthcare systems. Among them, *Gymnema sylvestre* (Retz.) R. Br. ex Sm., a perennial woody climber belonging to family Apocyanaceae is one of the most important medicinal plants used in Ayurveda for the management of diabetes mellitus and other metabolic disorders. The plant is commonly known as “Madhunashini”, “Gurmar” or “Sugar destroyer” due to its unique ability to suppress sweet taste perception and regulate blood glucose levels [1], [2]. The therapeutic significance of *G. sylvestre* is primarily attributed to its diverse phytochemical constituents, including gymnemic acids, gymnemasaponins, gurmarin, flavonoids, phenolic compounds and triterpene saponins. These metabolites exhibit antidiabetic, anti-oxidant, anti-inflammatory, antimicrobial, hepatoprotective and immunomodulatory activities which makes this plant an important pharmaceutical resource [3–5].

The increasing global demand for plant derived therapeutics has intensified the exploitation of natural populations of medicinal plants. In the case of *G. sylvestre*, extensive harvesting coupled with habitat destruction and slow natural propagation has raised concerns regarding sustainable utilization of the species. Furthermore, the production and accumulation of secondary metabolites in field grown plants is often influenced by environmental factors, geographical locations and seasonal variations, leading to inconsistent phytochemical yields. Consequently, the development of alternative strategies for sustainable and controlled production of valuable bioactive compounds are required [6–8].

Plant tissue culture technology offers a promising platform for the production of valuable phytochemicals under controlled conditions [9]. Among various *in vitro* systems, cell suspension cultures (CSC) are considered particularly advantageous due to rapid biomass accumulation, genetic uniformity, ease of scale up for commercial production and compatibility with bioreactor systems [10]. However, metabolite accumulation in cultured cells is often lower than that observed in *in vivo* plants thus limiting their commercial application and necessitating development of strategies to enhance the production of secondary metabolites.

Elicitation has emerged as one of the most promising approaches for enhancing the biosynthesis of bioactive compounds in *in vitro* cultures [11]. Elicitor is a substance which when introduced in small concentrations to a living system, initiates or enhances the production of specific compounds [12]. Elicitors when used in optimum concentrations, activate plant defense mechanisms through modulation of signal transduction pathway thereby triggering biosynthesis and accumulation of secondary metabolites [13]. Based on their origin elicitors may be classified as biotic or abiotic. Biotic elicitors include the crude extract of bacteria or fungal hyphae or the purified organic molecules such as polysaccharides, glycoproteins, chitin, methyl jasmonate, salicylic acid, etc. while abiotic elicitors often comprise of salts of heavy metals, mannitol, sorbitol, sodium chloride, potassium chloride, UV-radiation, temperature shifts, salinity, etc. Nevertheless, recent advances in nanotechnology have introduced use of nanoparticles as novel elicitors [14–16].

Nanoparticles are molecular structures which range in size from 1 to 100 nm [17]. These nanoparticles differ in their physical properties compared to their bulk counterparts due to difference in their surface to volume ratio [18]. This enables nanoparticles to efficiently penetrate the plant cells and modulate specific physiological and biochemical responses resulting in enhanced production of secondary metabolites. Among the range of nanoparticles, silver nanoparticles have attracted considerable attention due to their stability, catalytic and antibacterial activities, etc. [19]. Synthesis of nanoparticles can be done by physical, chemical, or biological methods. Physical and chemical methods are costly, laborious and require hazardous chemicals. While, biological methods which involve use of plant extracts or certain non-harmful bacteria, are eco-friendly. Such biosynthesized NPs are biocompatible making them suitable for applications in plant biotechnology along with other biological areas [19–22].

Despite the medicinal and pharmaceutical importance of *G. sylvestre* and widespread biological applications of nanoparticles, studies on the influence of phytosynthesized AgNPs on production of bioactive compounds in *G. sylvestre* cell suspension culture are limited. Therefore, the present investigation was carried out to study the effect of phytosynthesized AgNPs on the bioactive compound production in *G. sylvestre* cell suspension culture.

2. Materials and Methods

Collection of plant material: Plant material was collected from the Botanical garden, Department of Botany, Fergusson college, Pune. Its authentication was confirmed from Botanical Survey of India, Western Regional Circle (BSIWRC), Pune (voucher number: BSI/WRC/PI.Id.2026/PM01)

Cell Suspension culture

Callus culture was established using *G. sylvestre* leaf explant. Leaves were washed under running tap water for 10 min. it was then surface sterilized with 1% tween 20 for 3 min followed by 3% Bavistin treatment for 5 min. sterilization was then carried out using 70% ethanol followed by 0.1% mercuric chloride (HgCl_2). Leaf lamina was then cut into approximately 1 cm^2 pieces. These explants were incubated on basal MS media containing 8.0 g/L agar and 30 g/L sucrose. The media was supplemented with 2 mg/L 2,4-D and 1 mg/L BAP. Cultures were maintained at $25 \pm 2^\circ\text{C}$ temperature with a light/dark cycle of 16h/8 h for 5 weeks. Friable callus (0.25 g) was then transferred to basal MS media solution supplemented with 2 mg/L 2,4-D and 1 mg/L BAP. The flasks were aerated at 100 rpm along with aforementioned conditions.

Elicitation of CSC with AgNPs

AgNPs were phytosynthesized and characterized according to method modified from Limaye et.al. (2025). Green synthesized AgNPs (1, 3 and 5 ppm) were added to the CSC at day 1. The treatment was given for the period of 3, 5 and 7 days. After the completion of treatment period, growth analysis was carried out and the cells were dried at 60°C for LCMS analysis. Non elicited CSC were maintained for each treatment.

LCMS: Biochemical analysis of the treated and control samples was carried out by Liquid chromatography mass spectrometry technique with Agilent 6540 Q-ToF (Quadrupole Time-of-Flight) mass spectrometer. A Quadrupole Time-of-Flight (QTOF) mass spectrometer equipped with electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), and atmospheric solids analysis probe (ASAP) sources was used for high-resolution mass spectrometric analysis. It provided mass accuracy within 1 ppm (for $m/z < 500$) and MS/MS accuracy within 2 ppm, with a resolving power of 42,000 at m/z 2722 and a signal-to-noise ratio of 450:1 and the capability to simultaneously monitor approximately 50 precursor ions, enabling accurate qualitative and quantitative analyses of complex samples.

3. Results

3.1 Callus development: Callus cultures were successfully initiated from *G. sylvestre* leaf explants. Pale green to yellow friable callus was developed after 4 weeks of callus induction (Fig. 1).

3.2 Effect of AgNPs on growth index in CSC: As shown in Table 1 the growth index of *G. sylvestre* CSC increased steadily in the control treatment from 0.20 at inoculation to 0.74 after 7 days of cultivation, indicating active cell proliferation under normal culture conditions. In contrast, nanoparticle treated

cultures exhibited reduced growth throughout the experimental period. Among the treatments, T1 (1 ppm) maintained a relatively stable growth index between 0.23 and 0.27, whereas T2 (3 ppm) and T3 (5 ppm) showed a gradual decline with increasing culture duration. The lowest growth index (0.16) was recorded in T3 on day 7, suggesting a pronounced inhibitory effect on cell growth.

Table 1. Growth Index of AgNP treatment

AgNP treatment	Control	T1	T2	T3
3 days	1	0.35	0.4	0
5 days	1.8	0.15	0.15	0.1
7 days	2.7	0.35	0	-0.2

T1 = 1 ppm AgNP, T2 = 3 ppm AgNP and T3 = 5 ppm AgNP

3.3 Effect of AgNPs on bioactive compounds production in CSC: LCMS profiling revealed significant alterations in the secondary metabolite composition of *G. sylvestre* CSC following silver nanoparticle elicitation (Table 2) (additional data are given in Dataset 1, 2, 3 and 4). Several bioactive triterpenoids, saponins, particularly Gymnemic acid II, V, IX, XII, XV and XVII, exhibited enhanced accumulation compared with the untreated control. Gymnemic acid VI was detected exclusively in AgNP treated cultures, indicating elicitor induced activation of otherwise silent biosynthetic pathways. Furthermore, substantial increases in kaempferol and stigmasterol levels suggest stimulation of both phenylpropanoid and terpenoid metabolism. B-amyrin-related glycosides also indicated activation of otherwise silent biosynthetic pathway.

Conversely, reductions in certain gymnemosides and gymnemasaponins imply a redistribution of metabolic flux towards the synthesis of key bioactive metabolites.

Table 2. Abundance of bioactive compounds of *G. sylvestre* in control and AgNP treated cells

Sr. No.	Name of compound	Abundance in Control	Abundance in AgNP treated cells
1	Gymnemic Acid I	9292.32	6905.7
2	Gymnemic Acid II	2238.56	12500.8*
3	Gymnemic Acid V	13474.64	21834.96*
4	Gymnemic Acid VI	ND	2875.46*
5	Gymnemic Acid VII	17670.82	9309.51
6	Gymnemic Acid VIII	47658.44	12775.39
7	Gymnemic Acid IX	5671.74	11179.83*
8	Gymnemic Acid X	1753.92	2850.02*
9	Gymnemic Acid XII	6235.4	9366.43*
10	Gymnemic Acid XIII	19726.96	9373.56
11	Gymnemic Acid XIV	38358.7	28821.04
12	Gymnemic Acid XV	5387.52	9479.16*
13	Gymnemic Acid XVIII	8154.46	17013.47*
14	Gymnemagenin	2179.67	713.82
15	Gymnemasaponin II	27661.8	16192.75
16	Gymnemasaponin III	3257.24	7909.08*
17	Gymnemasaponin IV	13085.84	ND
18	Gymnemasaponin V	2953.7	ND
19	Gymnemoside C	45092.32	21297.56
20	Gymnemoside D	9481.7	2888.81
21	Gymnemoside F	4573.7	ND
22	Gymnemasin A	41974.24	8502.51
23	Gymnemasin B	11008.68	1941.44
24	Gymnemasin C	5545.1	5447.24
25	Gymnemanol	2725	4192.58*
26	Prosapogenin	4476.7	2305.98
27	Dihydroxy gymnemic triacetate	3258.54	3332.22*

Sr. No.	Name of compound	Abundance in Control	Abundance in AgNP treated cells
28	Kaempferol	5723.74	11484.58*
29	Stigmasterol	71138.56	32608.3
30	β -amyrin related glycoside	ND	2911.14*
31	Gymnamine (8-Hydroxy gymnamine)	1903.5	5677.42*
32	Deacyl gymnemic acid	75180.12	32778.32

ND = Not detected, * high abundance

4. Discussion

The observed reduction in biomass accumulation may be attributed to AgNP induced physiological stress, which can alter cellular metabolism diverting carbon fluxes and other cellular resources toward defense mechanisms. Nair & Chung, (2014) have also reported reduction in biomass in *Arabidopsis thaliana* due to stress caused by nanoparticles during elicitation treatment. Such growth suppression is commonly associated with elicitation responses and may contribute to enhanced secondary metabolite biosynthesis. Ghorbanpour & Hadian, 2015, have reported increased production of secondary metabolites associated with decreased biomass production. Although Nazir et. al. 2021 have reported contradictory results. Further, phytochemical and metabolomic analyses are required to establish the relationship between AgNP induced stress and bioactive compound production.

Gymnemic acids are the principle antidiabetic constituents of *G. sylvestre*. Their increased production indicates that AgNP stimulates the triterpenoid biosynthetic pathway, likely through activation of oxidative stress signalling and defense related mechanism. Substantial increase in Gymnema saponin III and disappearance of Gymnema saponins IV and V and also of gymnemosides after elicitation may be attributed to redistribution of metabolic flux towards specific saponins and gymnemic acids under elicitation stress. Increased production of Kaempferol which is a potent antioxidant flavonoid suggests activation of the phenylpropanoid pathway in response to AgNP induced stress. Similarly, elevated stigmasterol levels indicate increased phytosterol production which is often associated with membrane stabilization under stress conditions. Previously, studies conducted by Chung et al., (2018, 2018) in *Cucumis anguria* cell suspension culture and *Momordica charantia* hairy root culture, respectively, demonstrated that silver nanoparticles act as effective elicitors, significantly enhancing the accumulation of secondary metabolites. [28], reported a significant enhancement in biosynthesis and accumulation of secondary metabolites in *Linum usitatissimum* cell suspension cultures following elicitation with silver nanoparticles. [29], reported that AgNP mediated elicitation enhanced the production of secondary metabolites in *Caralluma tuberculata*.

5. Conclusion

The present study strongly demonstrates that AgNP elicitation improves the production of several antidiabetic bioactive compounds, particularly gymnemic acids, making it a promising strategy for metabolic enhancement in *G. sylvestre* CSC. Thus, nano-elicitation offers a promising, sustainable and scalable path for enhanced production of high value bioactive compounds.

Declarations

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Author contributions: Conceptualization and supervision A.L.; formal analysis, D.B.; investigation, P.M.; data curation, P.M.; writing—original draft preparation, P.M.; writing—review and editing, P.M. All authors have read and agreed to the published version of the manuscript.

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Data availability: The datasets generated during the current study are available from the corresponding author.

Ethics approval and consent to participate: Ethical approval was not required for this study because it involved only plant material and did not involve human participants and animals.

Consent for publication: Not Applicable.

Permissions for collection: The plant material (*Gymnema sylvestre*) was collected from Botanical Garden, Department of Botany, Fergusson College, Pune. Its identification was done by Ms. Madhuri Pawar, Botanist, Botanical Survey of India, Western Regional Circle (BSIWRC), Pune. Voucher specimen was deposited at BSIWRC (voucher number: BSI/WRC/Pt.Id.2026/PM01).

The plant material (*Gymnema sylvestre*) was collected in compliance with relevant local and national guidelines of India, including the regulations under the Biological Diversity Act. The plant material was obtained from a non-endangered species and non-protected area. Therefore, collection and use of the plant material did not require specific permission under applicable regulations.

Competing interest: Authors do not have any competing interest.

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Figures

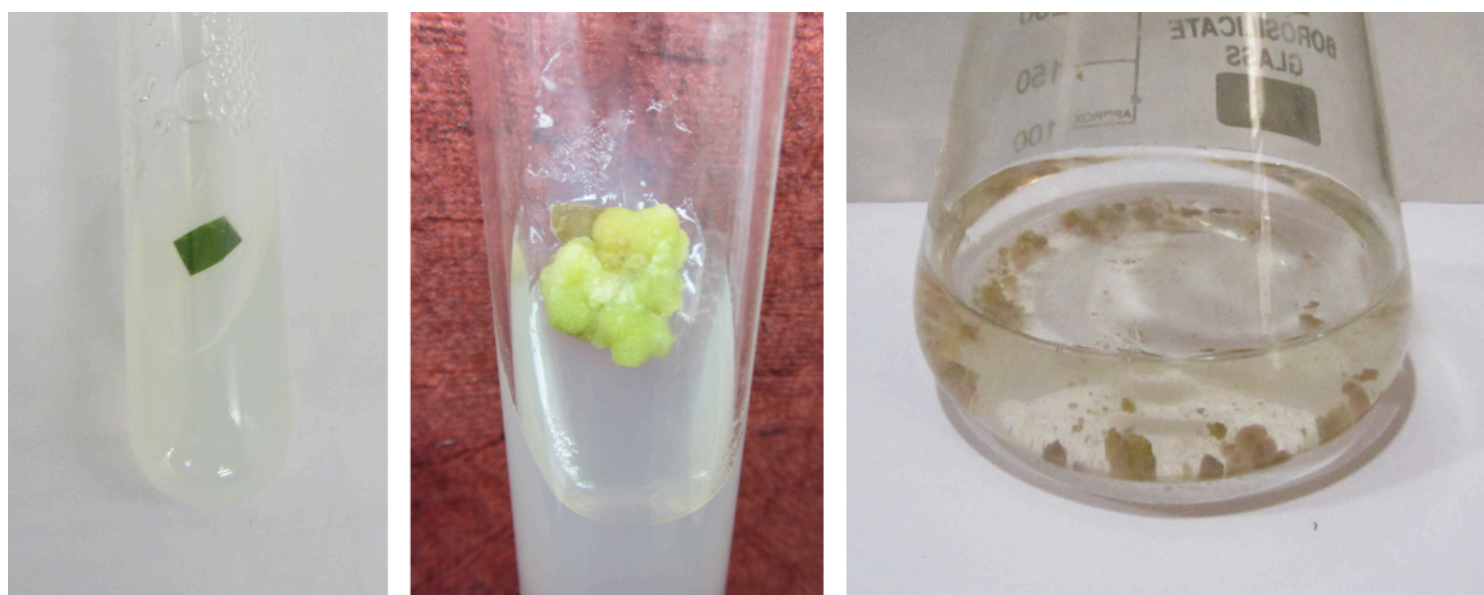


Fig. 1. a) *G. sylvestre* leaf explant b) friable callus c) cell suspension culture

Figure 1

See image above for figure legend

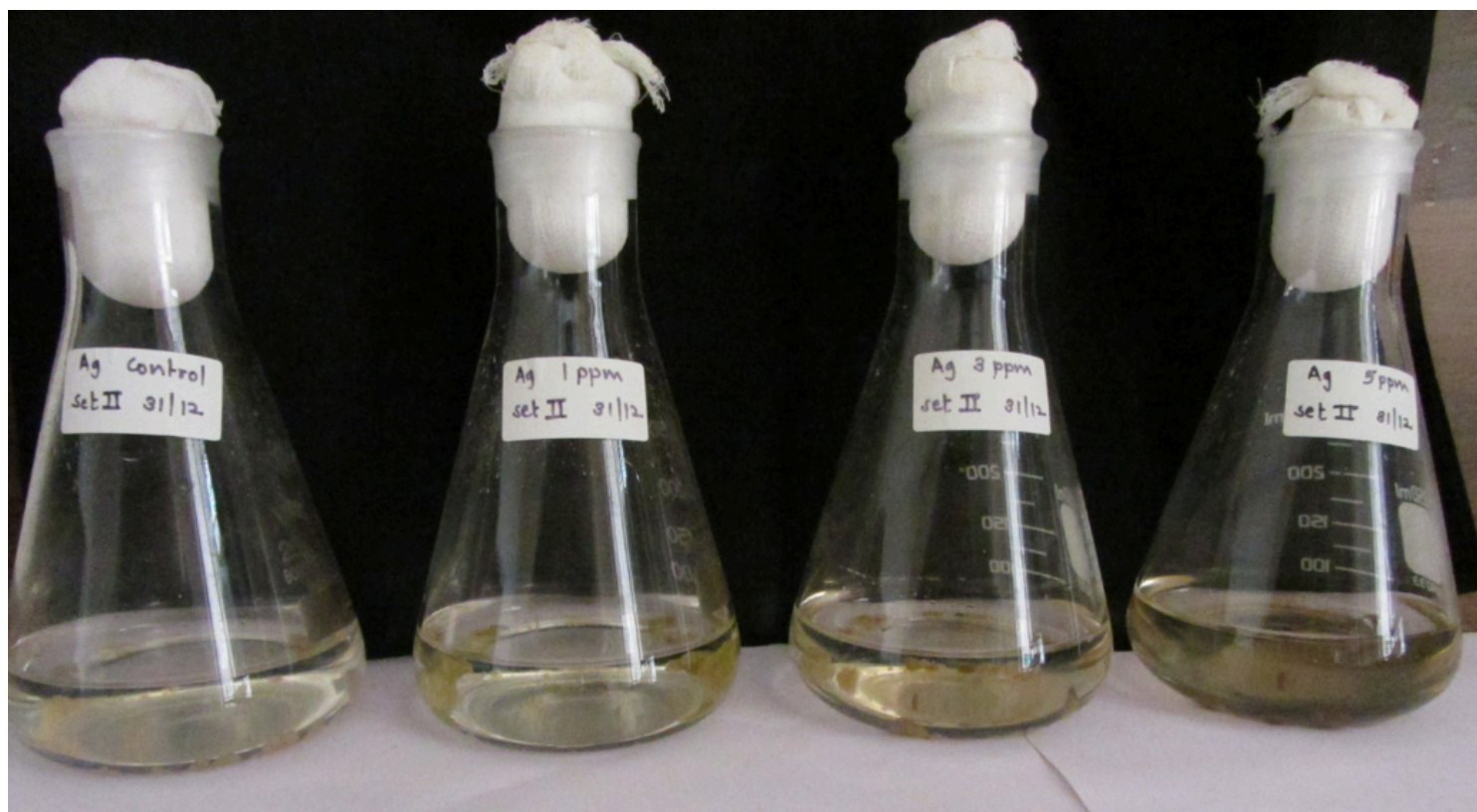


Fig. 2. AgNP treatment to CSC

Figure 2

See image above for figure legend

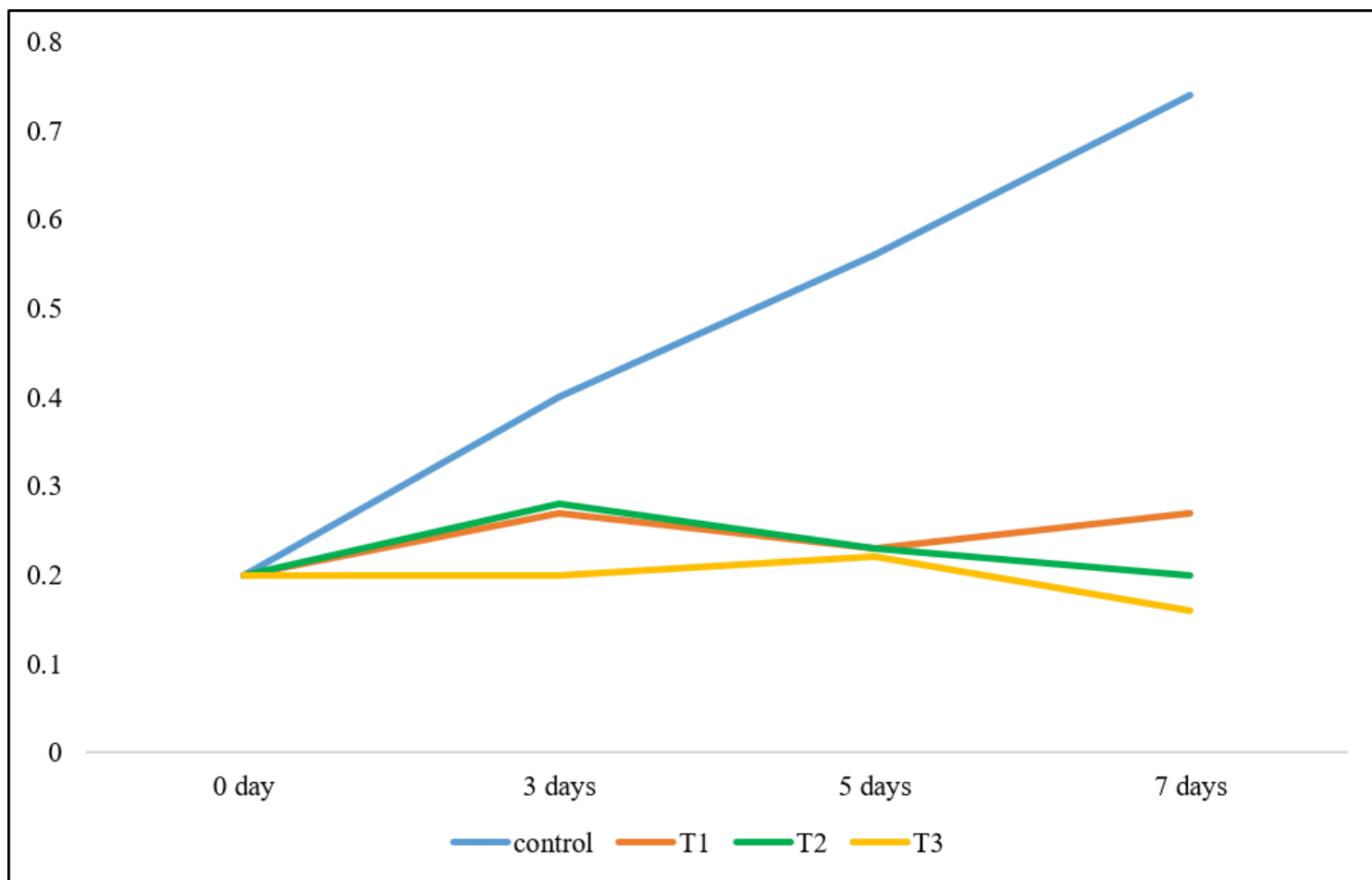


Fig. 3. Growth analysis of cells after AgNP treatment

Figure 3

See image above for figure legend

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